# Expression of glutathione S-transferase A1, a phase II drug-metabolizing enzyme in acute hepatic injury on mice

XIN MA[1], FANGPING LIU[1], MINMIN LI[1], ZHI LI[1], YUEXIA LIN[1], RUI LI[1], CHANGWEN LI[2], YICONG CHANG[1], CHANGWEI ZHAO[1], QING HAN[1], QIONG ZHOU[1], YULIN ZHAO[1], DENING WANG[1] and JINGLI LIU[2]

[1]College of Veterinary Medicine, Northeast Agricultural University, Harbin, Heilongjiang 150030;
[2]Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, Heilongjiang 150069, P.R. China

**Abstract.** In the present study, three models of acute liver injury in mice were induced via the administration of $CCl_4$ (35 mg/kg, 24 h), acetyl-para-aminophenol (APAP; 200 mg/kg, 12 h) and ethanol (14 ml/kg, 8 h) to study the effect of glutathione S-transferase A1 (GSTA1) on acute liver injury. The serum levels of alanine transaminase, aspartate transaminase and liver homogenate indicators (superoxide dismutase, glutathione and glutathione peroxidase) were significantly lower in model groups compared with the control group (P<0.01), whereas the liver homogenate indicator malondialdehyde was significantly increased (P<0.01). The expression of GSTA1 in liver was significantly decreased in the model groups compared with the control group (P<0.01). GSTA1 protein content was 3.8, 1.3 and 2.6 times lower in the $CCl_4$, APAP and ethanol model groups, respectively. Furthermore, GSTA1 mRNA expression levels decreased by 4.9, 2.1 and 3.7 times in the $CCl_4$, APAP and ethanol model groups, respectively. Among the three models, the injury induced by $CCl_4$ was the most marked, followed by ethanol and finally APAP. These results suggest that GSTA1 may be released by the liver and serve as an antioxidant in the prevention of liver damage.

## Introduction

Liver disease poses a serious threat to human health and food safety, as consumption of animals with liver disease may be detrimental to health (1). Acute liver injury is the common pathway and initiating factor of many liver diseases, such as acute liver failure (2). Three models of liver injury are typically used in research as they are representational and reflect the situation of hepatotoxicity comprehensively and intuitively (3). $CCl_4$ is a classical hepatotoxicant, which is able to induce reactive oxygen formation and deplete glutathione (GSH) (4). Acetyl-para-aminophenol (APAP) hepatotoxicity is induced by the electrophile N-acetyl-p-benzoquinoneimine (NAPQI), which is able to induce mitochondrial dysfunction and oxidative stress, leading to liver damage (5). The major etiological factors of hepatotoxicity in ethanol-induced hepatic injury are oxidative stress and inflammatory responses (6). Due to the complexity of liver function and the diversity of liver damage factors, experimental animal models are not able to accurately and fully reflect the nature of liver injury (7). Furthermore, existing animal models have various limitations such as non-standardized methods, lack of reproducibility and non-unified methods (8).

Glutathione S-transferases (GSTs) are enzymes that are able to protect cells from damage caused by reactive oxygen species (9). GSTA (α class GST) serves an important cytoprotective role in detoxifying reactive electrophiles and products of lipid peroxidation (10). GSTs including GSTA have previously been identified as inhibitors of stress-activated kinase activity, most notably c-Jun N-terminal kinase (11). This suggests that altered GST expression may be an important factor in modulating the cellular transition between proliferation, differentiation, and apoptosis, as well as in the pathogenesis of various inflammatory, degenerative and neoplastic diseases.

GSTA1 is a member of the α family GST gene superfamily. In human and rat liver cells, the dominant GST activity and content is GSTA, which is encoded by a gene cluster located on chromosome 6p12 (12). GSTA1 may be used as an indicator of hepatic injury in chronic hepatitis C, low platelet syndrome cystic fibrosis or liver transplant patients (13). Furthermore, GSTA1 serves as a crucial role in the GSH binding reaction. In normal liver and kidney tissues, GSTA1-encoded dimeric protein expression is high and serves an important role in the anti-oxidative defense system (14). It is able to catalyze many xenobiotics, such as carcinogens, environmental toxins and certain pharmacological agents, by combining with GSH to promote the degradation of these substances (15).

Previous studies have reported that GSTA1 is important for the early diagnosis and treatment of liver injury (16). The present study evaluated the expression of GSTA1 in acute liver injury, and explored the importance of GSTA1 variation between liver injury models. The aim of the present study

was to elucidate the protective effects and the molecular mechanisms of GSTA1 to provide a foundation for future investigations of hepatoprotective agents.

## Materials and methods

*Reagents*. $CCl_4$ and ethanol were purchased from the third Chemical Industry (Shanghai, China). APAP was purchased from Shanghai Aladdin Biochem Technology Co., Ltd. (Shanghai, China). Detection kits for alanine transaminase (ALT; cat. no. C009-1), aspartate aminotransferase (AST; cat. no. C010-1), superoxide dismutase (SOD; cat. no. A001-1), malondialdehyde (MDA; cat. no. A003-1), GSH (cat. no. A006-1) and GSH peroxidase (GSH-Px; cat. no. A005) were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The GSTA1 ELISA kit (cat. no. DRE30790) was purchased from RapidBio Systems, Inc. (Tucson, AZ, USA). TRIzol reagent was purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). PrimeScript™ RT reagent kit was purchased from Takara Bio Inc. (Otsu, Japan). For quantitative polymerase chain reaction (qPCR), the TransStart Top Green qPCR SuperMix kit was purchased from Beijing TransGen Biotech Co., Ltd. (Beijing, China).

*Animals and treatment*. A total of 412 8-week-old male Kunming mice (18-22 g), were purchased from the Harbin Pharmaceutical Group Co., Ltd. General Pharm. Factory Laboratory Animal Centre Harbin, China). Mice were raised in controlled conditions at 20±2˚C, 12 h light/dark cycle, 40-60% relative humidity and given *ad libitum* access to food and water, and were acclimated to laboratory conditions for at least 1 week prior to the experiment. All procedures where mice were used complied with the China National Institutes of Healthy Guidelines for the Care and Use of Laboratory Animals. All animal experimental procedures were approved by the Ethical and Animal Welfare Committee of Heilongjiang Province, China. Mice were divided into three cohorts for the optimal dose test, optimal time test and the replication of acute hepatic injury model experiments.

*Establishment of acute hepatic injury models*
*Optimal dose tests*. A total of 136 mice were used to establish the optimal dose. The mice were randomly divided into five $CCl_4$ model groups, six APAP model groups and six ethanol model groups (n=8 in each). All animals were fasted for 16 h prior to experiments. Mice in the $CCl_4$ dose groups were administered with 12.5, 25, 35 or 50 mg/kg $CCl_4$, or an equal volume of bean oil as a control. The APAP groups were administered with 1% (100 mg/kg), 1.5% (150 mg/kg), 2% (200 mg/kg), 2.5% (250 mg/kg) or 3% (300 mg/kg) APAP, or an equal volume of physiological saline as a control. Ethanol groups were administered with 10, 12, 14, 16 or 18 ml/kg ethanol, or an equal volume of physiological saline as a control. At the end of the experiment, mice were anesthetized with ether (Shenyang Chemical Reagent Factory, Shenyang, China) and sacrificed by cervical dislocation and serum was collected to detect transaminases.

*Optimal time tests*. A total of 176 mice were used to establish the optimal time. The mice were randomly divided into ten

$CCl_4$ model groups, six APAP model groups and six ethanol model groups (n=8 in each). All mice were fasted for 16 h prior to experiments. Experimental groups were treated with $CCl_4$ (35 mg/kg), APAP (200 mg/kg) and ethanol (14 ml/kg) respectively, whereas control groups were given equal volume of solvent (bean oil or physiological saline). Mice were anesthetized with ether and sacrificed by cervical dislocation at 2, 6, 12, 16, 20, 24, 28, 32 and 48 h for the $CCl_4$ model, at 4, 8, 12, 16 and 20 h for the APAP model and at 4, 6, 8, 10 and 12 h for the ethanol model, and serum was collected.

*Replication of acute hepatic injury models*. A total of 60 mice were randomly divided into two groups for each model ($CCl_4$, APAP and ethanol; n=10 in each group). The results of the optimal dose and optimal time tests were used to replicate three liver injury models. Mice were subsequently anesthetized with ether (Shenyang Chemical Reagent Factory) and sacrificed by cervical dislocation, following which serum and liver were harvested.

Of the mice, 40 were randomly divided into four groups ($CCl_4$, APAP, ethanol and control; n=10 in each group). The results of the optimal dose and optimal time tests were used to replicate three liver injury models. Mice were subsequently anesthetized with ether and sacrificed by cervical dislocation, following which livers were harvested to detect GSTA1.

*Serum ALT and AST measurement*. ALT and AST activities in mice serum were tested using detection kits according to the manufacturer's protocol.

*Liver homogenate indicators measurement*. The livers were prepared as homogenate using a homogenizer (Automatic Sample Quick Grinding Machine, Tissue lyser-24; Shanghai Industrial Development Co., Ltd, Shanghai, China), then SOD, MDA, GSH, GSH-Px were measured using detection kits according to the manufacturer's protocol.

*Histopathological analysis*. Liver tissues of mice were fixed in 10% formalin at room temperature for 24-48 h, embedded in paraffin and sectioned at 5 $\mu$m thickness. Tissues were subsequently stained with hematoxylin and eosin, observed under a light microscope (magnification, x400) and images were captured.

*GSTA1 content detection*. GSTA1 content in liver homogenate was detected using a mouse GSTA1 ELISA kit. The procedure was performed according to the manufacturer's protocol. The resultant color intensity (assessed using the generated standard curve) was proportional to the amount of GSTA1 in the sample.

*Detection of GSTA1 mRNA expression by RT-qPCR*. Total RNA was prepared from 50-100 mg of liver tissue using TRIzol reagent according to the manufacturer's protocol. Isolated RNA (1 $\mu$g/20 $\mu$l reaction volume) was used for first-strand cDNA synthesis using the PrimeScript™ RT reagent kit. The PCR products were separated by gel electrophoresis on 1% (w/v) agarose gels. qPCR studies were performed using an ABI PRISM 7500 Detection System (Applied Biosystems, ; Thermo Fisher Scientific, Inc.). qPCR reaction was carried out with a total reaction volume of 20 $\mu$l containing 10 $\mu$l SYBR

Table I. Primer sequences for reverse transcription-quantitative polymerase chain reaction.

| Name | 5' Forward Primer | 3' Reverse Primer |
|------|-------------------|-------------------|
| GSTA1 | TGGGAATTTGATGTTTGACC | CAGGGCTCTCTCCTTCATGT |
| β-actin | AGCGTCCTGGTCTTGATGTCTGT | GAGGTCCCAGGTAGATGGTGAAT |

GSTA1, glutathione S-transferase A1.

Table II. Changes of ALT and AST in serum and SOD, MDA, GSH and GSH-Px in liver following $CCl_4$-induced acute hepatic injury.

| Group | ALT (IU/l) | AST (IU/l) | SOD (U/mg) | MDA (nmol/mg) | GSH ($\mu$mol/g) | GSH-Px (U/mg) |
|-------|-----------|-----------|-----------|---------------|-----------|---------------|
| Control | 39.10±6.96 | 56.22±9.58 | 78.59±10.55 | 5.12±0.83 | 8.98±1.91 | 311.49±46.55 |
| Model | 925.85±170.77[a] | 650.79±127.85[a] | 53.83±10.97[a] | 8.65±2.73[a] | 5.29±1.55[a] | 221.11±36.48[a] |

Values are expressed as the mean ± standard deviation in each group. [a]$P<0.01$ vs. control. ALT, alanine transaminase; AST, aspartate transaminase; SOD, superoxide dismutase; MDA, malondialdehyde; GSH, glutathione; Px, peroxidase.

Premix Ex Taq, 0.4 $\mu$l ROX Reference Dye and 5 $\mu$M of each primer. Thermocycling conditions were as follows: 94°C for 5 min and then 40 cycles of 94°C for 30 sec, 58°C for 40 sec and 72°C for 40 sec, and a final extension step at 72°C for 5 min. The mRNA levels were normalized against β-actin mRNA. The results are expressed using the $2^{-\Delta\Delta Cq}$ method (17). Primer sequences are listed in Table I.

*Statistical analysis*. All numerical data are expressed as the mean ± standard deviation. Statistical significance was determined using SPSS 19.0 (IBM SPSS, Armonk, NY, USA). Tukey's test for multiple comparisons was used to determine statistical significance. $P<0.05$ was considered to indicate a statistically significant difference.

**Results**

*Optimal dose and time tests.*
*Liver injury induced by $CCl_4$*. The results of liver injury induced by $CCl_4$ are presented in Fig. 1. Serum levels of ALT and AST increased with $CCl_4$ administration in a dose-dependent manner. Mice treated with 25 mg/kg $CCl_4$ had significantly higher serum levels of ALT ($P<0.05$), whereas a significant increase in serum ALT and AST was observed in mice treated with 35 and 50 mg/kg $CCl_4$ compared with the control mice (both $P<0.01$). Serum levels of both ALT and AST reached a peak at 24 h following $CCl_4$ administration. Based on these results, 35 mg/kg $CCl_4$ for 24 h was selected as the optimal model.

*Liver injury induced by APAP*. The results of liver injury induced by APAP are presented in Fig. 2. Serum levels of ALT and AST increased following APAP administration in a dose-dependent manner. Serum levels of AST and ALT were significantly higher in mice treated with 150 ($P<0.05$), 200 and 250 mg/kg APAP (both $P<0.01$) compared with the control mice. Significant increases in serum levels of ALT and AST were observed from 8-20 h compared with control mice (all

$P<0.01$). Serum levels of both ALT and AST peaked at 12 h. Based on these results 200 mg/kg APAP for 12 h was selected as the optimal model.

*Liver injury induced by ethanol*. The results of liver injury induced by ethanol are presented in Fig. 3. Serum levels of ALT and AST increased following ethanol administration in a dose-dependent manner. Serum levels of AST were significantly higher in mice treated with 12 ml/kg ethanol ($P<0.05$), whereas ALT and AST levels were significantly increased with 14, 16 and 18 ml/kg ethanol (all $P<0.01$) compared with control mice. ALT and AST levels significantly increased at 8 h ($P<0.01$), and subsequently decreased. Based on these results, 14 ml/kg ethanol for 8 h was selected as the optimal model.

*Replication of acute hepatic injury models*
*Liver injury model induced by $CCl_4$*. The results of $CCl_4$ liver injury model replication are presented in Table II. A significant increase in serum ALT and AST was observed in the $CCl_4$ model group (35 mg/kg; 24 h) compared with the control group (both $P<0.01$). Furthermore, mice in the $CCl_4$ model group were found to have significantly lower levels of SOD, GSH and GSH-Px (all $P<0.01$) and significantly higher levels of MDA ($P<0.01$) compared with the control group.

*Liver injury model induced by APAP*. The results of APAP liver injury model replication are presented in Table III. A significant increase in serum ALT and AST was observed in the APAP model group (200 mg/kg; 12 h) compared with the control group (both $P<0.01$). Furthermore, mice in the APAP model group were found to have significantly lower serum levels of SOD, GSH and GSH-Px ($P<0.01$) and significantly higher serum levels of MDA ($P<0.01$) compared with the control group.

*Liver injury model induced by ethanol*. The results of ethanol liver injury model replication are presented in Table IV. A significant increase in serum ALT and AST was observed in the ethanol model group (14 ml/kg; 8 h) compared with

Table III. Changes of ALT and AST in serum and SOD, MDA, GSH and GSH-Px in liver following acetyl-para-amino-phenol-induced acute hepatic injury.

| Group | ALT (IU/l) | AST (IU/l) | SOD (U/mg) | MDA (nmol/mg) | GSH ($\mu$mol/g) | GSH-Px (U/mg) |
|---|---|---|---|---|---|---|
| Control | 50.45±6.55 | 60.26±10.85 | 88.64±15.10 | 8.31±0.53 | 12.98±2.01 | 283.24±29.11 |
| Model | 647.71±88.21[a] | 495.82±79.45[a] | 57.54±7.06[a] | 10.41±1.97[a] | 8.37±1.44[a] | 205.44±37.91[a] |

Values expressed as the mean ± standard deviation in each group. [a]P<0.01 vs. control. ALT, alanine transaminase; AST, aspartate transaminase; SOD, superoxide dismutase; MDA, malondialdehyde; GSH, glutathione; Px, peroxidase.
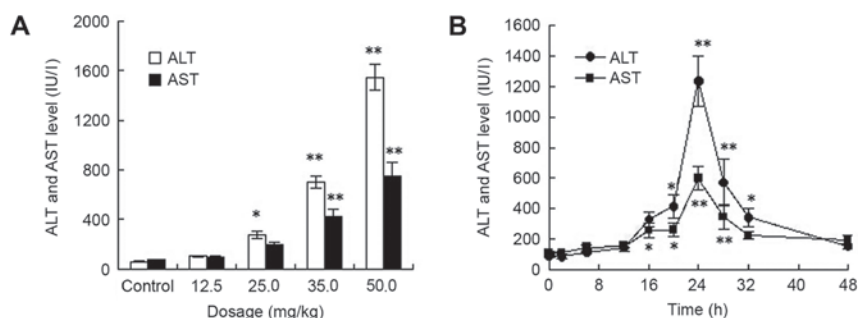


Figure 1. (A) Serum levels of ALT and AST with administration of CCl$_4$ (24 h) at various doses (0, 12.5, 25, 35 and 50 mg/kg) and (B) serum levels of ALT and AST at various times (0, 2, 6, 12, 16, 20, 24, 28, 32 and 48 h) following the administration of CCl$_4$ (0.35%). Values are expressed as the mean ± standard deviation in each group (n=8). *P<0.05, **P<0.01 vs. control. ALT, alanine transaminase; AST, aspartate transaminase.
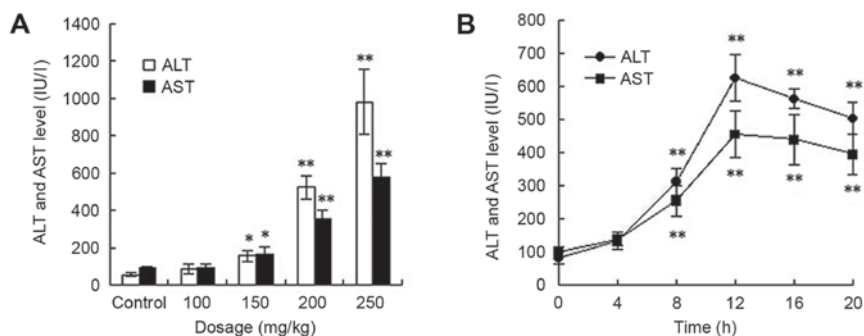


Figure 2. (A) Serum levels of ALT and AST with administration of APAP (12 h) at various doses (0, 100, 150, 200 and 250 mg/kg) and (B) the levels of serum ALT and AST at various times (0, 4, 8, 12, 16 and 20 h) following the administration of APAP (2%). Values are expressed as the mean ± standard deviation in each group (n=8). *P<0.05, **P<0.01 vs. control. ALT, alanine transaminase; AST, aspartate transaminase; APAP, acetyl-para-aminophenol.
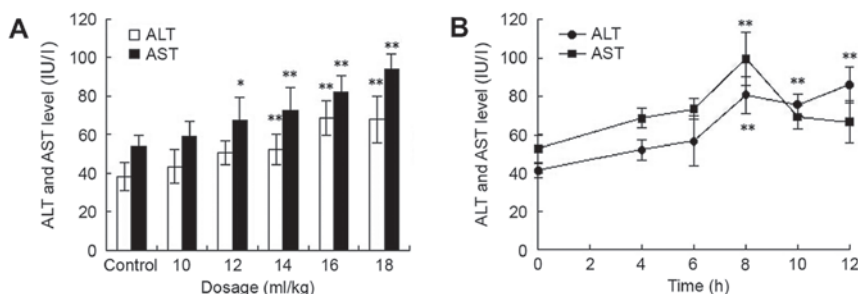


Figure 3. Serum levels of ALT and AST with administration of ethanol (8 h) at various doses (0, 10, 12, 14, 16 and 18 ml/kg) and (B) serum levels of ALT and AST at various times (0, 4, 6, 8, 10 and 12 h) following the administration of ethanol (50%). Values are expressed as the mean ± standard deviation in each group (n=8). *P<0.05, **P<0.01 vs. control. ALT, alanine transaminase; AST, aspartate transaminase.

the control group (P<0.01). Furthermore, mice in the ethanol model group were found to have significantly lower levels of SOD, GSH and GSH-Px (P<0.01) and significantly higher levels of MDA (P<0.01) compared with the control group.

Table IV. Changes of ALT and AST in serum and SOD, MDA, GSH and GSH-Px in liver following ethanol-induced acute hepatic injury.

| Group | ALT (IU/l) | AST (IU/l) | SOD (U/mg) | MDA (nmol/mg) | GSH ($\mu$mol/g) | GSH-Px (U/mg) |
|---|---|---|---|---|---|---|
| Control | 34.99±6.37 | 52.77±7.08 | 58.87±7.64 | 7.13±0.83 | 16.68±3.94 | 319.06±45.51 |
| Model | 47.52±7.21[a] | 94.14±16.95[a] | 44.81±6.69[a] | 10.12±1.36[a] | 9.35±1.01[a] | 193.16±38.84[a] |

Values expressed as the mean ± standard deviation in each group. [a]P<0.01 vs. control. ALT, alanine transaminase; AST, aspartate transaminase; SOD, superoxide dismutase; MDA, malondialdehyde; GSH, glutathione; Px, peroxidase.
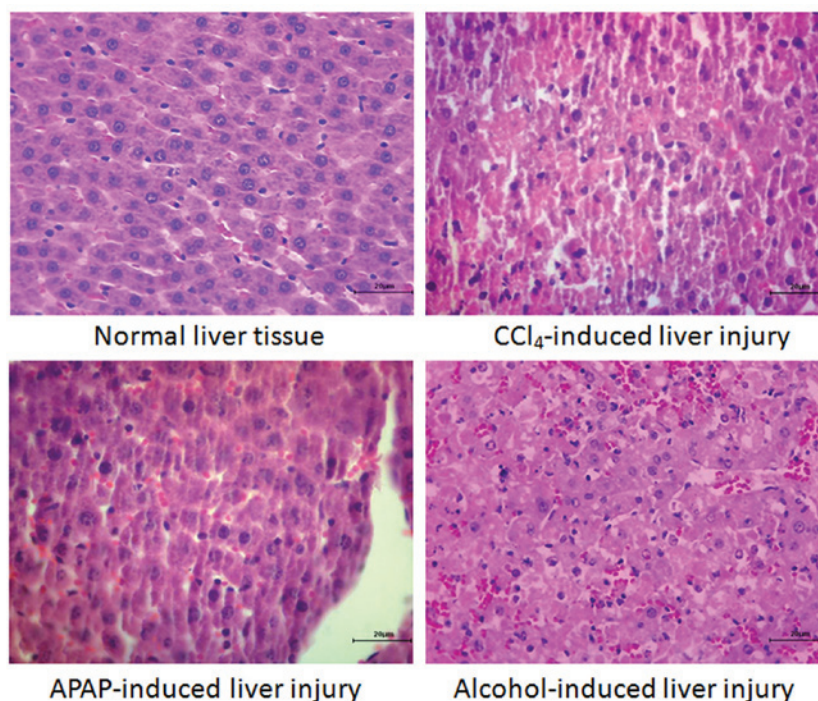


Figure 4. Hematoxylin and eosin staining of liver tissues in three models of liver injury (CCl$_4$, APAP and ethanol) were evaluated. Magnification, x400. APAP, acetyl-para-aminophenol.

*Histopathological analysis*. Pathological section results of each group are presented in Fig. 4. Histopathological analysis revealed blurred hepatic lobular boundaries, hepatic cord disorders, hepatocellular degeneration, cell swelling and inflammatory cell infiltration in the CCl$_4$-induced model. Central venous congestion, hepatic cord disorder, hepatocyte nucleus staining and hepatocellular necrosis were observed in the APAP-induced model. Hepatocellular hemorrhage, necrosis and inflammatory cell infiltration were observed in the ethanol-induced model.

*GSTA1 content in liver*. The GSTA1 content of liver homogenates from hepatic injury models are presented in Fig. 5A. Liver GSTA1 content was significantly decreased (P<0.01) in all model groups compared with controls. In the CCl$_4$ model, GSTA1 content was reduced from 16.21 to 4.28 ng/ml, a 3.8-fold decrease compared with the control group. In the APAP model, GSTA1 content reduced from 16.21 to 12.14 ng/ml, a 1.3-fold decrease compared with the control group. In the ethanol model, GSTA1 content reduced from 16.21 to 6.25 ng/ml, a 2.6-fold decrease compared with the control group.

*mRNA expression of GSTA1 in liver*. GSTA1 mRNA expressions in acute hepatic injury models are presented in Fig. 5B. Gel electrophoresis revealed the PCR amplification fragments of GSTA1 gene and β-actin gene, and the degree of injury was determined depending on the strength of the signal (Fig. 5C). The mRNA expression of GSTA1 in the liver significantly decreased in all model groups compared with controls (all P<0.01; Fig. 5B). In the CCl$_4$ model, GSTA1 mRNA expression reduced from 1.32 to 0.27, a 4.9-fold decrease compared with the control group. In the APAP model, GSTA1 mRNA expression levels were reduced from 1.32 to 0.62, a 2.1-fold decrease compared with controls. In the ethanol model, GSTA1 mRNA expression levels were reduced from 1.32 to 0.36, a 3.7-fold decrease.

**Discussion**

In the present study, the optimal dose tests of CCl$_4$, APAP and ethanol models of hepatic injury revealed that the degree of injury is dose dependent. Excessive doses are able to cause irreversible liver damage (18). The optimal administered
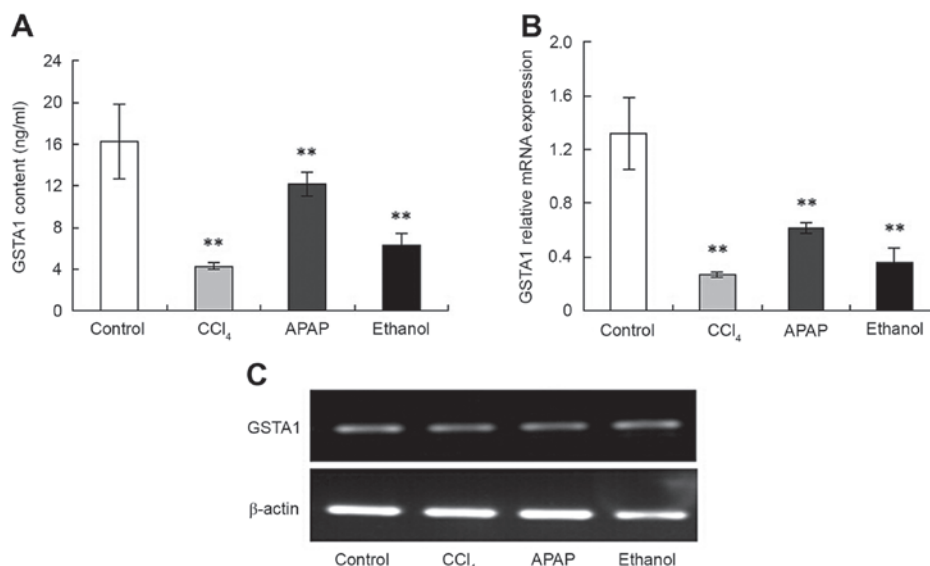
Figure 5. Changes in GSTA1 (A) protein and (B) mRNA expression in liver homogenates from three acute hepatic injury models. (C) Electrophoresis of polymerase chain reaction product. Values expressed as the mean ± standard deviation in each group (n=10). *P<0.05, **P<0.01 vs. control. GSTA1, glutathione S-transferase A1; APAP, acetyl-para-aminophenol.

dose was determined according to the minimum dose that was able to significantly increase ALT and AST levels in the serum. In exposure time tests, the levels of ALT and AST first increased and gradually decreased after reaching a peak. This may be associated with repair of the immune system. The difference between ALT and AST may be due to the different half-life (19).

ALT and AST levels in serum and histopathological changes in liver tissues are typically used to assess toxicity in experimental settings (20). Serum transaminase levels do not accurately reflect the degree of injury to liver function (21). Evaluations of the acute hepatic injury models should ideally use liver homogenate indicators, as they demonstrate changes in important factors associated with liver injury, such as oxidative stress (MDA) (22), antioxidant enzymes (SOD) (23), non-enzymatic antioxidant (GSH) (24) and selenium-containing enzymes (GSH-Px). The results of the optimal tests suggested that the experimental conditions (exposure dose and time) were appropriate to establish the acute hepatic injury models.

In all three models, the protein and mRNA expressions of GSTA1 were significantly decreased and the change in GSTA1 expression was consistent with the content in the liver. The results indicated the importance of GSTA1 in liver injury. GSTA1 serves an important role in hepatic injury, which is gradually being recognized (24). In the CCl₄ model, the GSTA1 protein content and mRNA expression significantly decreased, and the decrease was the greatest of the three models. The results suggest that liver cells release large amounts of GSTA1 to serve an antioxidant role and protect the liver when damaged (25). Liver cells release large amounts GSTAl to scavenge free radicals when facing oxidative stress so that the body's redox balance is maintained (26).

APAP-induced acute hepatic injury is typically caused by intermediate NAPQI, which may lead to the depletion of GSH (27). GSH is the substrate of an enzymatic reaction catalyzed by GSTA1 (28); therefore, GSTA1 may theoretically reflect the degree of liver damage. In the APAP model, the protein content and mRNA expression of GSTA1 significantly decreased in accordance with previous predictions that GSTA1 is affected byAPAP-induced hepatic injury (29). The significant decrease of GSTA1 suggests that it maybe released as an antioxidant to protect the liver from various hepatotoxins. The potential reason is that the injury primarily affected the central lobuli hepatis where GSTA1 is abundant and can be released easily, thus the variation of GSTA1 is relatively more sensitive (30).

GST levels may be used as an indicator of hepatic damage in the alcoholic liver (31). Because GSTA1 is a major component of GST, and increasing GST levels is will affect GSTA1 (32). In the ethanol model, GSTA1mRNA and protein levels significantly decreased, which was consistent with other oxidation index changes. The half-life of GSTA1 is about 1 h (33), suggesting that it may be a useful marker of hepatocellular injury (34). A previous study by the present authors demonstrated that GSTA1 is more sensitive and is able to be detected earlier than ALT (16); therefore, increasing GSTA1 content in liver is an important mechanism leading to the hepatoprotective effect.

Of the models used in the present study, the injury induced by CCl₄ was the most marked, followed by ethanol and finally APAP. The change of GSTA1 expression was positively correlated with its content. GSTA1 levels in all acute hepatic injury models were found to be significantly different from controls, which indicates that GSTA1 may serve a role in eliminating chemical mutagens and lipid peroxides. A previous study suggested that GSTA1 functions to remove toxic substances and carcinogens to protect the liver and prevent tumor formation (35).

In conclusion, the results of the present study suggest that GSTA1 in the liver may be released as an antioxidant to protect against liver injury. This research may serve as a

foundation for further investigations into the regulatory role of hepatoprotective agents such as GSTA1 and the molecular mechanisms of GSTA1 regulation.

## Acknowledgements

## References

1. Antoine DJ, Dear JW, Lewis PS, Platt V, Coyle J, Masson M, Thanacoody RH, Gray AJ, Webb DJ, Moggs JG, *et al*: Mechanistic biomarkers provide early and sensitive detection of acetaminophen-induced acute liver injury at first presentation to hospital. Hepatology 58: 777-787, 2013.
2. Reuben A, Koch DG and Lee WM; Acute Liver Failure Study Group: Drug-induced acute liver failure: Results of a U.S. multicenter, prospective study. Hepatology 52: 2065-2076, 2010.
3. Park SW, Lee CH, Kim YS, Kang SS, Jeon SJ, Son KH and Lee SM: Protective effect of baicalin against carbon tetrachloride-induced acute hepatic injury in mice. J Pharmacol Sci 106: 136-143, 2008.
4. Talwar S, Jagani HV, Nayak PG, Kumar N, Kishore A, Bansal P, Shenoy RR and Nandakumar K: Toxicological evaluation of Terminalia paniculata bark extract and its protective effect against CCl4-induced liver injury in rodents. BMC Complement Altern Med 13: 127, 2013.
5. Jaeschke H, Williams CD, McGill MR, Xie Y and Ramachandran A: Models of drug-induced liver injury for evaluation of phytotherapeutics and other natural products. Food Chem Toxicol 55: 279-289, 2013.
6. Wu D and Cederbaum AI: Alcohol, oxidative stress, and free radical damage. Alcohol Res Health 27: 277-284, 2003.
7. Hozumi H, Tada R, Murakami T, Adachi Y and Ohno N: Comparative analysis of hepatic CD14 expression between two different endotoxin shock model mice: Relation between hepatic injury and CD14 expression. PloS One 8: e53692, 2013.
8. Liong EC, Xiao J, Lau TY, Nanji AA and Tipoe GL: Cyclooxygenase inhibitors protect D-galactosamine/lipopolysaccharide induced acute hepatic injury in experimental mice model. Food Chem Toxicol 50: 861-866, 2012.
9. Frova C: Glutathione transferases in the genomics era: New insights and perspectives. Biomol Eng 23: 149-169, 2006.
10. Strange RC, Spiteri MA, Ramachandran S and Fryer AA: Glutathione-S-transferase family of enzymes. Mutat Res 482: 21-26, 2001.
11. Fadhel ZA and Amran S: Effects of black tea extract on carbon tetrachloride-induced lipid peroxidation in liver, kidneys, and testes of rats. Phytother Res 1 (Suppl): S28-S32, 2002.
12. Prabhu KS, Reddy PV, Jones EC, Liken AD and Reddy CC: Chrarcterization of a class alpha glutathione-S-transgerase with glutathione peroxidase acticity in human liver microsomes. Arch Biochem Biophys 424: 72-80, 2004.
13. Giannini E, Risso D, Ceppa P, Botta F, Chiarbonello B, Fasoli A, Malfatti F, Romagnoli P, Lantieri PB and Testa R: Utility of alpha-glutathione S-transferase assessment in chronic hepatitis C patients with near normal alanine aminotransferase levels. Clin Biochem 33: 297-301, 2000.
14. Suvakov S, Damjanovic T, Stefanovic A, Pekmezovic T, Savic-Radojevic A, Pljesa-Ercegovac M, Matic M, Djukic T, Coric V, Jakovljevic J, *et al*: Glutathione S-transferase A1, M1, P1 and T1 null or low-activity genotypes are associated with enhanced oxidative damage among haemodialysis patients. Nephrol Dial Transplant 28: 202-212, 2013.
15. Raza H: Dual localization of glutathione S-transferase in the cytosol and mitochondria: Implications in oxidative stress, toxicity and disease. FEBS J 278: 4243-4251, 2011.
16. Liu F, Lin Y, Li Z, Ma X, Han Q, Liu Y, Zhou Q, Liu J, Li R, Li J and Gao L: Glutathione S-transferase A1 (GSTA1) release, an early indicator of acute hepatic injury in mice. Food Chem Toxicol 71: 225-230, 2014.
17. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-tie quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408, 2001.
18. Fan G, Tang JJ, Bhadauria M, Nirala SK, Dai F, Zhou B, Li Y and Liu ZL: Resveratrol ameliorates carbon tetrachloride-induced acute liver injury in mice. Environ Toxicol Pharmacol 28: 350-356, 2009.
19. Shea TB, Rogers E, Ashline D, Ortiz D and Sheu MS: Apolipoprotein E deficiency promotes increased oxidative stress and compensatory increases in antioxidants in brain tissue. Free Radic Biol Med 33: 1115-1120, 2002.
20. van Beek JH, de Moor MH, de Geus EJ, Lubke GH, Vink JM, Willemsen G and Boomsma DI: The genetic architecture of liver enzyme levels: GGT, ALT and AST. Behav Genet 43: 329-339, 2013.
21. Mochizuki S, Kawashita Y, Eguchi S, Takatsuki M, Yamanouchi K, Tokai H, Hidaka M, Soyama A, Nagayoshi S and Kanematsu T: Liver repopulation by transplanted hepatocytes in a rat model of acute liver failure induced by carbon tetrachloride and a partial hepatectomy. Ann Transplant 15: 49-55, 2010.
22. Lykkesfeldt J: Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clin Chim Acta 380: 50-58, 2007.
23. Li G, Chen JB, Wang C, Xu Z, Nie H, Qin XY, Chen XM and Gong Q: Curcumin protects against acetaminophen-induced apoptosis in hepatic injury. World J Gastroenterol 19: 7440-7446, 2013.
24. Imai H, Narashima K, Arai M, Sakamoto H, Chiba N and Nakagawa Y: Suppression of leukotriene formation in RBL-2H3 cells that overexpressed phospholipid hydroperoxide glutathione peroxidase. J Biol Chem 273: 1990-1997, 1998.
25. Karahalil B, Yağar S and Ozin Y: Release of alpha-glutathione S-transferase (alpha-GST) and hepatocellular damage induced by helicobacter pylori and eradication treatment. Curr Drug Saf 2: 43-46, 2007.
26. Liang FQ, Alssadi R, Morehead P, Awasthi YC and Godley BF: Enhanced expression of glutathione-S-transferase A1-1 protects against oxidative stress in human retinal pigment epithelial cells. Exp Eye Res 80: 113-119, 2005.
27. Knight TR, Kurtz A, Bajt ML, Hinson JA and Jaeschke H: Vascular and hepatocellular peroxynitrite formation during acetaminophen-induced liver injury: Role of mitochondrial oxidant stress. Toxicol Sci 62: 212-220, 2001.
28. Loguereio C and Federico A: Oxidative stress in viral and alcoholic hepatitis. Free Radie Biol Med 34: 1-10, 2003.
29. Silvanto M, Munsterhjelm E, Savolainen S, Tiainen P, Niemi T, Ylikorkala O, Scheinin H and Olkkola KT: Effect of 3 g of intravenous paracetamol on post-operative analgesia, platelet function and liver enzymes in patients undergoing tonsillectomy under local anaesthesia. Acta Anaesthesiol Scand 51: 1147-1154, 2007.
30. Heijne WH, Slitt AL, van Bladeren PJ, Groten JP, Klaassen CD, Stierum RH and van Ommen B: Bromobenzene-induced hepatotoxicity at the transcriptome level. Toxicol Sci 79: 411-422, 2004.
31. Polimanti R, Piacentini S and Fuciarelli M: HapMap-based study of human soluble glutathione S-transferase enzymes: The role of natural selection in shaping the single nucleotide polymorphism diversity of xenobiotic-metabolizing genes. Pharmacogenet Genomics 10: 665-672, 2011.
32. Oakley A: Glutathione transferases: A structural perspective. Drug Metab Rev 43: 138-151, 2011.
33. Trull AK, Facey SP, Rees GW, Wight DG, Noble-Jamieson G, Joughin C, Friend PJ and Alexander GJ: Serum alpha-glutathione S-transferase-a sensitive marker of hepatocellular damage associated with acute liver allograft rejection. Transplantation 58: 1345-1351, 1994.
34. Beckett GJ and Hayes JD: Glutathione S-transferases: Biomedical applications. Adv Clin Chem 30: 281-380, 1993.
35. Coles BF, Chen G, Kadlubar FF and Radominska-Pandya A: Interindividual variation and organ-specific patterns of glutathione S-transferase alpha, mu, and pi expression in gastrointestinal tract mucosa of normal individuals. Arch Biochem Biophys 403: 270-276, 2002.