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# Establishment of a Standardized Animal Model of Chronic Hepatotoxicity Using Acetaminophen-Induced Hepatotoxicity in the Evaluation of Hepatoprotective Effects of Health Food

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## ABSTRACT

Hepatoprotection is an important issue in Taiwan. Currently, a toxic chemical substance, carbon tetrachloride ($CCl_4$), is used to induce liver damage in animal models that is employed to evaluate the hepatoprotective effects of health food in Taiwan. To provide a more flexible and accessible research model, this study was aimed to establish a standardized animal model of chronic hepatotoxicity using the common drug, acetaminophen; an overdose of this drug causes acute liver damage in experimental animals. Four animal strains from 2 species (ICR mice and BALB/c mice via intraperitoneal injection; Sprague-Dawley rats and Wistar rats via oral administration) were used as subjects in this study. Acetaminophen at various dosages was administered for 8 weeks, and then biochemical and histopathological examinations were performed. The results showed that acetaminophen significantly induced liver damage in mice by intraperitoneal injection, but not in rats by oral administration. The individual variation in BALB/c mice was less than that in ICR mice. Acetaminophen increased serum aspartate transaminase (AST), alanine transaminase (ALT), and glutathione (GSH) content. The glutathione reductase (GRd), superoxide dismutase (SOD), and catalase (CAT) activity were inhibited after acetaminophen injection in BALB/c mice in a dose-dependent manner. The highest dose of acetaminophen was lethal to mice. In terms of histopathological examination, acetaminophen caused hepatic necrosis and inflammation, consistent with the pathological progress found in humans. On the other hand, the liver protective effect of *N*-acetylcysteine (NAC) in BALB/c mice was analyzed. The data revealed that pretreatment with NAC significantly protected liver from damage induced by 400 mg acetaminophen/kg bw in BALB/c mice, and there was no significant difference between the 600 and 1200 mg NAC/kg bw groups. In conclusion, acetaminophen at the dose of 400 mg/kg bw could induce reproducible liver damage in BALB/c mice, and NAC (600 mg/kg bw) is a suitable protective drug in this animal model for the evaluation of hepatoprotective effects of health food.

Key words: acetaminophen, ICR mice, BALB/c mice, SD rats, Wistar rats, health foods, liver damage, NAC

## INTRODUCTION

Liver disease is one of the leading causes of death in Taiwan. Many animal models have been introduced to study hepatic fibrosis induced by hepatotoxic substances found in diet, drugs, or alcohol. Moreover, these animal models have been used to investigate possible mechanisms of hepatic damage, such as bile duct ligation or immunologic activation. Carbon tetrachloride ($CCl_4$) has been used widely in animal models to induce chronic liver injury[1,2]. However, $CCl_4$ is not a common cause of liver injury in the clinical setting, and furthermore, $CCl_4$ is cumbersome to use since it is a toxic chemical substance. Hence, it is necessary to establish an easily accessible and standardized animal model of chronic liver injury.

Acetaminophen is a widely used, safe, and effective analgesic and antipyretic. However, overdose of acetaminophen leads to liver failure and death in experimental animals and humans[3], and is a leading cause of drug-induced acute liver failure in the United States[4]. Acetaminophen is metabolized to a highly reactive and toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI) by cytochrome P450 2E1 (CYP2E1)[5]. Under normal conditions, NAPQI is detoxified by glutathione (GSH). However, under conditions of overdose, GSH is depleted and excess NAPQI binds to cellular proteins, thereby increasing oxidative stress and inducing liver necrosis[6-8]. Since oxidative stress is the main hepatotoxic factor in acetaminophen overdose[9], N-acetylcysteine

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(NAC), an antioxidant providing cysteine for glutathione synthesis[10,11], is used in the clinical setting for the treatment of acetaminophen overdose[12,13].

In experimental animal studies, acetaminophen is a common chemical used to induce liver injury. However, the results of these studies are controversial, owing to the fact that acetaminophen toxication and detoxification effects can vary with the species of experimental animals, the dose of acetaminophen, the route of drug administration, etc.[14,15] In short, acetaminophen has not been standardized as an experimental animal model of liver injury[16].

Therefore, the aims of this study were to establish a standardized animal model of acetaminophen-induced chronic liver injury, by comparing different animal species and strains with different doses of acetaminophen, and to further verify the suitable dose of the protective drug, NAC.

## MATERIALS AND METHODS

### I. *Materials and Chemicals*

Acetaminophen, reduced glutathione (GSH), $KH_2PO_4$, $K_2HPO_4$, KCl, NaCl, and NAC were obtained from Sigma-Aldrich Chemicals Company (St. Louis, MO, USA). Glutathione (GSH), glutathione reductase (GRd), superoxide dismutase (SOD), and catalase (CAT) assay kits were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). SPOTCHEMTM II GPT Reagent Strips and SPOTCHEMTM II GOT Reagent Strips were from Arkray Inc. (Kyoto, Japan).

### II. *Animals*

Male BALB/c mice, ICR mice, and Wistar rats were purchased from National Taiwan University Hospital (Taipei, Taiwan); Sprague-Dawley (SD) rats were obtained from BioLASCO Taiwan Co. (Taipei, Taiwan). All animals were housed in a controlled room (22 ± 3°C, 50 - 70% of relative humidity, and a 12-h light: dark cycle); standard rodent chow diet and water were provided *ad libitum*.

### III. *Experimental Design of Chronic Acetaminophen Hepatotoxicology*

BALB/c and ICR mice weighing about 18 g (7 weeks old) were randomly divided into 4 groups. Group 1 served as the normal control group, and groups 2 - 4 were the test groups injected with varying amounts of acetaminophen. Each group of animals were injected intraperitoneally twice a week for 8 weeks with isotonic 0.9% NaCl and acetaminophen, in the following amounts: 200 , 400 and 600 mg/kg bw for group 2, 3 and 4, respectively.

Sprague-Dawley and Wistar rats weighing about 200 g (7 weeks old) were randomly divided into 4 groups. Rats were orally given pure water, and 1.5, 2.5, or 3.5 g acetaminophen/kg bw twice a week for 8 weeks in group 1, 2, 3 and 4, respectively.

At the end of the first, third, and sixth weeks of the experiment, 2 rodents from each group were sacrificed for the observation of liver injury progress. At the end of the eight week, all animals were sacrificed for biochemical and histological analysis.

### IV. *Experimental Design of the Protective Effect of NAC on Acetaminophen-Induced Chronic Hepatotoxity*

Male BALB/c mice weighing about 20 g (8 weeks old) were divided into 4 groups. Groups 1 and 2 served as normal control and negative control groups, and were given 0.5% carboxymethyl cellulose (CMC) orally once daily for 9 weeks. The NAC groups 3 and 4 were treated orally with 600 and 1200 mg NAC/kg bw (in 0.5% CMC), respectively, once daily for 9 weeks. After the second week of the experimental period, groups 2, 3, and 4 were intraperitoneally injected with acetaminophen (400 mg/kg bw) twice a week for 8 weeks, 1 hr before NAC treatment, while group 1 received saline injection.

### V. *Analysis of Serum Parameters*

Blood from retro-ocular sinuses of mice or celiac arteries of rats were collected in heparin tubes. The blood samples were centrifuged at 12,000 rpm at 4°C for 5 min to separate the serum. Liver injury was determined by measuring aspartate transferase (AST) and alanine transaminase (ALT) activities in serum using SPOTCHEM$^{TM}$ II GOT Reagent Strips and SPOTCHEM$^{TM}$ II GPT Reagent Strips, respectively, on an automated biochemical analyzer (SPOTCHEM$^{TM}$ EZ SP-4430, ARKRAY Inc, Kyoto, Japan).

### VI. *Estimation of Hepatic GSH and Anti-Oxidative Enzymes*

As each rodent was sacrificed, the liver was immediately removed, washed with ice-cold saline, weighed, and stored at −80°C. A portion of each liver was homogenized (1 : 10, w/v) in phosphate buffer (8 mM $KH_2PO_4$, 12 mM $K_2HPO_4$, 1.5% KCl, pH 7.4), and the homogenate was centrifuged at 10,000 g for 30 min at 4°C. The resultant supernatant was used for analysis. Hepatic GSH and GRd levels and activities of CAT and SOD were determined by ELISA using commercial kits (Cayman Chemical, USA). GSH kit measures the content of 5-thio-2-nitrobenzonic acid produced from GSH and 5,5'-dithio-*bis*-2-(nitrobenzoic acid) for the quantification of GSH. GRd assay kit estimates GRd activity by measuring the rate of NADPH oxidation. CAT assay kit utilizes the peroxidatic function of CAT for confirmation CAT activity. The assay kit of SOD uses tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine.

### VII. *Histopathological Examination*

A portion of each liver tissue was fixed in 10% buffered

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**Table 1.** Effect of acetaminophen on serum contents of AST, ALT, and GSH, and hepatic enzyme activities of GRd, CAT, and SOD for different species of rodents

| Animal | Group | serum | | liver | | | |
|---|---|---|---|---|---|---|---|
| | | AST | ALT | GSH | GRd | CAT | SOD |
| | | (IU/L) | | (nmol/mg protein) | (nmol/mg protein/min) | | (U[1]/mg protein) |
| ICR mice | Control | 51 ± 26$^{b}$ | 170 ± 72$^{b}$ | 103 ± 8$^{a}$ | 56 ± 8$^{a}$ | 3.1 ± 0.3$^{a}$ | 11.1 ± 0.3 |
| | Acetaminophen 200 | 87 ± 47$^{b}$ | 164 ± 97$^{b}$ | 68 ± 17$^{ab}$ | 52 ± 17$^{ab}$ | 2.4 ± 0.8$^{a}$ | 11.1 ± 0.7 |
| | Acetaminophen 400 | 11798 ± 7600$^{a}$ | 7757 ± 3111$^{a}$ | 79 ± 4$^{bc}$ | 40 ± 4$^{bc}$ | 1.2 ± 0.9$^{b}$ | 10.2 ± 0.6 |
| | Acetaminophen 600 | 26540 ± 3167$^{a}$ | 10830 ± 1541$^{a}$ | 80 ± 7$^{c}$ | 30 ± 7$^{c}$ | 0.9 ± 1.0$^{b}$ | 11.1 ± 0.3 |
| BALB/c mice | Control | 38 ± 11$^{c}$ | 64 ± 17$^{c}$ | 83 ± 6$^{a}$ | 47 ±6$^{a}$ | 2.8 ± 0.8$^{a}$ | 11.2 ± 2.8$^{a}$ |
| | Acetaminophen 200 | 169 ± 60$^{bc}$ | 150 ± 76$^{c}$ | 66 ± 5$^{b}$ | 21 ± 5$^{b}$ | 1.4 ± 0.7$^{b}$ | 8.6 ± 0.4$^{b}$ |
| | Acetaminophen 400 | 319 ± 159$^{b}$ | 428 ± 288$^{b}$ | 69 ± 4$^{b}$ | 16 ± 4$^{b}$ | 1.6 ± 0.4$^{b}$ | 8.2 ± 0.2$^{b}$ |
| | Acetaminophen 600 | 6375 ± 771$^{a}$ | 3770 ± 410$^{a}$ | 38 ± 4$^{b}$ | 14 ± 4$^{b}$ | 1.2 ± 0.4$^{b}$ | 7.5 ± 1.6$^{b}$ |
| Wistar rats | Control | 90 ± 10$^{ab}$ | 44 ± 3$^{b}$ | 102.9 ± 1.7$^{d}$ | 8.6 ±1.7$^{d}$ | 18.0 ± 0.6$^{b}$ | 1.8 ± 0.1$^{b}$ |
| | Acetaminophen 1500 | 79 ± 15$^{b}$ | 59 ± 14$^{b}$ | 37.9 ± 3.4$^{c}$ | 24.8 ± 3.4$^{c}$ | 17.0 ± 0.3$^{c}$ | 1.9 ± 0.1$^{a}$ |
| | Acetaminophen 2500 | 61 ± 6$^{b}$ | 58 ± 7$^{b}$ | 36.9 ± 3.6$^{b}$ | 34.7 ± 3.6$^{b}$ | 18.3 ± 0.9$^{b}$ | 1.6 ± 0.2$^{c}$ |
| | Acetaminophen 3500 | 134 ± 72$^{a}$ | 155 ± 136$^{a}$ | 38.4 ± 2.4$^{a}$ | 47.9 ± 2.4$^{a}$ | 19.3 ± 0.3$^{a}$ | 1.2 ± 0.1$^{d}$ |
| SD rats | Control | 94 ± 20 | 53 ± 6 | 103.5 ± 3.4$^{a}$ | 24.4 ± 3.4$^{a}$ | 28.4 ± 0.6$^{ab}$ | 2.1 ± 0.08$^{b}$ |
| | Acetaminophen 1500 | 101 ± 16 | 76 ± 17 | 50.8 ± 3.6$^{a}$ | 26.1 ± 3.6$^{a}$ | 27.1 ± 0.8$^{c}$ | 2.1 ± 0.07$^{b}$ |
| | Acetaminophen 2500 | 109 ± 33 | 100 ± 32 | 40.5 ± 6.2$^{b}$ | 12.0 ± 6.2$^{b}$ | 27.4 ± 1.3$^{bc}$ | 2.1 ± 0.15$^{b}$ |
| | Acetaminophen 3500 | 109 ± 57 | 105 ± 75 | 32.4 ± 4.8$^{c}$ | 5.9 ± 4.8$^{c}$ | 29.5 ± 1.1$^{a}$ | 2.4 ± 0.12$^{a}$ |

Data are expressed as mean ± SD (n = 6 - 8).
[abc] Indicate groups with statistical difference ($p < 0.05$) by one-way ANOVA coupled with the Duncan's multiple range test. AST, aspartate aminotransferase; ALT, alanine aminotransferase; GSH, glutathione; GRd, glutathione reductase; SOD, superoxide dismutase; CAT, catalase
[1] One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

neutral formalin solution, embedded in paraffin, sectioned into 3 μm thick slices, and stained with hematoxylin and eosin (HE). The liver tissue injury was examined by a histopathological expert under optical microscope. Histology activity index, graded on severity scales from none (0), mild (1), moderate (2) to severe (3), is used to quantify the degree of liver inflammation[17].

VIII. *Statistical Analysis*

All data were expressed as a mean ± standard deviation (SD) (n = 6 - 8). The data were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for individual comparisons, using SAS 9.1 software (SAS Institute Inc., USA). Data were considered statistically significant when the *P* value was less than 0.05.

## RESULTS

I. *Species Differences of Chronic Acetaminophen-Induced Chronic Hepatotoxity*

As shown in Table 1, serum levels of ASL and ALT increased significantly in ICR and BALB/c mice groups injected with 200 or 400 mg/kg bw acetaminophen for 8 weeks as compared with the control group ($p < 0.05$). Serum AST and ALT levels in BALB/C mice were analyzed at different time points in the experiment (Table 2). After 1 week of acetaminophen treatment, AST and ALT increased significantly ($p < 0.01$), but stayed unchanged at week 3, 6, and 8. In rats, acetaminophen administration significantly elevated the serum levels of ASL and ALT in Wistar rats ($p < 0.05$) at the dose of 3500 mg/kg bw, but no significant change was observed in SD rats.

**Table 2.** Serum contents of AST and ALT in BALB/c mice after 400 mg acetaminophen/kg bw treatment

| | week | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 3 | 6 | 8 |
| AST (IU/L) | 86 ± 18$^{b}$ | 221 ± 117$^{a}$ | 263 ± 120$^{a}$ | 298 ± 137$^{a}$ | 428 ± 288$^{a}$ |
| ALT (IU/L) | 57 ± 14$^{b}$ | 318 ± 134$^{a}$ | 366 ± 308$^{a}$ | 423 ± 111$^{a}$ | 319 ± 159$^{a}$ |

Data are expressed as mean ± SD (n = 6 - 8).
[ab] Indicate groups with statistical difference ($p < 0.01$) by one-way ANOVA coupled with the Duncan's multiple range test.
AST, aspartate aminotransferase; ALT, alanine aminotransferase

The hepatic GSH content of BALB/c mice, Wistar rats and SD rats, treated with different doses of acetaminophen, depleted significantly to different degrees, as compared to control groups ($p < 0.05$).

In terms of the activities of anti-oxidative enzymes in liver, the higher doses (400 and 600 mg acetaminophen/kg bw) of acetaminophen significantly suppressed the GRd and CAT activities in ICR mice, but no effect was seen in SOD activity. In BALB/c mice, various doses of acetaminophen resulted in a dose-dependent and significant decrease of all enzyme activities compared with control groups ($p < 0.05$). On the other hand, acetaminophen boosted GRd and CAT activities significantly ($p < 0.05$) in Wistar rats. The GRd activity was diminished after 3,500 mg acetaminophen/kg bw treatment in SD rats, but SOD and CAT activities increased significantly ($p < 0.05$).

To confirm the hepatotoxicity of acetaminophen, histopathological examinations on each liver were performed and demonstrated the progress of liver damage in BALB/c mice induced by 400 and 600 mg/kg bw acetaminophen. The histological studies revealed that acetaminophen induced necrosis and inflammation in the liver tissue, especially at the end of the eight week. Similar results were observed in ICR mice. Histological examination of liver from Wistar and SD rats revealed increased tissue collagen in the periportal area, an increase in the number of bile ducts, and the vacuolation of hepatocytes around the central vein (data not shown). However, the severity of liver damage was generally low.

II. *Effect of NAC on Acetaminophen-Induced Chronic Liver Damage*

The protective effects of NAC on AST and ALT in serum and GSH level in liver from male BALB/c mice treated with acetaminophen are shown in Table 3. Oral administration of 600 and 1200 mg NAC/kg bw in BALB/c mice treated with 400 mg acetaminophen/kg bw, significantly lowered serum AST and ALT, while GSH levels increased ($p < 0.05$). No significant difference on AST, ALT, and GSH were found between the 600 and 1200 mg NAC/kg bw groups.

The histological examinations showed that acetaminophen treatment induced inflammation in the liver tissue, and blood congestion was observed (Figure 1B). Pretreatment with 600 or 1200 mg NAC/kg bw markedly mitigated the inflammation in hepatic lobules (Figure 1C & 1D). Histological activity indices of control, acetaminophen, NAC (600 and 1200 mg/kg) groups were 0 ± 0, 0.6 ± 0.2, 0 ± 0, and 0 ± 0 on hepatic lobules inflammation, respectively, which indicated that acetaminophen significantly induced inflammation ($p < 0.05$) while NAC significantly eased the acetaminophen-induced inflammation.

**Table 3.** Effect of pretreatment with NAC on serum AST, ALT, and hepatic GSH content after acetaminophen treatment in BALB/c mice

| Group | AST (IU/L) | ALT (IU/L) | GSH (nmol/ mg protein) |
|---|---|---|---|
| control | $72 \pm 17^{b}$ | $37 \pm 14^{b}$ | $112 \pm 19^{a}$ |
| Acetaminophen | $234 \pm 118^{a}$ | $206 \pm 107^{a}$ | $48 \pm 19^{b}$ |
| Acetaminophen + NAC 600 | $143 \pm 117^{b}$ | $100 \pm 58^{b}$ | $104 \pm 37^{a}$ |
| Acetaminophen + NAC 1200 | $101 \pm 20^{b}$ | $65 \pm 38^{b}$ | $108 \pm 11^{a}$ |

Data are expressed as mean ± SD (n = 6 - 8).
[abc] Indicate groups with statistical difference ($p < 0.05$) by one-way ANOVA coupled with the Duncan's multiple range test.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; GSH, glutathione

## DISCUSSION

The animal model of hepatotoxicity induced by $CCl_4$ is an official methodology in Taiwan for the evaluation of hepatoprotective effects of health foods. However, this model has little relevance to humans since hepatotoxicity induced by $CCl_4$, a toxic chemical substance, is a rare occurrence in humans. Therefore, acetaminophen, with similar metabolic and toxic responses in both rodents and humans[18], was planned to be the substitute drug to induce liver toxicity in animal models. Hence, the goal of this study was to build a standardized model of acetaminophen-induced liver injury.

Under therapeutic doses, acetaminophen is detoxified in the liver mainly by glucuronidation and sulfation[19,20]. Part of acetaminophen is metabolized by CYP450[21] CYP2E1, and is converted to a toxic metabolite NAPQI[22]. NAPQI is detoxified by conjugation with glutathione (GSH)[6]; thus, GSH content is the critical factor of acetaminophen toxicity. Under the condition of acetaminophen overdose, glucuronidation and sulfation become saturated, GSH is depleted, and NAPQI is bound to cellular proteins, resulting in oxidative stress and liver necrosis[23,24].

Mechanistically, acetaminophen is assumed to increase the serum levels of AST and ALT, and the indices of liver injury, and to decrease GSH content and GRd activity, which is an essential enzyme for the regeneration of GSH from glutathione disulphide (GSSG). The activities of CAT and SOD, the antioxidant enzymes, were expected to be decreased after acetaminophen treatment. In this study, in the majority of the cases, acetaminophen injection at the dose of 400 and 600 mg/kg bw significantly increased AST and ALT, decreased GSH, and decreased the activity of GRd, CAT and SOD in mice. There was one exception: the SOD activity in ICR mice was not statistically different from the control (Table 1). These findings are similar to previous studies[25-31]. Because the survival rate of BALB/c mice at the end of the experiment in 600 mg acetaminophen/kg bw group (19%) was significantly lower than that in 400 mg acetaminophen/kg bw group (90%), it is suggested acetaminophen at the dose of 400 mg/kg bw is optimal for hepatotoxicity induction studies.

Perivenular (zone 3) necrosis is a characteristic lesion induced by intrinsic toxins, including acetaminophen. In the case of acetaminophen overdose, confluent coagulative necrosis occurs in perivenular and mid-zones[32]. The

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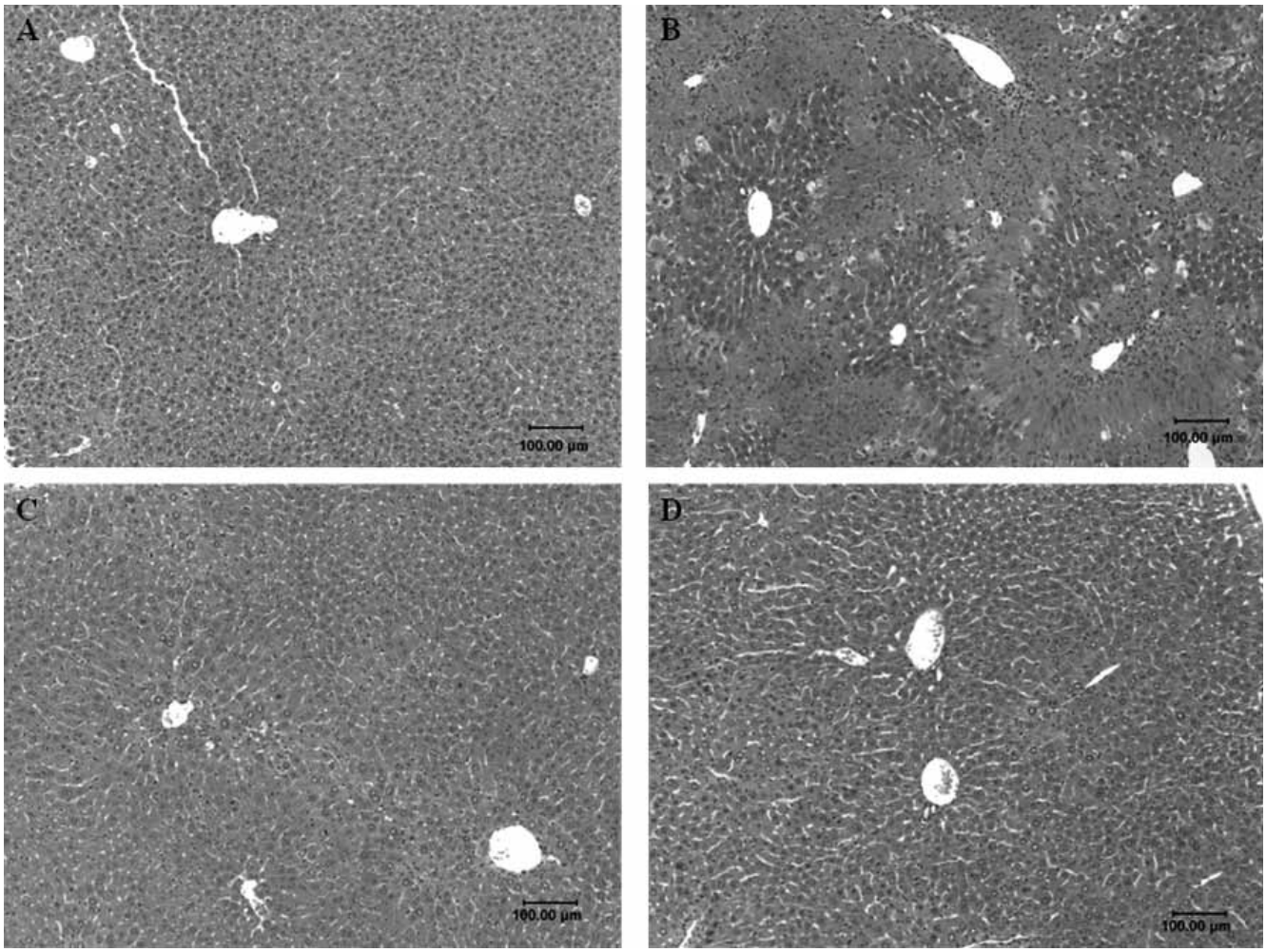


**Figure 1.** Hepatoprotective effect of NAC on acetaminophen-induced liver damage in BALB/c mice (H&E stain 100 X).
(A) Normal control group (B) Acetaminophen group (400 mg acetaminophen/kg bw, i.p.) (C) Acetaminophen + NAC (600 mg/kg bw, p.o.) (D) Acetaminophen + NAC (1200 mg/kg bw, p.o.)

progress of liver damage in the pathological examination of BALB/c mice induced by acetaminophen is consistent with that of human. Histological activity index scoring, a system for quantifying the degree of liver inflammation, supported the histological results (data not shown).

By reporting the effects of acetaminophen on the biochemical and histopathological parameters of liver damage, the present study demonstrated that acetaminophen induced liver injury to different species and animal strains to different extents. In both Wistar and SD rats, acetaminophen treatment did not induce severe injury even at the extremely toxic dosage (3500 mg/kg bw). The results of biochemical and histological examination results similar in BALB/c and ICR mice, but the individual variation was observed more often in ICR mice than in BALB/c mice. The different responses to acetaminophen by species and strains may be due to their different elimination pathways of acetaminophen. Previous studies have showed that mice and hamsters are sensitive, while rats, rabbits, and guinea pigs are less sensitive to acetaminophen toxicity. Strain specificities also exist[7,33]. In sensitive species or strains, acetaminophen is excreted mainly via toxication pathway-related metabolites, glutathione conjugate and its metabolites, by the CYP450 enzymatic reaction[14,34,35]. The ratio of toxication/detoxication-pathway metabolites of acetaminophen in liver slices is related to the response of acetaminophen in different species[36]. *In vitro* studies also demonstrate interspecies differences in sensitivity to acetaminophen [37,38]. These findings provide adequate explanation that acetaminophen treatment led to various levels of liver injury in different species and strains in our study.

GSH is an endogenous antioxidant and thus its presence is related to numerous diseases[39]. NAC, a water-soluble and easily absorbed chemical, is a precursor of GSH that provides cysteine, an amino acid that forms GSH[10,11]. Therefore, NAC is used to treat several critical illnesses, such as severe liver damage, renal failure, and acetaminophen overdose[40,41]. Hence, we used NAC as a protective drug in this animal model. The dosage of NAC used (600 and 1200 mg/kg bw) was converted from the oral dose (140

mg/kg, followed by 70 mg/kg every 4 h for 17 doses) that is typically used in the clinical setting[42]. The biochemical and histological examinations showed that NAC treatment via gavage significantly mitigated liver injury induced by acetaminophen in BALB/c mice. In this chronic model, there was no difference observed between these 2 doses.

In conclusion, the animal model of acetaminophen-induced chronic hepatotoxicity is standardized in this study. Acetaminophen administration (i.p.) at the dose of 400 mg/kg bw twice a week for 8 consecutive weeks on BALB/c mice is the optimal condition to induce liver injury. NAC at 600 mg/kg bw is an effective protective drug in this animal model. This model of acetaminophen-induced hepatotoxicity may be useful to evaluate the hepatoprotective effect of health foods.

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## REFERENCES

1. Rahman, T. M. and Hodgson, H. J. 2000. Animal models of acute hepatic failure. Int. J. Exp. Pathol. 81: 145-157.
2. Shi, Z., Wakil, A. E. and Rockey, D. C. 1997. Strain-specific differences in mouse hepatic wound healing are mediated by divergent T helper cytokine responses. Proc. Natl. Acad. Sci. U.S.A. 94: 10663-10668.
3. Thomas, S. H. 1993. Paracetamol (acetaminophen) poisoning. Pharmacol. Ther. 60: 91-120.
4. Larson, A. M., Polson, J., Fontana, R. J., Davern, T. J., Lalani, E., Hynan, L. S., Reisch, J. S., Schiødt, F. V., Ostapowicz, G., Shakil, A. O., Lee, W. M. and Acute Liver Failure Study Group. 2005. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology 42: 1364-1372.
5. Corcoran, G. B., Mitchell, J. R., Vaishnav, Y. N. and Horning, E. C. 1980. Evidence that acetaminophen and N-hydroxyacetaminophen form a common arylating intermediate, N-acetyl-p-benzoquinoneimine. Mol. Pharmacol. 18: 536-542.
6. Mitchell, J. R., Jollow, D. J., Potter, W. Z., Gillette, J. R. and Brodie, B. B. 1973. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J. Pharmacol. Exp. Ther. 187: 211-217.
7. Bessems, J. G. and Vermeulen, N. P. 2001. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Crit. Rev. Toxicol. 31: 55-138.
8. Albano, E., Rundgren. M., Harvison. P. J., Nelson, S. D. and Moldéus P. 1985. Mechanisms of N-acetyl-p-benzoquinone imine cytotoxicity. Mol. Pharmacol. 28: 306-311.
9. Jaeschke, H., Knight, T. R. and Bajt, M. L. 2003. The role of oxidant stress and reactive nitrogen species in acetaminophen hepatotoxicity. Toxicol. Lett. 144: 279-288.
10. Lauterburg, B. H., Corcoran, G. B. and Mitchell, J. R. 1983. Mechanism of action of N-acetylcysteine in the protection against the hepatotoxicity of acetaminophen in rats in vivo. J. Clin. Invest. 71: 980-991.
11. Corcoran, G. B. and Wong, B. K. 1986. Role of glutathione in prevention of acetaminophen-induced hepatotoxicity by N-acetyl-L-cysteine in vivo: studies with N-acetyl-D-cysteine in mice. J. Pharmacol. Exp. Ther. 238: 54-61.
12. Cotgreave, I. A. 1997. N-acetylcysteine: pharmacological considerations and experimental and clinical applications. Adv. Pharmacol. 38: 205-227.
13. Rumack, B. H., Peterson, R. C., Koch, G. G. and Amara, I. A. 1981. Acetaminophen overdose. 662 cases with evaluation of oral acetylcysteine treatment. Arch. Intern. Med. 141: 380-385.
14. Gregus, Z., Madhu, C. and Klaassen, C. D. 1988. Species variation in toxication and detoxication of acetaminophen in vivo: a comparative study of biliary and urinary excretion of acetaminophen metabolites. J. Pharmacol. Exp. Ther. 244: 91-99.
15. Newsome, P. N., Plevris, J. N., Nelson, L. J. and Hayes, P. C. 2000. Animal models of fulminant hepatic failure: a critical evaluation. Liver Transpl. 6: 21-31.
16. Tuñón, M. J., Alvarez, M., Culebras, J. M. and González-Gallego, J. 2009. An overview of animal models for investigating the pathogenesis and therapeutic strategies in acute hepatic failure. World J. Gastroenterol. 15: 3086-3098.
17. Knodell, R. G., Ishak, K. G., Black, W.C., Chen, T. S., Craig, R., Kaplowitz, N., Kiernan, T. W. and Wollman, J. 1981. Formulation and application of a numerical scoring system for assessing histological activity in asymtomatic chronic active hepatitis. Hepatology 1: 431-435.
18. Powell, C. L., Kosyk, O., Ross, P. K., Schoonhoven, R., Boysen, G., Swenberg, J. A., Heinloth, A. N., Boorman, G. A., Cunningham, M. L., Paules, R. S. and Rusyn, I. 2006. Phenotypic anchoring of acetaminophen-induced oxidative stress with gene expression profiles in rat liver. Toxicol. Sci. 93: 213-222.
19. Slattery, J. T. and Levy, G. 1979. Acetaminophen kinetics in acutely poisoned patients. Clin. Pharmacol. Ther. 25: 184-195.
20. Jollow, D. J., Thorgeirsson, S. S., Potter, W. Z., Hashimoto, M. and Mitchell, J. R. 1974. Acetaminophen-induced hepatic necrosis. VI. Metabolic disposition of toxic and nontoxic doses of acetaminophen. Pharmacology 12: 251-271.
21. Gibson, J. D., Pumford, N. R., Samokyszyn, V. M. and Hinson, J. A. 1996. Mechanism of acetaminophen-induced hepatotoxicity: covalent binding versus oxidative stress. Chem. Res. Toxicol. 9: 580-585.
22. Nelson, S. D. 1990. Molecular mechanisms of the hepatotoxicity caused by acetaminophen. Semin. Liver. Dis. 10: 267-278.

23. Moldéus, P. 1978. Paracetamol metabolism and toxicity in isolated hepatocytes from rat and mouse. Biochem. Pharmacol. 27: 2859-2863.
24. Moore, M., Thor, H., Moore, G., Nelson, S., Moldéus, P. and Orrenius, S. 1985. The toxicity of acetaminophen and N-acetyl-p-benzoquinone imine in isolated hepatocytes is associated with thiol depletion and increased cytosolic $Ca^{2+}$. J. Biol. Chem. 260: 13035-13040.
25. Banerjee, S. K., Mukherjee, P. K. and Maulik, S. K. 2003. Garlic as an antioxidant: the good, the bad and the ugly. Phytother. Res. 17: 97-106.
26. Kalantari, H. and Salehi, M. 2001. The protective effect of garlic oil on hepatotoxicity induced by acetaminophen in mice and comparison with N-acetylcysteine. Saudi. Med. J. 22: 1080-1084.
27. Ozdemirler, G., Aykaç, G., Uysal, M. and Oz, H. 1994. Liver lipid peroxidation and glutathione-related defence enzyme systems in mice treated with paracetamol. J. Appl. Toxicol. 14: 297-299.
28. Lee, S. C., Tsai, C. C., Chen, J. C., Lin, J. G., Lin, C. C., Hu, M. L. and Lu, S. 2002. Effects of "Chinese yam" on hepato-nephrotoxicity of acetaminophen in rats. Acta Pharmacol. Sin. 23: 503-508.
29. Wu, S. J., Wang, J. S., Lin, C. C. and Chang, C. H. 2001. Evaluation of hepatoprotective activity of legumes. Phytomedicine 8: 213-219.
30. Hu, J. J., Yoo, J. S., Lin, M., Wang, E. J. and Yang, C. S. 1996. Protective effects of diallyl sulfide on acetaminophen-induced toxicities. Food. Chem. Toxicol. 34: 963-969.
31. Lin, M. C., Wang, E. J., Patten, C., Lee, M. J., Xiao, F., Reuhl, K. R. and Yang, C. S. 1996. Protective effect of diallyl sulfone against acetaminophen-induced hepatotoxicity in mice. Biochem. Toxicol. 11: 11-20.
32. Lewis, J. H. and Kleiner, D. E. 2007. Hepatic injury due to drugs, chemicals and toxins. In "MacSween's Pathology of the Liver". 5th ed. pp. 649-765. Burt, A. D., Portmann, B. C., and Ferrell, L. D. ed. Churchill Livingstone. New York, U. S. A.
33. Plant, N. 2003. Role of genetics in toxic response. In "Molecular Toxicology". pp. 99-118. Plant, N, ed. BIOS Scientific Publishers. London, U. K.
34. Ioannides, C., Steele, C. M. and Parke, D. V. 1983. Species variation in the metabolic activation of paracetamol to toxic intermediates: role of cytochromes p-450 and p-448. Toxicol. Lett. 16: 55-61.
35. Lubek, B. M, Avaria, M., Basu, P. K. and Wells, P. G. 1988. Pharmacological studies on the in vivo cataractogenicity of acetaminophen in mice and rabbits. Fundam. Appl. Toxicol. 10: 596-606.
36. Miller, M. G., Beyer, J., Hall, G. L., deGraffenried, L. A. and Adams, P. E. 1993. Predictive value of liver slices for metabolism and toxicity in vivo: use of acetaminophen as a model hepatotoxicant. Toxicol. Appl. Pharmacol. 122: 108-116.
37. Jemnitz, K., Veres, Z., Monostory, K., Kóbori, L. and Vereczkey, L. 2008. Interspecies differences in acetaminophen sensitivity of human, rat, and mouse primary hepatocytes. Toxicol. In Vitro 22: 961-967.
38. Mitry, R. R., Hughes, R. D., Bansal, S., Lehec, S. C., Wendon, J. A. and Dhawan, A. 2005. Effects of serum from patients with acute liver failure due to paracetamol overdose on human hepatocytes in vitro. Transplant. Proc. 37: 2391-2394.
39. Hammond, C. L., Lee, T. K. and Ballatori, N. 2001. Novel roles for glutathione in gene expression, cell death, and membrane transport of organic solutes. J. Hepatol. 34: 946-954.
40. Demir, S. and Inal-Erden, M. 1988. Pentoxifylline and N-acetylcysteine in hepatic ischemia/reperfusion injury. Clin. Chim. Acta 275: 127-135.
41. Dodd, S., Dean, O., Copolov, D. L., Malhi, G. S. and Berk, M. 2008. N-acetylcysteine for antioxidant therapy: pharmacology and clinical utility. Expert. Opin. Biol. Ther. 8: 1955-1962.
42. Polson, J. and Lee, W. M. 2005. AASLD position paper: the management of acute liver failure. Hepatology 41: 1179-1197.