Original Article

THAI J GASTROENTEROL 2013
Vol. 14 No. 1
Jan. - Apr. 2013

Aloe vera Attenuated Acetaminophen Overdose-Induced Hepatitis in Mice

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ABSTRACT

Background: Acetaminophen (APAP) overdose result in hepatic damage via several mechanisms including increase production of cytokines, oxidative stress and decrease hepatic glutathione (GSH) which play an important role in detoxification. *Aloe vera (A. vera)* is a species of succulent plant. Extract of *A. vera* has been reported in antioxidative, anti-inflammatory effect. This study aim to determine the protective effect and mechanism of extract from *A. vera* on APAP-induced hepatotoxicity.

Methods: Twenty-four male mice (25-30 gram) were randomly divided into 3 groups; control, APAP and *A.vera*-treated group. In control group mice were fed with distilled water, while mice in APAP group were fed with APAP 400 mg/kg single dose and APAP 400 mg/kg plus *A.vera* 150 mg/kg single dose in *A.vera*-treated group. The serum and hepatic tissue was collected to identify activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), hepatic GSH, hepatic malondialdehyde (MDA) which is marker of oxidative stress and histopathology.

Results: In APAP group, serum AST, ALT and hepatic MDA were significant increased and hepatic GSH were significant decreased when compared to control group. The levels of AST, ALT, hepatic GSH and MDA in *A.vera*-treated group were obvious improved as compared with APAP group. In A.vera-treated group, the degree of hepatic necro-inflammation was lower than in APAP group.

Conclusion: *Aloe vera* can prevent hepatic damage from acetaminophen overdose primarily by restoring hepatic glutathione and decrease oxidative stress.

Key words : acetaminophen, *Aloe vera,* hepatitis, oxidative stress

[Thai J Gastroenterol 2013; 14(1):9-15.]

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INTRODUCTION

Acetaminophen [paracetamol, N-acetyl-*p*-aminophenol(APAP)] is a widely used analgesic and antipyretic agent. APAP has been generally considered safe and effective when consumed as recommended dose (1-4 g/day), however this drug has been reported as the major cause of drug-induced liver injury (DILI)[1]. According to toxicology centers in the United States, acetaminophen poisoning was responsible for more than 70,000 visits (in single agent use) and about 6,000 cases in combination with other drug to health-care facilities in 2010[2]. The United States Acute Liver Failure Study Group (USALF) report APAP overdose as the most common cause of acute liver failure (46%) with mortality rate approximately 300 deaths per year, divided in single overdose (intentional use or self-harm) group and therapeutic misadventure group in which the daily dose has not greatly exceeded recommend safe limit but had prolonged use of APAP, multiple hepatotoxic drugs or specific risk factors (chronic alcoholic consumption, malnutrition, fasting)[3]. At supratherapeutic dose (> 7-10 g/day) of APAP documented that cause severe liver injury secondary to massive hepatic necrosis[4,5]. Depletion of glutathione (GSH) stores via overdose of APAP, malnutrition or alcohol ingestion results in the ability of the liver to detoxify reactive metabolites and increase the risk of APAP toxicity[6]. N-Acetylcysteine (NAC) is the standard therapy for treatment of the APAP overdose patient by restoring hepatic GSH[7,8]. In addition, NAC improves hemodynamics and oxygen use, decreases cerebral edema and increases clearance of indocyanine green (a measure of hepatic clearance) in liver failure[9]. Recent studies represent other cellular mechanisms of APAP-induced hepatotoxicity such as lipid peroxidation, protein adduction binds to cell macromolecules, disruption of cytoskeleton, massive calcium influx, and oxidative stress that causes mitochondrial damage[10-12].

Aloe vera (Aloe barbadensis Mill) is classified in family of *Aloaceae*, originated in the dry areas of Africa, Asia, and Southern Europe, especially in the Mediterranean regions. *Aloe vera* and other species of Aloe are succulent and xerophytic plants that are adapted to living in areas with little water. These plants possess extensive water storage tissue in their leaves, the part of the plant which is used for its therapeutic properties[13]. The largest component (60%) in the dry matter, are the carbohydrates (soluble sugar and complex polysaccharides). *Aloe* is known well for its topical use as an anti-inflammatory and for curing wounds and burns[14]. Anti-oxidative, anti-inflammatory, and immunosuppressive activities of *Aloe vera* has been indicated[15,16]. Acemannan is a key compound that stimulates the immunity mediated by the cells[17].

In this study, we aimed to evaluate the effect of *Aloe vera* in APAP overdose-induced hepatotoxicity in mice.

MATERIALS AND METHODS

Chemicals

Aloe vera extract in powder form (Sigma®) was dissolved in distilled water (dH_2O) that was freshly prepared for the experiment. A single dose of 150 mg/kg of *Aloe vera* extract was administered to mice by oral gavage.

A single dose of 400 mg/kg of APAP (Tylenol®) which is freshly prepared by dissolving in distilled water (dH_2O) was administered to mice by oral gavage.

Animals

Male mice, weighing 25-30 gms (5-6 weeks of age) were purchased from the National Laboratory Animal Center, Salaya Campus, Mahidol University, Thailand and use as experimental animals. All animals were acclimated for at least 6 to 7 days to a 12-hour light-dark cycle in a humidity- and temperature-controlled room, were fed *Ad libitum* and water until experimental use. The study was performed in adherence to the National Institutes of Health guidelines for the experimental use of animals and followed a protocol approved by the Animal Care and Use Committee, Faculty of Medicine, Chulalongkorn University, Thailand. The mice were then fasted for 12 hours before experiments to sensitize mice to APAP.

Mice were divided into three groups.

Group I (control); mice were gavaged with dH_2O.

Group II (APAP group); mice were gavaged with a single dose of 400 mg/kg of APAP.

Group III (*A.vera*-treated group); mice were gavaged with a single dose of 400 mg/kg of APAP with a single dose of 150 mg/kg of *Aloe vera* extract.

Study design

Twenty four hours after APAP administration, all mice was anesthetized with intraperitoneal (i.p.) injection of thiopental sodium. The abdomen was opened

medially and the whole liver was rapidly removed and washed with cold normal saline (4-8˚C). The tissues were chopped into small pieces, frozen in liquid nitrogen, and stored at -80˚C for hepatic glutathione (GSH). The remaining liver tissue was fixed in 10% formalin solution for histopathology. Subsequently, the whole blood of mice was collected from the heart. The blood was allowed to coagulate at room temperature for 2 hours and then centrifuged for 20 minutes at 3,000 revolution per minute (r.p.m.) to obtain serum. The serum was collected to determine liver enzymes (AST and ALT).

Hepatic GSH assay

Cayman' GSH assay utilizes an optimized enzymatic recycling method, using GSH reductase, for the quantification of GSH. The sulfhydryl group of GSH reacts with 5, 5'-ditrio-*bis*-(2-nitrobenzoic acid) (DTNB), or Ellman's reagent and produces a yellow colored 5-thio-2-nitrobenzoic acid (TNB). The mixed disulfide, GSTNB (between GSH and TNB) that is concomitantly produced, is reduced by GSH reductase to recycle the GSH and produce more TNB. The production of TNB is directly proportional to this recycling reaction which is in turn directly proportional to the concentration of GSH in the sample. The optical density (O.D.) of TNB is then measured at 405-414 nm. using a microplate reader, which provides an accurate estimation of GSH in the sample.

Hepatic MDA assay

Lipid peroxidation of liver in mice using thiobarbituric acid (TBA) was measured by a modified method of Ohkawa *et al*[18]. One gram of liver tissue was homogenized in 3 milliliter (mL) of 50 millimolar (mM) potassium phosphate buffers (pH 7.0). To 0.3 mL of liver homogenated in test tube, 1.5 mL of 10% trichloroacetic acid (TCA) solution and 1.5 mL of 0.8% TBA solution were added. The mixture was boiled in waterbath at 95˚C for 60 minutes and then cooling with water at room temperature. After centrifugation at 3000 r.p.m. for 15 minutes, the absorbance of sample was measured at 532 nanometer (nm). 1, 1, 3, 3 tetramethoxy propane (TMP) was used as a standard of MDA. The MDA content was calculated in comparison with a standard MDA curve and was expressed as nanomole per milligram (nmol/mg) protein.

Histopathology

After the fixation of liver samples in 10% formalin solution at room temperature and then we used the standard method for preparation of histopathological specimens. Hepatic tissues were embedded in paraffin, sectioned at 5μm, and stained Hematoxylin & Eosin (H&E), and then picked up on glass slides. The histological slides were evaluated under light microscope (LM) by an experienced pathologist who is blinded to the experiment. All fields in each section were examined for grading of hepatic necroinflammation according to the criteria described by Brunt EM *et al*[19] from 0 to 3 as follow; score 0 = no hepatocyte injury/inflammation, score 1 = sparse or mild focal zone 3 hepatocyte injury/inflammation, score 2 = noticeable zone 3 hepatocyte injury/inflammation, and score 3 = severe zone 3 hepatocyte injury/inflammation.

Statistical analysis

All data will be presented as mean ± standard deviation (SD). Comparisons involving more than 2 groups will be analyzed by one-way ANOVA and Turkey PostHoc comparisons were employed. In all statistical comparisons, a *p*-value less than 0.05 will be used to consider a statistically significant. The data will be analyzed using the SPSS software version 17.0 for windows.

RESULTS

The serum AST and ALT were significantly increased in APAP group when compared with control group (AST; 1,361.2±290.3 vs. 107.8±38.7 U/L and ALT; 1,210.5±533.9 vs. 85.3±28.3 U/L, $p < 0.05$) and significantly decreased in *Aloe vera*-treated group compared with APAP group (AST; 458.6±228.2 vs. 1,361.2±290.3 U/L and ALT; 606.4±495.5 vs. 1,210.5±533.9 U/L, $p < 0.05$) (Figure 1 and 2).

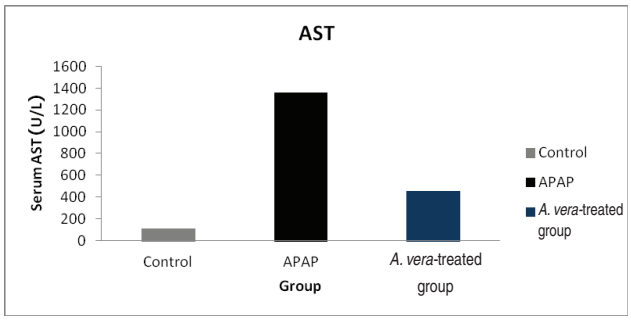

Figure 1. Serum AST: hepatic enzyme (AST) in serum was determined. The result (means ± SD) of "APAP" group was significantly increased from corresponding "control" group, $p < 0.05$, and was significantly different of *A.vera*-treated group.

Hepatic GSH was significantly decreased in APAP group when compared with control group (6.0±0.3 vs. 11.6±0.4 nmol/mg protein, $p < 0.05$). These were significantly restored in *A.vera*-treated group when compared with APAP group (10.0±0.2 vs. 6.0±0.3 nmol/mg protein, $p < 0.05$) (Figure 3).

Hepatic MDA which use as the marker of oxidative stress significantly increased in APAP group when compared with control group (3.6±1.5 vs. 1.4±0.1 nmol/mg protein, $p < 0.05$). These were significantly decreased in *A.vera*-treated group (1.5±0.6 vs. 3.6±1.5 nmol/mg protein, $p < 0.05$) when compared with APAP group (Figure 4).

In control group, hepatic necroinflammation score was 0 that showed normal (n=8) (figure 5A). In APAP group, the hepatic necroinflammation score was 2 (n=2), and 3 (n=6) that showed moderate and severe injury, consequently. In *A.vera*-treated group, the he-

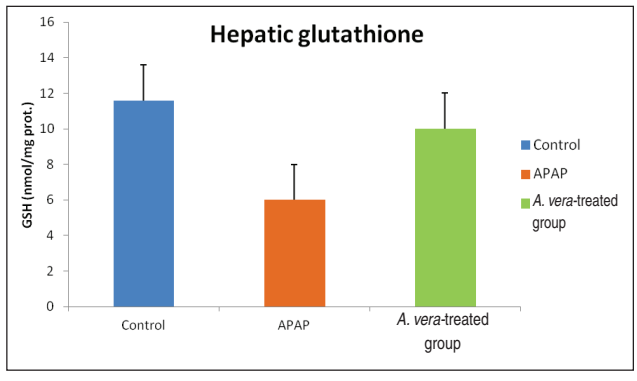

Figure 3. Hepatic glutathione (GSH) in serum was determined. The results are means ± SD of mice in " APAP" group significantly decreased when compared with "control" group, $p < 0.05$ and significantly different compared with *A.vera*-treated group ($p < 0.05$).

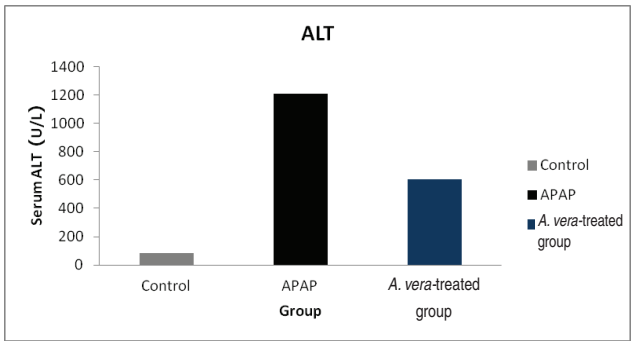

Figure 2. Serum ALT: hepatic enzyme (ALT) in serum was determined. The result (means ± SD) of "APAP" group was significantly increased from corresponding "control" group, $p < 0.05$, and was significantly different of *A.vera*-treated group ($p < 0.05$).

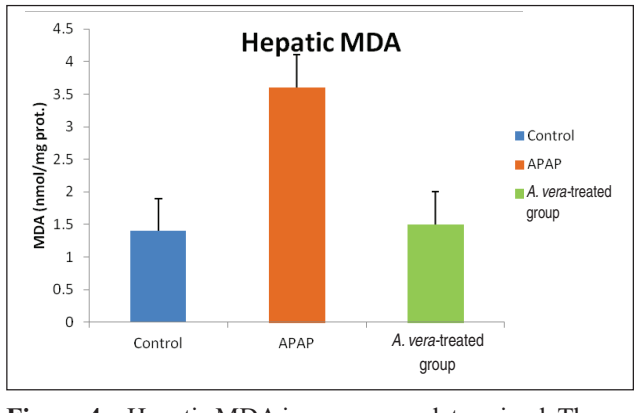

Figure 4. Hepatic MDA in serum was determined. The results are means ± SD of mice in "APAP" group significantly increased when compared with " control" group, $p < 0.05$ and significantly different compared with *A.vera*-treated group ($p < 0.05$).

Table 1. Summary of parameters in all groups.

Group	n	Parameters			
		AST (U/L)	ALT (U/L)	GSH (nmol/mg protein)	MDA (nmol/mg protein)
Control	8	107.8 ± 38.7	85.3 ± 28.3	11.6 ± 0.4	1.4 ± 0.1
APAP	8	1,361.2 ± 290.3*	1,210.5 ± 533.9*	6.0 ± 0.3*	3.6 ± 1.5*
A.vera-treated group	8	458.6 ± 228.2**	606.4 ± 495.5**	10.0 ± 0.2**	1.5 ± 0.6**

*$p<0.05$ compared with control.
**$p<0.05$ compared with APAP group.

Table 2. Summary of hepatic necroinflammation score in all groups. Data are reported as the number of mice exhibiting the grade of hepatic necroinflammation.

Group	Number	Hepatic necroinflammation			
		0= none	1= mild	2= moderate	3=severe
Control	8	8	-	-	-
APAP	8	-	-	2	6
A.vera-treated group	8	-	4	3	1

* All fields in each section were examined for grading of hepatic necroinflammation according to the criteria described by Brunt EM *et al*[19].
0 = no hepatocyte injury/inflammation
1 = sparse or mild focal zone 3 hepatocyte injury/inflammation
2 = noticeable zone 3 hepatocyte injury/inflammation
3 = severe zone 3 hepatocyte injury/inflammation

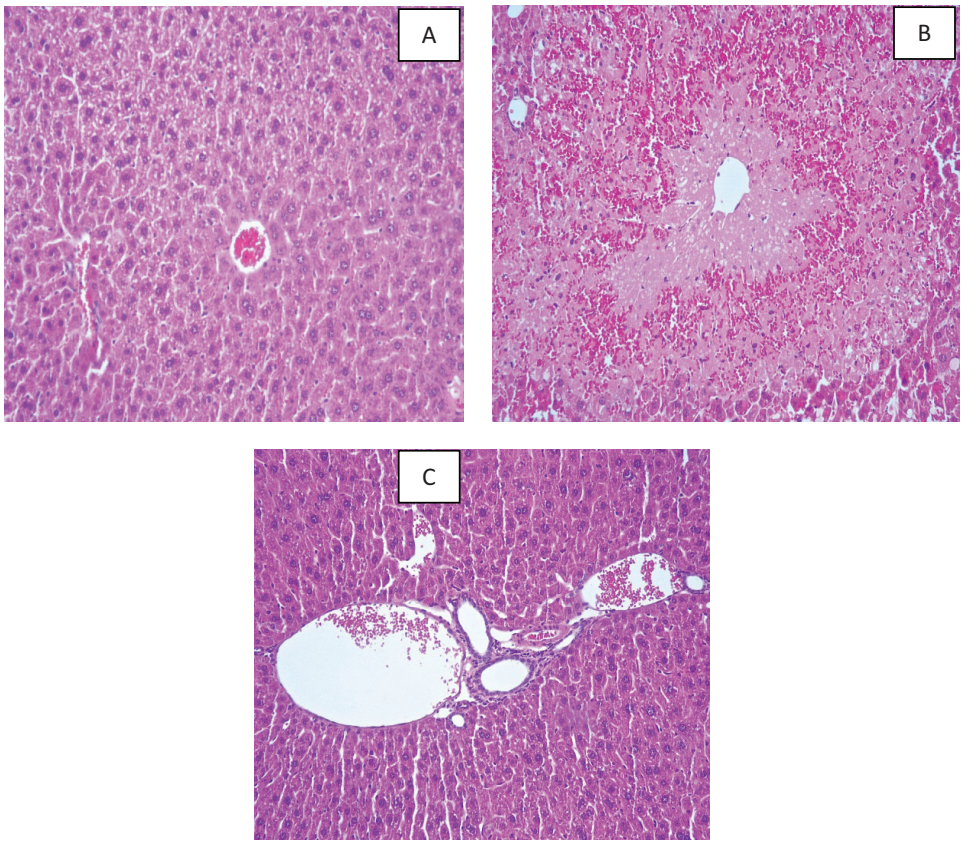

Figure 5. Liver histopathology (A); Control group, (B); APAP group, (C) *A.vera*-treated group, and were sacrificed at 24 h after the treatment. The picture represented X10 magnification. Liver sections were subjected to H & E staining.

patic necroinflammation score of was 1 (n=4), 2 (n=2), and 3 (n=1) that showed improvement of severity (Table 2). Histological examination of APAP group when compared with control group showed acute centrilobular hemorrhagic hepatic necrosis involving all zones (Figure 5B). The improvement of liver pathology revealed in *A.vera*-treated group when compared with APAP group that showed moderate focal necrosis with mild fatty changes and the classical hepatic architecture was preserved (Figure 5C).

DISCUSSION

APAP-induced liver injury has reported in sev-

eral mechanisms[10-12]. APAP undergoes metabolism to glucuronide and sulfate conjugates, which are excreted in the urine. Normally, only a small proportion(about 5%) of APAP is oxidized to NAPQI, that is detoxified by hepatic GSH. Excessive doses of APAP (> 7.5 g/day in adult or > 150 mg/kg in children) lead to the saturation of the glucuronic acid and/or sulfate pathways shunting more APAP into the CYP system[6]. The hepatorenal toxicity occurs when GSH reserves are depleted. NAPQI binds cellular proteins including oxidation of thiol groups in mitochondria leading to mitochondrial permeability transition. The ensuing mitochondrial dysfunction generates profound oxidative stress and also facilitates peroxynitrate formation. These events culminate in nuclear and cytoplasmic swelling, cytoplasmic vacuolization, and mitochondrial swelling of both hepatocytes and sinusoidal endothelial cells that finally cause apoptosis[21]. Immune mechanisms have been increasingly implicated in APAP-induced hepatotoxicity[22]. APAP-induced hepatocyte death leads to the release of free DNA and the subsequent activation of Toll-like receptor, increase production of interleukin-1(IL-1) and other cytokines leading to neutrophil recruitment. Lipid peroxidation of cell membrane results in decreasing of membrane fluidity, inability to restore ionic gradients that finally cause cellular swelling or tissue inflammation.

The present study demonstrates that APAP overdose results in hepatic GSH depletion corresponds to previous studies in animal model[23-24]. GSH is essential for detoxification of APAP metabolites and NAPQI that cause hepatic necroinflammation. Administration of *Aloe vera* extract in mice can restore hepatic GSH that results in decrease level of hepatic enzymes and improvement of liver histopathology similar to previous studies[25-26].

Aloe vera contains polysaccharide substance "Acemannan" and other antioxidative agents that prior animal-model studies reporting about hepatoprotective effect in alcoholic hepatitis or chemical-induced hepatotoxicity such as carbon tetrachloride, lindane, azoxymethane, etc. In this study, we found that *Aloe vera* extract results in minimization of hepatic MDA level, represent the antioxidant effect in the treatment group. The anti-inflammatory activity of *Aloe* is associated with the inhibition of the cyclooxygenase activity, which prevents the synthesis of prostaglandins and other inflammatory cytokines[20].

The current treatment of APAP-induced toxicity include N-acetylcysteine (NAC) administration, the antidote of APAP. However, reports of adverse reaction after use of this antidote anaphylactic, bronchospasm, nausea, vomiting or rash, especially in high dose of NAC[27]. *Aloe vera* extract may be used as adjunctive therapy in APAP toxicity, by hepatoprotective effect of this natural agent. Further study requires for confirm our hypothesis and benefit of *Aloe vera* attenuate APAP-induced hepatotoxicity.

CONCLUSIONS

In conclusion, this study suggest that APAP-induced liver injury is related to multiple mechanisms such as depletion of hepatic GSH concomitant with the liver inflammation, induction of oxidative stress, and the damage of liver pathology. Our results show that *Aloe vera* can prevent the hepatic necroinflammation induced by APAP in mice by induction of hepatic GSH, reduction of oxidative stress, attenuation of liver inflammation, and the improvement of liver pathology. In addition, 150 mg/kg of *Aloe vera* extract can prevent the effects of APAP toxicity. *Aloe vera* attenuate hepatic injury from APAP by restoring hepatic glutathione, minimizing of oxidative stress and might be a novel natural therapeutic agent in the future other than current standard treatment.

ACKNOWLEDGEMENT

This study had a partial financial support from the grant of "Gastroenterology Unit, Department of Internal Medicine, Faculty of Medicine, Chulalongkorn University" Bangkok, Thailand.

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