

Negative effects of *Aloe vera* gel on paracetamol-induced liver injury in rats

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ABSTRACT: The effect of *Aloe vera* gel on paracetamol induced liver injury in rats was studied using male Wistar rats divided into four groups. Group 1 (control) received 50% sucrose orally twice daily for 2 days. Group 2 (paracetamol) received a single dose (2.5 g/kg BW) of paracetamol dissolved in 50% sucrose. Groups 3 and 4 (*A. vera* 100 mg and 300 mg, respectively) received a single dose (2.5 g/kg BW) of paracetamol dissolved in 50% sucrose followed by 100 or 300 mg/kg BW of *A. vera* gel twice daily for 2 days. Blood was collected to determine alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymatic activities. Liver tissue samples were collected for hepatic glutathione, hepatic malondialdehyde (MDA), and histopathology. The results showed that the serum levels of ALT, AST, and hepatic MDA increased significantly in the paracetamol group compared to that of the control group. The liver histopathology in the paracetamol group revealed vacuolization, cell swelling, and mild infiltration of inflammatory cells around the central vein. Necrosis was observed predominantly around the centrilobular and midzonal regions. There were no differences in hepatic glutathione levels between groups and no difference in hepatic MDA levels between the paracetamol group and *A. vera* gel treated groups. This study concluded that neither 100 nor 300 mg/kg BW of *A. vera* protected rats from the liver damage induced by a high dose of paracetamol.

KEYWORDS: glutathione, malondialdehyde, serum aminotransferases, centrilobular necrosis, acetaminophen

INTRODUCTION

Paracetamol is a popular non-prescription analgesic and antipyretic drug. At therapeutic dose, it is believed to be safe but an overdose can cause poisoning and a potentially lethal hepatotoxicity¹. Paracetamol toxicity is the leading cause of acute liver failure in many countries^{2–4}. The toxicity depends on the metabolic activation of paracetamol via cytochrome P-450, which results in the formation of an electrophilic reactive metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI)⁵. NAPQI is quickly detoxified by hepatic glutathione to a nontoxic paracetamol-mercaptate compound that is renally excreted¹. Paracetamol overdose causes glutathione depletion and generation of reactive oxygen species resulting in the metabolite covalently binding to hepatic macromolecules⁵. In addition, oxidative stress, including lipid peroxidation can cause irreversible membrane injury and cell death^{6–8}.

Aloe vera, a perennial succulent plant belonging to the Liliaceae family, is widely distributed in tropical areas including Thailand. It has been used worldwide as a traditional medicine for many centuries.

The chemical compositions of *A. vera* leaf include glucomannans, acemannan, minerals, enzymes, inorganic compounds, flavonoid compounds, phenolic compounds, etc⁹. Several studies have demonstrated that *A. vera* extract has therapeutic properties such as anti-inflammatory effects, wound healing, anti-diabetic activities and antioxidant effects^{10–14}. In addition, some studies show a hepatoprotective effect of *A. vera*. The aqueous extract of *A. vera* has been shown to restore liver enzymes, triglycerides and improve liver histopathology in carbon tetrachloride induced hepatotoxicity¹⁴. Another study reported that fresh *A. vera* gel reduced serum γ -glutamyl transferase in petroleum-product induced hepatotoxicity¹⁵. The aim of this study was to test if *A. vera* has any hepatoprotective effects in rats treated with a high dose of paracetamol.

MATERIALS AND METHODS

A. vera gel preparation

A. vera grown in Nakhon Si Thammarat province, Thailand was used to prepare *A. vera* gel. *A. vera* gel was separated from the leaves and homogenized in

an electric blender. The extract was filtered and then lyophilized using a freeze dryer. *A. vera* gel powder was stored at -20°C until use.

Total phenolic contents determination

Total phenolic content in the *A. vera* gel powder was determined using the Folin-Ciocalteu method¹⁶ with some modifications, results are expressed as mg gallic acid equivalents per 1 g of *A. vera* gel powder. One gram of *A. vera* gel powder or standard solution was mixed with 1 ml of 2% sodium carbonated solution and 50 μl of Folin-Ciocalteu reagent. After incubation for 30 min at room temperature, the absorbance was measured at 750 nm on a visible spectrophotometer.

Animals

Male Wistar rats weighing between 220 and 250 g were purchased from the animal centre, Faculty of Science, Prince of Songkla University. The rats were kept in cages with access to food and water ad libitum in a room with controlled temperature ($23 \pm 2^{\circ}\text{C}$) and a 12-h light-dark cycle. The study protocol was approved by the Ethics Committee on Animal Experiment of Walailak University (protocol number 001/2011, approved on 18 April 2011). The rats were fasted for 12 h before the experiment. The rats were divided into 4 groups of 6 rats each. Group 1 (control) received 50% sucrose orally twice daily for 2 days. Group 2 (paracetamol) received a single dose (2.5 g/kg BW) of paracetamol dissolved in 50% sucrose. Groups 3 and 4 (*A. vera* 100 mg and 300 mg, respectively) received a single dose (2.5 g/kg BW) of paracetamol dissolved in 50% sucrose followed immediately by oral administration of 100 or 300 mg/kg BW of *A. vera* gel twice daily for 2 days.

The doses of *A. vera* gel in the present study based on the hepatoprotective activity of *A. vera* gel¹⁵. The 300 mg/kg BW of *A. vera* gel exhibited hepatoprotective activity in petroleum products-induced liver injury¹⁵. Furthermore, the 100 mg/kg BW of *A. vera* gel showed cardioprotective activity¹⁶. Hence, doses of 100 and 300 mg/kg BW of *A. vera* gel were used in the study.

Study design

Forty-eight hours after paracetamol administration, the rats were anaesthetized with intraperitoneal injection of thiopental sodium. Blood was collected via cardiac puncture and the rats were then sacrificed. The abdomen was opened and the whole liver was quickly removed and washed in ice-cold saline solution. A small section of the liver was stored at -80°C for hepatic MDA and hepatic glutathione analysis. The

remaining liver was fixed in 10% formalin solution for histopathology and embedded in paraffin.

Assay of serum ALT and AST

The blood samples were allowed to coagulate at room temperature for 30 min and then centrifuged at 3000g for 15 min at 4°C . The activity of serum ALT and AST were measured by an automated analyser (Siemens).

Estimation of hepatic MDA

Lipid peroxidation was measured using the method in Ohkawa et al¹⁷. A 200 μl aliquot of liver homogenate was added to a test tube containing 8% sodium dodecyl sulphate, 20% acetic acid, and 0.8% thiobarbituric acid. The mixture was boiled in a water bath at 95°C for 1 h and then cooled to room temperature using tap water. The mixture was centrifuged at 4000g for 10 min at room temperature, the supernatant was then collected and the absorbance measured at 532 nm on a visible spectrophotometer. Hepatic MDA content was determined by comparison with a standard MDA curve and expressed as nmol/mg protein.

Determination of hepatic glutathione

Hepatic glutathione was determined by using the Glutathione assay kit (Cayman Chemical Company). Briefly, livers were homogenized at 0°C in phosphate buffer containing 1 mM EDTA and centrifuged at 10000g for 15 min at 4°C . The supernatant was collected for a hepatic glutathione assay. Hepatic glutathione was expressed as nmol/mg protein.

Histopathological examination

Microtome sections of 5 μm thickness were prepared from the formalin fixed liver according to the standard protocol and stained with haematoxylin and eosin. The histological slides were examined under light microscope by a pathologist. The extent of necrosis was graded from score 0–3 as described previously¹⁸. Score 0 means normal liver morphology, 1 means 1–2 hepatocyte rows around central vein demonstrated swelling, necrosis, 2 means extensive necrosis confined to the centrilobular region, and 3 means necrosis extending from the central zone to the midzone or further to the portal triad.

Statistical analysis

Results were expressed as the mean \pm S.E.M. for continuous data and frequency for categorical data (score of necrosis). Data were analysed using ANOVA and the Levene test was performed to evaluate the

Table 1 Serum activities of ALT and AST enzymes in different experimental groups of rats (mean \pm S.E.M., $n = 6$).

Group	ALT (U/l)	AST (U/l)
Control	46 \pm 3	97 \pm 5
Paracetamol	860 \pm 180*	1400 \pm 340*
<i>A. vera</i> 100 mg	2550 \pm 640*	3400 \pm 890*
<i>A. vera</i> 300 mg	2280 \pm 700*	3240 \pm 100*

*significantly different ($p < 0.05$) from the control

variance homogeneity. When the variances were homogeneous, Tukey's test was used to compare among all groups. If the variances were not homogeneous, the data were compared using Kruskal-Wallis and Mann-Whitney test. A p -value < 0.05 was considered significant.

RESULTS

Total phenolic contents

The level of total phenolic contents was 4.19 mg gallic acid equivalents per 1 g of *A. vera* gel powder.

ALT and AST activities

The serum activities of ALT and AST enzymes were significantly increased in the paracetamol treated group when compared with the control group. The *A. vera* gel treatment groups (100 or 300 mg/kg BW) did not show the expected decrease in the serum activities of ALT and AST as compared with the paracetamol treated group and there was no statistically difference between either the *A. vera* groups or the paracetamol group (Table 1).

Hepatic MDA

The level of hepatic MDA, a marker of oxidative stress, was significantly increased in the paracetamol treated group when compared with the control group. *A. vera* gel treatment (100 or 300 mg/kg BW) showed no statistically significant difference in the level of hepatic MDA compared with the paracetamol treated group (Fig. 1).

Hepatic glutathione

The level of hepatic glutathione in the control group was 2610 \pm 170 nmol/mg protein, while the paracetamol treated group was 2760 \pm 470 nmol/mg protein. In rats treated with paracetamol and *A. vera* gel (100 or 300 mg/kg BW), the levels of hepatic glutathione were 2880 \pm 200 and 2240 \pm 350 nmol/mg protein, respectively. There was however was no significant difference among the groups (Fig. 2).

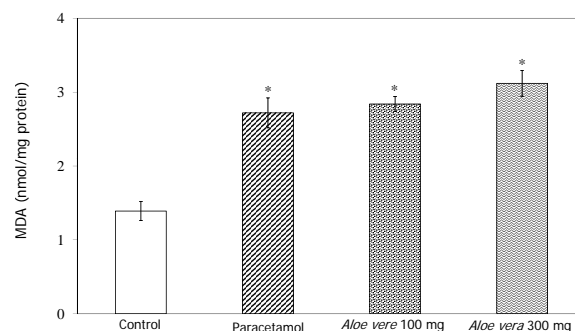


Fig. 1 Level of hepatic MDA in different experimental groups of rats (mean \pm S.E.M., $n = 6$); * indicates a statistically significant difference ($p < 0.05$).

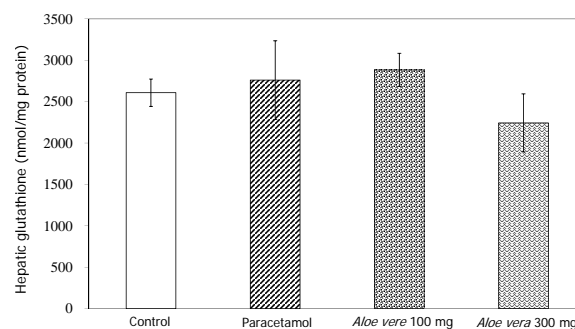


Fig. 2 Level of hepatic glutathione in different experimental groups of rats (mean \pm S.E.M., $n = 6$).

Histopathology

The histopathological examination of liver samples in the control group showed normal liver morphology, no inflammatory-cell infiltration, or necrosis (Fig. 3a). The paracetamol treated group showed vacuolization, cell swelling, and a mild infiltration of inflammatory cells around the central vein (Fig. 3b). The necrosis was observed predominantly around both the centrilobular and midzonal regions (Fig. 3b). *A. vera* gel treatment (100 or 300 mg/kg BW) however did not improve liver histopathology (Fig. 3c and 3d). Centrilobular necrosis, cell swelling and vacuolar degeneration also were found in this group (Fig. 3c and 3d). The summary of necrosis scores are shown in Table 2.

DISCUSSION

Paracetamol is widely used as an analgesic and antipyretic drug in many countries. In therapeutic doses, paracetamol is primarily metabolized via sulphation and glucuronidation and then the conjugated forms are eliminated from the body. In addition, a small proportion of paracetamol is oxidized by cytochrome

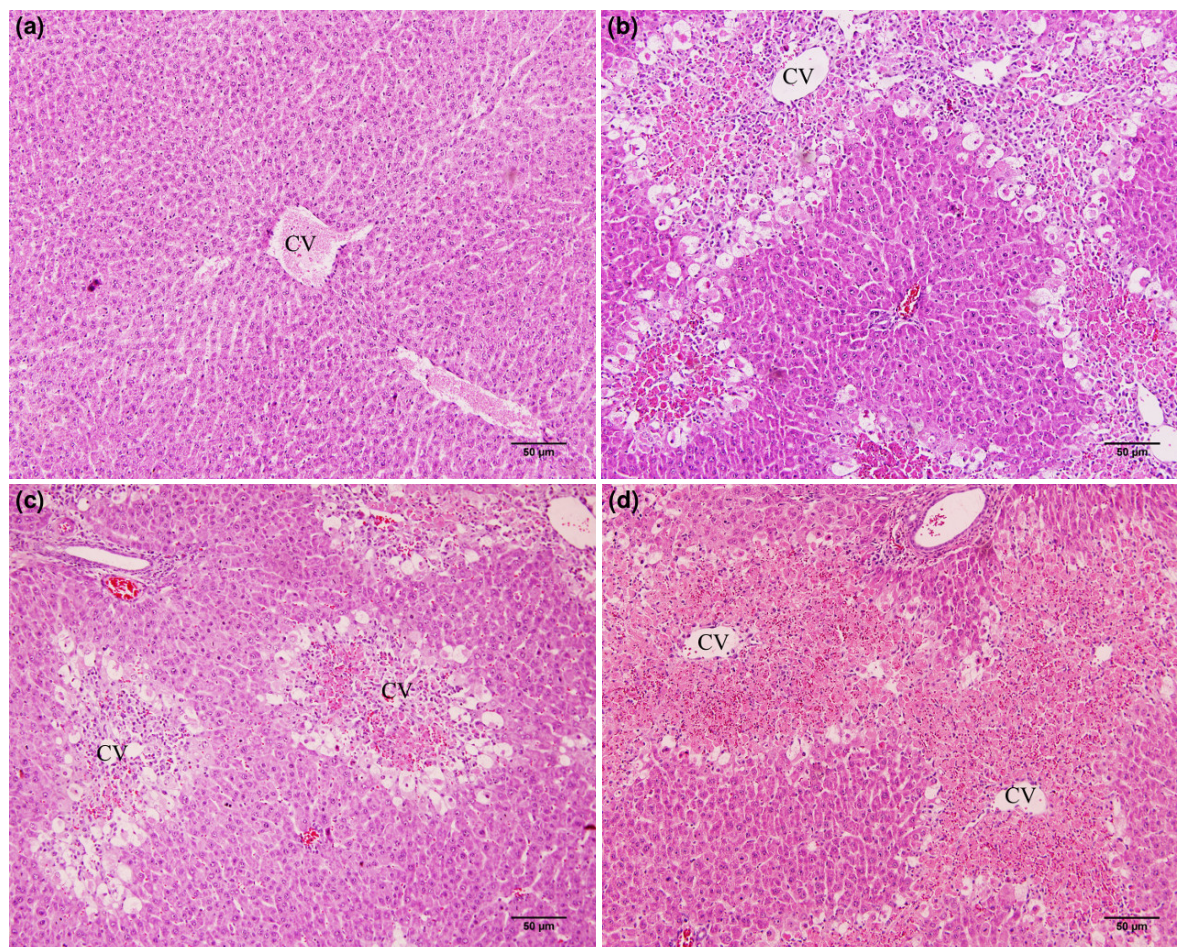


Fig. 3 Haematoxylin-eosin stained liver sections (20×): (a) control group showed normal liver histopathology; (b) paracetamol treated group, (c) and (d) *Aloe vera* gel treatment (100 or 300 mg/kg BW) showed vacuolization, cell swelling and centrilobular necrosis. CV: central vein.

P-450 (CYP2E1, CYP1A2 and CYP3A4) to a reactive metabolite NAPQI, which is quickly detoxified by hepatic glutathione to form a nontoxic paracetamol-mercaptate compound that is renally excreted. An overdose of paracetamol ingestion however generates NAPQI and depletes glutathione. When the hepatic stores of glutathione decrease to less than 30%, NAPQI binds to critical proteins which disrupts normal hepatocyte function and leads to liver injury and necrosis¹⁹. Our study showed that a single dose (2.5 g/kg BW) of paracetamol administration significantly elevated the ALT and AST activity. Leakage of ALT and AST into the blood circulation is a hallmark of hepatic injury in the paracetamol model^{7,8,19}. Furthermore, the histopathological changes such as severe centrilobular necrosis, cell swelling, and vacuolar degeneration were observed in the paracetamol-treated group. These results indicate that a single high

dose (2.5 g/kg BW) of paracetamol administration can cause severe liver damage.

Apart from the primary mechanism of paracetamol toxicity, it appears that biotransformation of paracetamol via cytochrome P-450 produces free radicals (superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals) leading to oxidative stress⁵. Free radicals formed within cells can oxidize biomolecules such as DNA, proteins, and lipids and this may cause cell dysfunction and death. One of the characteristic features of oxidative stress is an enhanced lipid peroxidation²⁰. The increase of hepatic MDA in the paracetamol group is consistent with previous studies^{6-8,21}, supporting a role of oxidative stress associated with lipid peroxidation in the development of paracetamol toxicity.

Glutathione is essential for detoxification of NAPQI to a nontoxic metabolite. Several studies

Table 2 Necrosis scores for each group ($n = 6$) expressed as the number of rats exhibiting the grade of necrosis.

Group	Necrosis score			
	0	1	2	3
Control	6	–	–	–
Paracetamol	–	–	1	5
<i>A. vera</i> 100 mg	–	–	1	5
<i>A. vera</i> 300 mg	–	–	1	5

Histopathology scores of necrosis: Score 0, normal liver morphology; Score 1, some of 1–2 hepatocyte rows around central vein demonstrated swelling or necrosis; Score 2, extensive necrosis confined to the centrilobular region; and Score 3, necrosis extending from the central zone to the midzone or further to the portal triad.

on the paracetamol model show paracetamol induced hepatic glutathione depletion^{7,19,21,22}. Some studies, measurement the hepatic glutathione levels at 48 h after paracetamol administration found a decrease hepatic glutathione levels^{21,22}. Hence, we evaluated hepatic glutathione levels at 48 h. The finding however indicates that hepatic glutathione levels did not decrease in the paracetamol-treated group. This study could be explained by a dynamic state of glutathione during paracetamol metabolism. Vendemiale et al observed the effects of paracetamol administration on hepatic glutathione content in rats and noted that the maximum hepatic glutathione depletion occurred 4 h after paracetamol administration, started to recover after 6 h, and at 24 h, had risen to approximately 73% of the basal value²³. Similarly, another study showed that glutathione levels were diminished in paracetamol treated mice at 2 and 4 h with signs of recovery at 6 h²⁴. It is not surprising therefore that the hepatic glutathione level did not change in the paracetamol treated group.

A. vera has therapeutic properties such as anti-inflammatory, wound healing, anti-diabetic, and antioxidant effects^{10–14}. The present study however demonstrates that *A. vera* gel treatment (100 or 300 mg/kg BW) did not decrease the liver enzyme levels, hepatic MDA levels, or improve liver histopathology in paracetamol-induced liver injury. This result may be due to differences in experimental models, dose of paracetamol, or duration of the *A. vera* gel treatment compared to other studies. For this reason, *A. vera* gel treatment, which exhibited a low total phenolic content (4.19 mg gallic acid equivalents per 1 g), could not protect liver injury.

A. vera gel has been reported as safe to the liver. Subacute toxicity study (300 mg/kg BW of

A. vera) showed the normal ALT and AST activities²⁵. The LD₅₀ of *A. vera* gel was found to be 4.8 g¹⁶. Some studies however have reported toxic effects of *A. vera* gel^{15,26}. *A. vera* gel treatment increased the γ -glutamyl transferase in mice¹⁵. A low molecular weight fraction from *A. vera* gel exerted cytotoxic effects in vitro study²⁶. *A. vera* contains quinones that react with protein thiol groups, and one should be aware of the possibility of adverse effects²⁶.

In conclusion, 100 or 300 mg/kg BW of *A. vera* gel treatment was not found to be hepatoprotective in paracetamol treated rats. The result of this study warrants additional examination of the effect of *A. vera* gel treatment before, during, and after paracetamol administration.

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