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Original Research Article

Protective effect of *Alhagi sparsifolia* against acetaminophen-induced liver injury in mice

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Abstract

Purpose: *To investigate the hepatoprotective effects of Alhagi sparsifolia extract against acetaminophen (APAP)-induced liver injury in mice.*
Methods: *Three doses of Alhagi sparsifolia (600, 300 and 150 mg/kg) were were administered to separate groups of mice orally once a day for three days. One-hour after the last dose, APAP (300 mg/kg) was intraperitoneally injected. Liver tissue was taken and tested for levels of serum aspartate aminotransferase (AST) and alanine transaminase (ALT) as biomarkers of liver injury; malonaldehyde (MDA); hydrogen peroxide (H_2O_2); glutathione (GSH) as an indicator of liver redox; and antioxidant enzyme activity using super oxide dismutase (SOD) assay. Additionally, western blotting was used to measure the expression of protein cytochrome P450 2E1 (CYP2E1) as the key enzyme of APAP metabolism.*
Results: *Blood serum of ALT and AST and levels of CYP2E1 were markedly reduced, while the levels of MDA, H_2O_2, and SOD were elevated in a dose-dependent manner in mice treated with Alhagi sparsifolia compared to control group treated with APAP alone.*
Conclusion: *The results demonstrate that Alhagi sparsifolia protects mice liver tissue against APAP-induced hepatic injury partly via decreased oxidative stress and inhibition of CYP2E1 expression.*

Keywords: *Alhagi sparsifolia, Polysaccharide, Acetaminophen, CYP2E1, Antioxidant*

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INTRODUCTION

Acute liver failure (ALF) is a devastating syndrome characterized by sudden loss of innate immunity which results in sudden cessation of normal liver function and multiple organ failures [1]. An intentional or accidental overdose is a critical factor in drug-induced liver injury (DILI), and the most clinically relevant drugs causing DILI is acetaminophen (APAP) [2]. APAP is one of the most widely used of the analgesic and antipyretic drugs; an overdose of APAP causes DILI in humans and animals [3,4]. It is also responsible for about 45,000 emergency cases per year in the United States [5].

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The liver is the primary location of APAP metabolism, using CYP enzymatic systems. Hepatic cytochrome P450 2E1, 12A, and 3A4 can potentially metabolize APAP to the toxic inter mediate product, N-acetyl-P-benzo-quinoneimine (NAPQI) [6], Which could in turn, combine with superoxide dismutase (SOD), glutathione (GSH), or cellularly important biological macro-molecules to induce liver cell death [7]. An APAP overdose increases mitochondrial dysfunction, and oxidative stress as well as increasing the formation of reactive oxygen species (ROS), which leads to acute liver injury with hepatocyte necrosis, mitochondrial DNA damage, apoptosis and nitration of mitochondrial proteins [8]. Previously, a large number of medicinal plants have been investigated for hepatoprotective effects against APAP-induced liver damage.

Alhagi sparsifolia (*Ci Tang* in Chinese, or *Taranjebin* in Uyghur) is also a well-known herb that is mainly distributed in the Xinjiang Uyghur autonomous region, it is widely used in Chinese traditional medicine to treat a number of disorders like arheumatism, cancer, and liver disorder and stomach disorders [9,10]. Uyghur ethno medicine describes, *Alhagi sparsifolia* as an antipyretic and anti-diarrhea that can be used without worries of toxicity [11]. Previous research has demonstrated that *Alhagi sparsifolia* possesses various pharmaceutical properties that provide benefits as an anti-inflammatory, antiviral, antibacterial, antihypertensive, hepatoprotective, and immunomodulatory agent [12-14]. To date, however, this herbal plant has only minimally been studied for its role as a hepatoprotective mechanism.

Hence, this study was conducted to focus on its novel effects of *Alhagi sparsifolia* on APAP-induced liver injury, exploring its potential mechanism.

EXPERIMENTAL

Plant material and preparation

Alhagi sparsifolia (harvested from the Turpan basin) was purchased from Xinjiang Uyghur Autonomous Region Uyghur Medicine Hospital in Xinjiang, China. Ten (10) grams of *Alhagi sparsifolia* was mixed with 200 mL of distilled water and boiled for 2 h in a round-bottomed flask. The supernatant was collected in a separate container, and the pellet was mixed with 100 mL of distilled water and again boiled for 1.5 h to collect supernatant. Finally, supernatants were mixed, filtered and the filtrate was lyophilized and stored at -20 °C.

Experimental animals

Five-week old Kun-Ming (KM) female mice (28 ± 2 g) were purchased from the Experimental Animal Center of the Fourth Military Medical University (Xi'an, China). All mice were maintained in a conventional sanitary facility, with the required consistent temperature and relative humidity. All animal experimental protocols were reviewed and approved by the Ethics Committee (no. 2015-mkrm01) of Northwest A&F University for the use of Laboratory Animals. We also followed the international guidelines for animal studies [15]. Mice were acclimatized to the laboratory conditions for two weeks prior to the experiment. Animals Unlimited access to standard food and distilled water (dH_2O), maintained at a laboratory temperature (22 ± 2 °C) and humidity (70 ± 4) % as well as 12 h light/12 h darkness cycle.

Animals and treatments

A total of 56 female mice were randomly divided into eight groups of seven animals each (n = 7). The negative control (NC) group received only 0.9 % physiological saline; the APAP group received 300 mg/kg APAP intraperitoneally; and the high, middle, and low dose Alhagi-honey groups (AH_H, AH_M, and AH_L, respectively) received 600, 300, and 150 mg/kg, respectively, of *Alhagi sparsifolia* by intragastric administration once a day for three consecutive days, with 300 mg/kg of APAP 1 h after the last administration of *Alhagi sparsifolia* Positive control groups received300 mg/kg silymarin (SL) and 300 mg/kg sodium salicylate (SS) followed by the same dosage regimen as the *Alhagi sparsifolia* groups.

The experimental animals were fasted overnight, weighed, and the mice were then anesthetized with chloral hydrate. Liver tissue samples were taken, and peritoneal venous blood was collected in 1.5 ml EP collection containers. The serum was separated from the blood samples by centrifugation at 2500 rpm for 10 min at 4 °C and stored at -20 °C until further analysis. The remaining liver tissues were thoroughly rinsed in cold 0.9 % physiological saline and divided into small portions, then frozen in liquid nitrogen and stored at -80°C for biochemical analysis.

Determination of total polysaccharides in *Alhagi sparsifolia*

Total polysaccharide content was measured in the *Alhagi sparsifolia* using the phenol-sulfuric acid method [16]. Also, 0.4 mL of *Alhagi sparsifolia* extract, 1.6 mL of distilled water, 1 mL

of 5 % phenol reagent and 5 mL of sulfuric acid solution were mixed in 10 mL test tubes. The mixture was eventually added to 10mL of distilled water and kept at room temperature for 10 min. The absorbance of the mixture was measured in a spectrophotometer at 490 nm. A standard curve for total polysaccharide content was prepared using glucose, and the total polysaccharide content was expressed as milligrams of glucose equivalents per gram of dry *Alhagi sparsifolia* extracts.

Serum alanine transaminase (ALT) and aspartate aminotransferase (AST) assays

Enzymatic activities of serum ALT and AST were estimated spectrophotometrically using commercial diagnostic kits per the manufacturer's protocol (Jiancheng Institute of Biotechnology, Nanjing, China).

Determination of hepatic MDA, H_2O_2, SOD, and GSH

Frozen liver tissues were thawed and homogenized in ice-cold PBS. The homogenate was centrifuged at 3,000 rpm for 10 min at 4 °C and the supernatants were assayed for MDA, H_2O_2, SOD, and GSH levels using commercial assay kits as per the manufacturer's instructions (Jiancheng Bioengineering Institute, Nanjing, China). The protein concentrations in tissue homogenates were measured using a Bradford protein assay with bovine serum albumin as standard (Tiangen Biotech, Beijing, China).

Western blot analysis

The microsomal preparation was carried out according to the method [15], and the protein concentration was using the BCA assay. Proteins were resolved by SDS-PAGE and transferred to PVDF membranes (Millipore Corporation, Boston, MA, USA). After blocking in TBST containing 5 % skimmed milk powder, the membranes were incubated overnight at 4 °C with primary antibodies against cleaved CYP2E1 (1:800) (Boster, Wuhan, China) and glyceraldehyde phosphate dehydrogenase (GAPDH, 1:1,000) (Tianjin Sungene Biotech, Tianjin, China). Blots were then incubated with a 1:1000 dilution of horseradish peroxidase conjugated secondary antibodies (Sungene Biotech, Tianjin, China) for 2 h at room temperature. Protein bands were visualized by ECL reaction (Genshare Biological, Xian, China). The protein levels were quantified using Gel-Pro Analyzer software (Media Cybernetics, Washington, DC, USA) and normalized to GAPDH.

Statistical analysis

All experimental data are expressed as mean ± SD. Significant differences from control in all experiments were assessed by one-way analysis of variance (ANOVA) using SPSS (IBM Corporation, Chicago, IL, USA), and $P < 0.05$ was considered statistically significant.

RESULTS

Total polysaccharide content

The values of the standard sample showed a linear relation: $y = 0.15x - 0.013$ ($R^2 = 0.991$) for glucose. The total polysaccharide content in the extract was 692.20 ± 28.16 mg/g.

Alhagi sparsifolia protects against APAP-induced hepatic dysfunction

Serum AST and ALT activities were significantly increased in the APAP-treated group compared with the control group, confirming the hepatotoxicity of APAP (Figure 1). In the group that received APAP following pretreatment with *Alhagi sparsifolia* serum (600, 300 and 150 mg/kg), ALT and AST activities were significantly reduced compared with the APAP group, indicating that *Alhagi sparsifolia* protected against APAP hepatotoxicity.

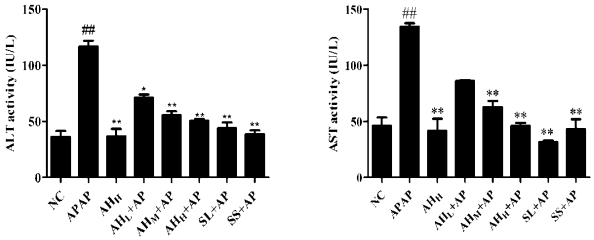

Figure 1: Effect of *Alhagi sparsifolia* on APAP-induced increase in serum transaminases levels. ***Note:*** Mice were administered different concentrations of Alhagi sparsifolia for three days before of 300 mg/kg APAP ip. Blood serum was collected 24 h after APAP injection. Each data expressed as mean ± SD (n = 7), $^{\#\#}p < 0.01$ compared with the NC group; $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the APAP administrated group

Alhagi sparsifolia inhibits APAP-induced oxidative stress in mice liver

APAP administration resulted in significant increases in MDA and H_2O_2 levels by 207.7 and 95.3 % respectively, when compared to the normal control group. Treatment with AH_H-APAP inhibited the increase of MDA and H_2O_2 level by 108.5 and 205.8 %, respectively (Figures 2A and 2B). In the APAP group, the concentration of

SOD and GSH were significantly reduced by 19.1 and 39.4 % when compared to the normal control group. Treatment with AH_H-APAP increased SOD and GSH levels by 8.3 and 40.8 %, respectively (Figures 2 C and D).

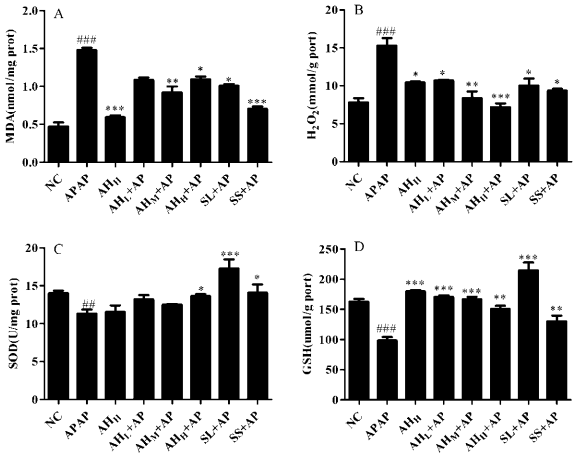

Figure 2: *Alhagi sparsifolia* reduced APAP-induced oxidative stress. ***Note:*** Mice were intragastrically administered different concentrations of *Alhagi sparsifolia* for three days before of 300 mg/kg APAP ip. Liver tissue was collected 24 h after APAP injection. Data expressed as mean ± SD (n = 7), $^{\#\#}p$ < 0.01 and $^{\#\#\#}p$ <0.001compared with the NC group; $^{*}p$ < 0.05, $^{**}p$ < 0.01, and $^{***}p$ < 0.01 compared with the APAP administrated group

Effect of .*Alhagi sparsifolia* on CYP2E1 protein expression

As shown in Figure 3, the expression levels of CYP2E1 in the APAP group were increased compared with the NC group ($p < 0.001$). The CYP2E1 level was decreased in the AH_H/AH_M/AH_L-APAP and SL/SS-APAP groups ($p < 0.001$).

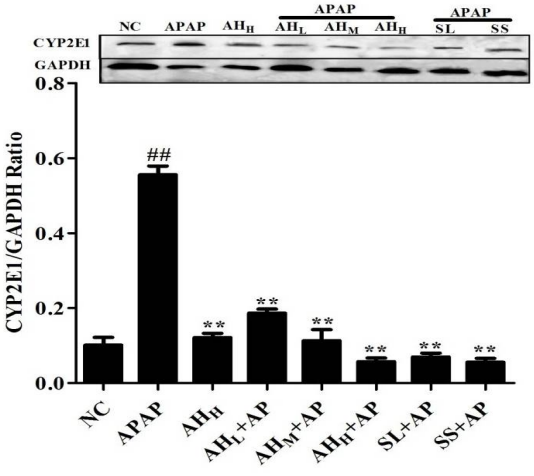

Figure 3: Effect of *Alhagi sparsifolia* on inhibition of CYP2E1 protein expression. ***Note:*** Mice were intragastrically administered different concentrations of *Alhagi sparsifolia* for three days, before of 300 mg/kg APAP ip. Liver tissue was collected 24 h after the APAP injection. Data are expressed as mean ± SD (n = 7), $^{\#\#}p$ < 0.01 and $^{\#\#\#}p$ < 0.001compared with the NC group; $^{*}p$ < 0.05, $^{**}p$ < 0.01, and $^{***}p$ < 0.01 compared with the APAP administrated group

DISCUSSION

Alhagi sparsifolia has been investigated for biological properties with therapeutic applications with various objectives. A previous study has illustrated that, *Alhagi sparsifolia* could attenuate carbon tetrachloride induced liver damage and drug induced acute liver failure. It has been speculated that the anti-oxidant properties of *Alhagi sparsifolia* might be responsible for its hepatoprotective effects [17].

In the present study, the levels of serum ALT and AST, as biomarkers of hepatic function, were significantly increased in the group treated with APAP-alone. ALT and AST are simple and widely accepted biomarkers for hepatic dysfunction, indicating that the APAP-induced liver damage model was successfully developed in mice [14]. In the *Alhagi sparsifolia* treatment group, the serum level of AST and ALT were markedly attenuate has compared to the APAP treatment group, suggesting that *Alhagi sparsifolia* has potential for use as APAP intervention.

Oxidative stress and lipid peroxidation play key roles in the occurrence and development of ALF. APAP oxidation in the liver produces many oxidative stress ROS products, such as superoxide anion radical, hydroxyethyl radicals, OH^- and H_2O_2. The decreased hepatic antioxidant status is related to oxidative stress and the elevation of lipid peroxidation; this could have resulted in the leakage of hepatic enzymes into serum in the animals that received only APAP [18]. A key protective action against APAP-induced liver injury is through anti-oxidant enzymes including GSH and SOD. These preventive antioxidants play a major role in protecting cells against oxidative stress during APAP metabolism, making them the first line of defense against oxidative injury. In this study, pretreatment with *Alhagi sparsifolia* significantly improved the survival rates of mice, inhibited the APAP-induced GSH and SOD depletion as well as improved the cell membrane stability.

Oxidative stress and lipid peroxidation play a key role in the occurrence and development of ALF. APAP oxidation in the liver produces a number of oxidative stress ROS products, such as superoxide anion radicals, hydroxyethyl radicals, OH^-, and H_2O_2. The accumulation of H_2O_2 exacerbates cell injury via lipid peroxidation, and

MDA is a reliable biomarker of lipid peroxidation [19]. In the present study, APAP-induced toxicity increased MDA and H_2O_2 levels in liver tissue as compared to the normal group. However, treatment with *Alhagi sparsifolia* significantly decreased the MDA and H_2O_2 levels in comparison with the negative control group.

In addition to the direct anti-oxidant properties, researchers have also focused on the influence of flavonoids on CYP450 enzymes [20]. CYP2E1 plays a key role in xenobiotic toxicity because it produces reactive metabolites and releases significant amounts of ROS during the course of its catalytic cycle [21]. Previous studies have shown that CYP2E1 plays etiological roles in the development of drug induced liver damage because the accumulation of mitochondrial CYP2E1 may be associated with the generation of ROS [22]. In addition, the increased mitochondrial CYP2E1 expression might have some harmful effects on hepatocytes [23]. A large number of natural products and their active substances could strongly inhibit the expression of CYP2E1 [24]. The findings in this study back that idea, where *Alhagi sparsifolia* treated groups had reduced CYP2E1 protein expression at a microsomal. It has also reported that polysaccharides and flavonoids are CYP450 inhibitor against protein expression [25]. Likewise, in this study, *Alhagi sparsifolia* dramatically reduced mitochondrial and microsomal CYP2E1 protein levels in APAP and *Alhagi sparsifolia* treated hepatocytes as compared to the group treated with only APAP.

CONCLUSION

In this study, *Alhagi sparsifolia* significantly attenuated APAP by enhancing the antioxidative defense and scavenging ROS, by decreasing the blood serum levels of transaminase (ALT and AST), and by reducing CYP2E1 expression. Consequently, *Alhagi sparsifolia* is a potential hepatoprotective agent against APAP-induced liver injury. However, further investigations are needed to ascertain its safe and effectiveness in humans.

DECLARATIONS

Acknowledgement

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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