Original Article

GASTROPROTECTIVE EFFECT OF ALHAGI MAURORUM ON EXPRIMENTAL GASTRIC ULCER IN RATS

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ABSTRACT

Objectives: *Alhagi maurorum* belongs to the family Leguminosae is used in Iranian folk medicine to treat gastric disturbances. The present study was undertaken to evaluate the *Alhagi maurorum* aqueous extract (AME) for anti-ulcer activity in rat.

Methodology*:* Male Wistar rats were pretreated with the AME (150, 300 and 450mg/kg, P.O.) before induction of gastric ulcer by water immersion restraint-stress (5 h, water immersion restraint stress at (20-22°C) or ethanol (100%; 1ml/200g of B.W, P.O). Negative control animals received saline (0.5ml/100g of B.W) & positive control animals received ranitidine (60mg/kg,P.O).

Results*:* The AME protected rats against water immersion restraint-stress and ethanol-induced ulcers in a dose-dependent manner. In water immersion restraint induced ulcerated rat, the AME increased pH and reduced gastric acid content. AME did not show any signs of toxicity and mortality up to10g/kg, P.O. in mice.

Conclusion*:* The results suggest that AME has significant mucosal protective and antisecretory effects on gastric mucosa in rats.

KEY WORDS: Alhagi maurorum, Antiulcer activity, Rat.

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INTRODUCTION

Gastric and duodenal ulcers are illnesses that affect a considerable number of people in the world. Some of the causes of these disorders are: stress, smoking, nutritional deficiencies and ingestion of nonsteroidal anti-inflammatory drugs.[1,2] The pathogenesis of gastroduodenal ulcers are influenced by various aggressive and defensive factors, such as acid-pepsin secretion, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents (prostaglandins and epidermal growth factor).[3] *Alhagi maurorum* (Syn. *Alhagi camelorum* and *Alhagi pseudoalhagi*) is a spiny plant with strong, stiff, abundant spines, belonging to Fabaceae family which locally known as Aqual. Traditionally, this plant is used for gastrointestinal disorders, gastric ulcer and rheumatism.[4,5] An aqueous extract of the whole plant of *Alhagi maurorum* is used in traditional medicine in southwest of Iran to treat heartburn resulted from gastric reflux. Phytochemical studies on this plant have revealed the presence of unsaturated sterols, triterpenes, tannins, carbohydrates, flavonoids,[6,7] flavanone glycosides such as alhagitin and alhagidin[8] and proanthocyanidins.[9] To validate the medicinal properties of *Alhagi maurorum*, we investigated the anti-ulcerogenic effects of an aqueous extract of *Alhagi maurorum* in two models of gastric ulcers induced by alcohol and water immersion restraint-stress in rats. The toxicity of the extract was also investigated in mice.

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MATERIALS AND METHODS

Extract preparation: Alhagi maurorum plant was collected in August 2005 from Ahwaz Jundishapur University of Medical Sciences

campus. The collected plant was dried in shade and then powdered. The powder was boiled in distilled water (1:10, W/V) for 60 min. The mixture was filtered through cheese cloth and centrifuged (3500 rpm, 15 min). The supernatant was concentrated and lyophilized for preservation (yield: 13%) which was stored at -4°C until used.

Animals: This study was carried out in Ahwaz Jundishapur University of Medical Sciences in August 2005. Male NMARI mice weighing 25-30g and male Wistar rats weighing 150-200g were obtained from the animal house of Ahwaz Jundishapur University of Medical Sciences. Animals were fed on conventional diets and water *ad libitum* and they were maintained under standard conditions of humidity, temperature (20-24°C) and light (12-h light: 12-h dark cycle). The rats were randomly assigned to control and different treatment groups, seven animals per group. All animal experiments were carried out in accordance with Ahwaz Jundishapur University of Medical Sciences, Ethical Committee acts.

Gastric ulceration: (a) In ethanol-induced gastric ulcer protocols, rats were starved of food but not for water 24 hours. Negative control group received saline and three groups received *Alhagi maurorum* aqueous extract (AME) at 150, 300 and 450mg/kg, p.o. 120 minutes before receiving ethanol and positive control group received ranitidine orally at 60 mg/kg 30 min prior were administered ethanol.[10] Ethanol was administered orally to these five groups at 1 ml/200g.[11] The volume of the saline, extract and ranitidine was 0.5ml/100g of body weight.

(b) Four groups of rats were used for restraint water immersion stress-induced gastric ulcer procedure, which starved of food but not for water 48 hours. Animals received saline (negative control group), extract (at 150 or 300mg/kg treated groups) or ranitidine at 60mg/kg (positive control group) orally 120 or 30 mintues prior to stress exposure in saline and extract groups and ranitidine group respectively. Rats were restrained individually in plexiglass restrainer and immersed up to their xiphoid in a water bath (20-22°C) for 5 hours.[12] Rats were then removed from restrainer and sacrificed by an overdose of diethyl ether and their stomachs removed and opened along the greater curvature and examined for gastric ulcers. The ulcer index in these rats was also scored by a person unaware of the experimental protocol. The sum of the area of all lesions in the corpus for each animal was measured and served as the ulcer index. The ulcer area was calculated using the formula area = length×width×ð/4.[13]

Gastric secretion study: To investigate the extract antisecretory activity, after removing rats from water, they were anesthetized by diethyl ether, stomach was ligated at lower esophageal sphincter and 2ml of saline (pH=7.0) infused in the stomach through the pylorus and then gastric content was drained for acid titration. Gastric washout (1ml) was titrated (Automatic Titrator Radiometer, Denmark) against 0.01 N of NaOH to endpoint 7.0 and acid content was expressed as µEq H^+.

Extract toxicity study: For acute toxicity study, the extract was administered in graded doses (1, 2, 4, 6, 8, and10g/kg, p.o.) to six groups of mice (6/group), while the control group received saline (2ml/kg, p.o.). All treated animals were closely observed for any abnormal or toxic manifestations and for mortality up to 48 h. Ethanol and diethyl ether were purchased from Merck (Germany), and ranitidine from Tolidaru (Iran).

Statistical analysis: The results were expressed as mean±SEM (n= number of animals in each group) and statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Student's *t*-test. *P* values of less than 0.05 were considered to indicate a significant difference between means.

RESULTS

Rat mucosal gastric injury induced by ethanol was reduced dose dependently (ANOVA, P<0.001) by aqueous extract of *Alhagi maurorum* (Figure-1). Administration of absolute ethanol to fasted rats resulted in severe gastric damage visible from the outside of the stomach as thick reddish-black lines. After opening, the gastric lesions were found in the

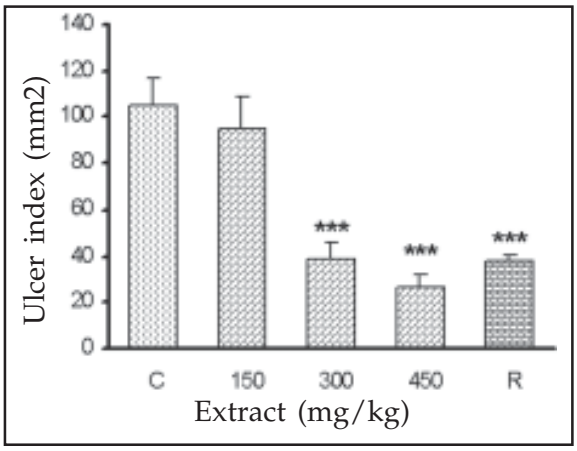

Figure-1: Effect of oral administration of *Alhagi maurorum* aqueous extract, and ranitidine (R; 60mg/kg) on gastric lesions induced by ethanol. The results are expressed as mean±SEM (n=7). Statistical comparison was performed using the ANOVA followed by *t*-test. ***P<0.001, when compared to control group (C; saline, 0.5ml/100 g, p.o.).

mucosa and consisted of elongated bands, 1–10mm long, usually parallel to the long axis of the stomach. They were located mostly in the corpus (the portion of the stomach secreting acid and pepsin). No gross lesions developed in the forestomach (nonsecretory part of the stomach). The *Alhagi maurorum* aqueous extract reduced the mucosal gastric lesion induced by water immersion restraint-stress test dose dependently (ANOVA, P<0.002). Ranitidine (60mg/kg, p.o.) protected the animals from ulceration significantly (Figure-2). In water immersion restraint-induced ulcerated

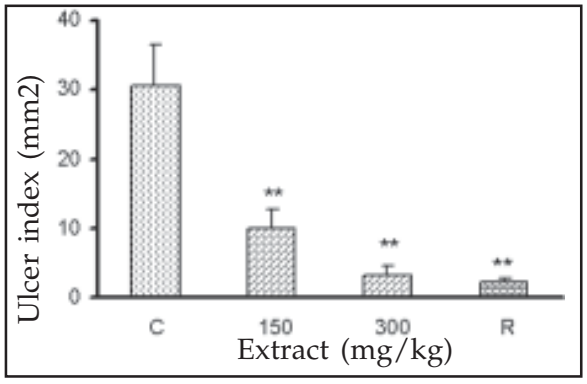

Figure-2: Effect of oral administration of *Alhagi maurorum* aqueous extract, and ranitidine (R; 60mg/kg) on gastric lesions induced by water immersion restraint. The results are expressed as mean±SEM (n=7). Statistical comparison was performed using ANOVA followed by *t*-test. **P<0.01 when compared to control group (C; saline 0.5ml/100 g, p.o.).

rats, the gastric acid content was lower and pH was higher (P<0.01) in animals pretreated with AME as compared to control rats (Table-I). On gross examination, all animals given AME at 1, 2, 4, 6, 8, and 10g/kg (p.o.) did not show any toxic symptoms and mortality up to 48 h after AME administration.

DISCUSSION

In the present study, we have indicated that the aqueous extract of *Alhagi maurorum* has an effective antisecretory and anti-ulcer activity against water immersion restraint- and ethanol-induced gastric ulcers. The examination of acute toxicity (LD_{50}) carried out on mice indicated that AME has no toxicity when administered orally (up to 10g/kg). The gastric protective effect of the extract may be related to an antacid effect or cytoprotective properties of the extract. The cytoprotective action against ethanol showed that the effect of extract is not only a simple acid neutralizing activity but the AME has a cytoprotective effect against the gastric mucosa in ethanol-induced gastric lesion in the rats. It is possible that the inhibitory effect of extract is due, at least partly, to the presence of terpenes in *Alhagi maurorum*.[6,7] Since, terpenes were associated to antiulcerogenic activity in other plants.[14,15] Some triterpenes are known as anti-ulcer drugs and their action has been suggested to be due to the reduction of mucosal prostaglandins metabolism, cytoprotective action and reduction of gastric vascular permeability.[16] Flavonoids have antiulcer and gastroprotective activity.[17-19] The aqueous extracts of

Table-I: Effect of oral administration of saline, *Alhagi maurorum* aqueous extract and ranitidine on gastric pH and acid content in rats exposed to water immersion-restraint stress.

Treatment	*Dose*	*gastric acid content (μEq H⁺)*	*pH*
Saline	0.5 ml/100g B.W	120.29±17.3	3.84±0.11
Extract	150 mg/kg	68.42±4.1**	5.65±0.12**
Extract	300 mg/kg	61.32±5.1**	5.88±0.19**
Ranitidine	50 mg/kg	50.20±4.4**	6.21±0.09**

The results are expressed as mean±SEM (n=7). Statistical comparison was performed using *t*-test. **P<0.01 when compared to control group.

Phoradendron crassifolium and *Franserio artemisiodes* that contain polyphenolic agents exert cytoprotective activity in rats.[20] Two flavonoids quercetin and catechin have been isolated from *Alhagi maurorum* with antioxidant activity which inhibit the lipid peroxidation and could counteract with free radicals.[21] This effect contributes the ulcer peptic prevention.[22] It has been shown that the flavanone glycosides have antiulcer activity.[23] Since, two flavanone glycosides, alhagidin and alhagitin, have been isolated from the whole plant of *Alhagi pseudoalhag*,[8] possibly these constituents implicated in the anti-ulcer activity of this plant. The extract provoked a marked decrease in gastric acid content and increase in pH values. It has been shown that the methanolic extract of this plant has calcium channel blocking activity on the gastrointestinal tract smooth muscle.[6] It is possible, therefore, that the extract acts via an anticholinergic mechanism and blocks the gastric acid secretion. At this stage, other mechanisms such as H_2 receptor antagonistic effect or the inhibition of gastric H^+/K^+-ATPase can not be excluded. In conclusion, *Alhagi maurorum* markedly inhibits acid secretion and the occurrence of lesions in stomach but exact mechanisms are not clear yet. The precise mechanism of action of *Alhagi maurorum* in protecting rats against induced gastric lesions is unknown. Further studies with isolated compounds are needed to elucidate the active principles and mechanisms involved in this activity.

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