

POSSIBLE ANALGESIC, ANTI-INFLAMMATORY AND ANTI-ULCEROGENIC EFFECTS OF *Alhagi maurorum* METHANOLIC EXTRACT IN RATS AND MICE

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Abstract: *Alhagi maruorum*, a species of family *leguminosae*, has high contents of flavonoids, triterpenes, sterols and glycosides. It is a woody perennial herb that was used in the folk medicine for gastric disturbance, rheumatic pains, liver disease and urinary tract infection. This study was designed to spot the light on some pharmacological activities of *Alhagi maurorum*. The fresh aerial parts of the plant were washed, dried at room temperature, crushed into fine powder, extracted with 80% methanol for three days with frequent agitation, filtrated and dried by rotatory evaporator under reduced pressure at 45°C. The concentrated filtrate then was lyophilized in lyophilizer. The extract was tested for some pharmacological activities (analgesic, anti-inflammatory and antiulcerogenic) in rats and mice. The current results proved that administration of *Alhagi maurorum* aqueous methanolic extract in mice at dose of 200 and 400 mg/kg BW exhibited analgesic effect that was noticed from a significant elevation in the reaction time of mice in a dose dependent manner when compared to the control mice by using hot plate method. Moreover, *Alhagi maurorum* aqueous methanolic extract demonstrated anti-inflammatory effect by using hind paw edema method in rats as confirmed by a significant dose dependent reduction in the thickness of the hind paw edema compared with the control group. Additionally, administration of *Alhagi* extract elicited a significant improvement in gastric damage induced by indomethacin in rats where mean ulcer score in *alhagi* 200 and *alhagi* 400 groups was 1.25 ± 0.045 and 0.25 ± 0.004 , respectively compared with 1.75 ± 0.054 of indomethacin group. It could be deduced from those results that *Alhagi maurorum* aqueous methanolic extract owns analgesic, anti-inflammatory, and antiulcerogenic activities.

Key words: *Alhagi maurorum*; aqueous methanolic extract; analgesic; anti-inflammatory; anti-ulcerogenic

Introduction

Natural products own valuable and advantageous biological activities (1). Researchers are designing several studies on natural products to find solutions toward tumor, viral

and microbial infections (2). *Alhagi marorum* belongs to the family *Leguminosae* commonly known as camelthorn (3). The family *leguminosae* contains about 550 genera and more than 13000 species (4). It supports us with

many edible plants. *Alhagi* species contain several compounds such as fatty acids and sterols (5,6), flavonoids (7-9), alkaloids (10), triterpenes resins, tannins and antipyretic, antioxidant and diuretic (11). *Alhagi marorum* is a foliage deciduous shrub. It grows up to 2 m and produces flowers in July. The flowers are small in size, bright pink to maroon in color and hermaphrodite. The plant grows in sandy and Loamy soils (12).

Alhagi maurorum has been used traditionally to treat polyps in nasal cavities, certain tumors (3) and kidney stones (13). It has been mentioned that *Alhagi maurorum* has many therapeutic properties as; laxative, gastroprotective, expectorant, antiseptic and antidiarrheal properties. Oil extracted from its leaves has been used for treatment of hemorrhoids and rheumatism, while its flowers have been reported to treat piles (12,14).

Alhagi was used as diuretic, antimicrobial and in treating of hemorrhoids and uterine diseases, moreover, its root was used as aphrodisiac (4,15,16). *Alhagi* is national to North Africa, Middle East and South East Europe. It is originating in wide areas including Asia (Bahrain, Kuwait, Afghanistan, India, Palestine, Jordan, Lebanon, Syria, Saudi Arabia, Iraq and Yemen); Africa (Egypt, Algeria, Libya, Niger, Sudan and South Africa); North America (USA); Europe and Australia (16).

Ethanollic extract of *Alhagi maurorum* gives gastroprotective effects against ulcers caused by phenylbutazone, indomethacin and ethanol (16). Antioxidant effect of the aqueous extract of *Alhagi maurorum* was assessed by measuring the concentration of lipid peroxidation marker malondialdehyde (MDA) and total antioxidant capacity (TAC). The test extract has shown a significant reduction in MDA level (17). *Alhagi maurorum* has been recorded to exert anti-inflammatory effect by inhibition the release of histamine and prostaglandin (pro-inflammatory mediators) of acute inflammation (18). The extract of *Alhagi maurorum* given to rats orally at dose of 100mg/kg body weight (BW) showed hepatoprotective and antioxidant activities, beside inhibition of fucosidase tumor marker and risky lipid ratio (19,20). Tamarixitin

3-o-dirhamnoside, isorhamnetin 3-o-rotinoside, isorhamnetin 3-o-robinoside, quercetin 3-o-rhamnoside, isorhamnetin 3-o-glucosylneohesperidoside and quercetin 3,7-diglycoside are examples of flavonoids isolated from *Alhagi maurorum* (21). Proceeding from the above, the current work was contrived to pore over the analgesic, antiinflammatory and antiulcerogenic activities of *Alhagi maurorum* aqueous methanolic extract.

Material and methods

Plant material

The parts above the ground (leaves, stems and flowers) were gathered during 2017 from Elsalihia village, Faqous, Sharkia Governorate, Egypt.

Animals

Forty-five adult male wister albino rats and 20 mice were obtained from Faculty of Veterinary Medicine, Zagazig University. Animals were kept under observation for two weeks for acclimatization to the laboratory conditions before starting the experiment. They were held under sanitary condition in metal cages and they had free access to standard laboratory diet. Housing and management of animals were conducted as stipulated in the guide for the care and the use of laboratory animal's guidelines of the National Institute of Health (NIH) and approved by local authorities of Zagazig University, Zagazig, Egypt.

Plant extraction

Three kilo grams of the fresh parts above the ground were washed completely with fresh water at room temperature to eliminate the dirt before the drying process and exposed to dryness for a week at room temperature. The plant was subjected to grinding into fine powder. Two hundred grams of the powder were put with 800 ml methanol and 200 ml water in a clean flask for 3 days with frequent agitation then filtration was done and the residue was taken for further maceration. The filtrate was dried by rotatory evaporator under reduced pressure at 45°C. The concentrated filtrate was lyophilized by using a lyophilizer

(Klee Lyophilizer, German) to obtain a stable cake dissolved in water when prepared to administration. The extract was placed in a refrigerator at 4°C in a glass container to protect it from the light until use. The extract was dissolved in sterile saline solution to be used in the experiment.

Drugs and chemicals

Indomethacin (Indomethacin100^R, MISR, Egypt), Ranitidine (Ranitidine150^R, MUP, Egypt), Carragenen (SIGMA-ALDRICH, USA) and Diclofenac sodium, voltaren 75mg/3mL^R (NOVARTIS, Switzerland) were dissolved in sterile saline solution to be used in this study.

Analgesic activity evaluation

Evaluation of the analgesic activity of the plant was performed using the hot plate method as described by Jacob and Bosovski (22). Twenty mature male albino mice weighing 20-25 g were allocated into 4 groups; each of five. The 1st group (control) was injected intraperitoneally (IP) with the solvent (sterile saline solution). The 2nd group (diclofenac) was injected IP by diclofenac sodium at a dose of 10 mg/kg BW (23). The 3rd group (*Alhagi* 200) received *Alhagi maurorum* methanolic extract at a dose of 200 mg/kg BW, per os (PO) (24). The 4th group (*Alhagi* 400) received *Alhagi maurorum* methanolic extract at a dose of 400 mg/kg BW, PO (24). Ten minutes later, each mouse was put in a two litres-beaker immersed in water bath thermostatically controlled at 56°C. The time passed away till the mouse licked its paw or jumped which was considered as the reaction time and was taken to evaluate the analgesic effect. Readings were recorded at 10, 20, 30, 60, 90 and 120 minutes after treatment.

Anti-inflammatory activity evaluation

Anti-inflammatory activity was assessed by using the method of rat hind paws edema (25) using diclofenac sodium as a standard. Twenty mature male albino rats weighing 150-200g were used. The animals were distributed into 4 equal groups; each of five. The 1st group (control) was treated IP with the solvent (sterile

saline solution). The 2nd group (diclofenac) was given diclofenac sodium IP at a dose of 10 mg/kg BW (23). The 3rd group (*Alhagi* 200) received *Alhagi maurorum* methanolic extract at a dose of 200 mg/kg BW, PO (24). The 4th group (*Alhagi* 400) received *Alhagi maurorum* methanolic extract at a dose of 400 mg/kg BW, PO (24). After an hour, carrageenan was injected in the right hind paw by dose of 0.1 ml of 10% to induce edema. The thickness of the paw was measured using a skin caliber at 1, 2, 3 and 4 hrs after the carrageenan injection to detect the anti-inflammatory activity of the tested extract.

Antiulcerogenic activity

Alhagi maurorum was tested for its antiulcerogenic activity using ranitidine as a reference drug at a dose of 100 mg /kg BW, PO once daily for rats (19) and indomethacin for induction of ulcer at a single dose of 100 mg/kg BW, PO (19). Twenty-five male albino rats of average weight 150-200g were allocated into five groups; each of five. The first group (control) received distilled water (0.5ml PO /animal) once daily for 10 days. The second group (ranitidine) received ranitidine (100 mg/kg BW, PO) once daily for 10 days then treated with a single dose of indomethacin (100 mg/kg BW, PO) one hour after the last dosing. The third group (indomethacin) received a single dose of indomethacin (100 mg/kg BW, PO) on the 10th day from the beginning of the experiment. The fourth group (*Alhagi* 200) received *Alhagi marorum* methanolic extract at dose of 200 mg /kg BW, PO once daily for 10 days then treated with a single dose of indomethacin (100 mg/kg BW, PO) one hour after last extract dosing. The fifth group (*Alhagi* 400) received *Alhagi marorum* methanolic extract at dose of 400 mg /kg BW, PO once daily for 10 days then treated with a single dose of indomethacin (100 mg/kg BW p.o) one hour after last extract dosing. Four hours after indomethacin administration. All rats were sacrificed, the stomachs were rapidly removed, opened along their curvature and examined for ulceration. The number and severity of discrete areas of damage in the glandular mucosa were scored. The ulcer score was calculated accor-

ding to the 1 to 5 scoring system devised by Wilhelmi and Menasse (26).

Ulcer index (U.I.) = Mean ulcer score of a group of animals similarly treated $\times$ % of ulcerated animals of this group (27).

Statistical Analysis

Repeated measure analysis of variance (ANOVA) test was done to determine the effect of time and different treatments on rats' hind-paw thickness and reaction time that represents the anti-inflammatory activity and the analgesic activity, respectively. Data were statistically described using mean $\pm$ SE. The results were considered significant with P value less than 0.05 ($P < 0.05$). All statistical analyses were done using IBM SPSS version 25.

Results

Analgesic activity

Mice treated orally with aqueous methanolic extract of *Alhagi maurorum* (200 and 400 mg/kg BW) revealed a significant ($P < 0.05$) dose dependent increase in the reaction time (till the mouse licked its paw or jumped) compared with the control group. However mice treated with diclofenac sodium (standard analgesic drug) demonstrated a significant ($P < 0.05$) rise in the reaction time compared with the control and *Alhagi maurorum* treated groups (Figure 1).

Anti-inflammatory activity

Oral administration of aqueous methanolic extract of *alhagi maurorum* in rats at dose of 200 and 400 mg/kg BW revealed a significant ($P < 0.05$) dose dependent decrease in the thickness of hind paw edema at 1st, 2nd, 3rd and 4th h after carragenan injection compared to the control group. Meanwhile, administration of diclofenac sodium (standard anti-inflammatory drug) in rats significantly ($P < 0.05$) reduced the thickness of hind paw edema at 1st, 2nd, 3rd and 4th h after carragenan injection compared with the control and *Alhagi maurorum* groups (Table 1).

Antiulcerogenic activity

Oral indomethacin administration in rats by a single dose of 100 mg/kg BW resulted in gastric mucosal damage confirmed by a significant ($P < 0.05$) elevation in mean ulcer score and ulcer index compared with the control group. Meanwhile, the aqueous methanolic extract of *Alhagi maurorum* at a dose of 200 and 400 mg/kg BW per os significantly ($P < 0.05$) improved the gastric damage induced by indomethacin in a dose dependent manner. However, rats treated with ranitidine orally at dose of 100 mg/kg BW for 10 days then were administered with a single dose of indomethacin after last extract dosing exhibited a significant ($P < 0.05$) reduction in mean ulcer score and ulcer index compared with indomethacin and *Alhagi maurorum* 200 treated groups (Table 2).

Table 1: Anti-inflammatory effect of *Alhagi maurorum* aqueous methanolic extract and diclofenac sodium against carragenan induced hind paw edema in rats. Mean $\pm$ SE N=5

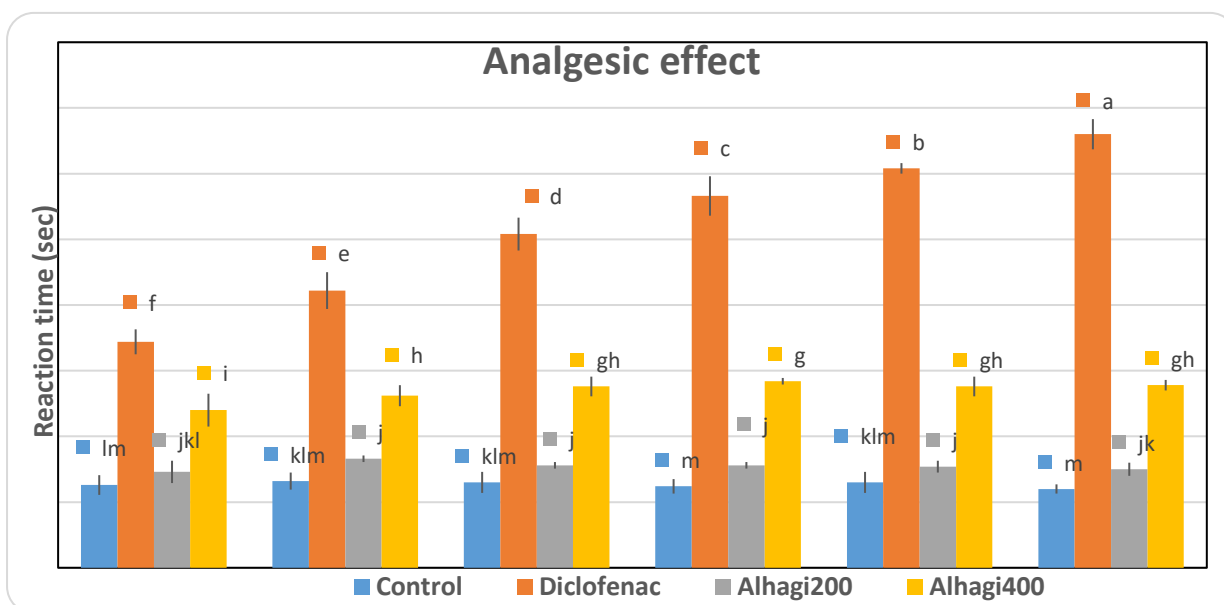
Group	Thickness of rat hind-paw (mm)					P-value
	Zero time	1 h	2 h	3 h	4 h	
Control	0.16 $\pm$ 0.01 ^j	0.65 $\pm$ 0.010 ^{de}	0.80 $\pm$ 0.08 ^{bc}	0.90 $\pm$ 0.03 ^{ab}	0.97 $\pm$ 0.05 ^a	0.000
Diclofenace	0.17 $\pm$ 0.02 ^j	0.24 $\pm$ 0.04 ^{ij}	0.34 $\pm$ 0.05 ^{hi}	0.38 $\pm$ 0.04 ^h	0.44 $\pm$ 0.04 ^{gh}	0.000
Alhagi200	0.18 $\pm$ 0.01 ^j	0.43 $\pm$ 0.02 ^h	0.55 $\pm$ 0.03 ^{ef}	0.63 $\pm$ 0.03 ^{ef}	0.74 $\pm$ 0.03 ^{cd}	0.000
Alhagi400	0.17 $\pm$ 0.02 ^j	0.37 $\pm$ 0.02 ^h	0.44 $\pm$ 0.02 ^{gh}	0.54 $\pm$ 0.03 ^{fg}	0.62 $\pm$ 0.02 ^{ef}	0.000

Means with different superscripts were significant at ($P < 0.05$).

Table 2: Antiulcerogenic activity of *Alhagi maurorum* methanolic extract and ranitidine against indomethacin induced gastric ulcer in rats. Means $\pm$ SE, N=5

Group	Mean ulcer score	Incidence of gastric ulceration (%)	Ulcer index
Control	0.00 $\pm$ 0.00 ^d	0.00	0.00
Ranitidine	0.25 $\pm$ 0.005 ^c	25	6.25
Indomethacin	1.75 $\pm$ 0.054 ^a	100	175
Alhagi 200	1.25 $\pm$ 0.045 ^b	75	93.75
Alhagi 400	0.25 $\pm$ 0.004 ^c	25	6.25

Means with different superscripts were significant at ($P < 0.05$)

**Figure 1:** Analgesic activity of *Alhagi maurorum* aqueous methanolic extract and diclofenac by using hot plate method in mice. Mean $\pm$ SE, N=5. Columns carrying different superscripts are significant at ($p < 0.05$)

Discussion

In the present work, administration of *Alhagi maurorum* aqueous methanolic extract in rats at dose of 200 and 400 mg/kg BW, PO revealed anti-inflammatory activity as confirmed by a significant reduction in the thickness of hind paw edema in a dose dependent manner compared with the control group. The anti-inflammatory effect of *Alhagi maurorum* may be due to presence of lupeol (bioactive anti-inflammatory component) (28). It has been reported that *Alhagi maurorum* had anti-inflammatory activity by inhibition the release of inflammatory mediators (histamine and prostaglandin) (19,29). The cause of pain

inhibition of *Alhagi maurorum* extract is like to anti-inflammatory drugs especially nonsteroidal (30). The anti-inflammatory activity of the extract would be explained by their antioxidant properties particularly radical scavenging activity toward diphenyl picryl hydrazyl (DPPH) radical (31). Our results are similar to previous studies which revealed that *Alhagi* has anti-inflammatory effect which examined by reducing the paw edema thickness (31-33).

In the present study, Diclofenac administration evoked a significant anti-inflammatory activity visible from the reduction in the thickness of paw edema in rats. It has been reported that the ability of diclofenac to show

a high degree of analgesic, anti-inflammatory, and antipyretic effects in various pharmacological studies. It has the ability to suppress prostaglandin biosynthesis *in vitro* and *in vivo* (34).

The current study showed that rats treated with aqueous methanolic extract of *Alhagi maurorum* at dose of 200 and 400 mg/kg BW PO demonstrated a significant analgesic effect compared with the control group. The analgesic effect could be imputed to presence of the high content of phenolics and flavonoids (35). *Alhagi maurorum* has been recorded to inhibit histamine, serotonin and prostaglandins that enable it to possess analgesic activity (19,29). Our data are consistent with a former study which showed that the aqueous methanolic extract of *Alhagi maurorum* showed analgesic effect after formalin administration (18).

The present study also demonstrated that *Alhagi maurorum* aqueous methanolic extract showed a significant dose dependent improvement in the gastric damage induced by indomethacin as confirmed by significant improvement in mean ulcer score and ulcer index. Quercetin and catechin (flavonoids) isolated from *Alhagi* have antioxidant effect by inhibiting lipid peroxidation and scavenging free radicals (36). This effect demonstrated the ulcer prevention (37). Alhagit in and alhagidin, flavanone glycosides, have been isolated from *Alhagi pseudoalhag* may contribute to the antiulcerogenic effect of the aqueous methanolic extract of *Alhagi maurorum* (38). *Alhagi maurorum* has been reported to contain terpenes (24,39). Terpenes have been illustrated to have antiulcerogenic activity in other studies (40,41). The mechanism of action of triterpenes is the decline of mucosal prostaglandins metabolism, cytoprotective effect and decrease gastric vascular permeability (42). It has been shown that the methanolic extract of *Alhagi* has calcium channel blocking activity on the gastrointestinal tract smooth muscle (24). Similarly, former studies have revealed the ability of *Alhagi maurorum* to protect gastric mucosa against phenylbutazone, indomethacin, ethanol (16), aspirin (19) and water immersion restraint stress (17) induced ulcer.

Conclusion

It might be concluded that, *Alhagi maurorum* aqueous methanolic extract possesses prophylactic anti-inflammatory, analgesic and antiulcerogenic activities in a dose dependent manner and further studies should be conducted to assess the phytochemical components of the extract.

Conflict of interest

None of the authors have any conflict of interest to declare.

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