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A Pharmacological Evaluation of Aqueous Extract of Alhagi Maurorum

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Abstract: The aqueous extract of Alhagi maurorum was evaluated in mice at doses of 125, 250 and 500µg/animal, for its anti-inflammatory, analgesic, antioxidant and antibacterial activities. The anti-inflammatory activity was examined for both test extract and (Diclofenac sodium 1 µg/animal) as a reference drug. The extract and reference drug significantly reduce the thickness of paw edema induced by formalin at dose -dependent manner in both phase Iand II. To evaluate the analgesic effect of alhaji water extract and diclofenac sodium(1µg/animal), licking frequency was estimated, in the phase 1 (0-5 min.) and in phase II (15-20 min) after formalin administration. Both anti-inflammatory and analgesic effects were compared to that of induced by distilled water (DW). Antioxidant effect was evaluated by estimating the level of MDA and also by Total antioxidant capacity (TAC) compared to acetylsalicylic acid antioxidant activity. The test extract seems to significantly reduce malondialdehyde level and potent antioxidant activity. However all doses of test extract have no antibacterial activity using Cup-plate diffusion method. In conclusion the aqueous extract of Alhagi maurorum may be useful in the protection against inflammatory diseases, especially if free radicals are a part of its pathophysiology. However, aqueous extract of Alhaji exert no antibacterial effects. More detailed phytochemical studies are necessary to identify the active principles and exact mechanisms of action.

Key words: Alhagi maurorum · Anti-inflammatory

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Medicine in Iraq can be traced back to the Sumerian period 3000-1970 B.C. and then to the Babylonian and Assyrian periods 1970-539 B.C. Later on, this knowledge was translated and enriched by the Arab physicians during Abbasid period 500-1038 A.D. Nowadays few of expert consumers of plant medicines have vast botanical knowledge and this knowledge is necessary because only an expert can knew the active ingredients, part of Plant used in treatment and they also know right time and method for collecting; right amount of plant parts as drug during the time in which the plant materials have bioactive Constituents and they also knew right method for drug preparation and their administration.

One of the medicinal plants in Iraqi folic medicine is Alhagi graecorum Bioss-Maurorum MEDI (Leguminosae) (Akool in Iraq, Camel Thorn, Persian Manna plant). it′*s used* for rheumatic pains, liver disorders, urinary tract infection and for various types of gastrointestinal discomfort. All parts of the plant drink, or incense, or coating, even though the cold water in which to treat hemorrhoids [1-4].

In our continuing efforts at identifying medicinal plants with anti-inflammatory activity and establishing scientific evidence for activity, the acclaimed potency of this plant in inflammatory conditions stimulated our interest to screen the water extract for effect on inflammation on paw edema induced by formalin in mice.

The search for better and safer ways of relieving pain is what led to herbology In this article analgesic effects of aqueous extract of the whole plant of Alhagi, also was examined on nociceptive activity induced by formalin.

The need for antioxidants becomes even more critical with increased exposure to free radicals. Pollution, cigarette smoke, drugs, illness, stress and even exercise can increase free radical exposure. Because so many factors can contribute to oxidative stress, As part of a healthy lifestyle and a well-balanced, wholesome diet, antioxidant supplementation is now being recognized as an important means of improving free radical protection[5]. the antioxidant capacity of test extract was,

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measured as either estimation of Malondialdehyde (MDA)level and by total antioxidant activity (TAC) MDA is a three carbon, low molecular weight aldehyde, that can be produced from free radical attack on polyunsaturated fatty acids of biological membranes. While The oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of hydrogen atoms from diallylic methylene groups of linoleic acid [6].

MATERIALS AND METHODS

Preparation of Plant Material: The whole green plant was used for in the present study, Akool was collected from the al-Zubair desert(south of Basrah) between April and May 2011, washed carefully with tab water then boiled in 3 L of distilled water (DW) for 1hr,dark brown solution was obtained(the plant still green and intact), then the solution dried by reduced pressure, the powder was kept in sterile container to be used later

Animal Preparation: The animals were housed in standard cages and were maintained on a standard pelleted Feed. Mice (22-25g) of either five were randomized into different groups of 5 mice each. They were used for the experiment after 16 hours fast and deprived of water only during experiment.

Analgesic Studies: Group A of the mice received distilled water (1ml/animal orally), which served as control. Group B mice were treated with diclofenac (1 µg/animal orally), which was reference drug. Groups C, D and E received (125, 250, 500 µg/mouse respectively of test water extract by oral administration[7,8]. Thirty minutes post administration of test extract, DW and diclofenac ; the mice were injected with 50 µl of 2.5% formalin into the sub plantar area of the hind limb [9,10]. The pain-related behavior was quantified by determining the incidence of spontaneous flinching of the injected paw, during the first phase (0 - 5 min) and second phase (20 - 25 min). Formalin-induced nociceptive behavior was assessed in an observer-blind manner [11,12].

Evaluation of Antiinflammatory Activity of the Extract: Formalin-Induced Paw Edema: The Formalin (10%) induced inflammation was used in a dose of 50µg/animal (9, 21). Mice were divided into 5 groups of 5 each. Thirty minutes before injection of formalin, group A, received 1ml normal saline each, group B received 1 µg/mouse of diclofenac (reference drug).Groups C,D and E received 125, 250 and 500 µg /animal respectively of alhaji extract. The Formalin (10%) induced inflammation was used in a dose of 50µg/animal [9],. The increase in paw diameter was measured in cm using Vernier caliper. Measurement of paw thickness was done immediately before and after 1 and 4 hours following formalin injection[13]. The percentage inhibition of the growth of oedema was calculated from the expression:

$$\text{Inhibion}\,(\%) = \frac{\text{mean control}\,(\text{N/S}) - \text{mean treated} \times 100}{\text{Mean control}\,(\text{N/S})}$$

Antioxidant Activity: It is essential to use more than one method to evaluate antioxidant capacity of plant materials because of the complex nature of phytochemicals [14].

Total Antioxidant Capacity (TAC): The antioxidant activity is determined by the conjugated diene method. then 100 µl of 250 - 500 µg/ml test extract were mixed with 2.0ml of 10 mM linoleic acid emulsion in 0.2 M sodium phosphate buffer (pH 6.6) in a test tube and kept in dark at 37°C to accelerate oxidation. After incubation for 15 hrs, 0.1 ml from each tube is mixed with 7.0 ml of 80% methanol in deionized water and the absorbance of the mixture is measured at 234 nm against a blank in a spectrophotometer [15]. The antioxidant activity is calculated as follows:

$$\text{Antioxidant activity (\%)} = \frac{\text{A0 - A1}}{\text{A0}} \times 100$$

Where: A0 is the absorbance of Ascorbic acid (a positive control) and A1 is the absorbance of test, tow doses 500,and 250 µg were used

Estimation of Malondialdehyde (MDA): The extent of lipid peroxidation is measured through malondialdehyde activity (MDA), a pro-oxidant factor, which determines the oxidative damage

The thiobarbituric acid assay of Beuge and Aust [19] was used to measure the serum MDA. The principle of the method was based on the spectrophotometric measurement of the coluor occurring during the reaction of thiobarbituric acid with MDA.

The mice in first group received distilled water (1ml/animal orally), which served as control. While in the second group mice were treated with 500µg/1ml of test water extract by oral administration [8]. Thirty minutes post administration ; the mice were injected with 50 µl of 10% formalin into the sub plantar area of the hind limb.

After 15 hrs,blood was collected from the experimental animals [16]. The serum was prepared by centrifuging the blood samples at 3000 rpm for 5 min [17] and collected by pipetting.

Concentration of thiobarbituric acid reactive substances was calculated by the absorbance coefficient of the malondialdehyde-thiobarbituric acid complex and expressed as µmol/ml, As a standard, MDA bis(dimethyl acethal)-TBA (thiobarbituric acid) complex was used. Absorbance was determined at 533 nm wavelength against blank.

MDA µmol/l = (? A/ 1.56) × 10 18,20]

Δ A=Absorbance of the test-Absorbance of the blank

Antibacterial Activity

Cup-Plate Diffusion Method: Antibacterial activity of plant extracts was carried out using cup-plate agar diffusion method [23] with some minor modifications. One ml from each standard bacterial stock suspension was mixed thoroughly with 20 ml of sterile Molten Muellur Hinton agar (45 - 50°C), poured into sterile Petri-dishes and left to solidify. Then, four cup-shape wells (10 mm diameter) were made in each plate using sterile cork-borer (No. 9). The agar disks were removed and four alternate cups were filled with extract using sterile adjustable pipettes. Four Petri-dishes with two alternate cups were used with the respective solvent instead of the extracts as control. The plates were then incubated in upright position for 18 to 24 hrs at room temperature. After incubation period, the inhibition zones diameters were measured [21-24]

Test Organisms: Gram-negative bacteria, :*Escherichia coli*, *Pseudomonas aeruginosa*,

Gram-positive Bacteria: *Staphylococcus aureus*, *Streptococcus byogenes*,

Statistical Analysis: Data were expressed as mean ± S.D. The results were statistically analysed by the students *t*-test; $P<0.05$ versus respective control was taken as significant.

RESULTS

Anti-inflammatory Activity: The results of anti-inflammatory studiesrevealed that the value obtained showed a significant reduction in the growth of edema in the hind paw in both phase I (Table 1) and phase II (Table 2). This inhibition appears to be dose- dependent. However, the percentage inhibition obtained for both 125, 250 and 500 µg/animal were less than the value obtained for Diclofenac 1 µg/mouse used as standard drug for the study.

Analgesic Activity: Treatment with the aqueous extract at doses of 125, 250, 500 µg/animal and diclofenac at 1µg/animal caused significant decrease in frequency of licking of the formalin- injected paw, as compared to D W treated group. The 500mg/kg dose and diclofenac showed the highest effect, in both phase I, phase Ii.The results are summarized in Tables 3 and 4.

Antioxidant Activity: The results of this study demonstrated that Alhaji extract in both doses have a significant antioxidant capacity in compared with Ascorbic acid activity. The inhibiting of studied plant on linoleic acid peroxidation, are presented in Table 5

Serum MDA: Significantly decreased ($p<0.01$) in Malondialdehyde MDA by the treatment of 500 µg/animal in comparison with negative control (received 1ml of DW).

Table 1: Anti-inflammatory effect of diclofenac sodium and the tested plant extracts against formalin-induced paw edema in mice after I hr from formalin injection. (n = 5).

Treatment	Dose (µg/animal)	Paw thickness cm (mean ± S.D)	Inhibition%	*P* value
D.w. (iml)		0.38±0.011		
Alhaji Extract	125	0.2525±0.06	33.55263	<0.01
Alhaji Extract	250	0.196±0.03	48.42105	<0.01
Alhaji Extract	500	0.146±0.05	61.57895	<0.01
Diclofenac	1	0.102±0.02	73.15789	<0.01

Table 2: Anti-inflammatory effect of diclofenac sodium and the tested Alhaji plant extracts against formalin-induced paw edema in mice after 4 hrs from formalin injection. (n = 5).

Treatment	Dose (µg/animal)	Paw thickness cm (mean ± S.D)	Inhibition%	*P* value
Formalin+Dw 1ml		0.43±0.12	-	-
Alhaji Extract	125	0.27±0.04	37.2093	0.041
Alhaji Extract	250	0.225±0.03	47.67442	0.018
Alhaji Extract	500	0.152±0.03	64.65116	0.006
Diclofenac	1	0.094±0.02	78.13953	0.003

Table 3: Antinociceptive effect phase I (0-5 min) of diclofenac and the tested Alhaji plant extracts using formalin in mice (n = 5).

Treatment	Dose (µg/animal)	Ferquancy of paw licking (mean ± S.D)	Inhibition% At time of 0- 5 min	*P* value
Dw 1ml		76.2±6.3		
Alhaji Extract	125	49.2±10.52	35.43307	*P*<0.01
Alhaji Extract	250	27.8±3.63	63.51706	*P*<0.01
Alhaji Extract	500	21±2.16	72.44094	*P*<0.01
iclofenac	1	15.6±3,36	79.52756	*P*<0.01

Table 4: Antinociceptive effect phase II(15-20 min) of diclofenac and the tested Alhaji plant extracts using formalin in mice (n = 5).

Treatment	Dose (µg/animal)	Ferquancy of paw licking (mean ± S.D)	Inhibition% At time of 5-20 min	*P* value
Dw 1ml		92.4± 8.65	-	--
Alhaji Extract	125	51.2± 7.33	44.58874459	<0.01
Alhaji Extract	250	36.6± 4.67	60.38961039	<0.01
Alhaji Extract	500	10.4± 2.30	88.74458874	<0.01
Diclofenac	1	10±2.55	89.17748918	<0.01

Table 5: Total antioxidant capacity of extract in 250,500µg/animal in comparison with ascorbic acid

	Mean±SD	Inhibition %	*P* value
Ascorbic acid	1.2652± 0.630928		-
Alhaji Extract 250µ	0.2166 ±0.055626	82.88018	<0.01
Alhaji Extact 500µ	0.1682 ±0.016037	86.70566	<0.01

Table 6: The level of serum MDA in both control and 500µg/animal group

Group	Mean ±SD	*P* value
Control	1.03±0.050662	-
Treated group500µg/animal	0.4875±0.067253	*p*<0.01

Anti Bacterial Activity: The test plant(Alhaji) of all doses that used in the present study had no antibacterial activity against both Gram negative and Gram positive bacteria that cultured in vitro

DISCUSSION

Folkloric treatment of inflammation of various etiologies, using medicinal plants, is well known to masters of the art of traditional medicine practice. *Alhagi maurorum* was used in folk medicine as a remedy for rheumatic pains, liver disorders, urinary tract infection and for various types of gastrointestinal discomfort

In the Anti-inflammatory studies, the Formaldehyde induced oedema is believed to be a multi mediated phenomenon that liberates diversity of mediators which could be in two phases, the first being the release of serotonin and histamine while the second after the one hour is mediated by prostaglandins. The cyclooxygenase products and the continuity between the phases are provided by kinins [25].

Pharmacological screening of extract of Alhaji has revealed that it possesses anti-inflammatory effect,the extract may have inhibited the release of pro-inflammatory mediators of acute inflammation such as histamine and prostaglandin [26].

The pain in the early phase of formalin test was due to the direct stimulation of the sensory nerve fibers by formalin while the pain in the late phase was due to inflammatory mediators, like histamine, prostaglandins, serotonin and bradykinins. This test is believed to be a more valid analgesic model which is better correlated with clinical pain. In this study, the extract caused a dose-dependent decrease in licking frequency by the mice injected with formalin signifying the analgesic effect of the extract [25,27].

Antioxidants are a type of complex compounds found in our diet that act as a protective shield for our body against certain diseases such as arterial and cardiac diseases, arthritis, cataracts and also premature ageing along with several chronic diseases. "

In vitro studies on medicinal plants and vegetables strongly supported this idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke. The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark. The oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of hydrogen atoms from diallylic methylene groups of linoleic acid [15].

Oxygen derived free radicals and reactive oxygen species interact with lipid bilayer of cell membrane resulting in lipid peroxidation. Malonaldehyde (MDA) is a stable end product of lipid peroxidation. It is well documented that MDA is a stable end product of free radicals induced by lipid peroxidation. Thus MDA serves as a reliable marker for the assessment of free radical induced damage to tissues. Determination of MDA allows

detection of the degree of lipid peroxidation and level of free oxygen radicals indirectly [28] reduction of lipid peroxide activity was observed in both treated group with 500 and 250 µg in comparison with negative control.

The present study suggest that tested plant extract have potent antioxidant activity and/or free radical scavenging activity. However, we do not know what components in the plant extracts show these activities. More detailed studies on chemical composition of the plant extract, as well as other *in vivo* assays are essential to characterize them as biological antioxidants which are beyond the scope of this study[28].The results of the present study are in agreement with that of ethanol extract of *Alhagi maurorum* [13]

Many solvents are used for extraction of bioactive compounds from the plants. The solvents used vary in their polarity. The most commonly used solvent is methanol. Traditionally, water is used for extraction but it is next to methanol. The use of non polar solvents is comparatively less indicating that the active constituents are soluble in polar solvents only.

Cuurent study the aqueous extract of *Alhagi maurorum* using all doses show that there was no inhibitory zone on tested bacterial growths. In contrast to methanolic extract showed inhibitory concentration against *Escherichia coli Salmonella typhi, Pseudomonas aeruginosa, Streptococcus byogens*, *Staphylococcus aureus* [2].

CONCLUSION

In conclusion the aqueous extract of *Alhagi maurorum* may be useful in the protection against inflammatory diseases, especially if free radicals are a part of its pathophysiology.However, more detailed phytochemical studies are necessary to identify the active principles and exact mechanisms of action.

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