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Anti-Inflammatory Activity of *Ipomoea reniformis* Methanolic Extract

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ABSTRACT
In the present study, methanolic extract of *Ipomoea reniformis* herb (MEIR) in acute, subacute and chronic models of inflammation was assessed in rats. Administration of MEIR (200, 400 mg/kg, p.o.) exhibited significant anti-inflammatory activity. In acute inflammation as produced by Carrageenan 59.55 % and 64.04 % protection was observed. While in subacute anti-inflammatory models using formaldehyde-induced hind paw edema (after 1.5 h) 38.36 % and 47.95 % and in chronic anti-inflammatory model using cotton pellet granuloma 15.02 % and 19.19 % protection from inflammation was observed. MEIR did not show any sign of toxicity and mortality up to a dose level of 1000 mg/kg, p.o. in rats. The results obtained suggest that the methanolic extract of *Ipomoea reniformis* herb (MEIR) is endowed with effective anti-inflammatory activity mediated via either by inhibition of cyclooxygenase cascade and by blocking the release of vasoactive substances (histamine, serotonin and kinins). These findings seem to justify the use of the plant in traditional Indian medicine in the treatment of inflammation, including arthritic conditions.

Keywords: *Ipomoea reniformis;* Anti-inflammatory; Carrageenan and formaldehyde-induced edema; Cotton pellet granuloma.

INTRODUCTION

Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Although rheumatism is one of the oldest known diseases of mankind affecting the majority of population, no substantial progress has been made in achieving a permanent cure. The greatest disadvantage in presently available potent synthetic drugs lies in their toxicity and reappearance of symptoms after discontinuation. Therefore, the screening and development of drugs for their anti-inflammatory activity is still in progress and there is much hope for finding anti-inflammatory drugs from indigenous medicinal plants. *Ipomoea reniformis* leaves resemble the ear of mouse (Musa - Mouse; Caini- Ear). In Chhattisgarh, it is a popular potherb called as Muscaini bhaji. [1] The whole plant has antipyretic activity and also useful in headache, bronchitis, paralysis, inflammation, troubles of nose, fevers due to enlargement of liver.

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The plant is bitter, acrid and pungent with cooling effect. Traditionally it is used in treatment of anthelmintic, as laxative, carminative, useful in diseases of the kidney, the bladder, the lungs, the uterus, It is also used as a remedy for pain, fever, urethral discharges, anaemia, fistula, leucoderma; useful in the disease of heart and the abdomen; reduces tumours. [2]. The present study is an attempt to study the methanolic extract of *Ipomoea reniformis* (MEIR) in acute, subacute and chronic models of inflammation in rats.

MATERIAL AND METHODS

A fresh plant specimen of *Ipomoea reniformis* for the proposed study was collected from field of Munjka village, nearby campus of Saurashtra University, Rajkot in the month of January 2008. The authenticity of the freshly collected plant was confirmed by comparing their morphological characters with the description mentioned in the different standard texts and floras. [2] The identification of the plant was also confirmed by Mr. Vishal Muliya, Botanist, Saurashtra University, Rajkot. A voucher specimen for this collection has been deposited in the herbarium of the Saurashtra
University, Rajkot.

Extraction

Air dried and coarsely powdered (350 g) of *Ipomoea reniformis* herbs were taken. Extraction was carried out in a soxhlet extractor using methanol. The extract was then concentrated to dryness under reduced pressure and controlled temperature. Final traces of methanol were removed under pressure by using rotary vacuum flask evaporator and they were preserved in a refrigerator. [3-4]

Chemicals

Carrageenan, acetylsalicylic acid (ASA) and phenylbutazone (PBZ) were purchased from Sigma Aldrich (Mumbai, India). Formaldehyde from Ranbaxy Fine Chemicals Ltd., (Mumbai, India).

Preliminary Phytochemical Studies

Preliminary phytochemical test of *Ipomoea reniformis* herb and methanolic extract *Ipomoea reniformis* (MEIR) was performed for the presence of phytochemical constituents. [3-5]

Animals

Male wistar rats weighing 200-300 g of either sex were procured from Cadila Health Care Ltd., Dholka, Ahmedabad. All the animals were kept in standard polypropylene cages and maintained under standard conditions: temperature (24 ± 1°C), relative humidity (45-55 %) and 12:12 light:dark cycle. The animals were allowed to acclimatize to laboratory conditions 48 hrs before the start of the experiment. Groups of 6 rats (200-300 g.) were used in all sets of experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment though, water was allowed *ad libitum*. All the experiments were conducted after obtaining permission from the Institutional Animal Ethics Committee (IAEC) of Department of Pharmaceutical Sciences, Saurashtra University, Rajkot.

Acute toxicity study

For the pharmacological tests the extract was suspended in double distilled water containing carboxy methyl cellulose (1 %, w/v, CMC). The acute toxicity was determined for the methanolic extract *Ipomoea reniformis* (MEIR) on Wister rats by fixed dose method of OECD Guideline no 425 given by CPCSEA respectively. [6] 400-4000 mg/kg of methanolic extract *Ipomoea reniformis* (MEIR) was administered by oral route to mice and rats. Mortality was observed for 3 days. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals. [7] Standard orogastric cannula was used for oral drug administration.

Anti-inflammatory Studies

Carrageenan-induced rat paw edema

The acute hind paw edema was produced by injecting 0.1 ml of carrageenan (prepared as 1% suspension in 1% CMC) locally into the plantar aponeurosis of the right hind paw of rats. [8] MEIR (100 mg/kg, 200 mg/kg and 400 mg/kg, p.o.) was administered to three different groups while the other two groups served as negative (received vehicle 1 ml/kg, p.o.) and acetylsalicylic acid (ASA, 300 mg/kg, p.o.) used as positive controls in this experiment inhibited COX-1, [9] respectively. MEIR and ASA were administered 1 h prior to the injection of carrageenan. The rat pedal volume up to the ankle joint was measured using plethysmometer (Ugo Basile, 7140 Comeriovarese, Italy) at 0 h (just before) and 3 h after the injection of carrageenan. Increase in the paw edema volume was considered as the difference between 0 and 3 h. Percent inhibition of edema volume between treated and control groups was calculated as follows:

$$\text{Percent inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where V_c and V_t represent the mean increase in paw volume in control and treated groups, respectively.

Formaldehyde-induced edema in the rat paw

The test was performed according to the technique developed by Brownlee. [10] Pedal inflammation was induced by injecting 0.1 ml of 4 % formaldehyde solution below the plantar aponeurosis of the right hind paw of the rats. The paw volume was recorded immediately prior to compound administration (0 h) and then at 1.5, 24 and 48 h after formaldehyde injection. Vehicle (1 ml/kg, p.o.), MEIR (100 mg/kg, 200 mg/kg and 400 mg/kg, p.o.) and standard drug, ASA (300 mg/kg, p.o.) were administered 1 h prior to formaldehyde injection.

Cotton pellet implantation

The effect of MEIR on chronic or proliferative phase of inflammation was assessed in cotton pellet granuloma in rat model. [11] Autoclaved cotton pellets weighing 35 ± 1 mg each were implanted subcutaneously through small incision made along the axilla or flank region of the rats anesthetized with ether. The different groups of rats were administered the MEIR (100 mg/kg, 200 mg/kg and 400 mg/kg, p.o) and ASA (300 mg/kg, p.o.) once daily for 7 consecutive days from the day of cotton pellet insertion. The control group received vehicle (1 ml/kg, p.o.). On the eighth day, all the rats were sacrificed and the cotton pellets covered by the granulomatous tissue were excised and dried in hot air oven at 60°C till a constant weight was achieved. Granuloma weight was obtained by subtracting the weight of cotton pellet on 0 day (before start of experiment) from the weight of the cotton pellet on eighth day.

Statistical analysis

All the data were presented as mean ± S.E.M. and analysed by Wilcoxon Sum Rank test [12] and unpaired Student's t-test for the possible significant interrelation between the various groups. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of methanolic extract *Ipomoea reniformis* (MEIR) both revealed the presence of carbohydrates, glycosides, saponins, tannins, phenolic compounds, proteins, free amino acids, flavonoids, gums and mucilage using specific reagents.

The present study demonstrates the potent anti-inflammatory activity of the methanolic extract of *Ipomoea reniformis* herb in different models of inflammation, i.e., acute exudative (Carrageenan-induced rat paw edema), subacute (Formaldehyde) and chronic proliferative inflammation (Cotton pellet granuloma), thereby indicating the possibility of developing MEIR as the cheaper, safer and potent anti-inflammatory therapeutic agent. *Ipomoea reniformis* herb provided no scientific evidence as regards to the anti-inflammatory activity till date. In acute and subacute models of inflammation, upon challenged by phlogistic stimuli, MEIR showed significant anti-inflammatory activity.

Carrageenan-induced rat paw edema

The mean increase in paw edema volume was about 0.89 ± 0.12 ml in the vehicle-treated control rats. MEIR (200 mg/kg and 400 mg/kg, p.o.) significantly ($P < 0.01$) reduced the

mean paw edema volume at 3 h after carrageenan injection. MEIR (100 mg/kg, 200 mg/kg and 400 mg/kg, p.o.) exhibited anti-inflammatory activity in a dose-dependent manner with the percent inhibition of paw edema of 32.58, 59.55 and 64.04, respectively, as compared with the control group. However, the standard drug, ASA (300 mg/kg, p.o.) showed highly significant ($p< 0.001$) anti-inflammatory activity with the percent inhibition of 75.28 as shown in Table 1.

The edema and inflammation induced by Carrageenan is shown to be mediated by histamine and serotonin during first 1 h. After which increased vascular permeability is maintained by the release of kinins upto 2.30 h, followed by the release of kinins and finally through the release of bradykinin, prostaglandin and lysosomes from 2.30 to 6 h. [13] The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents. [14] The mediators appear to be prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site. [15] The Carrageenan induced paw edema model in rats is known to be sensitive to cyclo-oxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents. [16-17] Though MEIR (200 and 400 mg/kg, p.o.) significantly reduced the granuloma formation the effect was of less intensity, when compared with ASA. The mechanism of anti-inflammatory activity of MEIR on proliferative phase of inflammation in a rat model of cotton pellet granuloma is not exactly known and needs further study.

Formaldehyde-induced edema in the rat paw

MEIR (200 mg/kg and 400 mg/kg, p.o.) significantly diminished the mean paw edema volume at 1.5 h (38.36% and 47.95%) ($p< 0.001$) and 24 h (24.24% and 27.27%) ($p< 0.05$; $p< 0.01$). The maximum inhibition of edema volume produced by MEIR (400 mg/kg, p.o.) Was almost comparable to that of ASA (300 mg/kg, p.o.) (47.95 % versus 50.68 % at 1.5 h). Interestingly, the effect of MEIR persisted up to a period of 24 h in contrast to ASA, the effect of which was significant only at 1.5 h as shown in Table 2.

MEIR showed significant inhibition of formaldehyde-induced rat paw edema. The formaldehyde injection into rat paw produces localised inflammation and pain. This nociceptive effect is biaphasic in nature: an early nerurogenic component followed by a later tissue-mediated response. [18] Inhibition of formaldehyde-induced pedal edema in rats is one of the most suitable tests to evaluate anti-proliferative activity and to screen anti-inflammatory activity. [19]

Cotton pellet implantation

The study of MEIR on proliferative phase of inflammation indicated that MEIR (200 mg/kg and 400 mg/kg, p.o.) slightly but significantly ($p<0.05$; $p<0.01$) reduced the granuloma formation with inhibition of 15.02 % and 19.19 % as compared with ASA (300 mg/kg, p.o.), which showed significant ($p<0.001$) inhibition on granuloma formation with the percent inhibition of 42.04 as shown in Table 1.

In case of cotton pellet-induced granuloma, there was significant reduction in granular tissue formation. This result is in confirmation with the anti-proliferative activity of extract observed in formaldehyde-induced paw edema in rats. [20]

From the acute, subacute and chronic studies, it is obvious that methanolic extract of *Ipomoea reniformis* (MEIR) was found to possess good anti-inflammatory activity mediated via either inhibition of cyclooxygenase pathway and by blocking the release of vasoactive substances (histamine, serotonin and kinins). These findings scientifically validated the traditional use of this plant Indian medicine in the treatment of inflammation, including arthritic. The advantages of MEIR, viz., better and safer anti-inflammatory profile deserves further studies (sub fractionation of MEIR and separation of active principles) to identify the possible mechanism of action as well as stablishing the therapeutic value in the treatment of inflammatory diseases.

Table 1: Effect of MEIR on carrageenan induced hind paw edema and cotton pellet granuloma in rats

Drug	Dose (mg/kg)	Carrageenan induced paw edema volume (ml)	Percentage Protection	Weight of Cotton pellet granuloma (mg)	Percentage Protection
Control	--	0.89 ± 0.12	--	109.53 ± 3.75	--
ASA	300	0.22 ± 0.33***	75.28	63.48 ± 2.14***	42.04
MEIR	100	0.60 ± 0.09	32.58	99.34 ± 2.92	9.30
MEIR	200	0.36 ± 0.06**	59.55	93.07 ± 3.93*	15.02
MEIR	400	0.32 ± 0.05**	64.04	88.5 ± 2.65**	19.19

Values are mean ± S.E.M. for six rats. *p < 0.05, **p < 0.01 and ***p < 0.001 compared to respective arthritis group

Table 2: Effect of MEIR on formaldehyde induced hind paw edema in rats

Drug	Dose (mg/kg)	Formaldehyde induce hind paw volume edema (ml)					
		1.5 h	% Protection	24 h	% Protection	48 hrs	% Protection
Control	--	0.73 ± 0.04	--	0.99 ± 0.05	--	0.55 ± 0.07	--
ASA	300	0.36 ± 0.04***	50.68	0.85 ± 0.03	14.14	0.54 ± 0.08	1.82
MEIR	100	0.53 ± 0.03**	27.39	0.91 ± 0.09	8.08	0.53 ± 0.06	3.64
MEIR	200	0.45 ± 0.02***	38.36	0.75 ± 0.06*	24.24	0.52 ± 0.09	5.45
MEIR	400	0.38 ± 0.03***	47.98	0.72 ± 0.04**	27.27	0.51 ± 0.05	7.27

Values are mean ± S.E.M. for six rats. *p < 0.05, **p < 0.01 and ***p < 0.001 compared to respective arthritis group

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