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Anti-inflammatory Activity of *Rotula aquatica* Lour. In Albino Rats

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ABSTRACT
The purpose of this study was to evaluate and compare the anti-inflammatory activity of different extracts of *Rotula aquatica* whole plant on acute inflammation (carrageenan-induced paw edema) and sub-acute inflammation (cotton pellet granuloma) in animal models. Five different groups of six animals each of either sex were used for acute and sub-acute experimental groups. Group I (control) received saline (5 ml/kg) and groups II rats were treated with indomethacin (10 mg/kg). Petroleum ether, ethyl acetate and ethanolic extracts of whole plant of *Rotula aquatica* at a dose of 200 mg/kg was given orally to group III, IV and V rats respectively. 1.0 ml of carrageenan was injected s.c. to plantar region of right hind paw of each rat, 1 hour after the drug administration. The change in paw volume was measured at 0, 1, 2 and 3 hours intervals. For sub-acute model of inflammation, sterilized cotton pellets, weighing 10 mg each, were implanted, one on each side of the groin, under light anaesthesia. Drug treatment was given for 7 days. On the eight day, cotton pellets along with granuloma were removed surgically, and wet pellets were weighed, after that dried at 60ºC overnight and then the dry pellets weight was taken. Petroleum ether and ethyl extracts at a dose of 200 mg/kg body weight exhibited significant inhibition (P<0.01) in acute and sub-acute inflammation models, which was comparable with standard drug (Indomethacin) whereas ethanolic extract showed marked inhibition (P<0.05). *Rotula aquatica* whole plant possesses anti-inflammatory effects in both acute and sub-acute inflammatory conditions and this may be possible to explain use of plant in traditional medicine.

Keywords: *Rotula aquatica,* Anti-inflammatory, carrageenan, granuloma, paw edema.

INTRODUCTION

Inflammation is a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a defensive mechanism; the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases. [1] Inflammation is a physiological reaction to injury or to infectious, allergic, or chemical irritation. Inflammatory processes are complex biochemical phenomena characterized by tissue edema, pain, and leukocyte infiltration. [2] Many components are involved in the inflammation process, to name few are edema formation, leukocyte infiltration and granuloma formation are widely noticeable. [3] The formation of edema in paw results a synergism between various inflammatory mediators which increases the vascular permeability and the blood flow. Carrageenan induced paw edema is used widely for determining the acute phase of inflammation. Histamine, 5-hydroxyl tryptamine and bradykinin are used as the first detectable mediators in the early phase of carrageenan induced inflammation and prostaglandins are used in the late phase of inflammation. Medicinal plants, those having a wide variety of chemicals, from which anti-inflammatory agents could be derived, can be scientifically proven for their efficacy and safety parameters. For centuries people have been trying to alleviate and treat disease with different plant extracts and formulations without or with minimal adverse effects. [4]

Rotula aquatica belongs to the family *Borogenaceae*, and is reported to contain baunerol, steroids and alkaloids. The roots of *Rotula aquatica* is also used as a laxative, and in treatment of piles and vineral disease. [5] Petroleum ether and alcoholic extracts of *Rotula aquatica* exhibited anthelmintic activity. [6] The effect of the alcoholic extract of *Rotula aquatica* against ethylene glycol induced urolithiasis is reported in albino rats. [7] The roots of *Rotula aquatica* is reported to contain antimitotic activity. [8]

However, literature review failed to offer any scientific validation on the anti-inflammatory activity of *Rotula*

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aquatica. Hence, this leads us to study anti-inflammatory activity of *Rotula aquatica* in different models.

MATERIALS AND METHODS

Plant Material

Preparation of *Rotula aquatica* whole plant was collected from Tunga River bed near Hariharapura of Koppa taluk in Chikamagalure district, Karnataka, India. The plant was authenticated by Dr. M Radhakrishna Rao, Visiting Professor of Botany, Department of Drava Guna, ALN Memmorial Ayurvedic College, Koppa, Chikamagalore district, Karanataka, India. A voucher specimen (RAL/MRCP/2011/012) was deposited in Malla Reddy College of Pharmacy, Maisammaguda, Dhulapally, Secunderabad, Andhra Pradesh. India.

Extraction of plant material

Around 3 kg of *R. aquatica* whole plant was shade dried, coarsely powdered and subjected for successive extraction process with three different solvents (petroleum ether, ethyl acetate and ethanol (70 %) into 5 batches of each 200 g in soxhlet extractor for 48 hours. After complete extraction, the solvents were distilled off and concentrated under reduced pressure to the dryness in a flash evaporator. The yield was found to be 1.25 %, 1.02 % and 1.28 % w/w respectively. The dried extracts were dissolved in dimethyl sulphoxide (DMSO) and subjected to anti-inflammatory activity.

Experimental animals

Albino rats (150-180 g) of either sex were housed under standard laboratory conditions. The animals had free access to food and water. Institutional Animal Ethical Committee (IAEC) approval was obtained from Malla Reddy Group of Institutions before conducting the experiments. The Animal Ethical Committee of the Institute approved all the protocols of the study (Registration No. 1201/ac/11/CPSCSE).

Acute toxicity studies

Different extracts (petroleum ether, ethyl acetate and ethanolic) of *Rotula aquatica* were orally administered as a single dose to different groups of mice (n=6) at a different concentrations (50, 100, 200, 500, 1000 and 2000 mg/kg, *p.o*). These animals were observed for 24 hours period, and then for 14 days. [9]

Anti-inflammatory activity

Carrageenan-induced hind paw edema

The rats were divided in to five groups containing six rats in each group. The different extracts (petroleum ether, ethyl acetate and ethanolic) were administered to the rats 1 hour before carrageenan injection. Different groups were treated as follows:

Group I: was treated with normal saline (5 ml/kg, p.o) and served as control.

Group II: was treated with Indomethacin (10 mg/kg b.w., p. o) and served as standard.

Group III, IV and V: was treated with petroleum ether, ethyl acetate and ethanolic extracts, at a dose of 200 mg/kg b. w., p. o. respectively and served as test groups.

Paw edema was induced by injecting subcutaneously 0.1 ml of 1 % carrageenan into sub-planter region of the hind paw of the rat according to the method of Winter *et al*. [10]

The paw volume was measured initially and at 0, 1, 2 and 3 hours after carrageenan injection, using Plethysmograph, and was compared with control rats.

The percentage inhibition of edema as calculated for each group with respect to its vehicle-treated control group. The anti-inflammatory activity was calculated by using formula

$$\text{Percentage of edema inhibition} = 1 - V_t / V_c \times 100$$

Where V_t = Volume of paw edema in drug treated groups

V_c= Volume of paw edema in control groups

Cotton pellet granuloma

Cotton pellet granuloma was produced in rats was studied according to the D' Arcy *et al*. [11] The rats were grouped in to five groups of six animals each. The pellets, weighing exactly 10 mg each, were made from 5 mm section of cotton rolls. The cotton pellets were sterilized in an autoclave for 30 min at 120ºC under 15 lbs pressure. Two pellets were inserted, one on each side of the groin, under light ether anaesthesia. After the insertion of the pellets, the skin was sutured.

Group I: treated with normal saline (5 ml/kg, p.o) served as control.

Group II: treated with Indomethacin (10 mg/kg, p.o) served as standard.

Group III, IV and V: treated with treated with petroleum ether, ethyl acetate and ethanolic extracts, at a dose of 200 mg/kg b. w., p. o., respectively for 7 consecutive days and served as test groups.

Animals were sacrificed on the 8th day and the cotton pellets along with granular tissue formed around were removed surgically and freed from extraneous tissue.

The wet pellets were weighted and dried in an oven at 60ºC for 24 hours to constant weight, after that the pellets were weighed again. Increments in dry weights of the pellets were taken as measure of granuloma formation. The anti-inflammatory effects of all the test groups were compared with control. The percent inhibition of granuloma tissue formation was calculated by the formula

$$\text{Granuloma inhibition (\%)} = W_c - W_t \times 100 / W_c$$

Where W_c and W_t represent the average weight of granuloma in the control and treated groups respectively.

Statistical analysis

Results are expressed as mean ± SEM. The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA) followed by Dunnett's test. The results were considered statistically significant when $P<0.01$, $P<0.05$.

RESULTS

Acute toxicity

From the acute toxicity study, no toxic symptoms was observed (50, 100, 200, 500, 1000 and 2000 mg/kg) in all the different extracts., the LD_{50} cut-off dose of different extracts was found to be safe up to 2000 mg/kg body weight. Hence, the therapeutic dose was selected $1/10^{th}$ of LD_{50}, i. e, 200 mg/kg body weight.

Carrageenan-induced paw edema

The effect of different extracts of *R. aquatica* whole plant against carrageenan induced paw edema was shown in Table 1. In this model, petroleum ether and ethyl acetate extracts were showed significant ($P<0.01$) inhibition at the first and second hours after the carrageenan treatment. The percentage inhibition was calculated one-way ANOVA followed by the Dunnett's test. The petroleum ether and ethyl acetate extracts showed significant inhibition 62.85 % and 60.10% respectively at third hour at a dose of 200 mg/kg body weight whereas ethanolic extract showed marked inhibition 37.14 % at third hour ($P<0.05$). The anti-inflammatory potential of different extracts were comparable with standard drug (Indomethacin, 10 mg/kg body weight) ($P<0.01$).

Table 1: Effect of different extracts of *Rotula aquatica* whole plant on carrageenan-induced paw edema in rats

Groups	Dose (mg/kg)	Increase in paw volume (Mean ± SEM) in ml			% inhibition at 3 hour
		1 hour	2 hour	3 hour	
Control	-	0. 33±0.01	0.37±0.03	0.35±0.07	-
Indomethacin	10	0.18±0.01**	0.14±0.06**	0.12±0.03**	65.71
Petroleum ether extract	200	0.19±0.04**	0.13±0.06**	0.13±0.03**	62.85
Ethyl acetate extract	200	0.21±0.03**	0.15±0.06**	0.14±0.05**	60.10
Ethanolic extract	200	0.27±0.03	0.24±0.02*	0.22±0.01*	37.14

All the values were expressed as mean±SEM, (n=6), ***P*<0.01, **P*<0.05

Table 2: Effect of different extracts of *Rotula aquatica* whole plant on cotton pellet-induced granuloma in rats

Groups	Dose (mg/kg)	Weight of cotton pellet (mg) (wet)	% inhibition	Weight of cotton pellet (mg) (Dry)	% inhibition
Control	-	142.0±1.91	-	41.5±2.11	-
Indomethacin	10	75.67±1.75**	46.71	23.83±0.95**	42.58
Petroleum ether extract	200	89.33±3.48**	37.09	25.33±1.76**	39.03
Ethyl acetate extract	200	91.17±1.83**	35.80	26.0±1.18**	37.35
Ethanolic extract	200	117.3±2.24*	17.39	34.77±1.42*	17.66

All the values were expressed as mean ± SEM, (n=6), ***P*<0.01, **P*<0.05

Cotton pellet granuloma

The effects of different extracts of *Rotula aquatica* and indomethacin on the proliferative phase of inflammation are summarized in Table 2. It was observed that, all the different extracts were responsible for anti-inflammatory effect, which would be calculated depending on the moist and dry weight of cotton pellets. The results indicate that, petroleum ether and ethyl acetate extracts of *R. aquatica* at a dose of 200 mg/kg body weight and indomethacin 10 mg/kg body weight were calculated as 37.09 %, 35.80 % and 46.71 % respectively (*P*<0.01). After they were dried, the anti-inflammatory effects were calculated for same extracts and indomethacin on the basis of dry weight pellets; the inhibition of inflammation was found to be 39.0 %, 37.35 % and 42.58 % respectively (*P*<0.01). Whereas the ethanolic extract in wet and dry, the moderate inhibition was found to be 17.39 % and 14.93 % respectively (*P*<0.05).

DISCUSSION

The anti-inflammatory activity of petroleum ether and ethyl acetate extracts of whole plant of *R. aquatica* was evaluated using acute (carrageenan-induced paw edema) and sub-acute (cotton pellet granuloma) models of inflammation. The present study revealed that petroleum ether and ethyl acetate extracts of whole plant at a dose of 200 mg/kg possessed significant anti-inflammatory activity in experimental animals.

Carrageenan-induced paw edema as *in-vivo* model of inflammation was selected to assess the anti-inflammatory activity of natural products, particularly in the acute phase of inflammation. [12] Carrageenan-induced inflammation is a biphasic phenomenon. The first phase, which occurs between 0 to 2.5 hours of injection of the phlogistic agent, has been attributed for serotonin. The second phase of inflammatory reaction which is measured at 3 hour is caused by the release of bradykinin, protease, prostaglandin and lysosome. [1]

The cotton pellet granuloma method has been widely used to assess the proliferative phase of sub-acute inflammatory reaction. The inflammatory granuloma is a typical feature of reaction. The events involved in this phase of inflammation are proliferation of macrophages, neutrophils and fibroblasts. The dry weight granuloma formed correlates with the amount of granulomatous tissue formed. [13] From Table 2, it can be seen that the petroleum ether and ethyl acetate extracts of whole plant significantly inhibited the granuloma tissue formation. The petroleum ether and ethyl acetate extracts of *R. aquatica* whole plant has effectively reduced the cotton pellet-induced granuloma, suggesting its activity in the proliferative phase of inflammation. This later phase of inflammation involves the proliferation of macrophages, neutrophils, and fibroblast, and multiplication of small blood vessels, which are the basic sources of the highly vascularized reddish mass termed granulation tissue. [14] This shows that, these are less effective in reducing leucocytes migration into areas of inflammation, since granuloma formation is due to leukocyte accumulation.

An extensive research in ethnopharmcology has taken place throughout the world. The plant *R. aquatica* was traditionally claimed for a large number of pharmacological action and medicinal uses. In the present investigation, the acute toxicity study in rats revealed that the all three extracts of *R. aquatica* whole plant is safe up to 200 mg/kg body weight. The significant anti-inflammatory action may be attributed to the phytoconstituents presents in it. The present study offered a scientific proof to the traditional use of *R. aquatica.* However, further phytochemical studies are needed to isolate the active compounds responsible for this pharmacological activity.

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