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Evaluation of Ant-inflammatory activity of *Rhododendron Arboreum* herb extract on experimental animal.

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Rhododendron Arboreum is a local medicinal plant used in ethnomedicine for the treatment of constipation, bronchitis and asthma. The aqueous decoction and the ethanolic extracts were subjected to anti-inflammatory activity using experimental animal model, in the presence of the positive control drugs. The inflammation was induced by carrageenan. From the results obtained the aqueous extract showed significant activity ($P < 0.001$) comparable to the reference drug used. At the different dose range used (40, 60 and 100 mg/kg), there was no significant differences in their anti-inflammatory activity hence they were not dose-dependent. However, the ethanolic extract did not show any appreciable activity and were also not dose-dependent. The results of the study showed the justification of the use of the plant in the treatment of inflammatory disease conditions, and the active chemical constituents when isolated will be added to the present anti-inflammatory agents.

Key words: Anti-inflammatory activity, carrageenan, *Rhododendron Arboreum.*

INTRODUCTION

The use of medicinal herb in the treatment and prevention of diseases is attracting attention by scientist's world wide. This is corroborated by World Health Organization in its quest to bring primary health care to the people. The plant kingdom has long serve as a prolific source of useful drugs, food, additives, flavoring agents, colorants, binders and lubricants. As a matter of fact, it has been estimated that about 25% of all prescribed medicines toady are substances derived from plants. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized countries has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies [1].

Rhododendron Arboreum (Earicaceae) is growing wildly in the forests. It contains Glycoside, alkaloids, fatty acids, resin, protein, tannins, phenol, flavonoids, saponins, and carbohydrate. *Rhododendron Arboreum* is used in supportive therapy. It has been reported to possess Astringent, diuretic, choleretic, antispasmodic, chronic eczema, diarrhoea, dysentery and menstrual disorders [2, 3]. The herbs are used for the treatment of inflammatory conditions as a household remedy on empirical basis. It was, therefore, decided to screen their extract for anti-inflammatory activity using animal models.

The aim of this study was to study their possible anti-inflammatory effect by using the carrageenan-induced oedema model. The extract was also studied for its acute toxicity effects and preliminary phytochemical screening. The study was therefore aimed at investigating the anti-inflammatory activity of the leaf extract with a view to justifying the use of the plant in the treatment of inflammatory disease.

MATERIALS AND METHODS

Plant material: Leaves of the plant *R. arboreum* were collected (in the month of October) from the surrounding fields of Meghalaya. The identification of plant was made by Mrs. J. M.Q. Lyngdoh, Lecturer, Department of Botany, K. N. G. College, and Jowai, India. The voucher specimen (Ref No: 14/P.colog/2008-2009) of the plant material has been deposited in the Department of Pharmacology. The plants were dried in shade at 4 to 5 days at 25 ºC, reduced to fine powder to particle size no 40. Around 1kg of herb of *R. arboreum* was subjected to continuous

hot extraction with Petroleum ether (60-80^{0}C) for 32 h. The same marc was successively extracted with Chloroform (60 - 70ºC) and Ethanol (72 – 86 ^{0}C) for 24 h. The extracts were concentrated and dried. The extracts were concentrated on water bath (50ºC). After concentrated preparation, the dried powder extract was stored at 4 ^{0}C. The yield of the petroleum extract, chloroform extract and ethanolic extract were found to be 4% (w/w), 2.6% (w/w) and 9.8% (w/w) respectively. Ethanolic extract were used for the experimental study.

Animals: Wister Albino rats (150 - 200 g) and Albino mice (20 – 25g) of either sex procured from Bioneeds animal house, Dhavas pet, Tumkur, were used for the study. The animals were kept in polypropylene cages and maintained at a temperature of 26 ± 2ºC. They were fed with standard diet supplied by Pranav agro industries Ltd. Sangli. The study has got the approval (Ref: IAEC/PP/14/2008-2009) from the Institutional Animal Ethical Committee (IAEC). All the animal experiments are conducted in accordance with the guidelines of the CPCSEA (Reg No. 997/c/06/CPCSEA) guide for care and use of laboratory animals. After procuring the animals were acclimatized for 10 days under standard husbandry conditions as: Relative humidity 45 - 55%, and 12 h light and dark cycle.

Preliminary phytochemical screening: The preliminary phytochemical Screening was carried out on the petroleum ether, chloroform, and ethanolic extracts of leaves of *R. arboreum* for qualitative identification. Tests for common phytochemicals were carried out by standard methods described in practical Pharmacognosy by Khandelwal[4,5].

Acute toxicity study The albino mice of 20 – 25 g body weight of either sex selected were selected to find out the acute toxicity study of ethanolic extract of *R. arboreum* leaves. The dose of 5, 50, 300 and 2000 mg/kg were selected based on the fixed dose (OCED Guideline No. 420) method of CPCSEA. The extract was administered by intraperitonially. The animals were continuously observed for 24 h to detect changes in autonomic or behavioral responses. Mortality in each group was observed for 7 days.

Evaluation of Anti-inflammatory activity: During the period of drug treatment the rats were maintained under normal diet and water *ad libitum*. Animal were divided into five groups of six Wistar albino rats (150 - 200 g) each of either sex. The animals were maintained under standard environmental conditions and had free access to standard diet and water (plant extracts were administered orally by distilled water at different dose levels). Anti-inflammatory activity was measured using carrageenan induced rat paw oedema assay [6]. Groups of 6 rats of both sexes (pregnant females excluded) were given a dose of the extract. After 1 h, 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured at hourly interval for 6 h. Four groups of drug treated rats and one control group were used the paw volume was first at 1,2,3,4,and 6h after drug administration of drugs , the mean paw oedema value for the test group being compared with its mean value for the control group[8]. Anti-inflammatory activity was measured as the percentage reduction in oedema level when drug was present, relative to control as shown in Table 1.

Activity = 100 – (100 x average drug treated /average for control). Indomethacin (10 mg/kg) was administered orally as reference drug while distilled water was used as negative control.

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Statistical analysis: The mean ± S.E.M. was calculated for each parameter. Total variations, present in a set of data were estimated by one way analysis of variance (ANOVA), followed by Dunnet's't' test. $P < 0.05$ was considered as statistically significant when compared to control group. The percentage of the protection is calculated as Activity = 100 – (100 x average drug treated /average for control).

RESULT

Preliminary phytochemical screening: Preliminary phytochemical investigations of different extracts of leaves of *R. arboreum* were studied. The petroleum ether extract contains phytosterols,

TABLE No. 1 - Anti-inflammatory activity of crude extract of *Rhododendron Arboreum* by carrageenan induced rat paw edema.

Groups & Dose	Increase in paw volume (mm) ± SEM and % Oedema inhibition relative to control at every hour till 6th hour					
	Time after Carrageenan injection					
	1 h	2 h	3 h	4 h	5 h	6 h
Carageenan Control (1%)	0.176 ±0.0031	0.292 ±0.293	0.389 ± 0.390	0.449 ±0.010	0.391 ±0.0017	0.359 ±0.0013
Extract treated (40mg/kg)	0.168 ± 0.007* (4.54 %)	0.214 ±0.176* (26.72 %)	0.280 ±0.282* (28.02 %)	0.308 ± 0.0015** (31.41 %)	0.243 ±0.0020** (37.85%)	0.163 ± 0.0014** (54.60%)
Extract treated (60mg/kg)	0.140 ±0.0012** (20.4 %)	0.196 ±0.163** (32.88 %)	0.256 ±0.226** (34.62 %)	0.244 ±0.0061** (45.66 %)	0.183 ± 0.0036*** (53.20 %)	0.139 ±0.0007*** (61.39 %)
Extract treated (100mg/kg)	0.131 ±0.0021** (25.57 %)	0.164 ±0.143** (45.84 %)	0.204 ±0.198** (47.56 %)	0.154 ±0.0125*** (65.71 %)	0.119 ±0.0041*** (69.57 %)	0.104 ±0.0012*** (71.04 %)
Indomethacin treated (10 mg/kg)	0.126 ±0.0010** (28.41 %)	0.160 ±0.141** (45.21 %)	0.200 ±0.200** (48.59 %)	0.155 ±0.0083*** (65.48 %)	0.118 ±0.0052*** (69.83 %)	0.103 ±0.0011*** (71.31 %)

Values are Mean ± SEM, (n = 6 in each group). Figures in parenthesis are percent protection as compared to Carrageenan control. Carrageenan control group was compared with normal group and all values were significantly different (P< 0.01). Experimental groups were compared with Carrageenan control: *P<0.05 and **P<0.01, ***P<0.01

saponins and fixed oils. The chloroform extract contains proteins. The ethanolic extract contains carbohydrates, saponins, flavonoids, phytosterols, tannins and phenolic compound.

Acute toxicity study: In the acute toxicity study ethanolic extract of leaves of *R. arboreum* were found to be toxic (2/3 mice died) at a dose of 300 mg/kg, intraperitoneally. Hence, LD50 cut off value of ethanolic extract was fixed as 300mg/kg body weight. So, that 1/7th, 1/5th and 1/3rd of the LD50 cut off value that is, 40, 60 and 100 mg/kg body weight were selected as screening dose for anti-inflammatory activity.

Carrageenan - induced rat paw oedema: The extract as well as indomethacin showed antiphleogestic activity. This anti-inflammatory activity was dose-dependant and found to be statistically significant at the higher concentration, 100 mg/kg, (Table 1). The anti-inflammatory activity of indomethacin, a standard reference drug, was also found to be significant. In the carrageenan-induced rat paw oedema test (Table 1) for acute inflammation, the extract of *R. arboreum* in doses of 40, 60 mg and 100 mg/kg body weight showed significantly inhibition of oedema respectively at the end of 6h.

DISCUSSION

Carrageenan induced hind paw oedema. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is the standard experimental model for acute inflammation and is devoid of apparent systemic effect [9]. Moreover, the model exhibits a high degree of reproducibility [10]. The probable mechanism of action of carrageenan-induced inflammation is biphasic; the first phase is attributed to the release of histamine, serotonin and kinins in the first hour; while the second phase is attributed to the release of prostaglandins and lysosome enzymes in the second to third hour [11, 12]. The principal therapeutic effects of NSAIDs derive from their ability to inhibit prostaglandin G/H synthase (cyclooxygenase or COX) which convert arachidonic acid to the unstable intermediates PGG2 and PGH2 and leads to the production of thromboxane A2 and a variety of prostaglandins [13]. Prostaglandins are also known to cause pain and NSAIDs are particularly effective when inflammation has caused sensitization of pain receptors to normally painless mechanical or chemical stimuli [14]. It is of interest therefore, that the extract behaved similar to the NSAIDs in this study and thus correlates well with the ethnomedical use of the plant in painful and inflammatory conditions. The ability of the extract to inhibit carrageenan induced paw oedema suggests it possesses a significant effect against acute inflammation. Flavonoids such as quercetin are known to be effective in acute inflammation [15]. Certain flavonoids possess potent inhibitory activity against a wide array of enzymes such as protein kinase C, protein tyrosine kinases, phospholipase A2, phosphodiesterases and others [16, 17]. Anti-inflammatory effect of the extract may be due to the presence of flavonoids, tannins, alkaloid and saponins, either of singly or in combination the significant level of anti-inflammatory activity of the ethanolic extract could be attributed to high amount of flavonoids present in the extract. This study also lends support to the fact that *R. arboreum*, unlike most euphorbia, does not cause inflammation or irritation to the skin during physical handling of the material. More importantly, the research work justified the traditional use of the plant in the treatment of inflammatory disease conditions such as asthma.

Further studies will be carried out to isolate and characterize other anti-inflammatory the plant chemical constituents present in the ethanolic extract. However, further studies are needed to isolate the active constituents responsible for the observed effect and to reveal the possible mechanisms of action responsible for the anti-inflammatory activity.

CONCLUSION

These findings suggest that the ethanolic herb extract of *R. arboreum* contain bioactive constituents with anti-inflammatory activities, and further support the ethnomedical claim of the use of the plant in the management of inflammatory conditions.

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