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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF ROOTS OF *ATALANTIA MONOPHYLLA*

B. GANGA RAO*, M. SANJITH NATH, G. V. SAMPATH KUMAR and M. SAMUEL

College of Pharmaceutical Sciences, Andhra University, VISAKHAPATNAM (A. P.) INDIA

ABSTRACT

The study was designed to evaluate the anti-inflammatory effect of methanolic and chloroform root extracts of *Atalantia monophylla (Rutaceae)*, in carrageenan induced albino wistar rats of either sex (175-225 g).The anti-inflammatory effects of chloroform extract of *Atalantia monophylla* 200,400 mg/kg p.o were found to be significant in reducing rat paw oedema induced by carrageenan, where as methanolic extract produced significant reduction of paw oedema at 400 mg/kg p.o. Ibuprofen (50 mg/kg) was used as the reference anti-inflammatory agent for comparison. The chloroform extract was highly significant in reducing rat paw oedema. The result of this experimental animal study indicated that the extracts possess anti-inflammatory activity, and thus, lend credence to the suggested use of these plants in the management or control of arthritis and other inflammatory conditions in certain communities of India.

Key word : Anti-inflammatory, *Atalantia monophylla,* Carrageenan, Ibuprofen.

INTRODUCTION

The word inflammation comes from the Latin word “inflammare”, means to burn. Inflammation is a response of tissue to an infection, irritation to foreign substances. It is a part of hosts defense for inflammation, a defensive reaction to injury with classical signs of warmth, reddening, pain, swelling and loss of function, which is of a acute or chronic type[1] this inflammation is also observed in cancer[2], bowel syndrome, hepatic and Alzheimer’s diseases. The characteristics of inflammation are humorous like reddening (visible), swelling (oedema), soreness (pain) and corresponding histological changes. Inflammation response has two facets, inflammation and repair. In turn, the inflammatory response sets into motion a complex series of events. Inflammatory reaction underlines the genesis of crippling rheumatoid arthritis, life threatening sensitivity reaction and some forms of

* Author for correspondence

glomerular diseases. Neutrophils are the first cells, which are recruited to sties of tissue injury to deal with the causes of inflammation. They destroy invading pathogens and compounds by phagocytosis or opsonisation and these processes involve the production of Reactive Oxygen Species (ROS), and release of tissue-damaging enzymes such as proteases and myeloperoxidase (MPO). Arachidonic acid (AA) is released from cell membranes by phospholipaseA_2 under the stimulus of several factors associated with inflammation[3]. The greatest disadvantage of the presently available potent synthetic drugs lies in their toxic symptoms in gastrointestinal tract and discontinuation of medication after the treatment.

The *Rutaceae* family consists of 140 genera and about 1300 species. In India, cultivated general like citrus, murraya, aegle etc represents the family. This is armed tree, up to 8 mtrs tall, branchlet spines 1 (or) 2, axillary on branches. It has been used as a folk medicine for several purposes such as the treatment of dysentery [4], chronic rheumatism[5] and paralysis[5] in India. However, literature survey indicated that there are no published reports on the anti-inflammatory activity of the roots of the plant and hence, to find out the traditional claim of these activities, the present work has been carried out on this plant.

EXPERIMENTAL

Materials and methods

Plant

The plant material of *Atalantia monophylla* was collected from the Chintapalli forest area, Visakhapatnam (District), A.P. India, in June 2006 and Dr. M. Venkaiah, Associate Professor, Department of Botany, Andhra University, Visakhapatnam confirmed identity. The specimen (Voucher No. BGR-NOV 2006) was kept in the herbarium of the pharmacognosy division of Andhra University, Visakhapatnam.

Animals

Laboratory breed Albino wistar rats of either sex weighing 175-225 g, were employed for the study. All animals were procured from National Institute of Nutrition, Hyderabad. The rats were maintained under standard laboratory conditions at 25 ± 2^0C, relative humidity 50 ± 15% and normal photo period (12 hr dark/12 hr light). Commercial pellet diet (Rattan Brothers, India) and water were provided *adlibitum*. The experimental protocol has been approved by the Institutional Animal Ethics Committee and by the regulatory body of the government (Reg.No. 516/01/A/CPCSEA).

Table 1. Percentage inhibition of carrageenan induced paw oedema in rats by prophylactic treatment with the methanolic extract of *Atalantia monophylla* and ibuprofen (Anti-inflammatory activity of methanolic extract of *Atlantia Monophylla*)

Treatment	Percentage inhibition of the maximal paw oedema during (6h)	Percentage inhibitions of total AUC paw oedema during (6h)
Group A	0.0 ± 4.19	0.0 ± 5.85
Group B	63.10 ± 3.87 ***	81.28 ± 1.50 ***
Group C	29.36 ± 1.10*	43.66 ± 6.26*
Group D	45.54 ± 5.07**	59.12 ± 3.96**
Group E	48.15 ± 5.90**	61.40 ± 6.06**
Significance : *P < 0.05, **P < 0.01, ***P < 0.001		

Table 2. Percentage inhibition of carrageenan induced paw oedema in rats by prophylactic treatment with the chloroform extract of *Atalantia monophylla* and ibuprofen (anti-inflammatory activity of chloroform extract of *Atlantia monophylla*)

Treatment	Percentage inhibition of the maximal paw oedema during (6h)	Percentage inhibitions of total AUC paw oedema during (6h)
Group A	0.0 ± 4.19	0.0 ± 5.85
Group B	63.10 ± 3.87 ***	81.28 ± 1.50 ***
Group F	43.66 ± 3.33 **	59.35 ± 3.75 * *
Group G	64.64 ± 1.15 ***	77.17 ± 0.95 ***
Group H	64.67 ± 2.92 ***	78.01 ± 1.39 ***
Significance : *P < 0.05, **P < 0.01, ***P < 0.001		

Extraction and isolation procedure

The freshly collected roots of the plant were shade dried and powdered. The powdered material was then subjected to cold and hot extractions.

Cold extraction

In the cold extraction process, all the powdered materials (roots) of the plant were initially macerated with methanol using maceration tank for 3 days. The methanolic extract was concentrated under vaccum (50^0C) by using rota vapor, dried completely and weighed. The residue of the plant material thus obtained after methanolic extraction was again macerated with chloroform for 3 days. The chloroform extract was then concentrated under vaccum (50^0C), dried completely and weighed.

Hot extraction

The dried powdered material roots of the plant were extracted with methanol is Soxhlet apparatus for 24 hrs. The extracts thus obtained were concentrated under vaccum (50^0C), dried completely and weighed. The methanolic extract was obtained as 66 g.

Acute toxicity study

The animals were divided into control and test groups containing six animals each. The control group received the vehicle 1% sodium CMC, whereas the test group consisting of methanolic and chloroform extracts of *Atalantia monophylla* and increasing doses of 200, 400, 800 and 1600 mg/kg body weight p.o were observed for mortality till 48 hours and LD50 was calculated[6].

Anti-inflammatory activity

The rats were divided into different groups (each contains 6) as follows – Group A received drug vehicle 1% sodium CMC. Group B received standard drug Ibuprofen at the dose of 50 mg/kg. Group C, D and E received methanolic extract of roots of *Atalantia monophylla* at doses 100, 200 and 400 mg/kg body weight *p. o*. Group F, G and H received chloroform extract of roots of *Atalantia monophylla* at the doses of 100, 200 and 400 mg/kg, respectively. Two hours after these administrations, each rat received in its right hind paw subplantar injection of 0.1 mL of saline and 0.1 mL of 1% carrageenan suspension to the left hind paw. The paw thickness of each rat was measured by using Zeitlins apparatus[7] before and at 1, 2, 3, 4, 5 and 6 hrs after carrageenan injection. Paw thickness was measured by subtracting the initial thickness from the obtained value at every hour after carrageenan injection. The percentage inhibition of paw oedema was

calculated by using the formula –

% Increase in paw thickness = $(Y_t - Y_0/Y_0) \times 100$

Y_t = Paw thickness at time t (1, 2, 3, 4, 5 and 6^{th} hr) after injection.

Y_0 = Paw thickness at 0 hr. (before injection)

Data were expressed in terms of mean values ± S.E.M.

Statistical analysis

All values were expressed as mean ± S.E.M. The differences were compared using one-way analysis of variable (ANOVA) followed by Dunnetts t-test and unpaired students t-tests. P values (< 0.05) were considered statistically significant.

RESULTS AND DISCUSSION

The roots of *Atalantia monophylla* were shade-dried and extracted by continuous hot extraction process using Soxhlet apparatus. Subplantar injection of 1% carrageenan (0.1 mL) produced marked, sustained and time related increase in the rat hind paw oedema of the control group. Plantar swelling and/or oedema reached its peak level of 4 hr after the injection of carrageenan and gradually decreased in the following hours. The chloroform extract of root of *Atalantia monophylla* at the dose of 100, 200, and 400 mg/kg produced time related, sustained and dose dependent significant reduction ($p < 0.05$-0.001) of carrageenan induced acute inflammation of the rat hind paw. A methanolic extracts of roots of *Atalantia monophylla* at the dose of 100, 200 mg/kg were not able to produce significant reduction where as doses of 400 mg/kg produced significant reduction ($P < 0.01$) in the inflammation produced by carrageenan.

From the observed values, the percentage of maximal paw oedema produced during 6 hrs was calculated for all the extracts of the plant. The present study was conducted to evaluate the anti-inflammatory activity of *Atalantia monophylla,* which is very new herbal drug that was firstly identified by us to get a berth in the group of anti-inflammatory herbal drugs. In the methanolic extract treated groups, a significant percentage inflammation reduction was produced by the extract at 400 mg/kg in inflammatory groups is highly significant ($P < 0.01$), when compared to the percentage reduction observed in ibuprofen (Standard) treated groups.

Different mechanisms of action to reduce inflammation with the help of plant extracts already exist. The development of oedema in the paw of the rat after the injection of carrageenan has been described as a biphasic event. The initial phase, observed around 1 hr, is attributed to the release of histamine and seratonin, the second, accelerating phase of swelling is due to the release of prostaglandin-like substances[8]. The inflammatory activity exhibited by the chloroform extract of the plant may due to the presence of flavonoids[9,10], which was confirmed to possess the anti-inflammatory activity, because of the presence of high amounts of the compounds in the chloroform extract. When compared to the methanolic extracts, they exhibited comparatively more activity. From these experimental findings, one can say that the plant is having anti-inflammatory activity. As the compounds were isolated from chloroform extract, further studies are under progress to find the activity for the pure molecules, chronic inflammation (adjuvant –induced arthritis model) and to establish the mechanism of action for the extract as well as pure compounds. It can be concluded that all the extracts have potential to be explored as anti-inflammatory agents. Further studies may reveal the exact mechanisms of action responsible for the anti-inflammatory activities of *Atalantia monophylla.*

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