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Preliminary Assessment of Anti-Inflammatory Activity of Callicarpa macrophylla Vahl. Leaves Extracts

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Abstract: Leaves of *Callicarpa macrophylla*, an indigenous plant of India, had been the plant of study for the current research work. Aqueous as well as ethanolic extracts of leaves of *C. macrophylla* were evaluated for their anti-inflammatory activity using carrageenan paw edema method using diclofenac sodium as standard. Results showed that ethanolic extract of *C. macrophylla* leaves have better anti-inflammatory profile than the aqueous extract and can be the choice to be used as anti-inflammatory drug. © 2011 IGJPS. All rights reserved.

Keywords: Callicarpa macrophylla; Leaves; Anti-inflammatory; Carregeenan Paw Edema Method.

INTRODUCTION

Callicarpa macrophylla Vahl. (fam-Verbenaceae) is an erect shrub which is globally distributed across India, Nepal, Bhutan, Myanmar, South East Asia, and China. In India it is distributed in Jammu & Kashmir, Himachal Pradesh, Uttar Pradesh, Bihar, Sikkim, West Bengal, Arunachal Pradesh, Assam, Meghalaya, Nagaland, Manipur, Mizoram, Tripura, and Andhra Pradesh, up to an altitude of 1800 meters. Leaves are 12.5-23 cm long, ovate or ovate-lanceolate, acuminate, base cuneate or rounded. Upper surface wrinkled, glabrate when mature, white-tomentose beneath with compound stellate hairs, Petiole 6-13mm long^[1]. It is flowering in August-November and fruiting October-December^[2].

The leaves are used in gout and rheumatic pain^[2]. Decoctions of the leaves are used in the treatment of diarrhoea, dysentery and arresting bleeding. A juice made from leaves used in gastric troubles, headache and stop bleeding^[3].

Literature data reveals that *Callicarpa macrophylla* Vahl have anti-inflammatory^[4], antimicrobial^[5]. *C. macrophylla* Vahl contains diterpene(Calliterpenone^[6]), diterpenoid(16 α , 17-Isopropylideno-3-oxo-phyllocladane^[7]; 3 β , 16 α , 17-trihydroxy-Phyllocladane^[8]), flavanoids(β -sitosterol, ursolic acid, luteolin and apigenin)^[9], 16, 17-dihydroxy-kauranoids^[10], fatty acids & other constituents^[11].

MATERIALS & METHODS

Collection & Authentification of Plant Material

The drugs were collected from Banaras Hindu University campus, Varanasi and authenticated by Dr. V.K. Joshi, Dean of Faculty of Ayurveda, Institute of Medical Science, B.H.U., Varanasi and also through National Botanical Research Institute (NBRI), Lucknow. A Voucher specimen of all the plants has been preserved in the Department of Pharmacognosy, College of Pharmacy, IFTM, Moradabad, for further references. The collected leaves were shade dried 15 days and size reduced by laboratory grinder in to coarse powder. The air dried coarse powder is used for preparation of extract.

Preparation of Extracts

The ethanolic and aqueous extracts were prepared according to the standard procedure. The filtered, extracts were dried in a vacuum evaporator and aqueous & alcoholic extracts were kept in desiccators until further use.

Animals

Male / female albino rats weighing between 120 to150 grams, from Animal House, College of Pharmacy, IFTM, Moradabad, were divided in ten groups of six animals each. The animals were kept in polypropylene cages, under standard condition of 12:12 light and dark cycle.

Evaluation of Anti-inflammatory Activity Using Carrageenan Paw Edema Method

Experimentally inflammation was produced by carrageenan paw edema method in albino rats^[12]. A volume of 0.01 ml of 1% (w/v) carrageenan suspension in (0.9 % w/v sodium chloride) was injected through a 26-gauge needle into the plantar side of the left hind paw. The prepared drug samples 200 mg/kg, 400 mg/kg of the ethanolic and aqueous extract orally were administered one hour before the carrageenan injection. The standard drug Diclofenac sodium was given orally in dose of 20mg/kg. Tween 80 (1% v/v) was used as suspending agent. The volume of the paw was measured at 60, 120, 180 and 240 minutes after injection. The ankle joint of the rats was marked with permanent marker and the paw was dipped in the mercury. The volume of hind paw of the rats up to the ankle joint was measured plethysmographically by the mercury displace method. The percent inhibition was calculated by following formula-

% Inhibition = $(1-Vt / Vc) \times 100$

Where, Vt and Vc are the mean change in paw volume of treated and control rats respectively

Data analysis and statistics

The values were expressed as mean \pm standard error mean (SEM). Statistical analysis of the data was carried out by two way ANOVA followed by bonferroni test to determine the significant between two groups p<0.05 was considered significant.

RESULTS & DISCUSSION

Carrageenan has been widely used as a noxious agent able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity. This phlogistic agent, when injected locally into the rat paw, produced a severe inflammatory

Indo-Global Journal of Pharmaceutical Sciences, 2011, Vol 1., Issue 3: Page No. 219- 222

reaction, which was discernible within 30 min. The development of edema induced by carrageenan corresponds to the events in the acute phase of inflammation, mediated by histamine, bradykinin and prostaglandins produced under an effect of cyclooxygenase[13]. The ethanolic and aqueous extracts (200 mg/kg, 400 mg /kg) of leaves of *Callicarpa macrophylla* showed significant (p< 0.05) antiinflammatory effect in the acute phase of the inflammation process as compared with standard drug, Diclofenac sodium (20 mg/kg) body wt. Further, the ethanolic and aqueous extracts were found to contain carbohydrates, steroids, flavonoids and tannins, through preliminary photochemical screening. The anti-inflammatory activity may be due to one/more group of above Phytoconstituents which may cause inhibition of histamine, serotonin or prostaglandin synthesis.

Increase in paw volume (in ml) after different times Treatment Dose %Reduction 30 min 1hr 2hr 3hr 4hr (mg/kg) (after 4 hr) 0.28 ± 0.02 0.36 ± 0.01 0.69 ± 0.03 0.73 ± 0.01 0.72 ± 0.01 Control _ $0.18 \pm 0.01^*$ $0.21 \pm 0.01^{\circ}$ $0.23 \pm 0.02^{*}$ $0.21 \pm 0.01^{*}$ $0.16 \pm 0.03^*$ 77.78 Diclofenac 20 Sodium $0.36 \pm 0.01^{\circ}$ LEE 200 $0.20 \pm 0.01^{*}$ $0.23 \pm 0.02^{*}$ $0.30 \pm 0.02^*$ $0.26 \pm 0.02^{*}$ 63.89 400 $0.19 \pm 0.02^*$ $0.22 \pm 0.03^{*}$ $0.31 \pm 0.03^*$ $0.26 \pm 0.01^{*}$ $0.17 \pm 0.02^*$ 76.39 0.24 ± 0.01 0.29 ±0.03* 200 $0.28 \pm 0.01^{\circ}$ $0.37 \pm 0.01^{\circ}$ $0.31 \pm 0.02^{*}$ 59.72 LAE 400 0.18 ± 0.01 $0.23 \pm 0.01^{\circ}$ $0.33 \pm 0.01^{\circ}$ $0.27 \pm 0.03^{\circ}$ $0.19 \pm 0.01^{\circ}$ 73.61

Table 1 Effect of ethanolic, aqueous extract of C. macrophylla Vahl. leaves on Carrageenan induced paw oedema in rats

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221

Indo-Global Journal of Pharmaceutical Sciences, 2011, Vol 1., Issue 3: Page No. 219- 222

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