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ANTI-INFLAMMATORY ACTIVITY OF WHOLE PLANT OF *BELOPERONE PLUMBAGINIFOLIA* (ACANTHACEAE)

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ABSTRACT: In the present study anti-inflammatory activity of ethanol extract of *Beloperone plumbaginifolia* whole plant were investigated. The anti-inflammatory activity of ethanol extract of whole plant of *B.plumbaginifolia* were evaluated by carageenan induced rat paw edema to determine its effect on acute phase of inflammation models in rats. The phytochemical screening of ethanol extract of whole plant of *B. plumbaginifolia* revealed the presence of alkaloid, catechin, coumarin, flavonoid, phenol, tannin, saponin, steroid, glycoside and terpenoid. Maximum inhibition (78.63%) was obtained at the dose of 400 mg/kg of *B.plumbaginifolia* whole plant after 3h of treatment in carrageenen induced paw edema, whereas indomethacin produced 77.34% of inhibition. The present study suggested that *B.plumbaginifolia* whole plant extract possess strong anti-inflammatory property.

INTRODUCTION: Inflammation is the first body's response to injection or injury, and is critical for both innate and adaptive immunity. It can be considered as part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. Uncontrolled inflammation often results in chronic diseases such as arthritis, autoimmune disorders, degenerative joint diseases, rheumatism, asterosclerosis, diabetes and even cancer [1 - 3]. Inflammation can be classified as either acute or chronic.

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Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system and the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation and characterized by the development of specific humoral and cellular immune response to pathogens, leads to a progressive degeneration of tissue and fibrosis [4].

Non steroidal anti-inflammatory drugs (NSAIDs), steroidal drugs and immune-suppressant drugs, which have been usually used in the relief of inflammatory diseases worldwide for a long time, are often associated with severe adverse side effects, such as gastrointestinal bleeding and peptic

ulcer [5]. As a result, a search for other alternatives is necessary. Recently, many natural medicines derived from plants, marine organisms etc, were considered effective and safer for the treatment of various diseases including inflammation and pain [6].

Beloperone plumbaginifolia (Acanthaceae) is a branched small shrub, which is being used by tribals in Kerala as an antidote for snakebite. In Brazil, its native place, it is grown as an ornamental [7]. In Tamil Nadu, it has been used to treat various ailments like antidote for scorption bite, psoriasis, *etc*, as the folklore medicine. Despite its rich pharmacological potential, so far no study has been conducted on this medicinal plant except shoot multiplication and callogenisis technique development [8].

In the present study, inhibitory effects of the ethanol extract of *Beloperone plumbaginifolia* whole plant were investigated in more detailed using acute inflammation model namely carrageenan-induced paw edema.

MATERIALS AND METHODS:

Plant material: The whole plant of *Beloperone plumbaginifolia* (Acanthaceae) were freshly collected from Agasthiarmalai Biosphere Reserve, Southern Western Ghats of Tamil Nadu. The plant specimen was identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu, India.

Preparation of plant extract for anti-inflammatory activity: The whole plant of *B. plumbaginifolia* was powdered in a Wiley mill. Hundred grams of whole plant powder was packed in a Soxhlet apparatus and extracted with ethanol. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extracts were used for preliminary phytochemical screening [9] and anti-inflammatory activity.

Animals: Adult Wistar Albino rats of either sex (150-200g) were used for the present investigation. Animals were housed under standard environmental conditions at temperature (25±2 °C) and light and dark (12:12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*. The study was carried out as per IAEC approval no. 1012/ C06/ CPSEA- Corres- 2008- 2009.

Acute toxicity study: Acute oral toxicity was performed by following OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study [10]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administrated orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administrated was assigned as toxic dose. If mortality was observed in one animal, then the same dose repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100 upto 2000 mg/kg body weight.

Anti-inflammatory activity of Carrageenan induced hind paw edema: Albino rats of either sex weighing 150 - 200 grams were divided into four groups of five animals each. The dosage of the drugs administered to the different groups was as follows. Group I - Control (normal saline), Group II - Ethanol extract of *B. plumbaginifolia* whole plant (200 mg/kg, p.o.); Group III - ethanol extract of *B. plumbaginifolia* whole plant (400 mg/kg, p.o.); and Group IV- Indomethacin (10 mg/kg, p.o). All the drugs were administered orally. Indomethacin served as the reference standard anti-inflammatory drug.

After one hour of the administration of the drugs, 0.1 ml of 1% w/v carrageenan solution in normal saline was injected into the sub plantar tissue of the left hind paw of the rat and the right hind paw was served as the control. The paw volume of the rats were measured in the digital plethysmograph (Ugo basile, Italy), at the end of 0 min, 60min, 120min and 180min. The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of the drugs was studied. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of the inflammation. Percentage inhibition was calculated using the formula;

$$\text{Percentage inhibition} = [(Vc-Vt)/Vc] \times 100$$

Where, Vt the percentage represents the percentage difference in increased paw volume after the administration of test drugs to the rats and Vc represents difference of increased volume in the control groups.

Statistical analysis: The data were analyzed using student's t-test statistical methods. For the statistical tests a *p* values of less than 0.001, 0.01 and 0.05 was taken as significant.

RESULTS: The phytochemical screening of ethanol extract of whole plant of *B. plumbaginifolia* revealed the presence of alkaloid, catechin, coumarin, flavonoid, phenol, tannin, saponin, steroid, glycoside and terpenoid. Acute toxicity study revealed the non-toxic nature of the ethanol extract of whole plant of *B. plumbaginifolia.* In the present study, the anti-inflammatory activity of ethanol extract of whole plant of *B. plumbaginifolia* was assayed in albino rats using carrageenan induced rat paw edema method. **Table 1** shows the anti-inflammatory activity of ethanol extract of *B. plumbaginifolia* whole plant significantly (P<0.001) inhibited the rat paw edema at 3rd hour post carrageenan were 74.50% and 78.63% for 200 mg/kg and 400 mg/kg of whole plant extract respectively. The results were compared with indomethacin at 10 mg/kg which shows paw reduction of 77.34%.

TABLE 1: EFFECT OF *B. PLUMBAGINIFOLIA* EXTRACT ON THE PERCENTAGE OF INHIBITION ON THE CARRAGEENAN INDUCED PAW EDEMA

Treatment	Edeme volume (ml)				% inhibition after 180min
	0 min	60 min	120 min	180 min	
Group I (Control)	38.16 ± 1.36	84. 31 ± 2.93	106.31 ± 2.65	124. 16 ± 2.92	-
Group II	38.92 ± 1.54	69.36 ± 2.26 ns	46. 21 ± 1.36**	31.65 ± 1.29***	74.50
Group III	37.16 ± 0.94	56.22 ± 1.38*	37.54 ± 1.23***	26.53 ± 1.56***	78.63
Group IV (Indomethacin)	36.54 ± 1.36	63.16 ± 1.09*	39.84 ± 1.16***	28.13 ± 1.58***	77.34

Each value is SEM ± 5 individual observations * P<0.05; **P<0.01; ***P<0.001, compared paw edema induced control Vs drug treated rats

DISCUSSION: Carrageenan induced rat paw edema is a suitable experimental animal model commonly used for the study of acute inflammation and is believed to be biphasic. In general, the first phase (1-2 h) involves inflammation mediated by the release of serotonin and histamine and increased synthesis of prostaglandins in the surroundings of the damaged tissues. The second phase (3-5 h) is the result of the release of kinins mainly prostaglandins [11].

In this study, the ethanol extract of *B. plumbaginifolia* whole plant exerted considerable inhibitory effects on carrageenan induced paw edema in rats starting from the first hour after administration. This effect was dose dependent and the maximum inhibition induced by the extract was 3h with the highest dose (400 mg/kg) of the *B. plumbaginifolia* extract. Similarly inhibitory effects were observed after carrageenan injection with indomethacin a potent non steroidal anti-inflammatory drug which acts by inhibitory cyclooxygenase. Therefore, our results suggest that the inhibitory effect of the extract of *B.plumbaginifolia* whole plant on carrageenan induced paw edema may be due to the suppression of the release of mediators including histamine, serotonin, bradykinin and prostaglandins responsible for the first and the second phase of acute inflammation induced by carrageenan. There are also evidences that compounds inhibitions the enzyme cyclooxygenases [12, 13]. Based on these reports, the inhibitory effect of *B. plumbaginifolia* extract on carrageenan induced inflammation could be mediated via this mechanism.

This study shows that ethanol extract of whole plant of *B. plumbaginifolia* possess significant anti-inflammatory effects. Phytochemical screening of the extract indicated the presence of flavonoids, terpenoids and steroids. It may be assumed that the anti-inflammatory effects of the ethanol extract of *B. plumbaginifolia* whole plant could be due to the presence of various flavonoids and terpenoids.

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