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Research Article

Evaluation of Antiinflammatory Activity of Ethanol Extracts of *Barleria courtallica* Nees (Acanthaceae)

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ABSTRACT
Ethanol extracts of *Barleria courtallica* stem, root and leaf were evaluated for its antiinflammatory activity at the dose levels of 150 mg/kg and 300 mg/kg body weight using a carrageenan induced paw edema method (acute inflammation). Results showed that all the studied ethanol extracts had potent and significant antiinflammatory activity. These results were also comparable with reference drug indomethacin.

Keywords: *Barleria courtallica*, carrageenan, paw edema, indomethacin.

INTRODUCTION

Inflammation is a pathophysiological response of living tissue to injury that leads to local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism that helps body to protect itself against infection, burns, toxic chemicals, allergens or other noxious stimuli, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases[1]. Inflammation can be classified as either acute or chronic and involves a cascade of biochemical events comprising the local vascular system, the immune system and different cell types found in the injured tissue. Acute inflammation is the initial response and is characterized by the increased movement of plasma and innate immune system cells, such as neutrophils and macrophages from the blood into the injured tissue. Chronic inflammation concerns a progressive change in the type of cells present at the site of the inflammatory reaction and is characterized by simultaneous destruction and healing of the injured tissue[2].

These diseases are mainly treated with nonsteroidal antiinflammatory drugs (NSAIDs) and steroidal drugs, which have proven effective but can have negative side effects. For instance, NSAIDs may induce gastric and intestinal ulcers, anaemia, platelet inhibition in uterine motility and in some reported cases, an increased risk of myocardial infarction[3]. Steroidal antiinflammatory drugs prevent or suppress inflammation but do not attack the root cause of the disease and the prolonged use of these compounds can inhibit the synthesis of the inducible isoform of nitric oxide synthase enzyme and cause pituitary-adrenal suppression, hyperglycaemia, glycosuria and an increased susceptibility to infections and peptic ulcers[4].

On the contrasy many medicines of plant origin had been used since ages without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop more effective and cheaper drugs. Plant represents a large natural sources of useful compounds that might serve as lead for the development of novel drugs[5,6].

Genus *Barleria* belongs to the family Acanthaceae. Whole plant extract *Barleria* contains a number of active compound like alkaloids, terpenes, flavonoids, glycosides, lignins, phenolics etc., which have shown potent therapeutic activities against several diseases[7-10]. *Barleria* also shows various pharmacological effects such as antimicrobial, anthelminthic, antifertility, antioxidant, antidiabteic, antiarthritic, hepatoprotective, diuretic, cytoprotective, antidiarrhoeal, analgesic, antileukemic, anti-inflammatory and hypoglycemic properties without any toxic efferts[11,12]. Taking into consideration of the medicinal importance of the plant the ethanol extract of *Barleria courtallica* was analyzed for the GC-MS. Till now, the investigation of phytocomponents by GC-MS has not been done on *Barleria courtallica* Nees. Up to date no pharmacological study has been systematically conducted to evaluate the antiinflammatory activity of ethanol extract of *Barleria courtallica* stem, root and leaf. Hence, the present study was undertaken to evaluate the antiinflammatory activity of *Barleria courtallica* Nees in wistar Albino rats using ethanol extracts.

MATERIALS AND METHODS

Plant material

The stem, root and leaf of *Barleria courtallica* Nees were freshly collected from Agasthiarmalai Biosphere Reserve, Southern Western Ghats of Tamil Nadu. The plant specimen was identified and authenticated in Botanical

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Table 1: Effect of *B.courtallica* stem, root and leaf extracts on the percentage of inhibition on the carrageenan induced paw edema.

Treatment	Edema volume (ml)					% Inhibition after 180 min
	Dose mg/kg	0 min	60 min	120 min	180 min	
Control Group-I	Normal saline	36.92±0.36	69.11±0.98	118.64±1.13	143.84±2.11	
Group-II	150 mg/kg	35.11±0.92	68.38±1.13ns	41.65±1.12***	32.86±1.31***	77.15
Group-III	300 mg/kg	37.26±0.16	51.63±0.15*	30.18±1.16**	21.56±0.81***	85.01
Group-IV	150 mg/kg	36.84±0.11	56.18±0.36ns	24.13±0.27**	20.51±0.28***	85.74
Group-V	300 mg/kg	34.81±0.26	51.93±0.67*	31.48±0.84**	18.36±0.11***	87.23
Group-VI	150 mg/kg	35.12±0.13	62.84±0.16ns	26.93±0.16***	18.12±0.16***	87.40
Group-VII	300 mg/kg	36.92±0.76	52.16±0.27*	22.31±0.26***	16.22±0.31***	88.73
Group-VIII	10 mg/kg	34.83±0.21	49.36±0.16**	24.63±0.84***	18.46±0.84***	87.13

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: ns-Not significant.

Survey of India, Southern Circle, Coimbatore, Tamil Nadu. A voucher specimen (VOCB4132) was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract for antiinflammatory activity

The stem, root and leaf of *Barleria courtallica* Nees were powdered in a Wiley mill. Hundred grams of stem, root and leaf powders were packed in a Soxhlet apparatus and extracted with ethanol. The ethanol extracs were concentrated in a rotary evaporator. The concentrated ethanol extracts were used for preliminary phytochemical screening[11] and antiinflammatory 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±20C) 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*.

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[13]. 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.

Antiinflammatory activity of Carrageenan induced hind paw edema

Albino rats of either sex weighing 150-200 grams were divided into eight 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 and III – Ethanol extract of *B.courtallica* stem whole plant (150 and 300 mg/kg, p.o.); Group IV and V- ethanol extract of *B.courtallica* root (150 and 300 mg/kg, p.o.); Group VI and VII- ethanol extract of *B.courtallica* root (150 and 300 mg/kg, p.o.); and Group VIII- Indomethacin (10 mg/kg, p.o). All the drugs were administered orally. Indomethacin served as the reference standard antiinflammatory 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, 180min, 240min, 360min, and 480min. 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;

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 extracts of stem, root and leaf of *B.courtallica* revealed the presence of alkaloid, catechin, coumarin, flavonoid, tannin,saponin, steroid, phenol, glycoside, terpenoid and xanthoprotein. Acute toxicity study revealed the non-toxic nature of the ethanol extracts of stem, root and leaf of *B.courtallica.*

In the present study, the antiinflammatory activity of ethanol extracts of stem, root and leaf of *B.courtallica* were assayed in albino rats using carrageenan induced rat paw edema method. Table 1 shows the antiinflammatory activity of ethanol extracts of stem, root and leaf of *B.courtallica* significantly inhibited the rat paw edema at 3rd hour post carrageenan were 77.15%, 88.01% for 150 mg/kg and 300 mg/kg of stem extract, 85.74%, 87.23%

for 150 mg/kg and 300 mg/kg root extract and 87.40 %, 88.73% for 150 mg/kg and 300 mg/kg of leaf extract respectively. The results were compared with indomethacin at 10 mg/kg, which shows paw reduction of 87.13%.

DISCUSSION

Carrageenan induced hind paw edema is the standared experimental model of acute inflammation. The time course of edema development in carrageenan induced paw edema model in rats is generally represented by a biphasic curve[14]. The first phase of inflammation occurs within an hour of carrageenan injection and is partly attributed to trauma of injection and also to histamine and serotonin components[15]. The second phase is associated with the production of bradykinin, protease, prostaglandin and lysosome[15]. Prostaglandins (PGs) play a major role in the development of the second phase of inflammatory reaction which is measured at 3rd hour[16].

The doses 150 mg/kg and 300 mg/kg of ethanol extracts of *B.courtallica* produced a significant inhibition of carrageenen induced paw edema at 3rd hour. Therefore, it can be inferred that the inhibitory effect of ethanol extracts of *B.courtallica* on carrageenan induced inflammation could be due to inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis. Significant inhibition of paw edema in the early hours of study by *B.courtallica* could be attributed to the inhibition of histamine and/or serotonin[17]. Prostaglandin-E_2, a powerful vasodilator, synergizes with other inflammatory vasodilators such as histamine and bradykinin and contributes to redness and increased blood flow in areas of acute inflammation. The significant ($P<0.001$) suppressive activity of the ethanol extracts of stem, root and leaf of *B.courtallica* in late phase shows its potent antiinflammatory effect. This result is quite similar to the one observed for indomethacin at 10mg/kg, which inhibited 87.13%. Therefore, it is suggested that the mechanism of action of the extract may be related to histamine and prostaglandin synthesis inhibition. At present, there are no reports on investigation to identify the active components present in ethanol extract of *B.courtallica*. Further investigations are anticipated to identify the active components and lead to their further clinical uses.

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