Research Article

Evaluation of Anti-inflammatory Activity of Aerial Part Extract of *Daphniphyllum neilgherrense* (Wt.) Rosenth

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ABSTRACT

Ethanol extract of *Daphniphyllum neilgherrense* was tested for antiinflammatory activity in animal model. Three doses, 100, 200 and 400mg/kg of the plant extracts were used. Acute inflammatory activity was studied in rats by carrageenan induced paw edema model. The standanrd drug used was indomethacin 10mg/kg. Three doses 100, 200 and 400mg/kg of plant extract exhibited significant (p<0.001) antiinflammatory activity in carrageenan model in comparison to control. This study established the antiinflammatory activity of aerial part of *Daphniphyllum neilgherrense.*

Keywords: *Daphniphyllum*, paw edema, acute, carrageenan.

INTRODUCTION

Inflammation is a pathophysiological response of living tissue to injury 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 are mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases[1].

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process[2].

Inflammatory diseases are mainly treated with non steroidal 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 affect 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, hyperglycemia, glycosuria and an increased susceptibility to infections and peptic ulcers[4]. Therefore, searching for new molecules with antiinflammatory activity but with fewer side effects is vital, and plants may represent a potential source of such compounds.

Several plants and their products are claimed and proved to possess antiinflammatory property. *Daphniphyllum neilgherrense* is a shrub or small tree found in Indo-Malaysian region. It is a type genus of the family Daphniphyllaceae.

The plants related to the genus *Daphniphyllum* are reported to be used in folklore medicines in South-East Asia and Southern China for the treatment of various ailments.

Many of the plants of this genus are used in the treatment of asthma, cough, rheumatism, inflammation, fever, fractures and snake bites[5]. Recently, few members of the genus become famous for their anti-tumour, antioxidant, anti-platelet aggregation, vasorelaxant and insecticidal properties[6]. Over 200 alkaloids have been isolated from the different species of the genus[7]. However, perusal of literature reveals that antiinflammatory activity of *Daphniphyllum neilgherrense* is totally lacking and hence the present investigation was undertaken. The main objective of the present study is to evaluate the antiinflammatory activity of *Daphniphyllum neilgherrense* aerial part.

MATERIALS AND METHODS

Collection of plant samples

The aerial parts of *Daphniphyllum neilgherrense* (Wt.) Rosenth were collected from Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu, India. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

Preparation of plant extract for antiinflammatory activity

The dried whole plant material of *D.neilgherrense* was powdered in a Wiley mill. Hundred grams of aerial plant powder was packed in a Soxhlet apparatus and extracted with ethanol. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract was used for 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[8]. 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 and 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 five groups of six animals each. The dosage of the drugs administered to the different groups was as follows. Group I - Control (normal saline), Group - II III and IV – Ethanol extract of *D.neilgherrense* aerial part (100, 200 and 400mg/kg p.o.), Group V – 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] × 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 plant extract did not exhibit any mortality upto the dose level of 2000mg/kg. So, the extract is safe for long term administration. In the present study, the anti-inflammatory activity of ethanol extract of *D.neilgherrense* was studied in Albino rats using carrageenan induced rat paw edema (acute inflammation) method. Table 1 shows that the antiinflammatory activity of ethanol extract of the aerial part of *D.neilgherrense* significantly (p<0.001) inhibited the rat paw edema at 3rd hr post carrageenan were 69.58%, 82.66% and 84.53% for 100, 200 and 400 mg/kg respectively. It shows that the plant extract have significant antiinflammatory effect and the results were compared with indomethacin (84.23%; p<0.01).

Table 1: Effect of *D.neilgherrense* extract on the Percentage inhibition of carrageenan induced paw edema

Treatment Groups	edema volume (ml)					% Inhibition after 180 min
	Dose mg/kg	0 min	60 min	120 min	180 min	
Group-I	Normal saline	27.31±1.34	73.56±1.34	101.35±1.26	139.36±2.54	-
Group-II	100 mg/kg	34.16±1.24ns	59.64±1.13*	51.26±1.18***	42.38±1.16***	69.58%
Group-III	200 mg/kg	29.18±1.37	51.56±1.86**	37.89±1.37***	24.16±1.04***	82.66%
Group-IV	400 mg/kg	26.34±0.98	37.25±1.03***	26.15±1.64***	21.55±0.96***	84.53%
Group-V	10 mg/kg	30.16±1.36	39.88±1.16***	28.76±1.21***	23.36±0.75***	83.23%

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

The carrageenan induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis[9].

Carrageenan induced hind paw edema is the standard experimental model for acute inflammation. The time course of edema development in carrageenan induced paw edema model in rats is generally represented by a biphasic curve[10]. 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[11]. The second phase is associated with the production of bradykinin, protease, prostaglandins (PGs) play a major role in the development of the second phase of inflammatory reaction which is measured at 3rd hr[12].

The doses 100, 200 and 400 mg/kg of ethanol extract of D. *neilgherrense* aerial part produced a significant inhibition of carrageenan induced rat paw edema at 3rd hr. Therefore, it can be referred that the inhibitory effect of ethanol extract of *D.neilgherrense* on carrageenan induced inflammation could be due to inhibition of prostaglandin synthesis. Significant inhibition of paw edema in the early hours of study by *D.neilgherrense* could be attributed to the inhibition of histamine and / or serotinin[13].

CONCLUSION

Antiinflammatory activities of many plants have been attributed to their high sterol/triterpenoid saponins[14].

Though at this stage it is not possible to identify the exact phytochemical constituent(s) responsible for antiinflammatory activity of *D.neilgherrense*, it may be assumed that the effects could be due to chemicals present in the ethanol extract examined by qualitative test. The result of present study indicates that ethanol extract of *D.neilgherrense* aerial part possess significant antiinflammatory activity of acute inflammation.

Further detailed investigation is underway to determine the exact phytoconstituents, which are responsible for the antiinflammatory activity.

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