Available online on www.ijcpr.com
International Journal of Current Pharmaceutical Review and Research; 7(2); 117-120

ISSN: 0976 822X

Research Article

Elucidation of Analgesic and Antipyretic Activities of *Daphniphyllum neilgherrense* (Wt.) Rosenth Aerial Part in Wistar Rats

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Available Online:27th February, 2016

ABSTRACT

The aim of this study was to investigate the analgesic and antipyretic properties of the ethanol extract from aerial part of *Daphniphyllum neilgherrense* in wistar albino rats. The analgesic activity of aerial part of *Daphniphyllum neilgherrense* was studied using hot plate method and tail immersion method in rats. The antipyretic activity of aerial part of *Daphniphyllum neilgherrense* was studied in Brewer's yeast induced pyrexia in rats. In analgesic activity by hot plate and tail immersion models, ethanol extract significantly (p<0.001) reduce the painful stimulus. This confirms central and peripheral effects of the drug. It also possess antipyretic activity, ethanol extract significantly (p<0.01) reduces fever at higher doses within 3 hours on Brewer's yeast induced pyrexia model in rats.

Keywords: *Daphniphyllum neilgherrense*, Analgesic, Antipyretic, Pyrexia, Diclofenac

INTRODUCTION

Pain is an ill-defined, unpleasant, sensation usually evoked by an external or internal noxious stimulus. It is a warning signal and primarily protective in nature, but causes discomfort. Analgesics are the drugs that selectively relieve pain by acting on the CNS (central nervous system) or on peripheral pain mechanisms, without significantly altering consciousness[1]. Due to having adverse side effects, like gastric lesions caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects. Fever or pyrexia is an elevated body temperature above the normal level characterized by an increase in thermoregulatory set-point, which results from the interaction of the central nervous or immune system. Fever is body's natural defense mechanism against infectious agents which can damage the tissue. This in turn triggers the enhanced formation of pro-inflammatory cytokines like tumour necrosis factor-α (TNF- α) and interleukin 1β, α and β, these pro-inflammatory mediators increase the synthesis of prostaglandian E2 (PGE2) near hypothalamus area and thereby trigger the hypothalamus to elevate the body temperature. The thermoregulatory system governed by nervous feedback mechanism alters the fever by vasodilation and vasoconstriction of blood vessels. Although fever is body's defensive mechanism, some studies have suggested that raising temperature may be harmful. Therefore, in clinical practices in which fever-associated risks offset benefits, antipyretic treatment is necessary[2,3]. Several plants and their products are claimed and proved to possess analgesic and antipyretic 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[4]. Recently, few members of the genus become famous for their anti-tumour, antioxidant, anti-platelet aggregation, vasorelaxant and insecticidal properties[5]. Over 200 *Daphniphyllum* alkaloids have been isolated from the different species of the genus[6]. To our knowledge no report on the effects of *Daphniphyllum neilgherrense* on analgesic and antipyretic activities. Therefore, the present study was undertaken to evaluate analgesic and antipyretic activity potential of ethanol extract of aerial part of *Daphniphyllum neilgherrense* on various animal models.

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.

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Table 1: Analgesic activity of ethanol extract of *Daphniphyllum neilgherrense* aerial part on the adult albino rats.

Groups	Response Time in sec (Mean ± SEM)	
	Eddy Hot Plate Method	Tail Immersion Method
Group I	3.95±0.165	2.92±0.026
Group II	17.46±0.159***	12.86±0.118***
Group III	10.16±0.054***	7.59±0.016*
Group IV	15.92±0.311***	11.24±0.268***
Group V	21.65±0.434***	19.36±0.316***

The data were expressed as mean ± S.E.M.; ANOVA followed by Tukey Kramer Multiple Comparison test: ***p<0.001, **p < 0.01 and *p<0.05(Extracts vs. control)

Table 2: Effect of ethanol extract of *Daphniphyllum neilgherrense* aerial part on the Antipyretic activity in Brewer's yeast induced pyrexia rats

Groups	Rectal Temperature in ^{0}C after 18hrs of Yeast Injection(Mean± SEM)					
	-18[a] hr	0[b] hr	1 hr	2 hr	3 hr	6 hr
Group I	36.19±0.13	38.21±0.16	39.16±0.12	39.62±0.13	39.78±0.11	39.96±0.67
Group II	37.06±0.18	39.54±0.18	37.98±0.16ns	36.84±0.15*	35.16±0.18*	34.16±0.13**
Group III	37.16±0.18	38.54±0.11	37.96±0.18ns	36.16±0.34*	35.18±0.13*	34.56±0.84**
Group IV	37.38±0.13	39.63±0.18	37.24±0.13*	35.16±0.92*	34.84±0.12**	34.34±0.18**
Group V	37.65±0.18	39.73±0.11	37.16±0.13*	35.65±0.13*	34.29±0.16**	34.04±0.36**

Data expressed in mean ± SEM; Level of significance: * P < 0. 05 when compared to control. ** P < 0.01 **p<0.01 when compared to control

[a] Temperature just before yeast injection

[b] Temperature just before drug administration

The final uniform powder was used for the extraction of active constituents of the plant material.

Preparation of plant extract for phytochemical screening, analgesic and antipyretic activities

The aerial part of the plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder, which was then subjected to extraction in a soxhlet apparatus using ethanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures[7-9]. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract was used for analgesic and antipyretic activities.

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^{0}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*.

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.

Evaluation of analgesic activity

Eddy's hot plate method

The wistar albino rats were divided in to five groups of 6 animals each. Group I served as control. Group II served as standard and were injected Diclofenac (10mg/kg) intraperitoneal. Group III, Group IV and Group V were treated orally with aerial part ethanol extracts of *D. neilgherrense* at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight respectively. The rats were individually placed on the hot plate maintained at 55^{0}C, one hour after their respective treatment. The response time was noted as the time at which rats reacted to the pain stimulus either by paw flicking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds[1].

Tail Immersion method

The wistar albino rats were divided into five groups of 6 rats each. Group I served as control. Group II served as standard and were injected Diclofenac (10mg/kg) intraperitoneal. Group III, Group IV and Group V were treated orally with aerial part ethanol extracts of *D. neilgherrense* at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight respectively. After one hour, the lower 5cm portion of the tail was immersed in a beaker of freshly filled hot water maintained at 55^{0}C ± 1.0^{0}C. The time taken to withdraw the tail was noted as reaction time. A cut of time of 10 seconds was maintained to prevent tissue damage. The time required for flicking of the tail was recorded to assess response to noxious stimulus[11].

Evaluation of antipyretic activity

Brewer's yeast induced pyrexia method

The antipyretic activity was evaluated by using Brewer's yeast induced pyrexia method in wistar albino rats. Fever was induced by injecting 2.0ml/kg of 20% aqueous suspension of Brewer's yeast in normal saline and 18 Hour after yeast injection the test drugs were administrated.

Rectal temperature was recorded by clinical thermometer at 0, 1, 2, 3 and 6 Hour after drug administration. The rats were divided in to five groups of 6 animals in each and were given the following treatment orally. Group I (control) received 1% normal saline. Group II received indomethacin (10 mg/kg) as standard drug. Group III, IV and V received (100, 200 and 400mg/kg) of ethanol extract of aerial part of *D. neilgherrense*. Before the experiment, the rats were maintained in separate cages with food *ad libitum* for 7 days and the rats with approximately constant rectal temperature (37.5 to 38.5^{0}C) were selected for the study. The mean rectal temperature was found out for each group and compared in to value of standard drug[11].

Statistical analysis

All values were expressed as mean ± SEM. The results were analysed for statistical significance using one-way ANOVA followed by Tukey Kramer multiple comparison test with ***p0.001, **p0.01 and *p<0.05 were considered as significant.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical screening of the aerial part ethanol extract showed the presence of alkaloid, coumarin, catechin, flavonoid, steroid, saponin, glycoside, phenol, tannin, terpenoid and sugar.

Acute toxicity

Oral administration of the ethanol extract of *Daphniphyllum neilgherrense* aerial part did not cause any acute toxicity in experimental rats at all the tested dosages, confirming that it has potentialy safe for consumption.

Analgesic activity

Eddy's hot plate method

Rats treated with ethanol extract of *D. neilgherrense* aerial part showed significant (p<0.001) and dose dependent analgesic activity in thermal stimulated pain (hot plate test) in rats. The reaction time at a dose of 400mg/kg (higher dose) was found to be 21.65 seconds after 90 minutes of drug treatment, whereas the standard drug diclofenac showed the tail flick latency17.46 seconds (Table 1).

Tail Immersion method

Rats treated with ethanol extract of *D. neilgherrense* aerial part showed significant (p<0.001) increase in the tail flick latency compared to control. The tail flick latency at a dose of 400mg/kg (higher dose) was found to be 19.36 seconds after 90 minutes of drug treatment, whereas the standard drug diclofenac showed the tail flick latency 12.86 seconds (Table 1). The activity was also found to be a significant activity.

Antipyretic activity

Brewer's yeast induced pyrexia method

The results of antipyretic activity of ethanol extract of *D. neilgherrense* aerial part was shown in Table 2. Ethanol extract reduced the hyperthermic at 100, 200 and 400mg/kg doses after 1 hour after administration. The initial and final rectal temperature in the groups treated with ethanol extract (400mg/kg) and indomethacin (10 mg/kg) were 39.73±0.11^{0}C and 34.04±0.36^{0}C; 39.54±0.18^{0}C and 34.16±0.13^{0}C respectively. Both ethanol extract and indomethacin showed significant (p<0.001) antipyretic activity throughout the test period of 6 Hour.

DISCUSSION

In the present investigation attempts were made to study detail phytochemical investigation and pharmacological action, particularly analgesic and antipyretic activity of aerial part of *D. neilgherrense*. The rats were subjected for hot plate and tail immersion analgesic activity. Ethanol extract of aerial part showed significant analgesic activity by hot plate and tail immersion analgesic activity. So the results obtained may be supported by the ability of the ethanol extract of the plant to have peripheral and central pain inhibition mechanism. The tested effective analgesic plant in the tail immersion assay may have the ability to modulate the action potential and signal transmission for pain relieving originated by heat[12]. In the present study, *D. neilgherrense* aerial part produced analgesic activity in a dose dependent manner and significant effect was observed at 400mg/kg. A number of flavonoids have been reported to produce analgesic activity. Also, there are few reports on the role of tannins and alkaloids in analgesic activity[13,14]. Hence, the present analgesic activity of *D. neilgherrense* aerial part may be attributed to the presence of alkaloids, flavonoids and tannins. Maintenance of human body temperature needs a proper balancing between loss and production of heat, which can be done by hypothalamus. Suspension of yeast remarkably increased rectal temperature following its subcutaneous injection. In the present study, the oral administration of ethanol extract of *D. neilgherrense* aerial part significantly (p<0.01) alleviated rectal temperature of yeast induced rats. The ethanol extract produced significant antipyretic effect in dose dependent manner. The phytochemical analysis of the ethanol extract of *D. neilgherrense* aerial part showed the presence of alkaloids, flavonoids, phenols, tannins, saponins and steroids. The flavonoid present in the ethanol extract of *D. neilgherrense* may also be responsible for its antipyretic activity by inhibiting prostaglandian synthesis in hypothalamus[15]. In many earlier studies, flavonoids have been reported to exhibit antipyretic effect[16-18]. Hence, the presence of flavonoids in the ethanol extract of *D. neilgherrense* may be contributory of its antipyretic activity. This study confirmed the biological significance of the ethanol extract of *D. neilgherrense* aerial part in terms of its potent analgesic and antipyretic activities. These findings conclude that the ethanol extract of *D. neilgherrense* aerial part may contain bioactive principles with pharmacological potential.

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