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Research Article

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Anti-nociceptive activity of methanolic extract of leaves of *Glycosmis pentaphylla*

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ABSTRACT

In the present investigation, the anti-nociceptive effect of methanolic extract of leaves of G. pentaphylla was studied using formalin test, radiant heat tail-flick test, hot plate test, writhing test & sensorimotor performance test at doses of 50-400 mg/kg, intra peritoneally. The extract produced a significant inhibition in the late phase of formalin induced pain. It did not exert any significant antinociceptive effect in tail flick test, but in the hot plate test, it significantly raised the pain threshold. In the writhing test it caused a significant decrease in the number of writhing in comparison with the control group (P<0.05). No significant change was observed in sensorimotor test. The results of pharmacological tests performed in the present study suggest that the methanolic extract of G. pentaphylla possess anti-nociceptive effect and these findings seem to justify the use of plant in traditional Indian medicine in the treatment of pain.

Key words: *Glycosmis pentaphylla*, Rutaceae, Anti-nociceptive activity.

INTRODUCTION

The plant *Glycosmis pentaphylla* Corr. belongs to the family Rutaceae. This plant is thornless shrub or small tree[1]. The plant is native to south-eastern Asia and north-eastern Australia. In India the plant is found in various states like Assam, Arunachal, Meghalaya, Nagaland and Mizoram[2]. This plant is used in indigenous medicine for cough, jaundice, inflammation, rheumatism and anemia[3].

A bibliographic survey showed that *G. pentaphylla* is traditionally used against various ailments, but till date it has not been scientifically explored for its anti-nociceptive potential. Therefore, in present study our efforts were devoted to explore this plant scientifically for its anti-nociceptive potential.

EXPERIMENTAL SECTION

Plant material

The plant material (leaves) of *Glycosmis pentaphylla* Corr. were collected from the campus of Dibrugarh University, Assam (India) and it was positively identified and authenticated from botanical survey of India, Shillong. A voucher specimen (DU/PSc/HRB-2/08) was deposited in the Herbarium of the institute.

Extraction
The leaves of *G. pentaphylla* were dried in the shade, powdered (100g) and extracted with methanol using soxhlet apparatus (Yield: 18.88%).

Animals
Albino rats (150-200g) and Swiss mice (20-30g) were maintained in standard environmental conditions. The animals were fed with standard food and water ad libitum. The experimental protocol was approved by the institutional ethical committee.

Anti-nociceptive evaluation
Before anti-nociceptive evaluation, all the animals were fasted overnight and maintained with free access to water. For formalin test, tail-flick test & hot plate test, the healthy Swiss mice or albino rats were divided at random into six groups of six animals each. Group I (normal control) was treated with propylene glycol & water (1:4, i.p.) used as vehicle. Group II to V were treated intra peritoneally with *G. pentaphylla* methanolic extract at doses 50, 100, 200, 400 mg/kg respectively, while group VI was treated with morphine (10 mg/kg, i.p.) used as a reference standard.

Formalin test
Fifteen minutes after treatment, 20µl formalin (2.5%) was injected to the dorsal surface of left hind paw. The mice were observed for 60 minutes after the injection of formalin, and the amount of time spent licking or biting the injected hind paw was recorded (Table-1). The first 5 minutes post formalin injection is known as the early phase and the period between 15 to 60 minutes as the late phase[4].

Table-1: Effect of methanolic leaves extract of *G. pentaphylla* in Formalin test

Groups	Dose (mg/ kg, i.p.)	Time in seconds, animals spent licking or biting the paw	
		Early Phase	Late Phase
Control	-	95 ± 0.04	206 ± 0.06
Methanolic Leaves extract of *G. pentaphylla*	50	110 ± 0.08	88 ± 0.06
	100	102 ± 0.05	64 ± 0.05
	200	112 ± 0.02	48 ± 0.04
	400	101 ± 0.03	28 ± 0.02
Morphine	10	10 ± 0.06	15 ± 0.04

Values are mean ± SEM, P < 0.05

Radiant heat tail flick test
Tail flick latency was assessed by analgesiometer. The strength of the current passing through naked nicrome wire was kept constant at 5 Ampere. The distance between heat source and the tail was 1.5 cm and the application site of the heat on the tail was maintained within 2 cm, measured from the root of the tail. Cut-off reaction time was 15 sec to avoid any tissue injury during the process. The tail-flick latency was measured before and 30, 45, 60, 75 & 90 minutes after the drug administration.[5] (Table-2)

Table-2: Effect of methanolic leaves extract of *G. pentaphylla* in Radiant heat tail-flick test

Groups	Dose (mg/kg, i.p.)	Latency time (seconds) at time (minutes)						
		0	15	30	45	60	75	90
Control	-	4.4 ± 0.06	4.5 ± 0.04	4.4 ± 0.03	4.8 ± 0.06	3.8 ± 0.02	4.7 ± 0.03	4.2 ± 0.05
Methanolic Leaves extract of *G. pentaphylla*	50	4.8 ± 0.02	5.4 ± 0.06	5.8 ± 0.05	5.6 ± 0.02	5.8 ± 0.03	5.4 ± 0.06	4.6 ± 0.03
	100	4.6 ± 0.04	5.8 ± 0.05	5.4 ± 0.06	5.8 ± 0.04	5.7 ± 0.05	5.8 ± 0.02	5.0 ± 0.03
	200	4.4 ± 0.03	5.8 ± 0.06	5.6 ± 0.04	5.5 ± 0.03	5.4 ± 0.06	5.8 ± 0.02	4.8 ± 0.04
	400	4.4 ± 0.03	5.3 ± 0.03	5.4 ± 0.05	5.6 ± 0.06	5.9 ± 0.03	5.4 ± 0.02	4.5 ± 0.04
Morphine	10	4.6 ± 0.04	7.2 ± 0.06	8.8 ± 0.04	10.4 ± 0.04	12.2 ± 0.03	10.4 ± 0.04	7.2 ± 0.02

Values are mean ± SEM, P<0.05

Hot-plate test

Rats were placed on an aluminium hot plate kept at a temperature of 55±0.5 ˚C for a maximum time of 30 seconds. Reaction time was recorded (when the animals licked their fore and hind paws and jumped) before and 15, 30, 45, 60, 75 & 90 minutes after administration of vehicle, extract & reference standard[6] (Table-3).

Table-3: Effect of methanolic leaves extract of *G. pentaphylla* in Hot plate test

Groups	Dose (mg/kg, i.p.)	Reaction time (seconds) at time (minutes)						
		0	**15**	**30**	**45**	**60**	**75**	**90**
Control	-	5.4 ± 0.04	5.5 ± 0.02	5.54 ± 0.03	5.62 ± 0.02	5.68 ± 0.04	5.72 ± 0.05	5.82 ± 0.04
Methanolic Leaves extract of *G. pentaphylla*	50	5.2 ± 0.04	5.4 ± 0.06	5.6 ± 0.05	5.7 ± 0 .06	5.7 ± 0.03	5.8 ± 0.02	5.4 ± 0.04
	100	4.8 ± 0.02	5.5 ± 0.04	5.5 ± 0.02	5.7 ± 0.04	5.8 ± 0.05	6.0 ± 0.04	5.6 ± 0.02
	200	4.2 ± 0.06	5.8 ± 0.03	5.8 ± 0.04	5.7 ± 0.04	5.8 ± 0.06	6.4 ± 0.06	5.4 ± 0.02
	400	4.4 ± 0.03	5.8 ± 0.06	6.2 ± 0.04	6.3 ± 0.04	10.6 ± 0.03	16.4 ± 0.04	10.5 ± 0.08
Morphine	10	4.5 ± 0.02	10.2 ± 0.02	17.6 ± 0.04	20.4 ± 0.06	22.6 ± 0.02	26.4 ± 0.04	16.2 ± 0.06

Values are mean ± SEM, P<0.05

Writhing test

The healthy Swiss mice were divided at random into seven groups of six animals each. Group I (normal control) was treated with propylene glycol & water (1:4, i.p.), used as vehicle. Group II to V were treated intra peritoneally with *G. pentaphylla* methanolic extract at doses 50, 100, 200, 400 mg/kg respectively, while group VI & VII were treated with morphine (1 mg/kg, i.p.) and indomethacin (5 mg/kg) used as reference standard. 30 minutes after treatment, the mice were given an intraperitoneal injection of 0.6% v/v acetic acid in a volume of 10ml/kg to induce the characteristic writhing. The number of writhing occurring between 5 and 15 minutes after acetic acid injection was recorded (Table-4). The response of the extract treated animals was compared with that of the animals receiving reference standards as well as with the control group[7].

Table-4: Effect of methanolic leaves extract of *G. pentaphylla* in Writhing test

Groups	Dose (mg/ kg, i.p.)	Numbers of writhing	Inhibition (%)
Control	-	44.44 ± 0.08	-
Methanolic Leaves extract of *G. pentaphylla*	50	32.20 ± 0.04	27.55
	100	24.64 ± 0.02	44.56
	200	18.24 ± 0.06	58.95
	400	12.42 ± 0.04	72.05
Morphine	01	7.24 ± 0.03	83.70
Indomethacin	05	16.5 ± 0.08	62.87

Values are mean ± SEM, P<0.05

Sensorimotor performance

The healthy albino rats were divided at random into six groups of six animals each. Group I (normal control) was treated with propylene glycol & water (1:4, i.p.), used as vehicle. Group II to V were treated intra peritoneally with *G. pentaphylla* methanolic extract at doses 50, 100, 200, 400 mg/kg respectively. For this test, a Rota rod apparatus was used at a rotating speed of 16 rpm. A preliminary selection of rats was made on the day of experiment to exclude those that did not remain on the Rota rod bar for two consecutive periods of 45 seconds each. Sensorimotor performance was assessed 30 minutes after injection of vehicle and different doses of extract in rats separately (Table-5). Results are expressed as percentage of animals that succeeded in remaining on the rod for 45 seconds, which was the cut off time[7].

Statistical analysis

Results obtained in the present investigation were expressed as mean ± SEM. The data were analyzed using Student's t-test and results were considered significant when P<0.05.

Table-5: Effect of methanolic leaves extract of *G. pentaphylla* in Sensorimotor performance test

Groups	Dose (mg/ kg, i.p.)	Animals with success in Rota rod test (%)
Control	-	97.5 ± 0.5
Methanolic Leaves extract of *G. pentaphylla*	50	98.7 ± 0.8
	100	97.3 ± 0.8
	200	98.2 ± 0.6
	400	97.2 ± 0.9

Values are mean ± SEM, P<0.05

RESULTS AND DISCUSSION

Methanolic extract was not effective during the first phase of the formalin test. However, the extract caused a significant inhibition of licking and biting of the hind paw responses during the late phases of formalin test. When morphine responses were compared with that of control, significant inhibitions of early and late phases were observed. The formalin test is a valid and reliable model of nociception, and it is sensitive for various classes of analgesic drugs. The test produces a biphasic response: early phase is thought to result from direct chemical activation of nociceptive afferent fibers, and peripheral inflammatory processes seem to be responsible for the late phase[8]. Drugs that act primarily as central analgesics inhibit both phases while peripherally acting drugs inhibit only the late phase[6]. In our investigation, extract produced a marked reduction of duration of licking in the late phase, consistent with the inflammatory reaction[9]. Inhibition of only the second phase of formalin test is a typical characteristic of cyclooxygenase inhibitors suggesting a peripheral analgesic activity of extract[10].

Methanolic extract did not increase the tail flick latencies when compared with control. When morphine responses were compared with that of control, it increased the latency significantly at 15, 30, 45, 60, 75, 90 minutes. At dose 400mg /kg, the extract increased the latency to hot plate test at 60, 75 & 90 minutes, while morphine increased the latency significantly to hot plate test at 15, 30, 45, 60, 75 & 90 minutes. The hot plate test is commonly used to assess narcotic analgesia. Hence it is assumed that extract does not exert a central analgesic effect, and this effect may probably be due to its peripheral analgesic effect[11].

In writhing test, the extract produced a significant decrease in the number of writhing in comparison with the control. The antinociceptive activity of the extract at 200mg/kg was comparable to that of indomethacin 5mg/kg and less than that of morphine 1mg/kg, while at 400mg /kg, it was more than that of indomethacin and less than that of morphine. The acetic acid induced writhing test is normally used to evaluate the peripheral analgesic effect of drugs. The response is thought to be mediated by the process or release of arachidonic acid metabolites via cyclooxygenase, and prostaglandin biosynthesis. The significant antinociceptive activity of the extract might be due to the involvement in prostaglandin biosynthesis[12].

Because sedation can affect the reaction to noxious stimuli, the integrity of motor coordination was assessed with Rota rod apparatus in sensorimotor performance test, no significant change was observed with the administration of the extract in any of the doses tested. In fact in the treatment groups, the percentage of animals with success in the Rota rod test ranged between 97.2 ± 0.9% and 98.7 ± 0.8%.[13]

The results of pharmacological tests performed in the present study suggest that the methanolic extract of *G. pentaphylla* possess anti-nociceptive effect and these findings seem to justify the use of plant in traditional Indian medicine in the treatment of pain.

REFERENCES

[1] Khare, C.P, Indian Medicinal Plants-An Illustrated Dictionary, Springer-Verlag Berlin Publishers, New Delhi, **2007**, 289.
[2] Islam, M., Weeds of North-East India, First Edition, The Assam paper industry, Tinsukia, **1996**,108,152.
[3] Kirtikar, K.R. and Basu, B. D., Indian Medicinal Plants, Vol. 3, International Book Publisher, Dehradun, **1993**, 1621–22.
[4] Handy R.L.C., Wallace P., Gaffen Z.A., Whitehead K.J., Moore P.K., *Br J Pharmacol*, **1995**, 116, 2349-50.
[5] D Amour F.E., Smith D.L., *J Pharmacol Exp Ther*, **1941**, 72, 74-79.

[6] Morteza semnani K., Saeedi M, Hamidian M, Vafamehr H, Dehpour A.R., *J Ethanopharmacol*, **2002**, 80, 181-86.
[7] Pieretti S., Piaz V.D., Matucci R., Giovannoni M.P., Galli A., *Life Sci*., **1999**, 65, 1381.
[8] Tjolsen A., Berge O.G., Hunskaar S., Rosland J.H., Hole K., *Pain*, **1989**, 38, 347-52.
[9] Hunskaar S., Hole K., *Pain*, **1987**, 30, 103-14.
[10] Rosland J.H., Tjoslen A., Maehle B., Hole D.K., *Pain*, **1990**, 42, 235-42.
[11] Rojas corrales M.O., Cases J., Moreno brea M.R., Gibert rahola J., mico J.A., *Eur Neuropsychopharm* **2003**, 13, 355-63.
[12] Franzotti E.M., Santos C.V.F., Rodrigues H.M.S.L., Mourao R.H.V., Andrade M.R., Antoniolli A.R., *J Ethanopharmacol*, **2000**, 72, 273.
[13] Karabay yavasoglu N.U., Karamenderes C., Baykan S., Apaydin S., *Pharm Biology*, **2007**, 45, 2, 162-68.