

**PRELIMINARY STUDIES ON ANTINOCICEPTIVE ACTIVITY OF
DENDROPTHOE FALCATA (L.F) ETTINGSH (LORANTHACEAE)**

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Summary

The plant *Dendrophthoe falcata* (L.f) Ettingsh (Loranthaceae) of the order Santalales, is used in folklore medicine for ailments including ulcers, asthma, impotence, menstrual troubles, asthma, psychic disorders, paralysis, skin diseases, and wounds. *Dendrophthoe falcata* bark is considered to be astrngent and narcotic. The present study was undertaken to investigate the antinociceptive activity of ethanolic extracts of the barks of *D. falcata* (DFEE) using formalin-induced nociception model, hot plate method (thermal stimulation), acetic acid-induced writhing test, *p*-benzoquinone-induced abdominal constriction test and Haffner,s tail clip method in rodents. Pre-treatment with DFEE with a dose of 200 and 400mg/kg body wt revealed significant ($p<0.05$) antinociceptive activity against all the tests performed as compared to the control group. The preliminary phytochemical investigation revealed the presence of steroids, terpenes, glycosides, tannins, proteins and flavonoids. The results of the present study substantiate the ethnopharmacological approach in selecting the plant as a remedy for nociceptive induced pain.

Keywords: *Dendrophthoe falcata* (L.f) Ettingsh, antinociceptive activity, phytochemical studies.

Introduction

Dendrophthoe falcata (Bengali-Baramanda, Oriya-Manda, Hindi-Bandai, Tam.– Pulluri) is a hemi-parasitic plant belonging to the family Loranthaceae (1). It is not a host specific and usually growing on variety of host plants throughout India. The entire plant is medicinally important. Bark is considered to be astrngent and narcotic. It is used in wounds and menstrual troubles, also as remedy for asthma and psychic disorders. It is also used as a remedy for consumption, mania, substitute for betel nut (2, 3). This study was undertaken to evaluate the antinociceptive potential of the ethanolic extract of *D. falcata* aerial parts (DFEE) to prove its narcotic use. The activity of DFEE was proved by using different nociceptive models.

Materials and methods

Plant Material

Fresh plants of *Dendrophthoe falcata* were collected in October 2006 from the thick forest areas of Similipal biosphere reservoir, Mayurbhanj district of Orissa. Taxonomic identification was performed by Dr. N. K. Dhal, Sr scientist, Dept. of Natural Products, Regional Research Laboratory (RRL), Bhubaneswar, India and the voucher specimen is deposited in the herbarium of RRL, vide access no. 9996.

Preparation of Extracts

The plant material was washed thoroughly with tap water and air dried in shade at room temperature. They were then mechanically powered and sieved. 1000gm of powered plant material was extracted with ethanolic soxhlation and dried in a rotary evaporator at 40⁰C.

Preliminary phytochemical screening

A preliminary phytochemical screening was carried out for the extracts employing the standard procedures to reveal the presence of alkaloids, steroids, terpenes, flavonoids, saponins, tannins, glycosides, carbohydrates and proteins (4).

Experimental animals

Sexually matured Wistar albino rats (100 – 150gm) and Swiss albino mice (20 25g) of either sex were obtained from M/s Ghosh Enterprises, Kolkata. 3 animals were housed per cage (polypropylene cage) and acclimatized for a period of 10 days. Light –dark cycle (light on 6am-6pm), and ambient temperature of 22 ±2 °C and relative humidity of 65% were also maintained on standard palettes (Amruth Laboratory animal feed, Nay-Maharashtra chakan oil mills Ltd., Pune, Maharashtra) and water *ad-libitum*. The experiment was carried out in between 10.00 to 17.00h. Free rice husk, which were used for the purpose of keeping as a bed to the animals were also autoclaved and maintained safety from infection. All the experiments were performed after obtaining prior permission from Institutuinan Anianal Ethical Committee.

Acute toxicity studies

Acute toxicity study was performed for the extract according to the acute toxic classic method as per OECD guidelines (5). Wistar albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extracts were, administered orally 400mg/kg and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the same dose was assigned to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose i.e. 2000mg/kg for the extract (DFEE).

Evaluation of antinociceptive activity

Assessment of antinociceptive activity using formalin-induced nociception in mice

The ethanolic extract (DFEE) was evaluated for its antinociceptive activity by the formalin-induced method (6). Male Swiss mice, 25-30g were used. 20 μ l of 2.5% formalin (0.92% formaldehyde) made up in phosphate buffer was injected under the paw surface (sub-plantar region) of the right hind paw. All animals were divided into four groups of 5 animals each [as control (3%w/v Na carboxy methyl cellulose (CMC), p.o.), morphine (5mg/kg, s.c), DFEE (200 & 400mg/kg, p.o.)]. The animals were observed simultaneously from 0 – 30 minutes following formalin injection. The time spent in licking the injected paw was noted. The initial nociceptive response normally peaked 0 – 5 mins after formalin injection (phase I) and 15 – 30 min after formalin injection(phase II), representing neurogenic pain and inflammatory pain respectively. The data are represented in table 1.

Hot plate method (thermal stimulation)

In the hot plate method (7), albino rats (200 – 250g) were divided into four groups each consisting of six animals. One group served as negative control (received 0.3w/v Na CMC, 2ml/kg), second group served as positive control (pentazocine, 10mg/kg, s.c.), third and fourth group received DFEE (200 & 400mg/kg, respectively, p.o.)the hot plate was maintained at 56 \pm 1⁰C. Animals were placed on the surface and the time between placement and jumping was recorded as response latency. The reaction time was recorded at 0,15, 30, 60, 90minutes. The test was terminated at 15sec to prevent the tissue damage (table 2).

Acetic acid induced writhing test (chemical stimulation)

Male Swiss mice, 25 – 30g, were divided into four groups, six animals in each group. First group was pretreated with vehicle (3%w/v NaCMC), second group was treated with ibuprofen (40mg/kg, i.p.), third and fourth group was treated with DFEE (200 & 400mg/kg, respectively, p.o.). After 30 minutes acetic acid (10ml/kg of 0.6% v/v solution) was injected intraperitoneally. The number of writhings was counted for 20 minutes after acetic acid (8). Antinociceptive activity was expressed as the reduction of the number of writhing between control animals, animals treated with test and standard (table 3). Percent protection is calculated as follows,

$$\% \text{ Protection} = 1 - (\text{Experimental/control}) \times 100$$

p-Benzoquinone-induced abdominal constriction test in mice

p-Benzoquinone-induced abdominal constriction test (9) was performed on mice for the determination of antinociceptive activity. According to the method evaluated, 60 min after the oral administration of the test samples (DFEE, 200 and 400mg/kg body wt), the mice were intraperitoneally injected with 0.1 ml/10 g body weight of 2.5% (w/v) *p*-benzoquinone (PBQ) solution in distilled water. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for the observation and the total number of the abdominal contractions (writhing movements) was counted for the following 15 min, starting 5 min after the PBQ injection. The data represent the average of the total number of writhes observed. Antinociceptive activity was then expressed as the percentage change from

writhing controls. Acetylsalicylic acid (ASA) at 200 mg/kg doses was used as the reference drug in this test.

Haffner,s tail clip method

The method was described as early as 1929 by Haffner who observed the raised tail (straub phenomenon) in mice treated with opioid drugs and found the tail to be less sensitive to noxious stimuli after drug treatment. Mice (25-30 g) of either sex were divided into four groups with six animals in each group. The animals were administered the control, standard (pentazocine, 10mg/kg) and extracts (200 and 400 mg/kg) by the oral route of administration 30min prior to testing. A bulldog clamp was applied to the root of the tail of the mice to induce pain. The animal quickly responded to this noxious stimulus by biting the clip 'or' the tail near the location of the clip. The time between the stimulation onset and the response was measured using a stopwatch (10).

Statistical analysis

Results of all the experiments are expressed as Mean \pm SEM. Total variation present in a set of data was estimated through one-way analysis of variance (ANOVA) followed by Benferroni's multiple comparison tests. Values of $p < 0.05$ were considered statistically significant using the statistical software Sigmastat 3.5 (trial version Dt. 15/ 01/ 08, Systat).

Results

Extractive value and preliminary phytochemical screening

The extractive yields were found to be 18.764%. The preliminary phytochemical investigation revealed the presence of steroids, terpenes, glycosides, tannins, proteins and flavonoids.

Acute toxicity test

The ethanolic extract did not cause any mortality up to 2000 mg/kg and were considered as safe and the animals showed no stereotypical symptoms associated with toxicity such as convulsions, ataxia, and diarrhoea.

Formalin-induced nociception in mice

In order to determine the anti-nociceptive activity of DFEE formalin-induced nociception model was used and the results were presented in table 1. Animals treated with vehicle showed response time of 52.2 ± 0.26 sec and 68.4 ± 0.04 sec in the first and second phases, respectively. Animals treated with morphine showed 34.6 ± 0.17 in first phase and very little duration (1.2 ± 0.06 sec) in second phase. Those animals treated with higher dose of the ethanolic extract of *D. falcata* illustrated 46.4 ± 0.74 & 18.8 ± 0.52 seconds for neurogenic and inflammatory pain, respectively, while the lower dose possessed 49.2 ± 0.46 and 32 ± 0.23 seconds for both the phases.

Table 1. Assessment of antinociceptive activity on formalin-induced nociception in mice for *Dendrophthoe falcata*

Group	Treatment	Dose	Paw licking time during 0 - 5 min in Sec. (Phase - 1)	Paw licking time during 15 - 30min in Sec. (Phase - 2)
I	0.3% w/v Na CMC	2ml/kg, p.o.	52.2±0.26	44.4±0.04
II	Morphine	5mg/kg, s.c.	34.6±0.17**	1.2±0.06**
III	DFEE	200mg/kg, p.o.	49.2±0.46*	32.24±0.23**
IV	DFEE	400mg/kg, p.o.	42.4±0.74**	18.8±0.52**
One way ANOVA		<i>F</i>	374.662	13217.997
		<i>df (B.t.,E)</i>	3, 20	3, 20
		<i>P</i>	<0.001	<0.001

Results are expressed as mean ± SEM from six observations; * $p < 0.05$, ** $p < 0.001$ as compared to control; DFEE – ethanolic extract of *Dendrophthoe falcata*; *df (B.t.,E)* = degree of freedom (Between treatment, Error within treatment).

Hot plate method

When the rat was placed on the radiant heat source i.e. hot plate analgesiometer, the nociceptors were stimulated and the vehicle treated group of animals jumped within 3 –5 seconds. But, the standard drug, morphine and test drug, DFEE revealed potential antinociceptive activity (represented in Table 2) in this thermal stimulation model. The two doses of the extracts had increased the reaction time in dose dependent manner. Higher dose i.e., 400 mg/ kg of methanolic extract of *D. falcata* had exhibited the higher anti-nociceptive effect than the lower dose.

Acetic acid induced writhing test

Dose dependent antinociceptive effect was noted with the ethanolic extract at the tested dose levels 200 and 400mg/kg (table 3). In the acetic acid induced writhing model DFEE with 400 mg/kg dose has exhibited a maximum of 44.2% inhibition of writhing and the activity was shown more than standard drug ibuprofen (64.4%) while lower dose 200 mg/kg have shown 27.98% reduction.

p-Benzoquinone-induced abdominal constriction test

DFEE at 400mg/kg p.o., showed an inhibition of 40.161% which was higher active than the lower dose 200mg/kg body wt (20.94%). The standard drug acetylsalicylic acid inhibited 59.735% of writhing induced by *p*-Benzoquinone (table 4).

Tail clip method

The extract of *D. falcata* (200 and 400mg/kg) produced significant ($p < 0.05$) increase in the mean latency of biting of the tail-clip after 30min and was dose dependent. The statistical data also shows that the extracts were comparable to that of standard pentazocin.

Table 2. Effect of *Dendrophthoe falcata* on thermal stimulus induced pain (hot plate test) in rats.

Group	Treatment	Dose	Reaction time after (in seconds)				
			0min.	15min.	30min.	60min.	90min.
I	0.3w/v NaCMC,	2ml/kg	3.56±0.09	3.98±0.16	3.68±0.12	4.14±0.18	4.64±0.06
II	Pentazocin	10mg/kg	3.76±0.18	6.28±0.15**	8.21±0.27**	11.36±0.04**	10.64±0.19**
III	DFEE	200mg/kg	3.96±0.09	3.88±0.19	4.32±0.01*	4.84±0.39*	5.98±0.91**
IV	DFEE	400mg/kg	4.16±0.14	4.28±0.06	4.92±0.21*	5.62±0.26**	7.12±0.12**
One way ANOVA		<i>F</i>	4.659	90.716	125.124	429.461	504.074
		<i>df (B.t.,E)</i>	3, 20	3, 20	3, 20	3, 20	3, 20
		<i>P</i>	<0.05	<0.001	<0.001	<0.001	<0.001

Results are expressed as mean ± SEM from six observations; * $p < 0.05$, ** $p < 0.001$ as compared to control; DFEE – ethanolic extract of *Dendrophthoe falcata*; *df (B.t.,E)* = degree of freedom (Between treatment, Error within treatment).

Table 3. Effect of *Dendrophthoe falcata* on acetic acid-induced writhing in mice.

Group	Treatment	Dose	Mean number of writhings ± SEM(20min)	Percentage inhibition of writhings(%)
I	0.3w/v NaCMC,	2ml/kg	68.46 ± 0.96	—
II	Ibuprofen	40mg/kg	24.34 ± 0.64**	64.4
III	DFEE	200mg/kg	49.3±1.06*	27.98
IV	DFEE	400mg/kg	38.22 ± 0.86**	44.2
One way ANOVA		<i>F</i>	737.320	
		<i>df (B.t.,E)</i>	3, 20	—
		<i>P</i>	<0.001	

Results are expressed as mean ± SEM from six observations; * $p < 0.05$, ** $p < 0.001$ as compared to control; DFEE – ethanolic extract of *Dendrophthoe falcata*; *df (B.t.,E)* = degree of freedom (Between treatment, Error within treatment).

Table 4. Antinociceptive effect of ethanolic extract of *Dendrophthoe falcata* bark with *p*-Benzoquinone-induced abdominal constriction model in mice.

Group	Treatment	Dose	Mean number of writhings \pm SEM(20min)	Percentage inhibition of writhings(%)
I	0.3w/v NaCMC,	2ml/kg	48.28 \pm 2.26	—
II	Acetylsalicylic acid (ASA)	200mg/kg	19.44 \pm 1.43**	59.735
III	DFEE	200mg/kg	38.17 \pm 2.1*	20.94
IV	DFEE	400mg/kg	28.89 \pm 1.09**	40.161
One way ANOVA		<i>F</i>	678.49	
		<i>df</i> (B.t.,E)	3, 20	—
		<i>P</i>	<0.001	

Results are expressed as mean \pm SEM from six observations; * p <0.05, ** p <0.001 as compared to control; DFEE – ethanolic extract of *Dendrophthoe falcata*; *df* (B.t.,E) = degree of freedom (Between treatment, Error within treatment).

Table 5. Antinociceptive effect of ethanolic extract of *Dendrophthoe falcata* bark with Tail Clip method in mice.

Group	Treatment	Dose	Latency period				
			0min.	30min.	60min.	90min.	120min.
I	0.3w/v NaCMC,	2ml/kg	3.12 \pm 0.02	3.02 \pm 0.12	3.51 \pm 0.01	3.34 \pm 0.29	3.46 \pm 0.35
II	Pentazocin	10mg/kg	3.56 \pm 0.28	5.88 \pm 0.86**	9.121 \pm 0.57**	13.36 \pm 0.09**	11.64 \pm 0.31**
III	DFEE	200mg/kg	3.82 \pm 0.79	4.09 \pm 0.11	4.49 \pm 0.07*	5.14 \pm 0.28**	7.12 \pm 0.84**
IV	DFEE	400mg/kg	4.01 \pm 0.15	4.48 \pm 0.06	5.52 \pm 0.51*	6.62 \pm 0.76**	9.12 \pm 0.12**
One way ANOVA		<i>F</i>	5.129	24.46	118.121	521.23	554.067
		<i>df</i> (B.t.,E)	3, 20	3, 20	3, 20	3, 20	3, 20
		<i>P</i>	<0.05	<0.001	<0.001	<0.001	<0.001

Results are expressed as mean \pm SEM from six observations; * p <0.05, ** p <0.001 as compared to control; DFEE – ethanolic extract of *Dendrophthoe falcata*; *df* (B.t.,E) = degree of freedom (Between treatment, Error within treatment).

Discussion and conclusion

Considering the active constituent(s) for nociception might be accumulated in the barks as it was used in folkloric practice, ethanol extract of dried barks of *D. falcata* was prepared and administered to animals, to check the potentiality. The antinociceptive effect of orally administered ethanolic extract of *D. falcata* barks (DFEE) was demonstrated in this study by five different chemical nociceptive tests and thermal nociceptive test. The DFEE (200 and 400mg/kg) has been effective in all the experimental designs, which is used to screen for both peripherally and centrally acting agents (11). The formalin induced paw licking test is a valid and reliable model for analgesic activity and it is sensitive for various classes of analgesic drugs. In the formalin test, the response to formalin is biphasic with an early and a late phase involving different mechanisms of nociception. The first phase is due to a direct effect of formalin on nociceptors and the second due to inflammation (12, 13). The ethanolic extract has been effective on the early phase as well as late phase, in the formalin test, indicating the possibility that it can alleviate efficiently the clinical pain associated with inflammatory conditions. In hot plate test, nociceptive reaction towards thermal stimuli in mice is a well validated model for detection of opiate like analgesic drugs where in pain response is from spinal origin (14). The hot plate method was originally described by Woolfe and Mac Donald (15). This test has been found to be suitable for the evaluation of centrally but not of peripherally acting analgesics. The validity of this test has been shown even in the presence of substantial impairment of motor performance (16). Acetic acid – induced writhing has been used as a model of chemonociceptive induced pain, which peripherally increase PG-E2 and PG-F2 (17). In both hot plate and acetic acid induced nociceptive models *D. falcata* barks extract exhibited antinociceptive activity which indicates both central and peripherally mediated anti-nociceptive properties. The central analgesic property of the extracts was corroborated by the first phase of formalin- induced pain, hot plate and tail- clip. The peripheral analgesic activity of DFEE was confirmed by the acetic acid and *p*-Benzoquinone-induced abdominal constriction model. Thus it can be concluded that the barks of the plant *Dendrophthoe falcata*, possess significant anti-nociceptive activity in experimental animals. The preliminary phytochemical analysis data revealed the presence of flavonoids, which are mostly responsible for the anti-nociceptive activity (18).

Results of the present study seemed correlate with the folkloric data. Further studies involving the purification of the phytochemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-nociceptive agent with a low toxicity and better therapeutic index.

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