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Research Article

ANTINOCICEPTIVE PROPERTY OF LEAVES EXTRACT OF *LITSEA GLUTINOSA*

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ABSTRACT

Antinociceptive property of leaves extract of *Litsea glutinosa* was evaluated by abdominal writhing and tail flick methods using mice. Acetyl salicylic acid was used as the standard reference. Ethanol extract of leaves was tested at three different concentrations 100 mg/kg, 200 mg/kg and 300 mg/kg body weight orally. Results showed that, among three different dosages, dosage of 300mg/kg (65%) significantly ($p<0.01$) inhibited the nociception induced by acetic acid when compared to dosage of 100 mg/kg (36.64%) and 200 mg/kg (52.79%). But less effective than the standard reference acetyl salicylic acid (84.06%). Tail-flick test showed that, the extract at the dosage 300 mg/kg showed significant ($p<0.01$) results in comparison with the standard acetyl salicylic acid. But less effective than the standard reference. This investigation suggested that the ethanol extract of leaves of *L. glutinosa* has significant antinociceptive property and supported the ethnomedicinal claims of *L. glutinosa.*

Keywords: *Litsea glutinosa* ; acute toxicity; abdominal writhing method; tail flick method.

INTRODUCTION

Litsea glutinosa (Lour.) C.B. Rob is an aromatic tree belongs to the family Lauraceae and is found to be sparsely distributed in the Western Ghats, India. *L. glutinosa* is an evergreen medium-sized tree and plant can attain a height of 20 meters. The traditional practitioners residing in the vicinity of forest of the Bhadra wild life sanctuary of the Western Ghats are using the leaves extract and the aromatic oil from the seeds for the treatment of rheumatic pain. Its barks and leaves are also used as a demulcent and mild astringent for diarrhoea and dysentery; the roots are used for poulticing sprains and bruises[1]. The leaves were reported for the treatment of the spontaneous and excessive flow of semen in young boys[2]. Recently, research disclosed that the bark extract of *L. glutinosa* significantly showed analgesic effect on mice and the results justified the reported uses in rheumatism[3].

Previous papers reported the presence of some alkaloids from the leaves[4] and barks[5] of *L. glutinosa.* Megastigmane diglycoside, (6S, 7E, 9R)-roseoside; (7'R, 8'R)-3, 5'-dimethoxy-9, 9'-dihydroxy-4, 7'-epoxylignan 4'-b-D-glucopyranoside; (7'R, 8'S)-dihydrodehydrodiconifenyl alcohol 9'-O-b-D-xylopyranoside; and Pinoresinol 3-O-b-D-glucopyranoside were reported from the EtOH extract of *L. glutinosa* leaves and twigs[6]. A new 2'-Oxygenated Flavone Glycoside, named Glutin was isolated from the leaves extract of *L. glutinosa*[7]. Tannin, β-sitosterol, and actinodaphnine are reported to be the common constituents of the species; and other constituents known are: Boldine, norboldine, laurotetanine, n-methyllaurotetanine, n-methylactinodaphnine, quercetin, sebiferine, litseferine etc. [8].The leaves of *L. glutinosa* possesses antibacterial[9], cardiovascular activities[10-12].

There are no reports available on antinociceptive property of leaves extract of *L. glutinosa.* In the present investigation, an effort has been made to establish the scientific validity of the antinociceptive property of *L. glutinosa* leaves.

MATERIALS AND METHODS

Plant material

The leaves of *L. glutinosa* were collected from the Bhadra wild life sanctuary of the Western Ghats, Karnataka, India and authenticated by Dr. Tariq Husain, Scientist, Biodiversity and angiosperm taxonomy, National Botanical Research Institute (NBRI), Lucknow, India. A voucher specimen was deposited in NBRI (Voucher no. No. 97294).

Preparation of the extracts

Shade dried and coarsely powdered leaves of *L .glutinosa* were subjected to solvent extraction in a Soxhlet extractor using ethanol solvent. The extract was vacuum dried using rotary flash evaporator (Buchi, Flawil, Switzerland) to obtain ethanol extract (14.12%).

Animals

Male Swiss albino mice weighing 25-30 g were procured from Central Animal House, National College of Pharmacy, Shivamogga, Karnataka, India and were maintained at standard housing conditions (temp. 23 ± 2^{0}C, humidity 55–60% with 12 h light and dark cycle). The animals were fed with commercial diet (Durga Feeds and Foods, Bangalore) and water *ad libitum* during the experiment. Animals were fasted, but allowed for water 12 h prior to the experiment. The Institutional Animal Ethical Committee (Reg. No. IAEC/CL/13/12/2010-11) permitted the study.

Acute toxicity study

The staircase method[13] was adopted for the determination of the acute toxicity. Healthy albino mice of either sex weighing 20-25 g were used to determine the safer dose. Water was used as a vehicle to dissolve the extract and was administered orally.

Acetic acid-induced writhing test

Antinociceptive property was evaluated using the acetic acid-induced writhing test[14]. Mice were divided into five groups of six animals each. The first group served as control and was treated with 0.6% acetic acid (dose 10 ml/kg) intraperitoneally. After 5 min of injection of acetic acid, number of writhes was counted for 20 min. The second group was administered the standard drug acetyl salicylic acid (100 mg/kg). The animals of the third to fifth groups were administered orally with the water dissolved ethanol extract in three different concentrations 100, 200 and 300 mg/kg body weight respectively. After one hour incubation of all the groups except group I animals were administered with acetic acid. After 5 min, each group mice were observed for the onset of writhing, and the number of writhing responses for the period of 20 min was recorded. The mean value for each group was calculated and compared with the control.

Tail flick method

Antinociceptive activity was evaluated using the tail flick method described by Sewell and Spencer (1976). Mice were divided into five groups of six animals each. The first group served as control and received only normal saline (10 ml/kg); the second group was administered standard drug acetyl salicylic acid (100 mg/kg, p.o). The animals of the third to fifth groups were treated with ethanol extract in three different concentrations 100, 200 and 300 mg/kg body weight respectively. One to two centimeter of the tail of experimental mice was immersed in warm water kept constant at 50°C. The pain reaction time was the time taken by the mice to deflect their tails. The first reading is discarded and the reaction time was taken as a mean of the next two readings. A cut off time of 10 sec was observed to prevent any tissue damage to the animal.

The latency period (reaction time) was noted when the animal responded with a sudden and characteristic flick or tail lifting. The latent period of the tail-flick response was taken as the index of antinociceptive property and the tail flick latencies were recorded at pre-drug, 15, 30, 60, 90, 120, 150 and 180 min after administration of drugs.

Statistical analysis

The data of antinociceptive activity was expressed as mean ± S.E.M of six animals in each group. The statistical analysis was carried out using one way ANOVA followed by Tukey's *t*-test. The difference in values at p≤ 0.01 was considered as statistically significant.

RESULTS

Acute toxicity study

After 72 h observation, a plot of mortality values *vs* log dose showed that the ethanol extract at the dose of 2000 mg/kg did not show any sign of mortality. One tenth of this dose (200 mg/kg.b.wt) was considered as safer dose for administration.

Writhing method

The number of writhes observed during 20 min period in control group was 80.50 ± 0.76. The crude extract at the dose of 100 mg/kg, 200 mg/kg and 300 mg/kg reduced the number of writhes to 51.07 ± 1.32 (with 36.64% protection), 38.00 ± 1.69 (with 52.79% protection) and 28.17 ± 1.49 (with 65.00% protection) respectively. These values indicated that the responses were dose dependent. Results also indicated that the leaves extract in different concentrations was showed to be less potent than standard drug acetyl salicylic acid which showed 05.23 ± 0.49 (with 84.06% protection) writhes. All the readings found to be significant (p<0.01, when compared to control). The results of acetic acid induced writhing test are depicted in Table 1.

Table 1:Antinociceptive property of leaves extract of *L. glutinosa* by writhing method.

Drug treatment	Dose	Number of writhes	% inhibition of writhings
Control (acetic acid)	10 ml/kg (i.p.)	80.50 ± 0.76	_
Acetyl salicylic acid	100 mg/kg (p.o.)	12.83 ± 0.48	84.06[b]
Ethanol extract	100 mg/kg (p.o.)	51.07 ± 1.32	36.64[b]
Ethanol extract	200 mg/kg (p.o.)	38.00 ± 1.69	52.79[b]
Ethanol extract	300mg/kg (p.o.)	28.17 ± 1.49	65.00[b]

Values are the mean ± S.E.M. of six mice. Symbols represent statistical significance.
[a] $p < 0.05$, [b] $p < 0.01$ as compared to control group.

Tail flick method

Throughout the 3h observation, animals pretreated with normal saline did not show significant effect on the latent period of tail-flick response. The antinociceptive effects of crude leaves extract in three different dosages were evident within 0.5 h following oral administration and the effect remained significant (p<0.01) throughout the 3h observation period. Comparatively, 300mg/kg showed highly significant antinociceptive activity. The antinociceptive activity of *L. glutinosa* leaves extract was dose dependent. The effects of crude extract on nociceptive responses induced by noxious heat (50°C) are shown in Table 2.

Table 2:Antinociceptive property of leaves extract of *L. glutinosa* on mice by tail flick method.

Group(N)	Dose	Reaction time in seconds					
		0.5h	1.0h	1.5h	2.0h	2.5h	3.0h
Control	10 ml/kg	2.70 ± 0.05**	3.0 ± 0.06**	2.8 ± 0.04**	3.30±0.08**	3.40± .02**	3.20± 0.05**
Aspirin	100 mg/kg	5.20±0.08**	6.40±0.05**	8.2±0.62**	8.1±0.14**	7.6±0.15**	5.7±0.10**
Ethanol extract	100 mg/kg	3.40 ± 0.13**	4.10± 0.08**	4.30 ± 0.07**	3.80±0.05**	3.10±0.04**	3.10± 0.05**
Ethanol extract	200 mg/kg	3.70 ± 0.08**	5.50 ± 0.04**	5.60 ± 0.04**	5.10±0.06**	3.90±0.04**	3.40± 0.04**
Ethanol extract	300 mg/kg	4.00 ±0 .04**	6.10 ± 0.02**	6.20 ± 0.05**	6.00±0.04**	4.70±0.02**	4.30± 0.13**

Values are the mean ±S.E.M. of six mice. Symbols represent statistical significance.
***$p < 0.05$, **$p < 0.01$, ns – not significant, as compared to control group.**

DISCUSSION

It is well established that chemical mediators are responsible for the inflammatory pain. Acetic acid produces nociception by liberating endogenous substances including serotonin, bradykinin, histamine and prostaglandin. Inflammatory mediators are released sequentially with histamine and serotonin released within the first 30 min, followed by kinins released approx. 1 hr. and prostaglandins released approx. 2 hr. after induction of inflammation[15]. It is known that the NSAIDs reduce pain by inhibiting synthesis and release of prostaglandins. Aspirin (acetyl salicylic acid), the reference in the current study, offers relief from pain by suppressing the formation of pain substances in the peripheral tissues where prostaglandins and bradykinins are suggested to play an important role.

Taking these points in view, the ethanol extract from leaves of *L. glutinosa* was studied using chemical and thermal methods. From this study, it is established that ethanol extract from leaves of *L. glutinosa* possesses significant analgesic effects. The analgesic property of *L. glutinosa* in the dosage of 300 mg/kg was significant in comparison with the aspirin, a standard NSAID. The observed analgesic activity in the ethanol extract of *L. glutinosa* is attributed to the presence of compounds including alkaloids, flavonoids, glycosides tannins and saponins. The presence of these compounds was confirmed by the phytochemical screening conducted during the study.

Flavonoids have been reported to have a role in analgesic activity by targeting prostaglandins and inhibiting prostaglandin synthetase, more specifically the endoperoxidase. The role of alkaloids[16] and tannins in anti-nociceptive activity has also been reported[17]. Many of the investigators have evaluated the antinociceptive property of various herbal extracts using experimental mice[18, 19]. The significant effect of the phytoextracts of *L. glutinosa* is due to the presence of a single active constituent in higher levels or due to the combined effect of more than one phytoconstituent.

CONCLUSION

This investigation revealed that the leaves extract of *L. glutinosa* possess significant antinociceptive property against chemical and thermal stimuli, and supported the ethnomedical claims of *L. glutinosa.*

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