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STUDY OF ANALGESIC ACTIVITY OF *LITSEA GLUTINOSA* (L.) ETHANOLIC EXTRACT ON SWISS ALBINO MICE

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ABSTRACT

Aim: *Litsea glutinosa* (L.) a medicinal plant is popularly used as herbal remedy for various ailments. But the scientific basis for its medicinal use especially in pain and inflammation remains unknown. Therefore, the present study was designed to evaluate analgesic potential of the ethanolic extract of the bark of the plant.

Approach: The analgesic activity of standard tramadol HCl was determined for its central pharmacological actions using hotplate method in mice. Simultaneously the ethanolic extract was also investigated for its analgesic action.

Results: The extract, at the dose of 100 and 300 mg/ Kg, has shown a significant ($p<0.05$) increase in pain threshold in hotplate, as compared with control group.

INTRODUCTION: Pain is a sensorial modality and primarily protective in nature, but often causes discomfort. It is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause [1]. Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. In this respect new compounds with improved pain management capacity and fewer side effects are being sought with urgency [2]. The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation has demonstrated the safety and efficacy of traditional medicine. However, scientific evaluation is needed to provide evidences of their safety and efficacy.

Narcotic analgesics are associated with addictive properties and numerous side effects including respiratory depression, drowsiness, decreased gastro intestinal motility, nausea and alteration in autonomic nervous system activities. The search for pharmacological agents to overcome these shortcomings has become a major goal in pain research [3]. For centuries, medicinal plants are the basis for the treatment of various diseases. Nearly 80% of people living in developing countries still depend on plant-based traditional medicine for their primary health care and almost three-fourths of the herbal drugs used worldwide are derived from medicinal plants. However, the quality control of herbal medicine remains a challenge owing to the fact that there is a high variability in the active constituents involved [4].

MATERIALS AND METHODS:

Materials: Borosil Soxhlet extractor, solvent evaporator, Analgesiometer or Eddy's hot plate [Purchased from INCO] Digital balance [ELB 300, Manufactured from SHIMADZU], Tramadol HCl inj. (Tramazac^{R}, Manufactured from Zydus Alidac Batch No. AFH 1063), Syringes and needles were purchased from local market.

Plant Material: The bark of *Litsea glutinosa* (L), which according to Kirtikar and Basu, "is one of the most popular among native drugs", is considered to be capable of relieving pain, arousing sexual power and also producing a soothing effect on the body, good for the stomach and are considered to be mildly astringent, other uses of the bark include treatment of diarrhoea and dysentery. The methanolic extract of the bark of *Litsea glutinosa* (L) showed antibacterial activity comparable to chloramphenicol, against 16 tested microorganisms [7]. *Litsea glutinosa* (L) is widely available throught India. The fresh dried stem barks were collected from the forests of Ananthanahalli near Harapanahalli, Karnataka in the month of June 2008 and herbarium was prepared by processing the plant for 10 days.

The specimen was further identified and authenticated in Department of Botany, by botanist Prof. S.A. Kappali, Basaveshwar Science College, Bagalkot, Karnataka. Voucher specimen (B.sc./Bot/07/08709) was deposited in the herbarium of the same college. The bark was subjected to coarse powdered (#: 44) to obtain uniform texture. All chemicals and reagents purchased were analytical grade. The sieved powder was stored in airtight and high density polyethylene containers before extraction. The sieved powder was subjected to hot continuous Soxhlet extraction with petroleum ether and ethanol for 24 hours cycle at 70^{0}C [8]. Excessive solvent was removed by solvent distillation apparatus and residue was concentrated by using Lyotrap dryer. The brownish solid masses of extract were preserved in aseptic condition before performing the experiment.

Morphology of Plant: An evergreen shrub or tree, up to 25 m in height and 1.5m in girth, branches and peduncles softly pubescent. Bark somewhat corky, viscid inside, brownish grey, and wood yellowish grey to grayish brown. Leaves aromatic, elliptic ovate or oblong lanceolate, pubescent, variable in size,10-12 pairs. Flowers: white or yellowish, fruit globose, black or purple, 0.64cm diameter [5].

Phytochemical Constituents: Tannins, β-sitosterols and actinodaphnine, boldine, nor-boldine, laurotetanine, N-methyl laurotetanine, N-methylactinodaphnine, quercetine, sebiferine, litsiferine [6], Kaempferol- 3- glucoside, amino acids, quercetin- 3- rhamnoside, Kaempferol- 7-aminoglucoside, Pelargonidine- 5- glucoside, naringenin- 7- monorhamnoside, mono and sesquiterpenes, β- amirine acetate [5].

Animals: For the experiment Swiss albino mice of either sex, 3-4 weeks of age, weighing between 20-25 g, were procured from Mahaveer Enterprises, Hyderabad, India were used in the studies. Animals were maintained under standard environmental conditions(temperature: (24.0±1.0°), relative humidity: 55-65% and 12 h light/12 h dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee (IAEC, Clearance/2007/1-8), HSK College of Pharmacy, Bagalkot, Karnataka.

METHODS:

Acute toxicity: Acute oral toxicity study was performed as per OECD-423 Guidelines. Female Swiss albino mice (20-25gm) were randomly distributed to 6 groups (n=6) the animals were fasted overnight and drug was administered orally at the dose of 100, 200, 400, 800, 1600 and 3200 mg/kg the animals were closely observed for 24 hr for a toxic symptom and 72hr for mortality rate [9].

Analgesic Screening:

Hot Plate Method: The animals were divided into four groups with five mice in each group. Group I animals received vehicle (normal saline), animals of group II were administered a 22.8 mg/kg (body weight i. p. route) dose of standard drug (Tramadol HCl) [10]. While animals of Group III and Group IV were treated with 100 and 300 mg/ kg body weight (p. o.) of the crude extract of *Litsea glutinosa* (L) respectively. The animals were placed on Eddy's hot plate kept at a temperature of 55±0.5°C. A cut off period of 15s, was observed to avoid damage to the paw.[11] Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90 min after oral administration of the samples [12, 13].

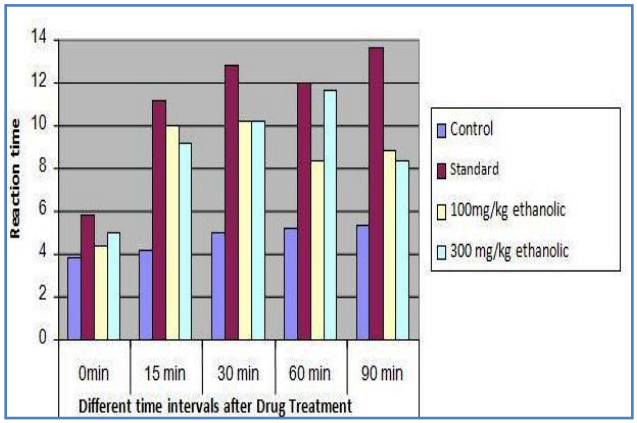
Statistical Analysis: Statistical analysis for animal experiments was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group. p-Value<0.05, 0.001 were considered to be statistically significant.

RESULTS: From the **table 1** and from **graph 1**, we found that, *Litsea glutinosa* (L) ethanolic extract of both doses (100mg/kg and 300mg/kg) shown significant increase in reaction time. 100 mg/kg ethanolic extract at 15 and 30 min interval has showed significant effect ($p<0.01$), after administration of 300 mg/kg 15 and 60 minute interval has shown significant effect ($p<0.001$) and 30 minute interval has shown $p<0.01$, as compared with control group both standard and extracted treated group.

TABLE 1: EFFECT OF *LITSEA GLUTINOSA* (L) ETHANOLIC EXTRACT ON LATENCY TO HOT PLATE TEST

Treatment group	Post drug Reaction time in sec (Mean ± SEM)				
	0min	15 min	30 min	60 min	90 min
Control	3.80 ± 0.20	4.2 ± 0.583	5.0 ± 0.547	5.2 ± 0.663	5.4 ± 0.979
Standard (Tramadol Hcl 22.8 mg/kg i. p.)	5.8 ± 0.80	11.2±0.374***	12.8 ± 0.489***	12 ± 0.707***	13.6 ± 0.509***
100mg/kg ethanolic extract (p. o.)	4.4 ± 0.509	10 ± 0.894**	10.2 ± 1.356**	8.4 ± 0.812	8.8 ± 1.158
300 mg/kg ethanolic extract (p. o.)	5 ± 1.14	9.2 ± 1.625***	10.2 ± 1.200**	11.6 ± 1.28***	8.4 ± 1.568

All values are expressed as Mean ± SEM, n= 5, One way Analysis of Variance (ANOVA) followed by Dunnet's test. The minimum value of $p<0.05$ was considered as significant. *$p<0.05$, **$p<0.01$, ***$P<0.001$ as compared with control group, both standard and extract treated group



GRAPH 1: ANALGESIC EFFECT OF *LITSEA GLUTINOSA* (L) BARK ETHANOLIC EXTRACT WHEN COMPARED WITH STANDARD GROUP (DIFFERENT TIME INTERVALS AFTER DRUG TREATMENT VS REACTION TIME)

DISCUSSION: The analgesic property of *Litsea glutinosa* (L) Can also probably due to the blockade of the effects or the synthesis and /or release of PGs and /or other endogenous substances that excite pain nerve endings [14]. The hot plate method test is considered to be selective to examine compounds acting through opoid receptor; the extract increased mean basal latency which indicates that it may act via centrally mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain [15,16]. Based on the results of the present study, we conclude that the plant extract possesses strong analgesic potential.

However, further studies are necessary to examine underlying mechanisms of analgesic and antioxidant effects and to isolate the active compound (s) responsible for these pharmacological activities.

CONCLUSION: Further Studies required identifying the active phytochemical constituents and evaluating their effectiveness in vivo so that they can be synthesized and commercial production begins in earnest.

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