

Analgesic Activity of Methanolic Extract of *Litsea deccanensis* Gamble Bark in Mice Model

Israt Jahan Bulbul^{1,2}, Mohammad Rashedul Haque² and Mohammad A. Rashid²

¹Department of Pharmacy, Southeast University, Banani, Dhaka-1213, Bangladesh

²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka Dhaka-1000, Bangladesh

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Abstract

Litsea deccanensis Gamble. (Family: Lauraceae) is used traditionally for sprained or swollen joints such as ankles or knee. Still, there is no report for its analgesic activity, therefore the present study was aimed to evaluate analgesic activity of the crude methanolic extract of *L. deccanensis* (MELD) bark in various mice models. Acetic acid-induced writhing, Eddy's hot plate and formalin induced paw licking tests were performed to evaluate the pain reducing/relieving effects. In acetic acid-induced writhing model, MELD showed significant reduction of squirming ($p < 0.001$, $p < 0.01$ and $p < 0.05$) in a dose dependent manner as compared to control. In the heat induced pain model (Eddy's hot plate method), MELD showed a slight increment of the latency time for the initiation of the reaction in the experimental animals, that revealed a moderate analgesic activity of MELD when compared to control group, but the results were insignificant. Whereas, in the formalin induced pain model, the reaction time was decreased significantly ($p < 0.05$) with the increment of doses for the studied plant extract (MELD) as well as standard in the late phase (20-30 min) of the study. So, the present study concluded a significant reduction of acetic acid-induced squirming or writhing, and formalin induced biting or licking in mice, provided us the evidence of having pain reducing potential.

Key words: *Litsea deccanensis*, acetic acid, formalin, hotplate, analgesic.

Introduction

Litsea deccanensis (Lauraceae), the name of plant comes from the Chinese word litse (LIT-see-a) or *li*, means small or little and (day-kahn-NEN-sis) means from Deccan peninsula, India. It is an evergreen small tree with spirally arranged leaves. Common names of *L. deccanensis* are Deccan litsea, Deccan tallow laurel, Ganapaty tree. It is called "gonopata" in bengali, Chikna, Kurak by Marathi, Narramamidi by Telegu, Mala-poenna, Pathali by Malayalam. The synonyms are *Litsea quinqueflora*; *Tetranthera tomentosa*; *Litsea tomentosa*; *Actinodaphne quinqueflora*. *L. deccanensis* is found in Chattogram, Bangladesh, Pakistan, Western India, Sri Lanka, Deccan Peninsula, Myanmar, Philippines,

Malaysia, Thailand, Indo-China, South China. Traditional healers of Kottayam district in Kerala use *L. deccanensis* for the remedy of inflammatory disorders. In Andhra Pradesh, leaves of this plant are used in chest pain. Traditionally this plant is used for sprained or swollen joints such as ankles or knees, sickle cell anemia, scabies and gastric acidity (van der Werff, 2001). Methanol extract of *L. deccanensis* was reported to have cardioprotective effect in animal models, *in vitro* antioxidant and reducing activities (Kumar *et al.*, 2011a; Kumar *et al.*, 2011b). The essential oil from *L. deccanensis* leaf was reported to have about 40 compounds among which caryophyllene epoxide, β -caryophyllene, germacra-3,9,11-triene, α -humulene, bicyclogermacrene and

Corresponding author: Mohammad A. Rashid; E-mail: r.pchem@yahoo.com

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limonene are the major volatile constituents (Irelandi et al., 2016). GC-MS study of *L. deccanensis* by Kumar et al. (2011a) reported the presence of quassin, stigmasterol, squalene, vitamin E and oleic acid in the extract. Several aporphine alkaloids including isocorydine, corytuberine, dicentrine, nordicentrine, boldine, norboldine, and magnoflorine have been isolated and characterized by Gupta and Bhakuni (1989). Considering the traditional uses of *L. deccanensis*, this study was aimed to investigate analgesic activity of this plant which is a continuation of our studies to find analgesic activity in medicinal plants of Bangladesh (Bulbul et al., 2016; Bulbul et al., 2017 and Munira et al., 2018).

Materials and Methods

Collection of plant material and extraction: *L. deccanensis* bark was collected from the hill tract region of Bangladesh. A botanist of Bangladesh National Herbarium, Mirpur-1, Dhaka-1216 identified the plant and an accession number was supplied for the plant *L. deccanensis* (Acc. No. DACB-35517). Before comminution of the plant material, the bark was washed with the help of a brush followed by running water and dried for a few days. Then the dried samples are ground into coarse plant powder. The crushed plant sample (500 g) of *L. deccanensis* bark was soaked in methanol, for 15 days, at room temperature, with 1 hour shaking every day. Then the methanolic extract was decanted through cotton plug followed by Whatman filter paper number 1. Excess of solvent was evaporated with the help of a rotary evaporator and the concentrated extract was further dried to get dried mass for this study. This extract was stored in refrigerator for further studies.

Test animals: The Swiss Albino mice were selected for this study and animals with both sexes were collected from Jahangirnagar University. They were aged of 4-5 weeks with 30 gm to 35 gm of body weight. Then the mice were adapted in the Pharmacology Research Laboratory of Southeast University for about 7 days at temperature $24.0 \pm 1^\circ\text{C}$ and at 55-65% of humidity and 12 hrs dark/light cycle

was maintained. The animals were handled according to the institutional protocol of Southeast University.

Peripheral analgesic activity by acetic acid induced writhing test: The analgesic test by counting writhing in mice was accomplished according to the method of Koster (1959). In this method, 0.7% v/v acetic acid was administered to the investigational animals through intra-peritoneal route to produce pain sensation. Two different doses (100 and 200 mg/kg bw) of the methanol extract of *L. deccanensis*, were coded as MELD-1 and MELD-2. For the acetic acid induced writhing model, the experimental animals were allocated into four groups (each group contains four mice). Group I was control group, indicated to administer only vehicle i.e. saline water & tween 80. Group II was standard control, specified to administer Indomethacin, 10 mg/kg bw while Group III and IV were treatment control groups, designated to administer 100 and 200 mg/kg bw of MELD. At zero hour of the experiment, saline water to group I, indomethacin (10 mg/kg) to group II and MELD (100 and 200 mg/kg bw) to group III and IV, respectively were administered orally by means of a feeding needle. After 30 minutes of the treatment, 0.7% v/v acetic acid was administered through intraperitoneal route to each animal of all the groups. After 5 minutes of the intraperitoneal administration of acetic acid, the total number of twists or writhing was counted for every mouse for 30 minutes. The total number of acetic acid-induced squirms in the mice of test groups' i.e. selected plant extracts treated mice was compared with individuals in the control and standard group mice. Complete squirming or writhing was not always taking place by the experimental animals, sometimes they started to squirm or twist but they could not complete. This type of incomplete writhing was considered as half-writhing and two half-writhing were considered as one complete writhing.

Central analgesic activity by Eddy's hot plate method: The method was formerly established by Woolfe and MacDonald (1944). They developed the method depending on the basis that the paws of mice or rats are sensitive to heat at 55°C temp, which does not damage the skin. The response may be jumping,

paws withdrawal from the hot surface or paws licking. This original method was updated by Eddy and Leimback (1953) which was further modified by Toma *et al.* (2003). Three different doses (100, 200 and 400 mg/kg bw) of the methanol extract of *L. deccanensis*, were coded as MELD-1, MELD-2 and MELD-3. For the Eddy's hot plate method, the experimental mice were grouped into five groups (each contains four mice). Group I or the control group was indicated to administer only vehicle i.e. saline water & tween 80, group II or the standard control group was allowed to administer tramadol, 10 mg/kg bw, whereas group III, IV and V were treatment control groups and specified to administer 100, 200 and 400 mg/kg bw MELD. At zero-hour saline water to group I, tramadol (10 mg/kg bw, p. o.) to group II and three different doses of each plant extract were administered to group III, IV and V orally by means of a feeding needle. One hour after peroral administration of MELD at three different doses and standard tramadol, the experimental animals were positioned on the hot plate with maintained temperature at $55^{\circ} \pm 0.5$ C. To avoid damage of the paw the animals were not kept for more than 15 s. A stopwatch was used to note the reaction time as well as the form of response. The showed responses were noted as the reaction time which were measured after 30, 60, 120 and 180 min following peroral administration of 100, 200 and 400 mg/kg of each of the extract to different groups. Then mean values of the reaction times were determined and the % of pain inhibition was calculated by using the equation mentioned below:

$$\% \text{ of pain inhibition} = (\text{drug latency} - \text{baseline latency}) / \text{baseline latency} \times 100$$

Central analgesic activity by formalin-induced paw licking test: This experiment was completed according to Tjolsen *et al.*, 1992, where pain is induced by a chemical formalin which is given to the sub-plantar region, hind paw of the investigational animals. Consequently, a pain sensation is induced in animals and the animals produce responses in the form of paw licking or biting. Two different doses (100 and 200 mg/kg bw) of the methanol extract of *L.*

deccanensis, were coded as MELD-1 and MELD-2. For the formalin induced paw licking and biting model, the experimental animals were allocated into four groups (each group contains four mice). Group I was control group, indicated to administer only vehicle i.e. saline water & tween 80. Group II was standard control, specified to administer Indomethacin, 10 mg/kg bw while Group III and IV were treatment control designated to administer 100 and 200 mg/kg bw of MELD. At zero-hour saline water to group I, indomethacin (10 mg/kg bw) to group II and two different doses of MELD were administered to group III and IV peroral by means of a long feeding needle. After 30 minutes of oral administration of standard indomethacin as well as two different doses of each plant extracts, 2% formalin was administered to every animal of all the groups. The total time of licking and biting the particular formalin injected paw by the experimental mice was recorded by using stopwatch. The response was measured in two different phases of rigorous licking periods stated as early phase (0–5 min after formalin injection) and the late phase (20–30 min after formalin injection) was documented for further data analysis. The latency of paw licking in the first 5 minutes indicates response to neurogenic pain while the latency of paw licking in between 20-30 minutes indicates inflammatory pain.

The % inhibition of paw biting and licking was calculated by the formula:

$$(\text{PL}_C - \text{PL}_T) / (\text{PL}_C) \times 100;$$

Where, PL_C denotes the mean value of paw licking of the control group;

PL_T denotes the mean value of paw licking of the treated group.

Results and Discussion

Peripheral analgesic activity by acetic acid induced writhing test: The obtained data from both the doses of methanol extract of *L. deccanensis* (MELD) showed a decrease in pain sensation induced by acetic acid in a dose dependent manner and the results were highly significant with $p < 0.001$. The % pain inhibition for 100 mg/kg bw of MELD was

measured as 37.28% which was measured as 79.66% for 200 mg/kg bw of MELD (Table 1).

Table 1. Analgesic activity of methanolic extract of *L. deccanensis* (MELD) by acetic acid induced writhing test.

Treatment groups	Dose (mg/kg, p.o.)	No. of writhing	% of inhibition
Control		14.75±5.56	
Indomethacin	10	3.00±2.16***	79.66 %
MELD_1	100	9.25±1.26***	37.28 %
MELD_2	200	3.00±2.94***	79.66 %

All the values are stated as mean ± STDEV. (Where, n=4); significance at ***p<0.001, **p<0.01, *p<0.05 as compared to control

Central analgesic activity by Eddy's hot plate method: The central analgesic effect of *L. deccanensis* was investigated by Eddy's hot plate method, where pain is induced by heat and analgesia was assessed by counting the time required for the initiation of the reaction. When various doses (100, 200 and 400 mg/kg bw) were given to animals and were subjected to induce pain by heat, animals produced an increased reaction time in a dose dependent manner compared to the control group. The pain-relieving activity data (Hot plate method) were presented as reaction time in seconds at 0, 30, 60, 120 and 180 min after treatment

with standard tramadol 10 mg/kg bw and three different doses of methanol extract of *L. deccanensis* (MELD).

The present research revealed a moderate analgesic activity of MELD when compared to control group, but the results were insignificant. In control group, the animals were untreated and only allowed for the vehicle, so the latency time was very short, while the latency time for the animals treated with standard (tramadol 10 mg/kg) increased significantly ($p < 0.05$) at 30 mins, 60 mins, 120 mins and 180 mins of the study period. The % pain inhibition for tramadol 10 mg/kg bw was 81.23%. In the present study, methanol extract of *L. deccanensis* (MELD) revealed 15.56%, 18.27% and 40.25% pain inhibition at 180 mins at 100, 200 and 400 mg/kg bw doses, respectively.

At 180-minute time period of pain sensation, percent inhibition was 40.25% for methanol extract of *L. deccanensis*, while for standard tramadol it was 81.23%. Maximum analgesia was revealed for 400 mg/kg bw of the plant extract. Percent inhibition was observed in a dose dependent manner, for methanol extracts of *L. deccanensis* which were 15.56%, 18.27% and 40.25%.

Table 2. Effect of methanolic extract of *Litsea deccanensis* (MELD) by Eddy's hot plate.

Treatment groups	Dose (mg/kg, p.o.)	Reaction time in seconds at time (min)					% inhibition at 180 min
		0	30	60	120	180	
Control	-	5.06±0.92	3.44±1.31	4.97±1.80	4.00±0.70	4.05±1.32	
Tramadol	10	3.84±1.45	5.06±1.17*	7.22±1.84*	7.26±2.12*	7.34±1.93*	81.23
MELD_1	100	3.45±0.69	3.32±1.25	3.76±0.41	4.50±0.92	4.68±1.42	15.56
MELD_2	200	3.72±1.34	3.29±0.47	4.15±0.60	7.65±0.83	4.79±1.24	18.27
MELD_3	400	3.81±0.74	3.73±1.11	4.85±1.67	5.17±1.46	5.68±1.55	40.25

All the values are stated as mean ± STDEV. (Where, n=5); significance at ***p<0.001, **p<0.01, *p<0.05 as compared to control.

Central analgesic activity by formalin induced paw licking test: The central analgesic effect of *L. deccanensis* was investigated by formalin induced paw licking test, where pain was induced by formalin and pain inhibition effect was investigated by calculating the total time of licking and biting their

paws. To investigate analgesia by using formalin test is advantageous because it includes responses in two different phases named as "an early phase" and "a late phase". The early phase indicates neurogenic while the late phase indicates inflammatory pain (Hunnskaar and Hole, 1987) and the by using these models the

studied plant extract may also can be classified as neurogenic or inflammatory pain reliever. Animals produced a decreased licking and biting time in a dose dependent manner compared to the control group.

The pain-relieving activity data (Formalin method) were presented as licking and biting time in seconds at early and late phases after treatment with standard indomethacin 10 mg/kg bw and plant extracts of the studied *Litsea* species. The paw licking time was significantly reduced in the inflammatory

(late) than as neurogenic (early) phase, which indicates the plants may reduce inflammatory pain.

In both the early phase and late phase, reaction times for licking and biting hind paw were decreased with the increment of the doses (from 100 mg/kg bw to 200 mg/kg bw), but in the late phase (20-30 min) the reaction time was decreased significantly ($p < 0.05$) with the increment of doses for all the studied plant extracts as well as standard indomethacin at 10 mg/kg bw.

Table 3. Effect of methanolic extract of *L. deccanensis* (MELD) formalin induced paw licking test.

Treatment groups	Dose (mg/kg, p.o.)	Paw licking at Early Phase (0-5 min)		Paw licking at Late Phase (20-30 min)	
		Time spent to lick or bite (sec)	Percentage (%) inhibition	Time spent to lick or bite (sec)	Percentage (%) inhibition
Control	-	103.00 ± 0.02		111.00 ± 0.02	
indomethacin	10	84.00 ± 0.01	18	48.00 ± 0.01	57
MELD_1	100	93.00 ± 0.01	10	104.00 ± 0.04	6
MELD_2	200	87.00 ± 0.02	16	69.00 ± 0.02	38

All the values are stated as mean ± STDEV. (Where, n = 5); significance at * $p < 0.05$ as compared to control.

Licking and biting reactions for normal control group were lasted for 103.00 ± 0.02 s at early phase while the responses at late phase lasted for 111.00 ± 0.02 s. For standard indomethacin the response time was measured 84.00 ± 0.01 s at early phase and 48.00 ± 0.01 s at late phase. For *L. deccanensis* (MELD), the response time (69.00 ± 0.02 s) was significantly decreased by the treatment with 200 mg/kg bw at the late phase ($P < 0.05$).

At the early phase, the percent inhibitory effects for MELD were 10% and 16%; On the other side, at the late phase the percent protection for MELD were 6% and 38%; at 100 mg/kg and 200 mg/kg bw, respectively.

Conclusion

The methanolic extract of *L. deccanensis* (MELD) was found to have very good peripheral and a moderate central analgesic activity in the present study. This finding helped us to support the traditional used of this plant in the treatment of joint pain. Further studies in mechanism level will confirm the

plant to have pain reducing ability. So, our future aim of the study is to explore the phytoconstituents by isolation, purification and characterization of the plant extract.

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