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Evaluation of *in vivo* analgesic, antiemetic and anxiolytic effect of methanolic extract of *Litsea monopetala* in animal model



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ABSTRACT

Objective: The present study was conducted to investigate *in vivo* analgesic, antiemetic and anxiolytic effect of methanolic extract of *Litsea monopetala* in animal model.

Materials and Methods: Analgesic activity was performed by formalin induce method. The antiemetic activity was conducted by using chick animal model where mean decrease in number of retches were calculated and anxiolytic activity was done by using Elevated Plus Maze (EPM) and Hole board method.

Results: The methanolic extract of *Litsea monopetala* at a dose of 400 mg/kg exhibited statistically significant ($P < 0.05$) and produced

66.67% of inhibition of paw licking in mice where standard drug showed 50 % inhibition. The extract further showed dose dependent and statistically ($P < 0.05$) significant antiemetic activity at a dose of 200 mg/kg and 400 mg/kg respectively. In addition the extract has showed excellent CNS depressant activity in both Elevated Plus Maze (EPM) and Hole board method in compare to standard drug diazepam.

Conclusion: From our current study it is obvious that the extract has good analgesic, antiemetic and anxiolytic effect which may be due to the presence of different chemical constituents like terpenes, flavonoids, tannins, saponin and sterols.

Keywords: *Litsea monopetala*, analgesic, antiemetic, anxiolytic.

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INTRODUCTION

From the beginning of civilization, life and diseases are closely inter linked with each other. The need for treating newer diseases is always a burning issue when people are greatly suffered. Then the search for new approach of treatment is becoming mandatory. At present about 200 plants are being used for treatment purpose although approximately over 14000 has been considered as medicinal plants.¹ Scientists are relentlessly working hard to launch a newer drug that have less side effect and cost effective. *Litsea monopetala* (Family: Lauraceae) a well known medicinal plant locally known as Bara-kukurchita widely distributed in Chittagong, Sylhet, Noakhali, Gazipur and Dinajpur district of Bangladesh. The plant has 136 species, 18m in height, 60 cm in diameter and having 7.5 to 13 cm long leaves.² The leaves and barks of this plant has been used as a tonic, antioxidant, analgesic and anti diarrheal agent. Here the main objective of our study is to evaluate the antidepressant, analgesic and the antiemetic activity of methanolic extract of *Litsea monopetala* leaves in animal models.

MATERIALS AND METHODS

Drugs and Chemicals

The analytical grade chemicals used in this work were purchased from Active Fine Chemicals, Bangladesh and the standard drug acetylsalicylic acid, metoclopramide and diazepam were purchased from Square Pharmaceuticals, Bangladesh.

Plant collection and identification

In order to conducting the experiment the leaves of *Litsea monopetala* were collected from remote rural areas of Noakhali, Bangladesh. Later, the identity conformation of the plant was done by the Bangladesh National Herbarium, Mirpur-1, Dhaka, Bangladesh (accession number- 45413).

Extract Preparation

At first the leaves of the plant were washed gently and cut into small pieces. Then the leaves were kept under the sun for drying purpose about 2 weeks and transferred into mechanical dryer having temperature 50-55°C. In order to make powder from drying leaves of the plant were put into a grinding and blending machine. About 200g of

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the powder material was added into a flat bottom container and extracted with 1500 ml of 99% methanol (Mark, Germany). The mixture of powder material and methanol (99%) was preserved for 2 weeks with regular shaking and stirring. Filtration of the plant extract was done by using Whatman filter paper. After filtration the methanolic extract of *Litsea monopetala* was concentrated by using Rotary evaporator. Finally, the crude extract of the plant was kept up to an airtight vial (15ml).

Formalin induced Analgesic activity test

In order to evaluate analgesic activity of methanolic extract of *Litsea monopetala* leaves 20 μ l of 2.5% formalin was administered into the planar surface of the left hind paw of mice.³ For experiment we divided 16 mice into four groups each having four mice. Group I was given only distilled water (10 ml/kg per body weight, *i.p* control), group II was given acetylsalicylic acid (100 mg/kg body weight, *s.c*), group III and IV were given methanolic extract (200 and 400 mg/kg body weight, *i.p*) respectively. The analgesic activity was measured for separate way, the first 5 minutes after formalin administered is considered as early phase (0-5min) and the time between 15-30 min was considered as late phase. Index of nociception time is defined as time that mice spent during paw licking.

Copper sulphate induced anti-emetic activity test

To evaluate the anti-emetic activity of methanolic extract *Litsea monopetala* leaves the one day old 16 chicks were divided into four groups each having 4 chicks. The chicks were kept in a large beaker at 25°C for 15 min. Group 1 was treated as control group, and given only saline 0.9% (10 ml/kg per body weight, orally). Group 2 (positive control) was given the standard drug 50 mg metoclopramide/kg body weight *i.p* (intraperitoneally). The remaining groups were treated with methanolic extract 200mg/kg and 400mg/kg respectively. About 1 hour later 50mg/kg copper sulphate (anhydrous) was orally administered to each chick. After that, an emetic action without vomiting known as retches was counted for 10 minutes. The antiemetic effect of extract was evaluated as the reduction in number of retches in treated group in comparison to control group. The inhibition (%) was calculated as follows: Inhibition (%) = [(A-B)/A] x 100 Where A is the control frequency of retching and B is the frequency of retching of the treated group.

Anxiolytic activity test Elevated Plus Maze Test

According to the Lister^{4,5} the Elevated plus maze apparatus consist of two open (40 \times 10 \times 1) and two

closed (40 \times 5 \times 15) closed arms. This test apparatus was elevated to a height of 40 cm above the floor. For experiment mice were divided into four groups (n=5) where the negative control group received only distilled water (10 ml/kg, *p.o*), the positive control group (standard) received the standard drug diazepam (1mg/kg, *i.p*) and the remaining two group received the plant extract 200 mg/kg and 400 mg/kg respectively. To evaluate the effect of the treated dose each mice was placed individually in the centre of elevated plus maze for a period of 5 minutes. The time that mice spent in the open and closed arms and the number of open-arm entries was considered as a measure of anxiety.⁵

Hole board Test

The hole board was adopted in this test.⁶ The hole board having 16 holes (each having 3cm in diameter and 2.2cm depth) was made of a wooden box (40 \times 40 \times 25 cm). Mice were divided into four groups where the negative control group received distilled water (10 mL/kg, *p.o*) the positive control group received diazepam (1 mg/kg *i.p.*), and the rest of the groups received methanolic extract 200 mg/kg and 400mg/kg respectively. About one hour later the mice were individually placed at one corner of the hole board and their number of head dipping in a period of five minutes was recorded.

Experimental animals

Both sex of adult Swiss albino mice (20-30g), aged 4-5 weeks were purchased from the animal house of Jahangirnagar University, Bangladesh. The experimental mice were kept under standard laboratory condition at Manarat International University research lab for 10 days and fed with ICDDR, B standard rodent food and water (*ad libitum*).

RESULT

Formalin induced hind paw lick test

The result of methanolic extract of *L. monopetala* leaves on formalin induced paw licking test in mice model are shown in the Table 1. Here the crude extract of *L. monopetala* showed the significant inhibition in both early and late phase at a dose dependent manner (200 mg/kg and 400 mg/kg) respectively.

Anti-emetic activity test

The effect of methanolic extract of *L. monopetala* leaves on copper sulphate induced test in mice model are shown in the Table 2. In this experiment the extract of *L. monopetala* at a dose of 200 mg/kg and 400 mg/kg showed the significant percent of inhibition in a dose dependent manner.

Table 1 Effect of *Litsea monopetala* leaf extract on formalin induced hind paw licking mice (Early and late phase)

Group	Dose	Early Phase(0-5 min)	% of inhibition	Late Phase(15-30 min)	% of inhibition
GroupI	10ml/kg	20±1.83	-	6±0.41	
GroupII	100mg/kg	11±0.41	45	3±0.41*	50
GroupIII	200mg/kg	7.5±0.29*	62	3±0.41*	50
GroupIV	400mg/kg	4.5±0.96*	77.5	2±0.41*	66.67

Each value represents the mean ± SEM (n=4). *P<0.05 compared with control. (One way ANOVA followed by Dunnett's't'-test).

Table 2 Effect of the *Litsea monopetala* leaf extract on copper sulfate induced antiemetic in mice

Group	Dose	Mean No. of retches ± S.E.M	Inhibition of emesis (%)
GroupI	10 ml/kg	110±2.08	-
GroupII	50 mg/kg	34.25±1.25*	68.86
GroupIII	200 mg/kg	28.75±2.72*	73.86
GroupIV	400 mg/kg	16.75±2.5*	84.77

Each value represents the mean ± SEM (n=4). *P<0.05 compared with control. (One way ANOVA followed by Dunnett's't'-test).

Table 3 Effect of methanol extract of *Litsea monopetala* on mice in the open arm and close of the EPM

Group	Dose	Time spend in open arm (s)	Time spend in closed arm (s)
Group I	10 ml/kg	17.5±3.22	282.5±3.22
GroupII	1 mg/kg	225.5±5.85*	74.5±5.85*
GroupIII	200mg/kg	62.75±5.57*	237.25±5.57*
GroupIV	400 mg/kg	78.5±4.36*	221.5±4.36*

Each value represents the mean ± SEM (n=4). *P<0.05 compared with control. (One way ANOVA followed by Dunnett's't'-test).

Table 4 Effects of methanol extract on mice stay in the hole board

Group	Dose	Number of head dipping
GroupI	10 mL/kg	36.2±1.16
GroupII	1 mg/kg	53.4±1.08*
GroupIII	200 mg/kg	64.4±1.29*
GroupIV	400 mg/kg	74.0±1.0*

Each value represents the mean ± SEM (n=4). *P<0.05 compared with control. (One way ANOVA followed by Dunnett's't'-test).

Anxiolytic activity test

The result of methanolic extract *L. monopetala* leaves on EPM and Hole board test in mice model are shown in the [Table 3 and 4](#).

DISCUSSION

The formalin induced paw licking test has been considered as an important method for searching new analgesic.⁶ In this experiment the methanolic extract of *L. monopetala* significantly inhibited the licking time in both phases. The first phase of the

test indicate the direct effect of formalin on nociceptors which also known as non-inflammatory pain and the second phase of the test define the inflammatory pain.^{3,7} As the extracts (200 mg/kg and 400 mg/kg) showed the significant inhibition at both phases, it may suggest that the methanolic extract of the plant possessed the analgesic effect.

The action of antiemetic effect of the extract is ambiguous. During copper sulphate induced emesis peripheral 5HT₃, 5HT₄ or NK₁ receptors are thought to be involved.⁸⁻¹⁰ It could be said that methanolic extract of *L. monopetala* provide

protection against emesis and which may be due to the presence of terpenes and flavonoids.¹¹ If these two vital constituents are present in the methanolic leave extract of this plant, it could be considered that these constituents are responsible for antiemetic activity.

The EPM is thought to be an important method of anxiety as it uses natural trigger.¹² However, it is validated that the anxiolytic agents increase the time spent in open arm of the EPM [4]. Normally mice prefer to spend their time in closed arm and are reluctant to explore in open arm. So, anxiolytic agent increases the open arm exploration.¹³ In our current study we showed that the leaves methanolic extract of *L. monopetala* significantly (*P<0.05) increase the spend in open in 200 mg/kg and 400 mg/kg respectively. Here the anxiolytic activity of the extract may be due to the presence of flavonoids, tannins, saponin and sterols as it was reported earlier.¹⁴⁻¹⁶

The hole board is another method to evaluate the anxiolytic effect in which anxiolytic state may be expressed by an increase in head dipping behaviors.¹⁷ Here in this test our extract 200 mg/kg and 400 mg/kg significantly showed the increase number of head dipping in a dose dependent manner. Gamma-amino-butyric acid (GABA) is major inhibitory neurotransmitter in the brain.¹⁸ The anxiolytic effect of the drug is explained by that drugs that act on the GABA/Benzodiazepine receptor complex. As a result majority of the anxiolytic drugs show their effect through opening of GABA-chloride channel in activated state.¹⁹

CONCLUSION

According to our experimental result we can claim that the methanolic extract of *Litsea monopetala* has strong analgesic, antiemetic and anxiolytic effect. So further pharmacological study are required to identify the active constituents so that the extract could be used for analgesic, antiemetic and anxiety disorders.

DISCLOSURES REGARDING REAL OR PERCEIVED CONFLICTS OF INTEREST

No competing interests in this scientific work.

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