



## CENTRAL ANALGESIC ACTIVITY OF *Litsea polyantha* Juss. BARK EXTRACT

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### ABSTRACT

*The sensation of pain is initiated in peripheral pain receptors (nociceptors) and its purpose is to draw attention to tissue damage. In order to test analgesic activity, it is obviously necessary to induce pain in the subject and then modify the response to, or perception of, this pain. Analgesic studies of the methanol (90% v/v) extract (MELP) of Litsea polyantha Juss. bark (Yield: 11.79% w/w) was carried out using healthy adult Swiss albino mice of either sex weighing between 20 to 25 g respectively. The experiment protocols were approved by the Institutional Animal Ethical Committee (621/02/ac/CPCSEA) prior to the conduct of the animal experiments. The animals were divided into 6 groups (n=6). Group I and II were used as control, received 10% v/v propylene glycol (PG) and distilled water (DW) at the dose of 10 ml/kg b.w. Group III, IV & V were treated with MELP (50, 75 and 100 mg/kg b.w., i.p.), respectively; Group VI received Morphine sulphate (10 mg/kg b.w., s.c.) an opioid analgesic as standard drug. A reduction in the tail withdrawal as compared to the control group was considered as evidence for the presence of analgesia. Tail flick latency was measured 30 min after the drug administration and Pain Inhibition Percentage (PIP) was calculated. MELP given by intraperitoneal route in mice showed significant and dose-dependent central analgesic activity ( $P < 0.001$ ) at all dose levels. MELP showed 22.2% – 60.4% increase in PIP in tail flick test and 21.2% – 67.8% increase in PIP in tail immersion method.*

**Keywords:** Litsea polyantha, analgesic, bark extract, pain



## INTRODUCTION

The sensation of pain is initiated in peripheral pain receptors (nociceptors) and its purpose is to draw attention to tissue damage. The impulses are transmitted to the dorsal horn, spinal cord, reticular formation and thalamus and finally to the cerebral cortex. Thus many parts of the brain are involved in the perception of pain.

Analgesics can therefore work in several ways, and it is for this reason that they are often used in combination, mainly a narcotic-type with an anti-inflammatory or paracetamol. Narcotic analgesics work by mimicking natural neurotransmitter peptides known as endorphins and enkephalin and others. There are several opioid receptors known, the main CNS receptors being the  $\delta$  (delta),  $\kappa$  (kappa) and  $\mu$  (mu), with others including the  $\sigma$  (sigma) and  $\epsilon$  (epsilon) receptors.

Morphine, the oldest and one of the most widely used of the opiate analgesics, is known to act primarily at  $\mu$  receptors. Naloxone antagonizes drug action at  $\mu$ ,  $\delta$  and  $\kappa$  receptors. In order to test for analgesic activity it is obviously necessary to induce pain in the subject and then modify the response to, or perception of, this pain (anesthesia, where the passage of pain impulses to the CNS is inhibited). This causes some difficulty in animal experiments; it is assumed that the animal responds to a pain stimulus in a similar manner to that which a human would, which cannot be proved.

The main methods of inducing pain experimentally are:

- Thermal                      Hotplate Test, Tail-Immersion Test, Tail-Flick Test
- Mechanical                 Tail-Clip Method
- Chemical                    Writhing (Squirming) Test
- Electrical                  Stimulation of Tooth-Pulp in Man
- Ischemic                     Application of a Tourniquet to the Arm, In Man.

The estimation of pain is either by quantal response, in which the percentage of animals responding to a fixed stimulus, e.g. heat, is determined; or a threshold response, in which the stimulus, e.g. pressure/heat, is increased until each animal responds.



## Experimental Animals

Studies were carried out using healthy adult Swiss albino mice of either sex weighing between 20 to 25 g respectively. They were obtained from the animal house, Department of Pharmaceutical Sciences, BIT, Mesra, Ranchi, India. The animals were grouped and housed in polyacrylic cages with not more than six animals per cage and maintained under standard laboratory conditions (temperature  $25 \pm 2$  °C) with dark and light cycle (14/10 h). Animals were allowed free access to standard pellet (Hindustan Lever, Mumbai, India) food and drinking water *ad libitum*. The animals were acclimatized to laboratory condition for 10 days before commencement of experiment. The experiment protocols were approved (BIT/PH/IAEC/19/2008) by the Institutional Animal Ethical Committee (621/02/ac/CPCSEA) prior to the conduct of the animal experiments.

## Plant Materials

The methanol (90% v/v) extract (MELP) of *Litsea polyantha* Juss. bark (Yield: 11.79% w/w) was concentrated in rotary evaporator followed by lyophilization. The completely dried sample was then reconstituted with 10% v/v propylene glycol (PG) for pharmacological activities.

## Methods

Analgesic activity of the methanol (90%) extract of *Litsea polyantha* Juss. was determined by both thermal and chemical methods in mice.

- ❖ Thermal method: Central analgesic activity
  - Tail flick method
  - Tail immersion method
  - Eddy's hot plate method

## 1 Central Analgesic Activity

### 1.1 Tail Flick Method

The animals were divided into 6 groups (n=6). Group I and II were used as control, received 10% v/v propylene glycol (PG) and distilled water (DW) at the dose of 10 ml/kg b.w.



Group III, IV & V were treated with MELP (50, 75 and 100 mg/kg b.w., i.p.), respectively; Group VI received Morphine sulphate (10 mg/kg b.w., s.c.) an opioid analgesic as standard drug. Before administration of the test compound or the standard drug the normal reaction time was determined. Basal reaction time of all the animals to radiant heat was recorded by placing the tip of the tail on the radiant heat source. A reduction in the tail withdrawal as compared to the control group was considered as evidence for the presence of analgesia. Tail flick latency was measured 30 min after the drug administration and Pain Inhibition Percentage (PIP) was calculated according to the following equation:  $PIP = [(T_1 - T_0) / T_0] \times 100$ ; where,  $T_1$  is post drug latency and  $T_0$  is predrug latency.

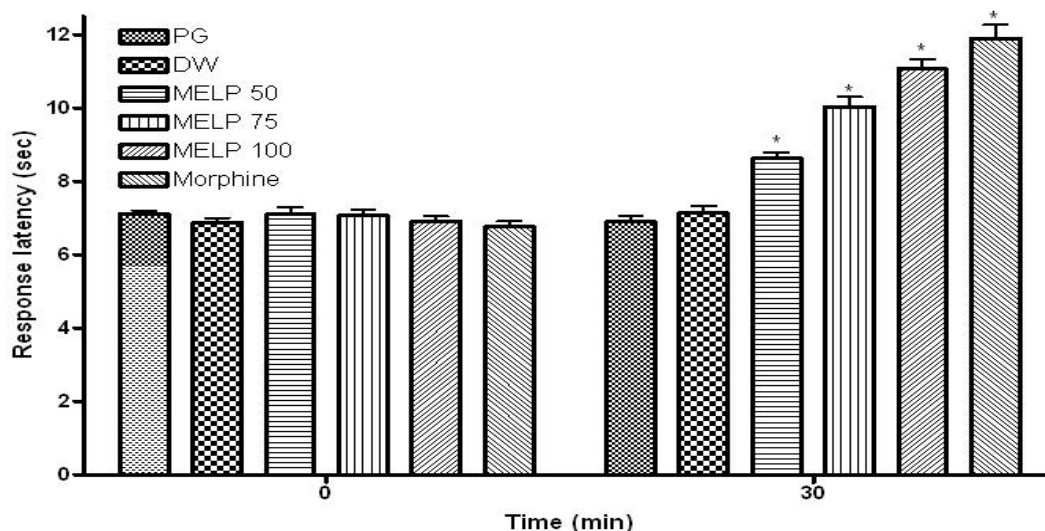
## Results

MELP given by intraperitoneal route in mice showed significant and dose dependent central analgesic activity ( $P < 0.001$ ) at all dose levels (Table 1 and Figure 1). MELP showed 22.2% – 60.4% increase in PIP in tail flick test.

**Table 1: Effect of MELP on tail flick response in Swiss albino mice**

Time (min)	Response Time (s) Mean $\pm$ SEM (n=6)					
	PG	DW	MELP 50	MELP 75	MELP 100	Morphine
0	7.12 $\pm$ 0.09	6.87 $\pm$ 0.13	7.10 $\pm$ 0.20	7.06 $\pm$ 0.17	6.91 $\pm$ 0.14	6.78 $\pm$ 0.15
30	6.90 $\pm$ 0.16	7.14 $\pm$ 0.20	8.63 $\pm$ 0.16*	10.03 $\pm$ 0.26*	11.06 $\pm$ 0.28*	11.90 $\pm$ 0.38*
PIP	-2.91 $\pm$ 3.38	3.93 $\pm$ 2.33	22.21 $\pm$ 5.09	42.68 $\pm$ 5.96	60.42 $\pm$ 4.77	76.28 $\pm$ 5.12

Values reported as Mean  $\pm$  SEM (n=6). The data were analyzed by two way ANOVA followed by Bonferroni's Multiple Comparison Test. Asterisk indicated statistically significant values from control. \* $P < 0.001$ . PG: Propylene Glycol; DW: Distilled Water; MELP: Methanol (90% v/v) extract of *Litsea polyantha* Juss. bark; PIP: Pain Inhibition Percentage.



**Figure 1: Effect of MELP on tail flick response in Swiss albino mice**

### 1.2 Tail Immersion Method

The animals were divided into 6 groups (n=6). Group I and II were used as control, received 10% v/v propylene glycol (PG) and distilled water (DW) at the dose of 10 ml/kg b.w. Group III, IV & V were treated with MELP (50, 75 and 100 mg/kg b.w., i.p.), respectively; Group VI received Morphine sulphate (10 mg/kg b.w., s.c.) an opioid analgesic as standard drug. Before administration of the test compound or the standard drug the normal reaction time was determined. The water in a beaker was kept at a temperature of  $55 \pm 0.5$  °C. Mice are held in position in a suitable restrainer with the tail protruding out. The tail up to 5 cm was dipped in the beaker of hot water. The time taken to withdraw the tail clearly out of water is taken as the reaction time. Tail withdrawal response was measured starting 30 min after the challenge with the treatments. A cut off period was kept 15-18 s to prevent the damage of the tail. Tail withdrawal latency was measured 30 min after the drug administration and Pain Inhibition Percentage (PIP) was calculated.



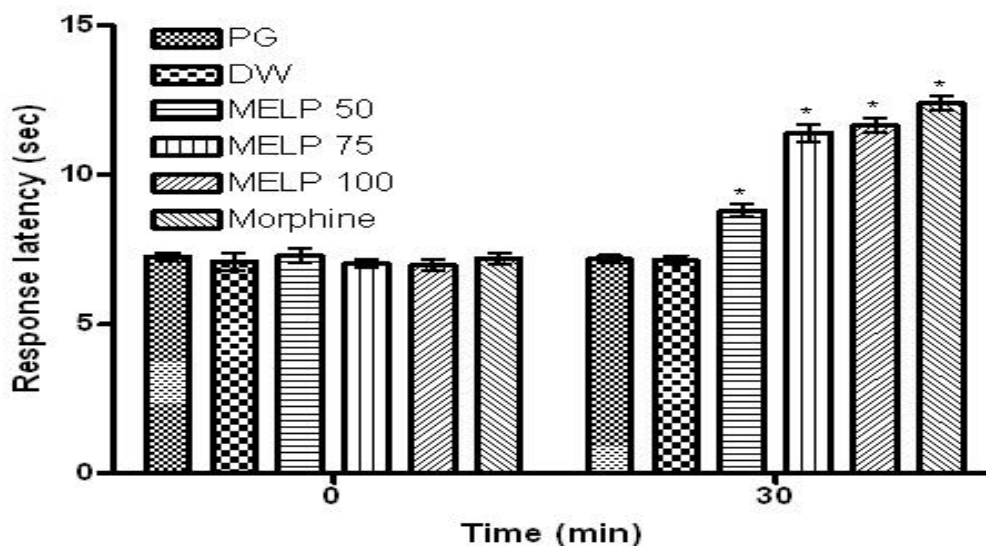
## Result

MELP given by intraperitoneal route in mice showed significant and dose dependent central analgesic activity ( $P < 0.001$ ) at all dose levels (Table 2 and Figure 2). MELP showed 21.2% – 67.8% increase in PIP in tail immersion method.

**Table 2: Effect of MELP on tail immersion response in Swiss albino mice**

Time (min)	Response Time (s) Mean $\pm$ SEM (n=6)					
	PG	DW	MELP 50	MELP 75	MELP 100	Morphine
0	7.23 $\pm$ 0.14	7.08 $\pm$ 0.29	7.27 $\pm$ 0.24	6.82 $\pm$ 0.19	6.95 $\pm$ 0.18	7.19 $\pm$ 0.19
30	7.16 $\pm$ 0.13	7.12 $\pm$ 0.15	8.79 $\pm$ 0.23*	11.39 $\pm$ 0.30*	11.63 $\pm$ 0.24*	12.39 $\pm$ 0.23*
PIP	-0.81 $\pm$ 2.76	1.73 $\pm$ 5.95	21.18 $\pm$ 2.71	62.24 $\pm$ 6.14	67.85 $\pm$ 5.86	72.96 $\pm$ 5.81

Values reported as Mean  $\pm$  SEM (n=6). The data were analyzed by two way ANOVA followed by Bonferroni's Multiple Comparison Test. Asterisk indicated statistically significant values from control. \* $P < 0.001$ . PG: Propylene Glycol; DW: Distilled Water; MELP: Methanol (90% v/v) extract of *Litsea polyantha* Juss. bark; PIP: Pain Inhibition Percentage.



**Figure 2: Effect of MELP on Tail immersion response in Swiss albino mice**



### 1.3 Hot plate method

The animals were divided into 6 groups (n=6). Group I and II were used as control, received 10% v/v propylene glycol (PG) and distilled water (DW) at the dose of 10 ml/kg b.w. Group III, IV & V were treated with MELP (50, 75 and 100 mg/kg b.w., i.p.), respectively; Group VI received Morphine sulphate (10 mg/kg b.w., s.c.) an opioid analgesic as standard drug. Before administration of the test compound or the standard drug the normal reaction time was determined. Mice were screened by placing them on Eddy's hot plate maintained at  $55 \pm 0.5$  °C and recorded the reaction time in seconds for licking of hind paw or jumping. The mice which reacted within 15 s and which did not show large variation, when tested on four separated occasions, were selected for studies. Response was measured 30 min after the drug administration and Pain Inhibition Percentage (PIP) was calculated.

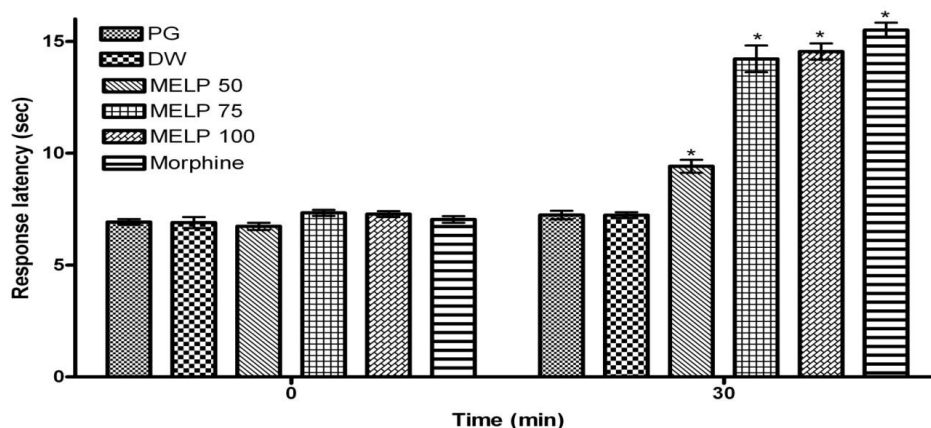
### Results

MELP given by intraperitoneal route in mice showed significant and dose dependent central analgesic activity ( $P < 0.001$ ) at all dose levels (Table 3 and Figure 3). MELP showed 39.9% – 100% increase in PIP in hot plate method.

**Table 3: Effect of MELP on hot plate response in Swiss albino mice**

Time (min)	Response Time (s) Mean $\pm$ SEM (n=6)					
	PG	DW	MELP 50	MELP 75	MELP 100	Morphine
0	6.93 $\pm$ 0.12	6.90 $\pm$ 0.25	6.74 $\pm$ 0.16	6.94 $\pm$ 0.16	7.28 $\pm$ 0.13	7.04 $\pm$ 0.15
30	7.24 $\pm$ 0.19	7.23 $\pm$ 0.13	9.42 $\pm$ 0.29*	14.22 $\pm$ 0.59*	14.55 $\pm$ 0.36*	15.51 $\pm$ 0.33*
PIP	1.14 $\pm$ 1.02	-1.77 $\pm$ 1.73	39.89 $\pm$ 2.80	94.49 $\pm$ 9.99	100.08 $\pm$ 5.25	120.78 $\pm$ 7.35

Values reported as Mean  $\pm$  SEM (n=6). The data were analyzed by two way ANOVA followed by Bonferroni's Multiple Comparison Test. Asterisk indicated statistically significant values from control. \* $P < 0.001$ . PG: Propylene Glycol; DW: Distilled Water; MELP: Methanol extract (90% v/v) of *Litsea polyantha* Juss. bark; PIP: Pain Inhibition Percentage.



**Figure 3: Effect of MELP on hot plate response in Swiss albino mice**

### 3 Discussions

Folkloric treatment of nociceptive of various etiologies, using medicinal plants, is well known to masters of the art of traditional medicine practice. *Litsea polyantha* Juss. has been indicated in pain and inflammatory conditions in folklore due to its high therapeutic potency.

MELP exhibited marked inhibition on thermally induced hyperalgesia. The MELP possesses significant ( $P < 0.001$ ) activity at all dose levels. Morphine (10 mg/kg b.w., s.c.) being standard drug, showed more potent activity. Therefore, the result of our study showed the central mediation in the antinociceptive activity of MELP. The possible mechanism may be inhibition of  $\mu$ -opioid receptor.

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