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

Manik Ghosh* and Barij Nayan Sinha

Department of Pharmaceutical Sciences and Technology

Birla Institute of Technology, Mesra,

Ranchi, Jharkhand (835215), INDIA

**Corresponding Author's E-mail: manik@bitmesra.ac.in*

Tel.: + 916512276247; Fax: + 916512275290

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.

INTRODUCTION

Natural Products

- Natural products have been the single most productive source of leads for the development of drugs.
- Over a 100 new products are in clinical development, particularly as anti-cancer agents and antiinfectives.
- Comparisons of the information presented on sources of new drugs from 1981 to 2007 indicate that almost half of the drugs approved since 1994 are based on natural products.

Harvey AL. Natural products in drug discovery. Drug Discov. Today 2008; 13(19/20): 894-901

Natural Product-Derived Drugs at Different Stages of Development

Therapeutic area	Pre-clinical	Phase I	Phase II	Phase III	Pre-registration	Total
Cancer	34	15	26	9	2	**86**
Anti-infective	25	4	7	2	2	**40**
Neuropharmacological	6	3	9	4	0	**22**
Cardiovascular / gastrointestinal	9	0	5	6	0	**20**
Inflammation	6	2	9	1	0	**18**
Metabolic	7	3	6	1	0	**17**
Skin	7	1	2	0	0	**10**
Hormonal	3	0	2	1	0	**6**
Immunosuppressant	2	2	0	2	0	**6**
Total	**99**	**30**	**66**	**26**	**4**	**225**

Harvey AL. Natural products in drug discovery. Drug Discov. Today 2008; 13(19/20): 894-901

Folklore Medicines

- Large numbers of medicinal plants have been advocated in folklore medicines of Jharkhand for treating various diseases and disorders.
- One of such a plant is *Litsea polyantha* Juss., locally known as Pojo.
- Tribal of Chotanagpur region are using bark of this plant for treatment of different diseases and ailments like pains, inflammation, bruises & contusions, cuts, wounds, diarrhea and fractures in animals.

Litsea

- *Litsea,* a large genus comprising of around 700 species of evergreen trees or shrubs, distributed chiefly in tropical and subtropical Asia, Australia and the Pacific Islands. About 43 species are found in India. It belongs to the family Lauraceae.

The Wealth of India (CSIR), 1985; (VI):152-156
http://www.tropicos.org/TaxonomyBrowser.aspx?nameid=40007934&conceptid=1

Ethnopharmacology

- **Parts used**:
  - In Folklore medicines - Bark, Stem and Roots are used to treatment various diseases and disorders

- **Properties and uses**
  - The bark of *Litsea polyantha* Juss. is mildly astringent and is reported to be used for diarrhea.
  - Powdered bark and roots are used for treatment of cuts, wounds, pains, bruises and contusions.
  - The Powdered bark is also used to cure fractures in animals.
  - The seed fat is use in ointments for rheumatism.

1. The Wealth of India (CSIR), 1985; (VI):154-155
2. Kirtikar KR and Basu BD. Indian Medicinal Plants, M/S periodical Experts, Delhi, 2nd ed., 1935 (Reprint 1975); (III): 2160-2161

EXPERIMENTAL

Collection and Authentication

- The bark of *Litsea polyantha* Juss (Lauraceae) were collected from BIT, Mesra of Ranchi District.
- The parts were authenticated by Dr. S. Jha, Professor, Department of Pharm. Sciences, BIT, Mesra and Dr. P. Venu, Scientist 'F' & HOO, Botanical Survey of India, Central National Herbarium, Howrah.
- The voucher specimen (BIT 417) was preserved in the Department of Pharmaceutical Sciences, BIT, Mesra.

Drying and Size Reduction

- The bark of *Litsea polyantha* Juss were dried in shade for about a week followed by drying at 35 °C – 40 °C in oven for 1 day. The dried barks were then grinded to coarse powder in an iron mortar and pestle. This powdered material was again dried in the oven at 35 °C . 40 °C for 1 hour and used for extraction.

Extraction

- The dried and powdered plant material (Bark) was subjected to successive hot extraction in a soxhlet apparatus with solvents of increasing polarity viz. petroleum ether (60-80), chloroform, ethyl acetate and methanol.
- The average time period for extraction was 48 hours. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was distilled followed by evaporation in a vacuum rotary evaporator. Methanol extract was also subjected to lyophilization.

- In another extraction method the defatted bark of *Litsea polyantha* Juss. was subjected to hot extraction in a soxhlet apparatus with mixture of methanol and water (90:10).

- The average time period for extraction was 72 hours. The extract was then filtered using Whatman filter paper No. 1, the filtrate was distilled and evaporated in a vacuum rotary evaporator followed by lyophilization.

Extractive Values and color of methanol (90% v/v) extract of *Litsea polyantha* Juss. bark

Name of the Extract	% Yield (Hot) w/w	Color of Extract	Color at 365 nm	Color at 254 nm	Consistency
Petroleum ether (60-80) – PLP	1.03	Pale White	White	Yellow	Greasy Waxy
Methanol (90 % v/v) – MELP	11.79	Reddish Brown	Black	Light blue	Amorphous

PHARMACOLOGICAL INVESTIGATIONS

SELECTION OF EXTRACT FOR PHARMACOLOGICAL INVESTIGATIONS

- All the five extracts were concentrated in rotary evaporator followed by lyophilization as and when required. The completely dried samples were then reconstituted with 10% v/v propylene glycol (PG) for pharmacological experiments.

- Guided by the ethnopharmacological literatures on *Litsea polyantha* Juss., all the five extracts were subjected to pharmacological screening. Results suggested that methanol (90% v/v) extract (MELP) was pharmacologically more potent than other extracts.

- The percentage yield of methanol (90% v/v) extract (MELP) was also appreciably high (11.79 % w/w). This extract answered positive for major phytoconstituents like alkaloids, flavonoids, etc present in *Litsea* species. ***This is how MELP was selected for detail pharmacological and phytochemical investigations.***

Central Analgesic Activity

- Tail Flick Method
- Tail Immersion Method
- Eddy's Hot Plate Method

Tail Flick Method

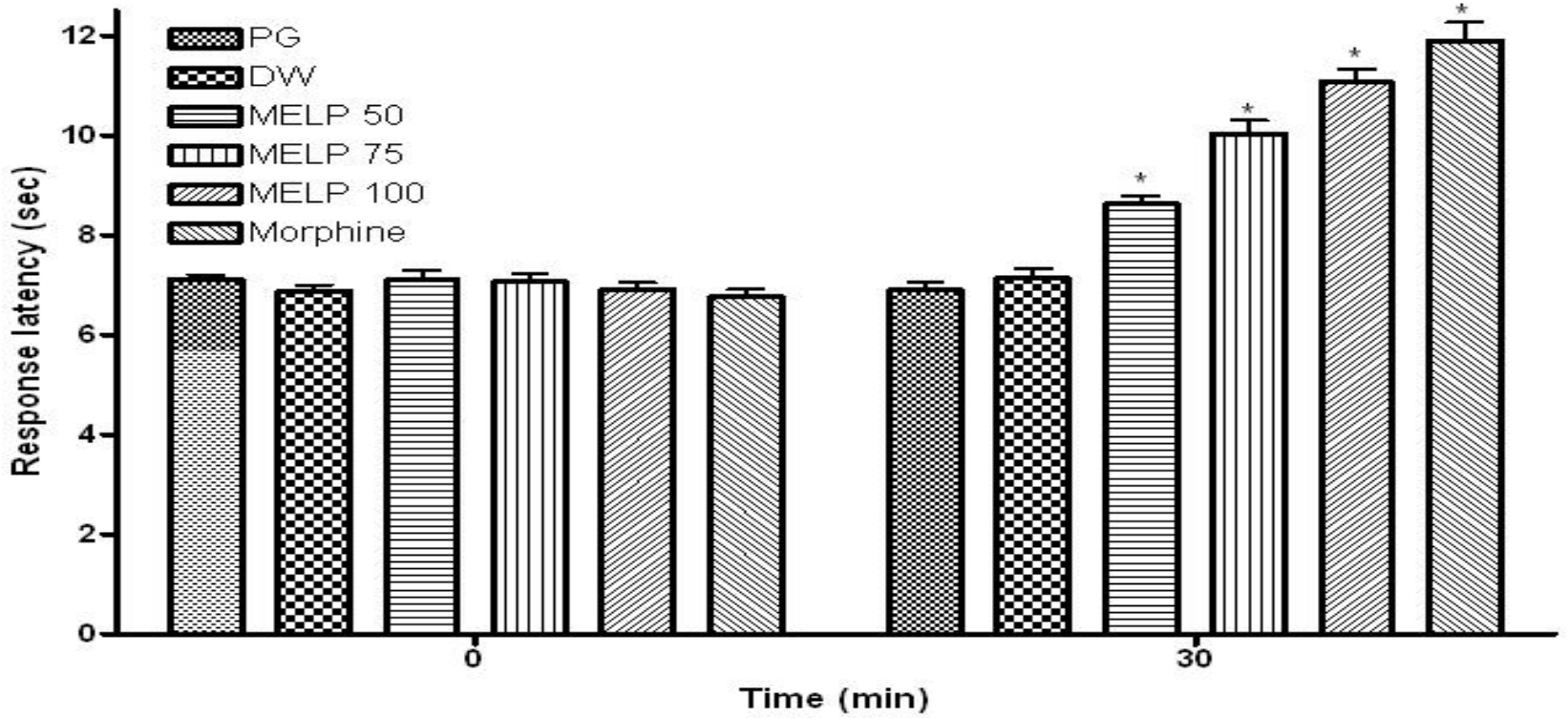
Effect of MELP on Tail flick response in Swiss albino mice.

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

Values reported as Mean ± 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.

Turner, R.A., 1965. In: Turner, R., Ebborn, P. (Eds.), Analgesics: Screening Methods in Pharmacology. Academic Press, New York.

Tail Flick Method



Effect of MELP on tail flick response in Swiss albino mice.

Values reported as Mean ± 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.

Tail Immersion Method

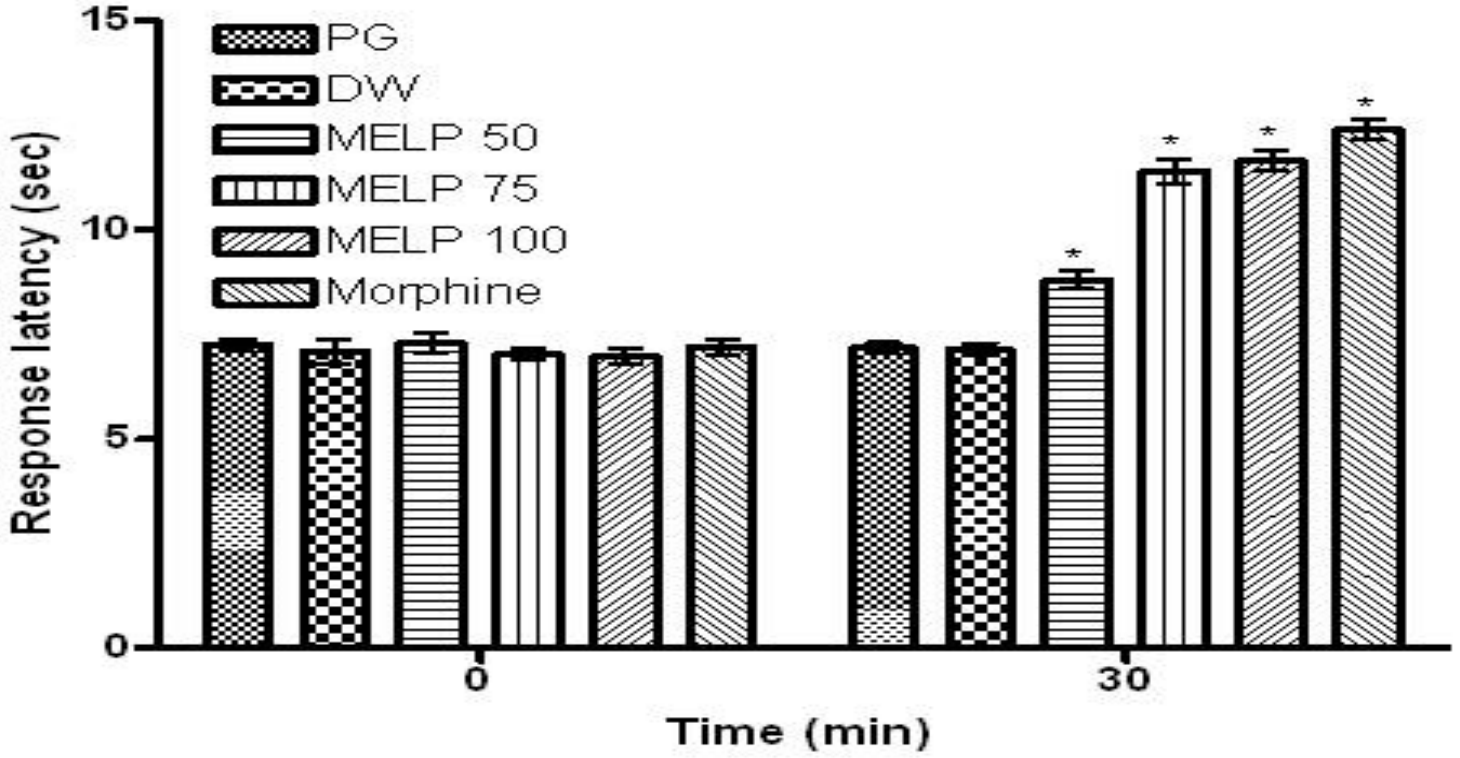
Effect of MELP on Tail immersion response in Swiss albino mice.

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

Values reported as Mean ± 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.

Aydin, S., Demir, T., Ozturk, Y., Baser, K.H.C., 1999. Analgesic activity of Nepeta italica L. Phytotherapy Research 13, 20–23.

Tail Immersion Method



Effect of MELP on tail immersion response in Swiss albino mice.

Values reported as Mean ± 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.

Hot Plate Method

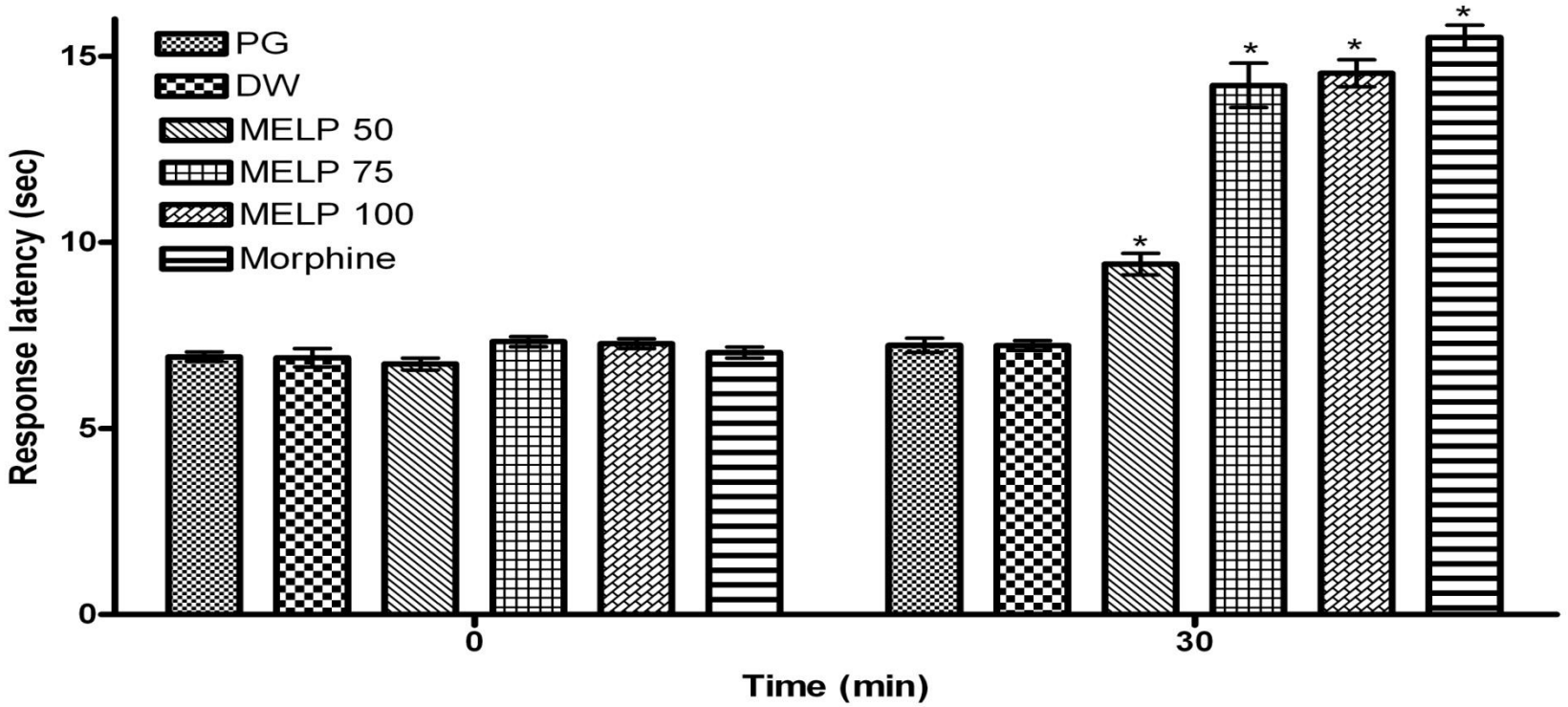
Effect of MELP on Hot plate response in Swiss albino mice.

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

Values reported as Mean ± 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.

Dar, A., Faizi, S., Naqvi, S., Roome, T., Zikr-Ur- Rehman, S., Ali, M., Firdous, S., Moin, T.S., 2005. Analgesic and antioxidant activity of mangiferin and its derivatives: the structure activity relationship. Biological Pharmaceutical Bulletin 28, 596–600.

Hot Plate Method



Effect of MELP on Hot plate response in Swiss albino mice .

Values reported as Mean ± 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.

Comparative Study

Comparison of all three methods of Analgesic Activities

Tests	Pain Inhibition Percentage (%)					
	PG	DW	MELP 50	MELP 75	MELP 100	Morphine
Tail Flick	*-2.91 ± 3.38*	*3.93 ± 2.33*	*22.21 ± 5.09*	*42.68 ± 5.96*	*60.42 ± 4.77*	*76.28 ± 5.12*
Tail Immersion	*-0.81 ± 2.76*	*1.73 ± 5.95*	*21.18 ± 2.71*	*62.24 ± 6.14*	*67.85 ± 5.86*	*72.96 ± 5.81*
Hot Plate	*1.14 ± 1.02*	*-1.77 ± 1.73*	*39.89 ± 2.80*	*94.49 ± 9.99*	*100.08 ± 5.25*	*120.78 ± 7.35*

Values reported as Mean ± 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;

Discussion

- *Litsea polyantha* Juss. has been indicated in pain and inflammatory conditions in folklore due to its high therapeutic potency.

- MELP showed marked antinociceptive activity in various pain models including tail-flick, tail immersion and hot plate test.

- MELP exhibited marked inhibition on thermally induced hyperalgesia. The MELP possesses significant ($P<0.001$) activity at all dose levels. The possible mechanism may be inhibition of μ-opioid receptor.

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Thank You