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COMPARATIVE STUDIES ON ANALGESIC POTENTIALS OF *PREMNA TOMENTOSA* WILLD AND *PREMNA LATIFOLIA* ROXB. USING DIFFERENT MODELS

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ABSTRACT

The comparative studies on analgesic potentials of whole plants methanol extract of *Premna tomentosa* Willd (MEPT) and *Premna latifolia* Roxb. (MEPL) using Formalin induced pain model and Hot plate reaction time in mice. Standard drugs such as indomethacin and morphine used in above methods. The results showed dose-dependent and significant ($P < 0.01$) increases in pain threshold at 30 min post-treatment with doses of 200 and 400 mg/kg of MEPT and MEPL in Formalin induced pain model and Hot plate reaction time in mice. The MEPT and MEPL (200 and 400 mg/kg) exhibited a dose-dependent inhibition of hot plate reaction time with centrally acting analgesic effect and also showed a significant ($P < 0.01$) inhibition in both phases of the formalin pain test, but with a less intense effect in the first phase with peripherally mediated analgesic effect. The results indicate that the analgesic potency of *Premna tomentosa* Willd and *Premna latifolia* Roxb. is both centrally and peripherally mediated. *Premna latifolia* Roxb. extract showed more potent analgesic activity in above models than *Premna tomentosa* Willd extract.

Key words: *Premna tomentosa* Willd, *Premna latifolia* Roxb., Analgesic activity, Formalin test, Hot plate method.

INTRODUCTION

Premna tomentosa Willd and *Premna latifolia* Roxb. belongs to the family Verbenaceae. *Premna tomentosa* commonly called as "Krishnapalai" is a medicinal plant. It is moderately sized deciduous tree with shoots, leaves and inflorescence densely clothed with a tawny yellow stellate tomentum. The bark is light grayish colour, greenish yellow (Haines, 1961). The genus premna comprised of 50-200 species, is distributed in tropical and subtropical Asia, Africa, Australia and Pacific islands (Kadereit, 2004). The leaves of *P.tomentosa* possess diuretic, anti-inflammatory, antinociceptive, hypnotic effects, cytoprotective and immunomodulatory activities (Devi, 2002).

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Premna latifolia Roxb. is widespread in India along the coastal regions of plains and hills. Traditionally the paste of *P. latifolia* bark is applied to cure boils; tender leaves are diuretic, anti-inflammatory (Mahire NB *et al*., 2009), anticancer (Suresh G *et al*., 2001), antirheumatic and acute dropsy (Reddy KN *et al*., 2010). In Tamil Nadu it is mentioned as a sacred tree and is used to cure swellings (Ganesan S *et al*., 2009). The major phytoconstituents of the plant are alkaloids, irdoids and oils. The stem bark shows the presence of irdoid glucosides and geniposidic acid (Khare CP, 2007). Premna latifolin A, a novel dimeric diterpene was isolated from the stem bark of *P.latifolia* (Kadereit, 2004) along with hexadecanoic acid and 1-octen-3-ol (Kumar A *et al*., 2001). Healing of wound is an intricate process in which the skin repairs itself after injury (Nguyen DT *et al*., 2009). The current researches were attention towards extracts of medicinal plant species which can be used as herbal drugs.

The present study was undertaken to evaluate the comparative studies on analgesic potentials of whole plants extract of *Premna tomentosa* Willd and *Premna latifolia* Roxb. using Formalin induced pain model and Hot plate reaction time in mice.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The whole plants of *Premna tomentosa* Willd and *Premna latifolia* Roxb. (Family: Verbenaceae) were collected from Tirumala hills, Tirupati (Andhra Pradesh, India) during December 2012, identified and authenticated by Dr.Madhavachetty, Assistant Professor, Department of Botany, Sri Venakateswara university, Tirupati 517 502, A.P., India.

Extraction of Plant Material

The whole plants of *Premna tomentosa* Willd. (*Family* - Verbenaceae) and *Premna latifolia* Roxb (*Family* - Verbenaceae) were collected and botanically identified, cut into small pieces and then dried in the shade for 2 weeks. The dried drug was powdered and passed through No.40 sieve and used for the extraction by using methanol. The plant materials were extracted with methanol (95%) by continuous hot percolation using soxhlet apparatus until the extraction was completed. After the completion of the extraction the extract was filtered and the solvent was removed by distillation under reduced pressure.

Preliminary phytochemical screening

The phytochemical qualitative chemical constituents of methanol extract of whole plants of *Premna tomentosa* Willd and *Premna latifolia* Roxb. using commonly employed precipitation and coloration to identify the major natural chemical groups such as alkaloids, steroids, phenolic compounds, saponins, tannins, flavonoids, amino acids and glycosides were performed by the standard methods (Harbone JP, 1973). General reactions in these analyses revealed the presence or absence of these compounds in the crude extracts tested.

Animals used

Male albino mice (20-25g) were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA. The research work was done in Research Center, Sri Venkateswara College of Pharmacy, Chittoor, Andhra Pradesh, India.

Analgesic activity by Formalin induced pain

The formalin test was carried out as described by Hunskaar and Hole (Hunskaar S and Hole K, 1987). Male Albino mice weighing 20-25g were divided into five groups of six animals each. All groups of animals were injected subcutaneously with 20µl of formalin into the dorsal hind paw.

Experimental design

Group I - Received vehicle control (1% v/v tween 80, 1ml/100 g)
Group II - Received methanol extract of whole plant of *Premna tomentosa* Willd. (MEPT) (200mg/kg body weight p.o) suspended in 1% v/v tween 80
Group III - Received methanol extract of whole plant of *Premna tomentosa* Willd. (MEPT) (400mg/kg body weight p.o) suspended in 1% v/v tween 80
Group IV - Received methanol extract of whole plant of *Premna latifolia* Roxb. (MEPL) (200mg/kg body weight p.o) suspended in 1% v/v tween 80
Group V - Received methanol extract of whole plants of *Premna latifolia* Roxb. (MEPL) (400mg/kg body weight p.o) suspended in 1% v/v tween 80
Group VI- Received standard drug (Indomethacin, 10mg/kg) p.o, respectively 30 min before formalin injection.

The time the mice spent licking or biting the injected paw or leg was recorded. On the basis of the response pattern described by Tjolsen *et al.* (Tjolsen A *et al*., 1992) two distinct periods of intensive licking activity were identified and scored separately. The first period (early phase) was recorded 1- 5 min after the injection of formalin and the second period (late phase) was recorded 20–40 min after the injection.

The percentage inhibition of licking was calculated by the formula:

$(C-T)/C \times 100$

where C represents the vehicle treated control group value for each phase and T represents the treated group value for each phase.

Analgesic activity by Eddy's Hot plate method

The *hot plate* method was carried out as described by Turner (Turner RA, 1965). Male Albino mice weighing 20 to 25 gm were divided into five groups of six animals each.

Experimental design

Group I - Received vehicle control (1% v/v tween 80, 1ml/100g)
Group II - Received methanol extract of whole plant of *Premna tomentosa* Willd. (MEPT) (200mg/kg body weight p.o) suspended in 1% v/v tween 80
Group III - Received methanol extract of whole plant of *Premna tomentosa* Willd. (MEPT) (400mg/kg body weight p.o) suspended in 1% v/v tween 80
Group IV - Received methanol extract of whole plant of *Premna latifolia* Roxb. (MEPL) (200mg/kg body weight p.o) suspended in 1% v/v tween 80

Group V - Received methanol extract of whole plants of *Premna latifolia* Roxb. (MEPL) (400mg/kg body weight p.o) suspended in 1% v/v tween 80

Group VI - Received standard drug morphine sulphate (5mg/kg s.c) 30 min before the thermal pain stimulus.

Mice were screened by placing them on a hot plate maintained at 55 ± 1 °C and recorded the reaction time in seconds for licking of hind paw or jumping. A cut-off time of 40s was selected to avoid tissue damage.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett's test p values less than 0.05 were considered as significance.

RESULTS

Preliminary phytochemical screening

The phytochemical qualitative analyses revealed the presence of steroids, phenolic compounds, tannins, flavonoids and absence of saponins, glycosides in methanol extract of whole plant of *Premna tomentosa* Willd. Whereas methanol extract of whole plant of *Premna latifolia* Roxb. revealed the presence of steroids, alkaloids, phenolic compounds, tannins, saponins, glycosides, flavonoids.

Formalin induced pain

MEPT (200 & 400mg/kg) and MEPL (200 & 400mg/kg) produced significant ($P < 0.01$) inhibition in the late phase of formalin induced pain (49.78, 60.35 and 58.17, 67.72%) respectively (Table 1 and Figure 1). The positive control indomethacin (10 mg/kg) also produced significant ($P < 0.01$) inhibition in the late phase (71.49%).

Hot plate reaction time in mice

As shown in Table 2 and Figure 2, the MEPT (400mg/kg) and MEPL (400mg/kg) showed significant ($P < 0.01$) analgesic activity. Morphine sulphate at 5 mg/kg, *s.c.* significantly showed its maximum protective effect of 28.50 ± 0.4282 (P <0.01) after 30min. Maximum protective effect was achieved by (P <0.01) 400 mg/kg of MEPL (24.33 ± 0.5578) and 400 mg/kg of MEPT (21.67 ± 0.4216) compared than MEPT (200mg/kg) and MEPL (200mg/kg) (14.67 ± 0.4216 & 17.33 ± 0.3333) from thermal induced pain.

DISCUSSION AND CONCLUSION

The methanolic extract of whole plants of *Premna tomentosa* Willd and *Premna latifolia* Roxb. showed a dose-dependent and significant (P < 0.01) increase in the pain threshold at 30 min post-treatment with doses of 200 and 400 mg/kg of extract in formalin test and eddy's hot plate methods. Peripheral and central analgesic properties of *Premna tomentosa* Willd and *Premna latifolia* Roxb. was confirmed in present study.

Table 1. Analgesic Effect of MEPT & MEPL in the formalin test[a]

Groups	Design of treatment	Licking(sec)[b]		Inhibition (%)
		0-5min	15-40min	
I	Control (1% v/v tween 80, 1ml/100 g)	58.50± 0.8466	115.17±0.9458	–
II	MEPT (200mg/kg b.w, *p.o*)	57.50±0.6191	57.83±0.6009**	49.78
III	MEPT (400mg/kg b.w, *p.o*)	54.33 ±0.3333**	45.67±0.4944**	60.35
VI	MEPL (200mg/kg b.w, *p.o*)	53.33±0.3333**	48.17±0.5426**	58.17
V	MEPL (400mg/kg b.w, *p.o*)	51.17±0.4773**	37.17±0.6009**	67.72
VI	Indomethacin (10mg/kg b.w, *p.o)*	50.33 ±0.4216**	32.83± 0.3073**	71.49

[a] After 30 minutes MEPT & MEPL treatment, mice were injected *s.c* with 20µl of formalin into the dorsal hind paw. [b]Values are expressed as mean±S.E.M. (N=6). * P<0.05, ** P<0.01 significant compared with control.

Table 2. Effect of MEPT & MEPL on Hot plate reaction time in mice

Group	Design of treatment	Mean Latent (Sec)	
		Initial	After 30 min[a]
I	Control (1% v/v tween 80, 1ml/100 g)	11.84 ± 0.3416	11.17 ± 0.3073
II	MEPT (200mg/kg b.w, *p.o*)	11.33 ± 0.3333	14.67 ± 0.4216**
III	MEPT (400mg/kg b.w, *p.o*)	11.17 ± 0.2108	21.67 ± 0.4216**
VI	MEPL (200mg/kg b.w, *p.o*)	11.50 ± 0.3073	17.33 ± 0.3333**
V	MEPL (400mg/kg b.w, *p.o*)	11.66 ± 0.2108	24.33 ± 0.5578**
V	Morphine (5mg/kg, s.c)	11.22 ± 0.3651	28.50 ± 0.4282**

[a]After 30 minutes MEPT & MEPL treatment, mice were placed on hot plate. Values are expressed as mean ± S.E.M. (N=6) and units are in sec. * P<0.05, and ** P<0.01 significant compared with control values.

Figure 1. Analgesic Effect of MEPT & MEPL in the formalin test

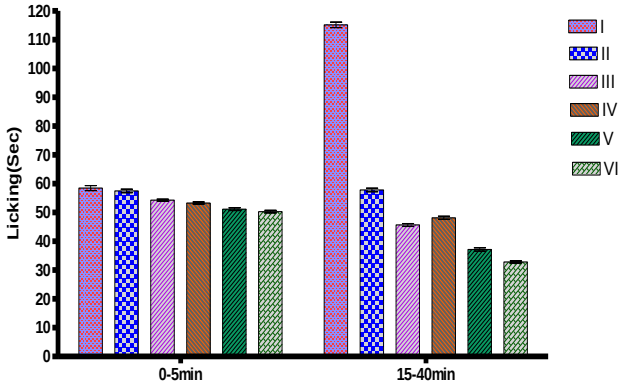
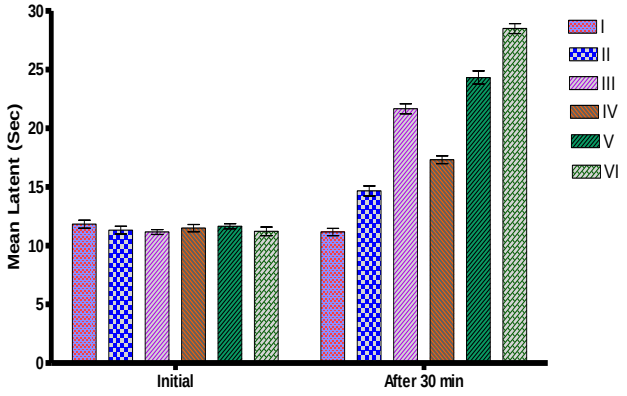


Figure 2. Effect of MEPT & MEPL on Hot plate reaction time in mice



The effects of the extract were significantly ($P < 0.001$) lower than those produced by standard drug (Indomethacin and morphine) in the same tests. The MEPT & MEPL (200 and 400 mg/kg) exhibited a dose-dependent inhibition in both phases of the formalin pain test, but with a less intense effect in the first phase.

The hot plate test has been found to be suitable for evaluation of centrally acting analgesics (Dhara AK *et al*., 2000). It is well known that NSAID's usually do not increase the pain threshold in normal tissues, whereas narcotics drugs and local anesthetics are increase the pain threshold (Ferreira SH *et al*., 1978). In these experiments result indicated that the pharmacological actions were mediated by mu (μ) opioid receptors rather than kappa (κ) and delta (δ) receptors (Schmauss C *et al*., 1984; Aydin S *et al*., 1999).

This showed that the MEPT & MEPL was a weaker opioid receptor agonist. The fact that the neurogenic (post 30 min) algesia was significantly blocked by the extract meant that it also acted through opioid receptors which were more centrally located than peripheral. Due to their central location, a higher therapeutic concentration (400 mg/kg) of the extract was required for the analgesia as revealed by the first phase of formalin-induced pain test.

The effect of MEPT & MEPL could be promoted by a group of substances which were extracted during the preparation of methanolic extract, such as flavonoids, alkaloids, diterpenes and phenolic compounds. Based on these observations it is possible that the antinociceptive effect of MEPT & MEPL in the present study is related, in part, to these active ingredients.

The MEPT & MEPL inhibited both phases of the formalin-induced pain with a more potent effect observed during the second phase. The formalin pain test is very useful for evaluating the mechanism of pain and analgesia (De Lima AB *et al*., 2009; Aydin S *et al*., 1999). Drugs which act mainly centrally, such as narcotic analgesics, inhibit both phases of pain in this model, while peripherally acting drugs, such as aspirin or indomethacin, only inhibit the late phase (De Lima AB *et al*., 2009). The results obtained in this study indicate that the extract possesses analgesic properties which are mediated via peripheral and central inhibitory mechanisms. Finally, this study provides a rationale for the use of these plants in pain disorders in folk medicine.

We used formalin test and eddy's hot plate methods for the determination of analgesic activity. The methanolic extract of whole plants of *Premna tomentosa* Willd and *Premna latifolia* Roxb. possess good analgesic activity by increase in the reaction time (increase threshold potential of pain) may be due to the above mentioned mechanism.

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