

ANTIINFLAMMATORY EFFECT OF *PREMNA LATIFOLIA* LEAVES

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Summary

Inflammatory disorder like arthritis and rheumatism are major group of prevalent diseases. Conventional treatment uses drugs to either control symptoms or relieve the discomfort but can never eliminate the ailment. *Premna latifolia* has been ethno pharmacologically documented to be used in treating dropsy and also used as diuretic. Literature review shows that other species of *Premna* are useful in chronic inflammation. On the basis of traditional claims the present study is planned to evaluate its anti-inflammatory activity. The methanolic extract is prepared and dose of 125 mg/kg, 250 mg/kg and 500 mg/kg body weight are selected and evaluated for antiinflammatory activity in different animal models like paw edema, vascular permeability and cotton pellet granuloma, The phytochemical constituent like iridoid glycosides, diterpenes and saponins are reported to be present in the plant. Thus the present study reveals that the plant *Premna latifolia* may be used in the management of inflammation.

Keywords: *Premna latifolia*, chronic inflammation, vascular permeability

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Introduction

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is body defense reaction in order to eliminate or limit the spread of injurious agent as well as remove the consequent necrosed cells and tissues. Inflammation and repair may be potentially harmful¹. The drugs commonly in use for the treatment of Arthritis include glucocorticoids (for example, cortisone and prednisone), non-steroidal anti-inflammatory drugs (NSAIDs; for example, ibuprofen and naproxen), disease-modifying anti-rheumatic drugs (DMARDs; for example, methotrexate (MTX) and leflunomide), and biological response modifiers. However, besides their high cost, the prolonged use of many of these drugs is associated with severe adverse reactions and toxicity, including some risk of infections in subsets of patients being treated with biological response modifiers². As a result, alternative treatments based on natural plant products and herbal mixtures belonging to complementary and alternative medicine (CAM) are becoming increasingly popular in the US and other countries. *Premna latifolia* is a low bushy tree with trunk up to 1.2 m girth sometimes elliptic, leaves 5-17 cm long, entire, pubescent beneath or both sides. The leaves are diuretic in nature, judicious use of which can mobilize interstitial edema without significant reductions in plasma volume. The milk of the bark is applied to boils³. The leaves of *Premna latifolia* are used in folk medicine for treating dropsy i.e. accumulation of fluid or edema⁴. Also, an infusion of the leaves and coriander in boiling water has been used in acute dropsy. Iridoid glycosides, isolated from other species of *Premna* like *Premna integrifolia*, *Premna tomentosa* and *Premna herbacea* have reported anti-inflammatory activity. Hence, taking into consideration the traditional claims and reported pharmacological activities of *Premna latifolia* the need was felt to assess the therapeutic efficacy of this plant as a useful candidate in treating arthritis.

Plant material

The leaves of *Premna latifolia* were collected from Victoria Botanical garden, Byculla, Mumbai. The medicinal plant specimen was identified and authenticated by and deposited at "Botanical Survey of India" Pune. (Voucher specimen no: VRUPLV1).

Preparation of extract

After 10 days of drying under shed, the leaves were powdered using a mixer. The methanolic extract was prepared by soxhlet extraction method (Hot method). 100 gm of dried powder of leaves was extracted with 1500 ml methanol. The extract was concentrated and dried and suspension is made using acacia.

Drugs and chemicals

Carrageenan, acacia, Evans blue, indomethacin, petroleum ether, methanol were purchased from Research Lab fine chem. Industries, Mumbai.

Animals

Albino male Swiss mice (18-25 g) & Wistar Rat (150-180 g) were housed (5 animals per cage) under the standard laboratory conditions (light period of 12h/day, temperature $25 \pm 2^\circ$ C and humidity $55 \pm 5\%$) with free access to food (standard pellets chow, Lipton, India) and water *ad libitum*. Food but not water was deprived overnight and during the experiment. The experiments were carried out according to the CPCSEA (committee for the purpose of control and supervision of experiments on animals) the national guidelines on the proper care and use of animals. The institutional animal ethics committee (IAEC) approved the experimental protocol.

Preliminary chemical tests

The extract was subjected to preliminary screening, for various active phytochemical constituents such as flavonoids, alkaloids, steroids, carbohydrates, glycosides⁵.

Acute oral toxicity studies

Healthy adult albino mice of either sex, starved overnight were divided into six groups ($n = 6$) were orally fed with the methanolic extract of *Premna latifolia* in increasing dose levels of 100, 500, 1000, 3000 and 5000 mg/kg body weight. The mice were observed continuously for 2 h for behavioral, neurological and autonomic profiles and after a period of 24 and 72 h for any lethality or death⁶.

Carrageenan induced paw edema

The anti-inflammatory activity was determined in rats by measuring the mean increase in hind paw volume after the subplantar injection of inflammatory agents such as carrageenan⁷. Male or female Sprague-Dawley rats with a body weight between 100 and 150 g are used. The animals are starved overnight. To insure uniform hydration they were marked at the tarsal junction to facilitate uniform dipping at subsequent reading. Carrageenan induced rat paw edema is a gold model for screening of acute anti-inflammatory activity of test compound. In the present study, 0.1 ml of 1% carrageenan solution was injected into the left hind paw of the rat. The pretreatment time was 1 hr. before carrageenan injection. The paw volume was recorded at 0, 30, 60, 120 and 180 min by using plethysmometer (UGO Basile 7140). Anti-inflammatory effect of test drug was evaluated by measuring the percentages of inhibition obtained for each group using the following formula:

$$\frac{[(V_T - V_0)_{\text{control}} - (V_T - V_0)_{\text{treated}}]}{(V_T - V_0)_{\text{control}}} \times 100$$

Where V_T is the average volumes for each group after 30, 60, 120 and 180 min
 V_0 the average volume obtained for each group at 0 min after carrageenan injection⁸. Animals were divided into five groups viz, Vehicle Control (10ml/kg), Indomethacin (10 mg/kg) and MEPL (125 mg/kg 250 mg/kg 500 mg/kg)

Cotton pellet induced granuloma

This method is useful for determination of chronic inflammation in which foreign body granulomas were provoked in rats by two autoclaved cotton pellets weighing 10 ± 1 mg were implanted subcutaneously into both sides of the groin region of each rat. The animals were divided into five groups. Group 1 served as control and received the vehicle. The MEPL extract at concentrations of 125, 250 and 500 mg/kg was administered orally daily to three groups of animals for 7 days. Another group received indomethacin daily at a dose of 10 mg/kg orally for 7 days. After 7 days the animals were sacrificed and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60°C, weighed and compared with control⁹. The amount of newly formed connective tissue can be measured by weighing the dried pellets after removal. The average weight of pellets of control group as well as test group is calculated. The percentage change of granuloma weight relative to vehicle control group is determined.

Acetic acid-induced vascular permeability in mice

Test drugs or vehicles were intragastrically administered to mice. One hour later each mouse was given an intravenous injection of 0.5% Evans Blue solution at 0.1ml/10 g body weight followed by an intraperitoneal injection of 0.6% acetic acid at 0.1ml/10 g body weight. Mice were sacrificed by cervical dislocation 30 min after acetic acid injection and the peritoneal cavity of each animal was washed three times with a total of 10 ml of saline. Saline washes from the same animal were combined and centrifuged for 10min. Supernatants were collected and their absorbance at 602 nm was measured with a spectrophotometer. The amount of Evans Blue extruded into the peritoneal cavity was estimated from a standard curve, preparations of various concentration 2,4,6,8,10,12,14,16,18 and 20 ppm Evans blue solution and measured absorbance by spectrophotometer^{10, 11}.

Results

Carrageenan induced edema in rats

Subplantar injection of carrageenan in rats showed a time- dependent increase in paw volume; this increase was observed at 30, 60, 120 and 180 min after administration of carrageenan injection in vehicle treated groups. However, carrageenan induced inflammation was significantly reduced in later phase of the experiment by treatment with MEPL and indomethacin. ($p < 0.05$, $p < 0.01$). Indomethacin 10.0 mg/kg and MEPL at dose of 125, 250 and 500 mg/kg

caused significant inhibition of paw edema, by 51.7,25.8,27.5 and 39.6% respectively, 180 min after carrageenan administration. (Table-2).

Table-1 Effect of MEPL on carrageenan-induced paw edema in rats

Paw volume (ml \pm SEM) in rats						
Groups	Dose mg/kg	0 min	30 min	60 min	120 min	180 min
Control	vehicle	0.94 \pm 0.0103	1.20 \pm 0.0189	1.33 \pm 0.0133	1.44 \pm 0.0173	1.52 \pm 0.0135
Indomethacin	10	0.96 \pm 0.0237	1.10 \pm 0.0162**	1.18 \pm 0.0281**	1.22 \pm 0.0242* *	1.24 \pm 0.0213* *
MEPL	125	0.98 \pm 0.0457	1.21 \pm 0.0291	1.31 \pm 0.0251	1.34 \pm 0.0296*	1.41 \pm 0.0288**
MEPL	250	0.94 \pm 0.0180	1.15 \pm 0.0154	1.23 \pm 0.0224*	1.32 \pm 0.0111**	1.36 \pm 0.0054* *
MEPL	500	0.95 \pm 0.0085	1.12 \pm 0.0212*	1.21 \pm 0.0311**	1.27 \pm 0.0309**	1.3 \pm 0.0321* *

Values are expressed as mean \pm S.E.M ($n = 6$). * $P < 0.05$ * * $P < 0.01$ compared with vehicle control (ANOVA followed by Dunnet's t-test).

Table-2- Percentage edema inhibition by MEPL in rats

% oedema inhibition in rats					
Groups	Dose mg/kg	30 min	60 min	120 min	180 min
Control	vehicle	0.0	0.0	0.0	0.0
Indomethacin	10	46.15	43.58	48	51.72
MEPL	125	11.53	15.38	28	25.86
MEPL	250	19.23	25.64	24	27.58
MEPL	500	34.61	33.3	36	39.65

Cotton pellet-induced granuloma formation in rats

The effect of MEPL on cotton pellet-induced granuloma formation was shown in Table 3. Indomethacin at 10 mg/kg reduced granulomatous tissue formation by

27.1%. MEPL at 125, 250 and 500mg/kg significantly inhibited granulomatous tissue formation by 8.17, 12.9 and 22.3%, respectively. The potency of 500mg/kg MEPL was comparable to that of 10 mg/kg indomethacin. ($p < 0.01$)

Table-3 Effect of MEPL on Cotton pellet induced granuloma in rats

Groups	Dose mg/kg	Granuloma wet weight (mg)	% Reduction	Granuloma dry weight (mg)	% Reduction
Control	vehicle	61.45 ± 0.7621	0	30.23 ± 0.7141	0
Indomethacin	10	37.88 ± 0.4047**	38.35	22.03 ± 0.4357**	27.12
MEPL	125	53.64 ± 0.575**	12.70	27.76 ± 0.3034**	8.17
MEPL	250	49.57 ± 0.4562**	19.33	26.31 ± 0.9475**	12.96
MEPL	500	41.84 ± 0.3492**	31.91	23.46 ± 0.3721**	22.39

Values are expressed as mean ± S.E.M (n = 6). * $P < 0.05$ * * $P < 0.01$ compared with vehicle control (ANOVA followed by Dunnet's t-test).

Acetic acid-induced vascular permeability in mice

As shown in Fig.1, indomethacin at 10 mg/kg inhibited acetic acid-induced dye extrusion into the peritoneal cavity by 54.3%. MEPL produced a dose-dependent inhibitory effect on dye extrusion at 125, 250 and 500mg/kg, the inhibition rates of MEPL were 11.8, 26.3 and 44.5%, respectively as shown in Table 4.

Table-4- Effect of MEPL on acetic acid induce vascular permeability

Groups	Dose mg/kg	Evan's blue (ppm)	% inhibition
Control	Vehicle	13.59±0.3883	0
Indomethacin	10	6.21±0.3634**	54.30
MEPL	125	11.98±0.5289*	11.84
MEPL	250	10.01±0.3246**	26.34
MEPL	500	7.54±0.2140**	44.51

Values are expressed as mean ± S.E.M (n =6). * $p < 0.05$ * * $p < 0.01$ compared with vehicle control (ANOVA followed by Dunnet's t-test).

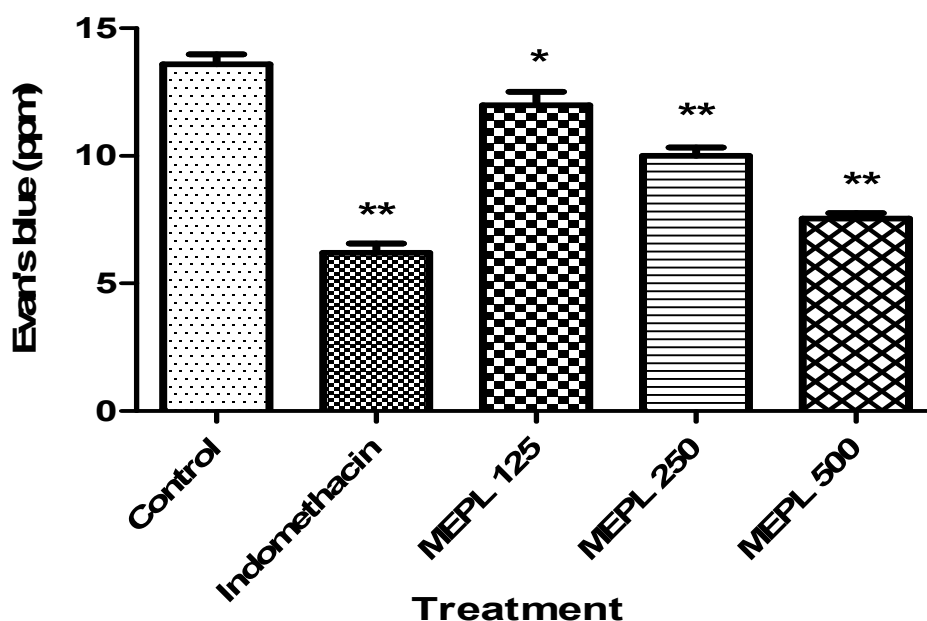


Figure 1. Effect of MEPL on acetic induced vascular permeability in mice

Discussion

Carrageenan-induced paw edema is a useful model in assessing the contribution of mediators involved in vascular changes associated with acute inflammation. In 0-2 hrs after carrageenan injection, there is a release of histamine, serotonin, and bradykinin. The inflammatory edema reached its maximum level at the third hour and after that it started declining. The late phase of the inflammatory response has been shown to be due to the potentiating effect of bradykinin on mediator release and prostaglandins, producing edema after mobilization of the leukocytes¹².

The significant reduction as well as inhibitory effect of the MEPL extract on the carrageenan-induced edema paw volume is an indication of the anti-inflammatory potentials of the plant. The MEPL extract shows inhibition after the third hour indicating an effect on the inhibition of prostaglandin release or biosynthesis. While indomethacin shows significant activity from the first hour indicating effect on both phases of inflammation.

The inflammatory granuloma is a typical feature of established subacute and chronic inflammatory reaction. The cotton pellet granuloma method has been widely employed to assess the transudative, exudative and proliferative component of subacute and chronic inflammation.

The fluid absorbed by the pellet greatly influences the weight of granuloma and dry weight correlate with amount of granulomatous tissue formed^{13, 14}. In order to assess the efficacy of MEPL against proliferative phase of inflammation in which tissue degeneration and fibrosis occur, the widely used cotton pellet granuloma test was employed. During the repair process of inflammation, there is proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels, which are the basic sources of forming a highly vascularised reddish mass, termed granulomatous tissue. MEPL reduced the dry weights of implanted cotton pellets, indicating that it may inhibit the proliferative phases of inflammation.

In the above acute inflammatory models, MEPL showed antiinflammatory activity similar to the positive control drug indomethacin, a known nonselective COX inhibitor. These data suggest that MEPL has an anti-inflammatory property probably like indomethacin, acting through the inhibition of the inflammatory mediators of the acute phase of inflammation. Inhibition of the cotton pellet-induced granuloma formation may suggest that MEPL exerts the anti-inflammatory activity also through its possible antiproliferative effect.

The vascular permeability was induced by acetic acid, which causes an increase in peritoneal fluids of prostaglandin E2 (PGE2), prostaglandin F2 α (PGF2 α), serotonin, and histamine. This leads to a dilation of the capillary vessels and the increase in vascular permeability. As a consequence, fluid and plasma proteins are extravagated, and edema forms^{15,16}.

MEPL markedly inhibited the acetic acid-induced increase in vascular permeability in mice. This result suggests that MEPL may effectively suppress the exudative phase of acute inflammation.

Conclusion

The results of the study have demonstrated that *Premna latifolia* possesses antiinflammatory activity on the animal models investigated. This provides a rationale for its use in traditional medicine for the management of inflammation and contains pharmacologically active substance(s) with antiinflammatory activity.

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