



**ANTI INFLAMMATORY AND ANALGESIC  
POTENTIAL OF METHANOLIC EXTRACT OF  
LEPTADENIA PYROTECHNICA**

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**Abstract**

In the present study the anti-inflammatory and analgesic potential of methanolic extract of *Leptadenia pyrotechnica* was investigated. The methanolic extracts of *Leptadenia pyrotechnica* were ingested orally in the form of suspension in two different doses, 100 and 200 mg/kg body weight. The anti-inflammatory effect of *Leptadenia pyrotechnica* was observed in carragenan -induced paw oedema in wistar albino rats and formalin-induced paw oedema in swiss albino mice and compared with the standard, indomethacin (5 mg/kg body weight). The analgesic effect was evaluated in swiss albino mice by Eddy's hot plate method and compared with the standard, aspirin (25 mg/kg body weight). The methanolic extract of *L. pyrotechnica* produced significant reduction ( $p \leq 0.01$ ) in inflammation i.e. 64.65 % (100 mg/kg body weight) and 76.75% (200 mg/kg body weight) as compared to the standard drug, indomethacin, which was 86.88%. The analgesic effects, produced a significant ( $p < 0.01$ ) reduction in the paw licking and jumping response for *Leptadenia pyrotechnica* (200 mg/kg) and aspirin (25 mg/kg) when compared to control. These results indicate that the extracts have analgesic and anti-inflammatory properties.

**Keywords:** - : Anti-inflammatory, Analgesic, Indomethacin, Aspirin, *Leptadenia pyrotechnica*.

**INTRODUCTION**

Plants are the rich sources of medicines. Several thousands of medicinal plants are available in the different bioclimatic zones. Inflammation include rheumatoid arthritis are still one of the main health problems of the world's population. Modern drugs are used to treat these disorders but their continuous use may cause severe adverse effects.<sup>1</sup> consequently there is a need to develop new anti-inflammatory agents with minimum side effects. The use of natural remedies for the treatment of inflammatory and painful conditions has a long history, starting with Ayurvedic treatment. The herbal drugs are playing a vital role in the management of inflammatory diseases.<sup>2</sup> *Leptadenia pyrotechnica* (Forsk.) belonging to family Asclepiadaceae, commonly known Khimp. It is an erect, ascending, leafless shrub up to 0.5 meter to 2.6 meter high with green stem and yellowish green alternating much branched with a valuable desert plant.<sup>3</sup>

*Leptadenia pyrotechnica* is an erect, ascending, shrub up to 0.5 meter to 2.6 meter high with green stem and pale green alternating bushy branches with watery sap.

Leaf is rarely found and are deciduous when present are 2.6-6.5 X 0.2-0.3 cm, sessile, narrowly linear to linear lanceolate, caduceus.<sup>5</sup> Flowers are in cluster lateral umbellate cymes, greenish yellow. Corolla lobes valvate, outer corona is of 5 scales, stamina corona of raised undulate fleshy ring.<sup>6</sup> Each flower is bisexual pentamerous actinomorphic, sepals joined as base only, corolla sympetalous.<sup>7</sup> Follicles 7.0-14.0X0.5-0.8 cm, terete, lanceolate, tapering to slender beak, glabrous.<sup>8</sup> Seeds are 5-7 mm long, ovate lanceolate, glabrous, hairy with tufted hairs 2.6-3.7 cm long.<sup>9</sup> Flowering and fruiting occurs from August to January.<sup>10</sup>

**MATERIALS AND METHODS**

**Collection And Authentication Of Plant Material**

Fresh plant of *Leptadenia pyrotechnica* was collected in the month of April from Venkateswara University, Tirupati, Andhra Pradesh, India. The plant was identified and authenticated by Dr. K. Madhava Chetty, Asst. Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. A voucher specimen has been deposited at the College of Pharmacy, TMU Moradabad

(TMU/Consult/2011-12/206) for future reference.

### PREPARATION OF THE EXTRACT

Fresh whole plant along with roots were shade dried reduced to moderately coarse powder, loaded into soxhlet extractor and was subjected to successive extraction with Petroleum Ether, Benzene, Chloroform, Methanol and distilled water by hot extraction using Soxhlet apparatus at a temperature of 60°C. The extracts were concentrated under reduced pressure using a rotary vacuum evaporator to constant weight and preserved in desiccator for further studies.

### ANIMALS

Wistar albino male rats (200-250 g) and Swiss albino mice 25-30 g, were grouped and housed in polyacrylic cages and maintained under standard laboratory conditions (temperature 24-28°C, RH, 60-70% and 12 h light dark cycles). All the experimental procedures and protocols involving animals were reviewed by the Institutional Animal Ethics Committee in accordance with the guidelines of CPCSEA.

### CARRAGENAN - INDUCED PAW OEDEMA IN RATS

Anti-inflammatory activity of *Leptadenia pyrotechnica* was assessed by carragenan induced paw oedema method.<sup>11</sup> Rats were divided into 4 groups. Animals of all the groups were injected with 0.1 ml of 1% carragenan in 0.9% normal saline, under the plantar aponeurosis of the right hind paw. Group I animals (carragenan control) received methanolic extract suspension in 0.5% of Tween 80 p.o., 30 min prior to carragenan injection. Group II, the standard reference group was given p.o., an aqueous solution of indomethacin (5 mg/kg), 30 min prior to carragenan injection. Group III and Group IV received p.o., 100 and 200 mg/kg of *Leptadenia pyrotechnica* methanolic extract suspension in 0.5% of Tween 80, 30 min prior to carragenan injection, respectively. The paw volume of the rats was measured by plethismograph just before and 3 h after carragenan injection. The anti-inflammatory activity was determined as the percentage of inhibition of inflammation. The percentage inhibition was calculated by using the formula.

$$\% \text{Inhibition} = \frac{\text{Mean paw inflammation of test}}{\text{Mean paw inflammation of control}} \times 100$$

### FORMALIN INDUCED OEDEMA

Anti-inflammatory activity was evaluated by formalin induced paw oedema method. Swiss albino mice were divided in to 4 groups. Animals of all the groups were injected with 0.1 ml of 1% formalin in 0.9% normal saline, under the plantar aponeurosis of the right hind paw. Group I animals (formalin control) received a plain aqueous suspension in 0.5% of Tween 80 p.o., 30 min prior to formalin injection. Group II, the standard reference group was given p.o., an aqueous solution of indomethacin (5 mg/kg), 30 min prior to formalin injection. Group III and Group IV received p.o., 100 and 200 mg/kg of *Leptadenia pyrotechnica* methanolic extract suspension in 0.5% of Tween 80, respectively, 30 min prior to formalin injection. The paw volume of the rats was measured by plethismograph just before and 3 h after formalin injection. The percentage inhibition of the oedema was calculated for each the vehicle treated control group.

### EDDY'S HOT PLATE METHOD

Analgesic effect of *Leptadenia pyrotechnica* was assessed by the Eddy's hot plate in Swiss albino mice. Mice were divided into 4 groups. Group I control received a plain aqueous suspension in 0.5% of Tween 80 (0.5 ml) p.o., 20 min prior to placement of the animal in hot plate. In group II the standard control group received a single dose of aspirin 25 mg/kg, before 20 min to hot plate. Group III and Group IV received a single dose of 100 and 200 mg/kg of *Leptadenia pyrotechnica* methanolic extract suspension in 0.5% of Tween 80, respectively, before 20 min to hot plate.<sup>12</sup> The number of paw licking and jumping per animal recorded during the 20 min period

### STATISTICAL ANALYSIS

The calculation of the average oedema for the anti-inflammation and number of paw licking and jumping were based on the expression of numerical data as mean  $\pm$  S.D. The statistical significance between control and treated groups were analyzed using analysis of variance (ANOVA), where  $p \leq 0.01$  were taken to be significant.<sup>13</sup>

### RESULTS AND DISCUSSION:

Oral administration of *Leptadenia pyrotechnica* significantly inhibited ( $p \leq 0.01$ ) the carragenan induced paw oedema in rat at both doses (100 and 200 mg/kg) studied. At 100 mg/kg dose, 64.65% inhibition and at 200 mg/kg dose, 76.75% inhibition was observed. The group treated with indomethacin (5 mg/kg) showed maximum inhibition of oedema, which was 86.88% as

shown in Table 1. Oral administration of *Leptadenia pyrotechnica* significantly inhibited the formalin induced paw oedema in mice at both doses (100 and 200 mg/kg) and indomethacin showed maximum inhibition of oedema in Table 2. Animal produced paw licking and jumping in the control group, and *Leptadenia pyrotechnica* at both doses used in the study significantly inhibited the jumping and licking response in mice. Aspirin at 25 mg/kg dose was significantly reduced the paw licking and jumping response when compared to the control Table 3.

Inflammation is a complex process and various mediators e.g. prostaglandins, leukotrienes and kinins, platelet activating factor, etc. have been reported to be involved in the development of inflammatory diseases. Carrageenan assay is well studied for comparative bioassay of anti-inflammatory agents, since the relative potency estimates obtained from most drugs tend to reflect clinical experience.<sup>14</sup> The time course of oedema development on carrageenan induced paw oedema model in rats is generally represented by a biphasic curve. The first phase occurs within an hour of injection and also due to the serotonin component.<sup>15</sup> Prostaglandins play a major role in the development of the second phase of reaction which is measured around 3 h time.<sup>16</sup> The presence of prostaglandin in the inflammatory exudates from the injected foot has been well demonstrated previously by other workers.<sup>17</sup>

The carrageenan induced paw oedema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit cyclooxygenase involved in prostaglandin synthesis.<sup>18</sup> Based on these reports, it is inferred that the inhibitory effect of *Leptadenia pyrotechnica* on carrageenan induced inflammation in rats in the present study may be due to inhibition of the enzyme cyclooxygenase, leading to inhibition of prostaglandin synthesis. Based on these reports, it is inferred that the inhibitory effects of *Leptadenia pyrotechnica* on carrageenan induced inflammation in rats in the present study may be due to inhibition of the enzyme cyclooxygenase, leading to inhibition of prostaglandin synthesis. Basal reaction time is recorded as mentioned in the method using analgesiometer.

Here the reaction may be hind paw licking or jump response. Hind paw licking appears within 4-6 sec and after 2-3 sec jumping may start. One has to observe both these response before and after administration of drug like Morphine (5 mg/kg). It is well known that inhibition of formalin-induced paw oedema in rats is one of the most suitable test procedure to screen anti-inflammatory agent as it closely resembles human arthritis injection of formalin subcutaneously into hind paw of rats produces localized inflammation and pain. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response. Thus formalin induced arthritis is a model used for the evaluation of an agent with probable antiproliferate activity. This experiment is associated with the proliferate phase of inflammation.

**Table 1**

Evaluation of anti-inflammatory effect of methanolic extract of *Leptadenia pyrotechnica* (MELP) on carrageenan induced paw oedema in Wistar albino rats.

S. N.	Treatment Groups	Dose	Difference in paw volume at 3h	Percentage inhibition of oedema
1	Carrageenan control	--	0.43±0.01	--
2	Indomethacin	5 mg/kg	0.04±0.02**	86.88
3	MELP	100 mg/kg	0.14±0.05**	64.65
4	MELP	200 mg/kg	0.10±0.01**	76.75

All values are given in mean±SD, (n=6) ANOVA \*\*p<0.01, when compared to carrageenan control group

## CONCLUSIONS

The findings of the present study have demonstrated that *Leptadenia pyrotechnica* has potent anti-inflammatory and analgesic activity and justify its use in traditional medicine to treat inflammatory and painful conditions. The results also furnish evidence that the beneficial effects of this plant may be due to its free radical scavenging activity.

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**Table 2**

Evaluation of anti-inflammatory effect of methanolic extract of *Leptadenia pyrotechnica* (MELP) on formalin induced paw oedema in Swiss albino mice.

S.N.	Treatment groups	Dose	Oedema diameter (cm)			
			1h	2h	3h	4h
1	Normal saline	10 mL/kg	0.72±0.16	0.80± 0.12	0.77±0.1	0.77± 0.12
2	Indomethacin	5 mg/kg	0.58±0.04	0.60±0.04	0.60±0.02	0.62±0.7
3	MELP	100 mg/kg	0.42±0.04**	0.39±0.07**	0.38±0.07**	0.38±0.69**
4	MELP	200 mg/kg	0.40±0.07**	0.34±0.05***	0.34±0.60**	0.34±0.6**

All values are given in mean±SD, (n=5) ANOVA \*p<0.05, \*\*p< 0.01, \*\*\*p<0.001, when compared to normal saline group.

**Table 3**

Evaluation of analgesic effect of methanolic extract of *Leptadenia pyrotechnica* (MELP) by Eddy's hot plate method.

S.N.	Treatment groups	Dose	Reaction time			
			0 min	30 min	60 min	120 min
1	Control	--	2.0±0.1	2.1±0.2	2.32±0.21	2.27±0.1
2	Aspirin	25 mg/kg	3.1±0.2	7.5±0.3	10.1±0.3**	12.8±0.80**
3	MELP	100 mg/kg	2.4±0.3	2.6±0.1	4.31±0.3333	4.7±0.7*
4	MELP	200 mg/kg	2.1±0.5	4.6±0.2*	7.32±0.3333**	8.7±0.5**

All values are given in mean±SD, (n=6) ANOVA \*p<0.05, \*\*p< 0.01, \*\*\*p<0.001, when compared to control group

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