

## Analgesic Activity of *Phyllanthus lawii* Extract in Swiss Albino Mice

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**Abstract:** The analgesic activity of the methanol extract of *Phyllanthus lawii* (MEPL) was investigated in Swiss albino mice with hot plate and acetic acid induced writhing models. The results suggested that MEPL possess potent analgesic activity. The MEPL produced a significant decrease in mice writhing induced by acetic acid suggesting peripheral analgesic activity and elevated mean basal reaction time in hot plate method suggesting central analgesic activity. The results are in agreement with its traditional use for pain treatment of *P. lawii*.

**Keywords:** *Phyllanthus lawii*, methanol extract, acetic acid induced, analgesic activity, swiss albino mice.

### INTRODUCTION

Some species of *Phyllanthus* L. (Euphorbiaceae) are reported to be bitter, astringent, stomachic, diuretic, febrifuge, and antiseptic and have been used as hepatoprotective agents in Ayurvedic medicine [1]. The chemical and pharmacological properties and clinical studies of some *Phyllanthus* species have been summarized in a review article [2]. Of the *Phyllanthus* market samples, about 76% contains *P. amarus* Schum. & Thonn. and the remaining percentage is shared by several other species [3]. *Phyllanthus lawii* Grah. is a glabrous shrub usually growing along the bank of rivers towards the Konkan Ghats. It is also found in Nilatwar near Kaladghi, bank of the Gatpraba River, Bihar, Central Provinces and Western Peninsula [4]. Treatment of *P. lawii* together with powdered sugar candy is taken orally for 7 days in stomach and other cancers barring brain and lung cancer [5]. The aerial parts of *P. lawii* was tested for hepatoprotective activity against CCl<sub>4</sub> in rats and it has shown significant activity, lowering the serum enzymes like SGOT and SGPT in rats intoxicated with CCl<sub>4</sub> [6]. Diuretic activity in rats and hypothermic effect in mice of *P. lawii* has been reported earlier [7]. The present study is to investigate and evaluate the pharmacological basis for the use of *P. lawii* in the folk medicine for the treatment of pain.

### MATERIALS AND METHODS

#### Plant Material

The aerial parts of the plant *Phyllanthus lawii* was collected from Mangalore of Karnataka, India in December 2007. The plant material was identified and authenticated by

Dr. Gopalkrishna Bhat, Department of Botany, Poorna Pragna College, Udupi, Karnataka, India. A voucher specimen has been kept in the Herbarium of the college. A voucher specimen CH/NGSM-IF 47 is deposited in the Herbarium of NGSM Institute of Pharmaceutical Sciences.

#### Extraction

The plant material was dried in shady place and then powdered with a mechanical grinder and stored in airtight container. About 500 g of the powder was extracted using soxhlet apparatus for 12 h using 5.0 L of methanol as solvent. The extract was made free from solvent by keeping it on water bath at 50-60°C for about 6 h and gave a yield of 9.25%.

#### Animals

Male Swiss albino mice weighing 20-25 g were obtained from an animal colony of NGSM Institute of Pharmaceutical Sciences, Paneer, Deralakatte, Mangalore, India. They were kept in departmental animal house in polypropylene cages in an air-conditioned area at 25±2°C with 10:14 h light and dark cycle and maintained on Amrut brand balanced animal feed and water ad libitum. The experimental protocols were approved by the Animal Ethical Committee of Nitte University.

#### Chemicals

The drugs and chemicals used were Aspirin (Research Lab, Mumbai), gum acacia (SD fine chemicals Limited, Mumbai), pentazocin (Pure Pharma Ltd, Mumbai) and Methanol (BDH, Mumbai).

#### Toxicity Assay

The LD<sub>50</sub> was determined using the graphical method [8] in mice. Briefly, geometric doses of the extract (100–2,000 mg/kg) were administered i.p. to 10 groups of mice each

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consisting of six animals. Control group received normal saline (5 mL/kg i.p.). Signs of toxicity and mortality within 24-72 h were noted. Confirmatory test was carried out and the LD<sub>50</sub> was calculated from the graph of percent mortality against profit log dose of the extract.

## Analgesic Activity

### Hot plate Method (Thermal Stimulation)

In the hot plate method, albino mice (20-25 g) were divided into 3 groups each consisting of six animals. One group served as negative control (received 5% gum acacia 5mL/kg. Second group served a positive control (pentazocin 5 mg/kg). While the third received MEPL (200 mg/kg, p.o.). The basal reaction time was noted 1, 2, and 3 h after the treatments [9].

### Acetic acid Induced Writhing Test (Chemical Stimulation)

In acetic acid induced writhing model [10], albino mice (20-25 g) were divided into three groups each consisting of six animals. One group served as negative control (received 2% gum acacia mL/kg), Second group served as positive control (received aspirin 100 mg/kg), while third group received MEPL (200 mg/kg) orally. The writhing movements were observed and counted for 30 min after acetic acid administration.

## Statistical Analysis

Statistical analysis of data was presented using Student's t test [11].

## RESULTS AND DISCUSSION

The MEPL treatments (100–2,000 mg/kg) did not cause significant changes in behavior or weight of mice. The extract is considered to be of low toxicity according to the reference of Lorke [12]. The results of hot plate test indicated a

significant increase in reaction time at 2 and 3 h with 200 mg/kg of MEPL whereas reference drug pentazocin significantly increased the reaction time at 1 to 2 h (Table 1). In acetic acid induced writhing test, MEPL (200 mg/kg) reduced writhing count significantly, inhibiting pain by 69.00% compared to control (Table 2).

Pain and inflammation are associated with pathology of various clinical conditions like arthritis, cancer, and vascular diseases [13]. In various traditional medicinal systems a number of natural products are used to relieve the symptoms of pain. The MEPL exhibited a significant analgesic activity in different animal models of pain. The hot plate method has been found to be suitable for evaluation of centrally acting analgesics [14]. The nociceptors seem to be sensitized by sensory nerves. The involvement of endogenous substances such as PGs may be minimized by this model. In centrally acting analgesic methods, the extract of 200 mg/kg dose was found to be significantly effective. Acetic acid induced abdominal contraction method has been used to evaluate peripherally acting analgesics. In acetic acid induced method pain is generated indirectly via endogenous mediators like prostaglandin, which stimulates peripheral nociceptive neurons. These neuronal fibers are sensitive to both narcotics and non steroidal anti-inflammatory drugs [15]. MEPL inhibited the acetic acid induced pain with potency compared to the aspirin. The standard drug aspirin produced inhibition of writhing movement inhibiting peripheral pain induced by direct action of acetic acid. MEPL is dominated by pentacyclic triterpenoids [16]. Pentacyclic triterpenoids have potent analgesic activity [17]. Triterpenoids are reported to be inhibitors of NF- $\kappa$ B signaling [18] just like aspirin and other NSAIDs [19]. These evidences reveal that pentacyclic triterpenoids may be responsible for the analgesic activity of MEPL.

## ABBREVIATIONS

**Table 1. Effect of the Methanol Extract of *Phyllanthus lawii* (MEPL) on Thermal Stimulus Induced Pain (Hot Plate Test) in Mice**

Treatment	Dose (mg/kg)	Reaction Time (Seconds)			
		0	1 h	2 h	3 h
Control (5% gum acacia)	5	4.45±0.11	5.11±0.12	5.12±0.15	4.55±0.05
Pentazocin	5	4.62±0.22	9.33±0.25**	11.34±0.34**	8.23±0.24**
MEPL	200	4.36±0.64	6.47±0.46*	9.47±0.45**	7.89±0.12**

\*\*P<0.001; \*P<0.05 when compared to control. Values are expressed as mean±SEM.

**Table 2. Effect of the Methanol Extract of *Phyllanthus lawii* (MEPL) on Acetic acid Induced Writhing in Mice**

Treatment	Dose	Number of Writhes (Per 30 Min)	Percentage Inhibition of Writhes
Control (5% gum acacia)	5 mL/kg	89±0.22	—
Aspirin	50 mg/kg	24±0.45**	74.00
MEPL	200 mg/kg	31±0.24**	69.00

\*\*P<0.001 when compared to control. Values are expressed as mean±SEM.

MEPL	=	Methanol extract of <i>Phyllanthus lawii</i>
NGSM	=	Nitte Gulabi Shetty Memorial
SEM	=	Standard Error Mean
NSAIDS	=	Nonsteroidal antiinflammatory drugs

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