

STUDIES ON THE ANTI-INFLAMMATORY, ANALGESIC AND
ANTIPYRETIC PROPERTIES OF *PAVONIA ZEYLANICA*

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Summary

The purpose of this investigation was to study the anti-inflammatory, analgesic, and anti-pyretic properties of methanol extract of *pavonia zeylanica* in rats and mice. The plant material was extracted with methanol. Dose of 200, 400 mg/kg of each extracts were used in carrageenan-induced paw edema, cotton-pellet granuloma in rats, writhing nociception in mice, and yeast induced hyperpyrexia in rats. All compounds reduced paw edema in comparison to the control group at 5 h post carrageenan injection. The methanol extract of *p.zeylanica* were similar to phenylbutazone in reduction of paw edema and cotton-pellet granuloma. The extract as well as Paracetamol induced antinociception in writhing test in comparison to control. Positive results for flavanoids and phenolic compounds were investigated by phytochemical analysis of the extract. The higher antinociception effects of the extract might be due to the presence of flavanoids, and phenol compounds. The methanolic extract produced a significant dose dependent inhibition of temperature elevation. These data suggest that the extract of *p.zeylanica* produce antinociceptive, anti-inflammatory, and anti-pyretic activities that could be due to the effect of one or a combination of the bio active components in each extract.

Key words: *pavonia zeylanica*, anti-inflammatory, antinociception, anti-pyretic, mouse and rats.

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Introduction

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Herbal therapy is used to treat a large variety of ailment and symptoms, e.g. inflammation, fever, and pain; however, there are no adequate experimental evidences about their effectiveness [1-4]. Some species of *pavonia* like *pavonia odorata* have been reported to possess astringent, anodyne properties and is helpful in bronchitis, swellings, and itches [5]. The group of flavanoid is famous for its anti-inflammatory, anti-allergic, antithrombotic, vasoprotective, and protection of gastric mucosa properties. These properties have been attributed to influence of flavanoids on production of prostaglandins and their antioxidant effects [6]. Till now *p.zeylanica* has not been the subject of any pharmacological research. Therefore, aim of this study was to carry out a pharmacological evaluation of methanol extract of *p.zeylanica* for its anti-inflammatory, analgesic, and antipyretic properties.

Materials and Methods

Plant material:

The fresh aerial parts of *p.zeylanica* were collected during the flowering stage from Siddha and Ayurvedic Medicines India Pvt Ltd, Erode Dist, Tamil nadu, in Aug 2006, and identified by G.V.S. Murthy, Botanical Survey of India, Coimbatore, Tamil nadu, voucher specimen [No.BSI/SC/5/23/06-07/TECH.835] has been deposited at the Herbarium of the Department of Pharmacology, Bharathi College of Pharmacy, Karnataka. The plant material was air dried, powdered and extracted twice with methanol (80%) in percolator. The combined methanol extracts were evaporated to dryness under reduced pressure.

Animals:

In breed Albino male Wistar rats(150-200 g) and Swiss Albino mice(20-25 g) were used for the experiments. All the animals were obtained from the laboratory animal centre, Bharathi College of pharmacy, Karnataka. The animals were maintained under standard environmental conditions and fed with standard diet and water *ad libitum*. The experimental was approved by the Experimentation Ethics Committee (1135/a/07/CPCSEA).

Drugs and Chemicals:

The drugs and fine chemicals were purchased from Sigma Chemical Co., St. Louis, USA. All other chemicals and solvents were obtained from local firms (India) and were of highest purity and analytical grade.

Studies on inflammation

Acute inflammation study

Carrageenan-induced paw oedema in rats:

Pedal inflammation in male Wistar rats (150-200 g) was produced according to the method described earlier [7]. An injection (s.c.) was made of 0.1 ml of 1% carrageenan into the right paw of each rat under the sub plantar aponeurosis. The test groups of rats were administered intraperitoneally with 200 and 400 mg/kg of the methanol extract of *p.zeylanica* 1 h before carrageenan injection.

At the same time, the control group received 5 ml/kg of 5% gum acacia and the reference group received 100 mg/kg phenyl butazone (i.p.). The paw value was measured immediately after carrageenan injection and at 1,2,3,4 and 5 h intervals after the administration of the edematogenic agent using a plethysmograph-apparatus up to the anatomical hairline on lateral malleolus [8], and compared with the control animals, which received only the vehicle. The inhibitory activity was calculated according to the following formula [9].

$$\text{Percent inhibition} = 100 - \frac{(\text{oedema volume in the treated})}{(\text{oedema volume in the control})} \times 100$$

Sub-acute inflammation study

Cotton-pellet granuloma in rats:

This study was carried out by cotton-pellet implantation method in rats [10]. This method used here was adopted from Sheth et al., (1972) [11], with a slight modification of using only male rats [12]. Under light ether anaesthesia, sterile cotton-pellets (10 mg) were implanted subcutaneously in the axilla and groin regions of the rats. The animals were treated orally with methanol extract at different doses of *p.zeylanica* (200 and 400 mg/kg) daily for 7 consecutive days. Animals in the control group received either normal saline or the vehicle gum acacia. Phenyl butazone (100 mg/kg, orally) was given to animals in the reference groups.

They were sacrificed on day 8, the cotton-pellet removed, freed from extraneous tissue and dried overnight at 60 °C and weighed. The percent inhibition of the dry weight of the granuloma were calculated and compared.

Antinociceptive activity

Effect on acetic acid-induced writhing in mice:

Analgesic activity was evaluated on the acetic acid-induced writhing according to Koster et al. (1959) [13]. The writhes were induced by intra-peritoneal injection of 0.6% acetic acid (v/v) (10 ml/kg). Two different doses (200 and 400 mg/kg) of the extract of *p.zeylanica* were administered orally to different groups of six animals each, 30 min before chemical stimulus. The numbers of writhing movements were counted 10, 20, 30, and 40 min after acetic acid injection. Antinociception (analgesia) expressed as the reduction of the number of abdominal constrictions between control animals (acetic acid treated mice) and mice pretreated with the extract.

Antipyretic activity

Yeast induced hyperthermia: Four groups of six rats each were injected subcutaneously with 10 ml/kg b.wt. Yeast suspension (15% aqueous suspension) to induce pyrexia, after measuring the basal rectal temperature (0°C) of each animal. Nineteen hours after yeast injection, the rectal temperature was recorded again and animals showing a rise in temperature of <0.6°C were discarded [14]. Thereafter, treatment was carried out as follows:

Group I: Distilled water (10 ml/kg; p.o.),

Group II-Methanol extract of *p.zeylanica* (200 mg/kg: p.o)

Group III- Methanol extract of *p.zeylanica* (400 mg/kg: p.o)

Group IV: Paracetamol (100 mg/kg: p.o.).

Rectal temperatures were then recorded at 20, 21, 22, 23 and 24h (T°C) after yeast injection.

Statistical analysis:

The data were analyzed for significance using the unpaired two-tailed student's t-test

Results

Studies on inflammation

Acute inflammation study

Carrageenan – induced paw oedema in rats:

Carrageenan – induced rat paw oedema was markedly inhibited by intraperitoneal treatment with either the extract (200, 400 mg/kg) or phenyl butazone (100 mg/kg). The extract of *p.zeylanica* showed highly significant ($p < 0.001$) acute inflammatory effect in a dose related manner, more or fewer equals to the effect were produced by phenyl butazone. The results were shown in the Fig-1.

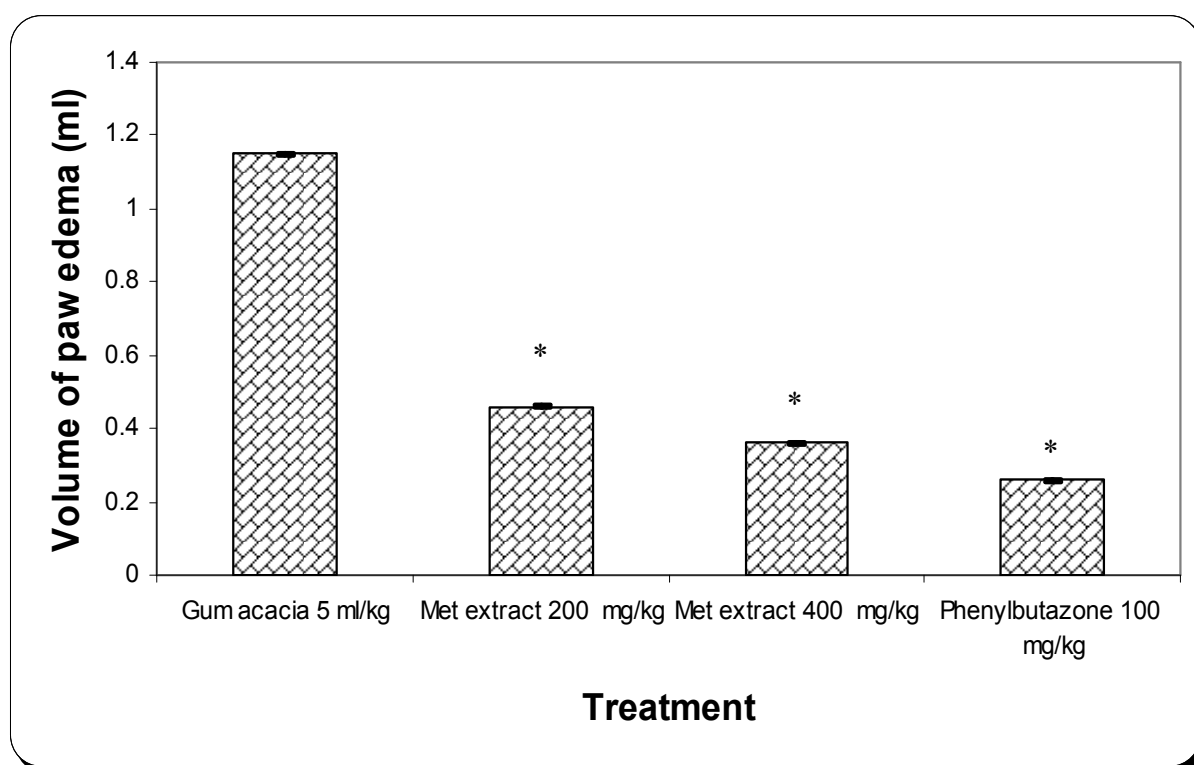


Fig 1: Effect of *p.zeylanica* on Carrageenan induced Paw edema in rats

Sub- acute inflammation study

Cotton Pellet granuloma in rats:

In sub acute studies Methanol extract of *p.zeylanica* shows highly significant sub acute anti-inflammatory effect (Fig-2).

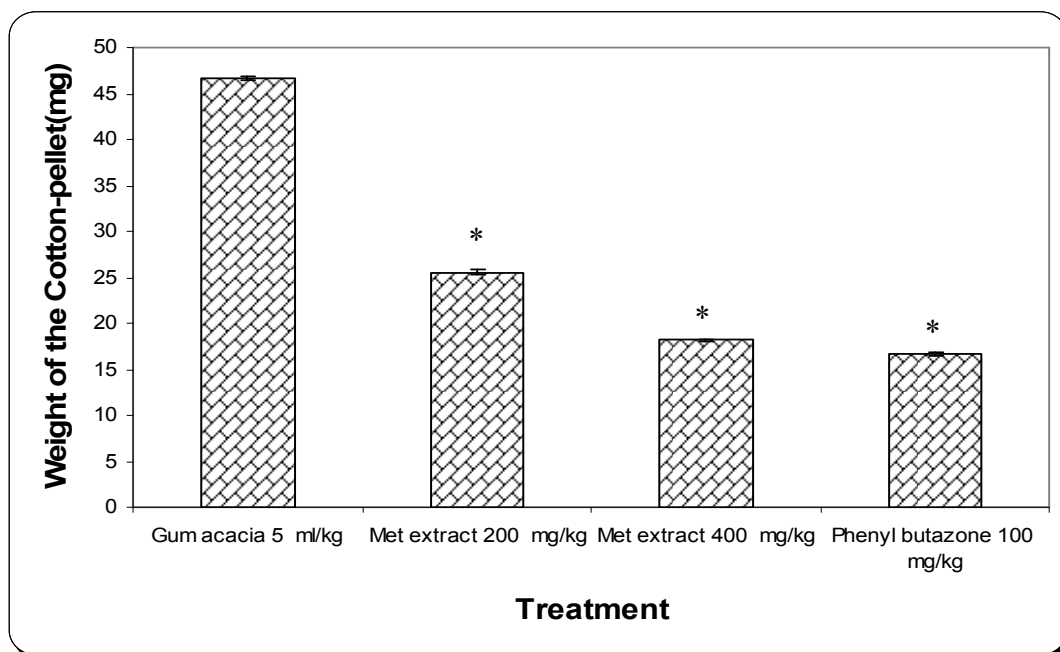


Fig 2: Effect of *p.zeylanica* on Cotton-pellet granuloma in rats.

Antinociceptive activity

Acetic acid induced writhing in mice:

The Methanol extract of *p.zeylanica* (200-400 mg/kg, i.p.) were significantly reduced ($p < 0.001$) acetic acid-induced abdominal constrictions and stretching of hind limbs in a dose-dependent manner (Fig-3).

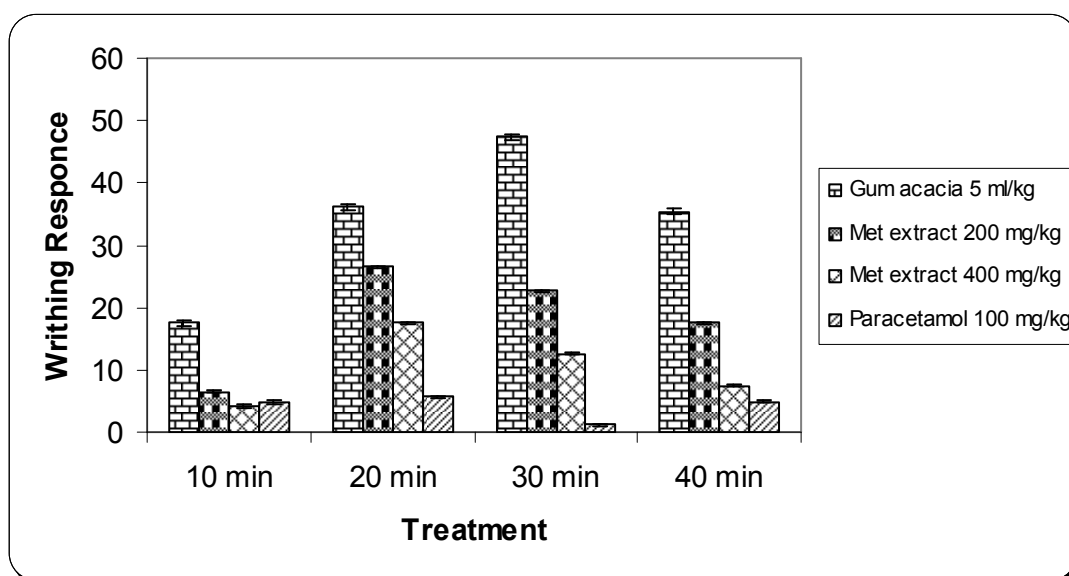


Fig 3: Effect of *p.zeylanica* on Acetic acid induced writhing in mice

Antipyretic activity:

As shown in (Fig-4) subcutaneous injection of yeast caused elevation of rectal temperature in control rats 19h after administration. Oral administration of the extract produced a significant ($P < 0.001$) dose dependent inhibition of temperature elevation. Peak inhibitory effect was observed at 1 h post-therapy, i.e., 20 h post-yeast injection ($p < 0.001$).

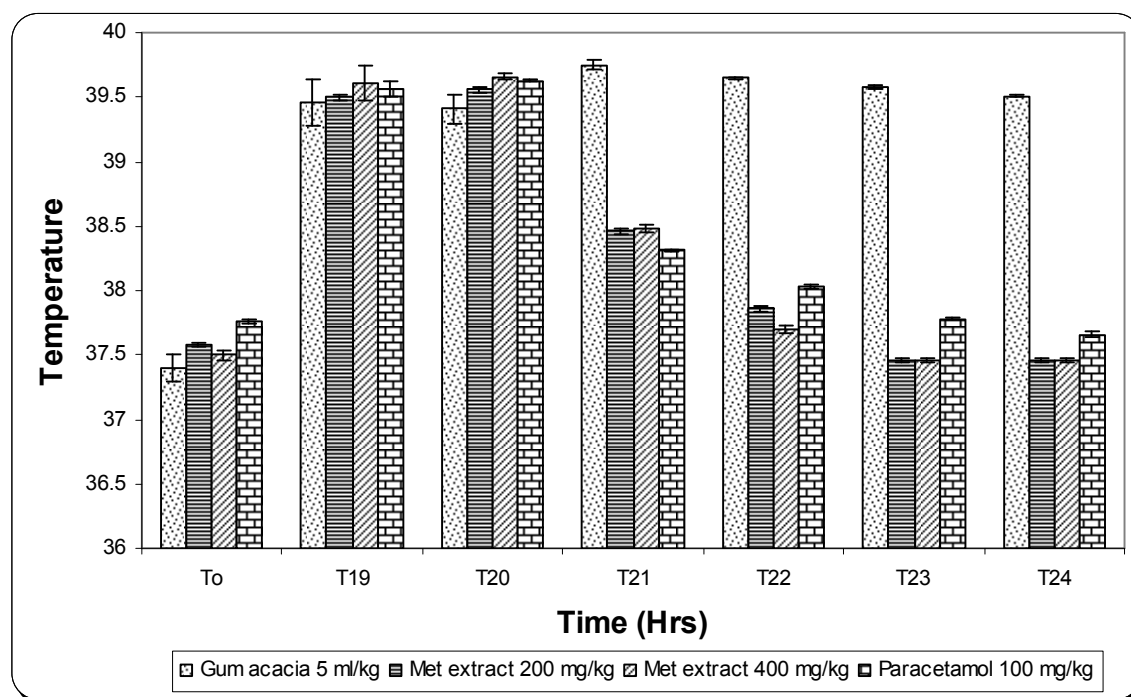


Fig 4: Effect of *p.zeylanica* on yeast induced Hyperpyrexia in rats

Discussion

Among several traditional claims, the usefulness of *p.zeylanica* in fever, inflammation and pain have been emphasized more in literature. Hence it was considered that investigations for these medicinal properties might give scientific authentication to the traditional claims. Moreover, this plant has not been subjected to above mention systemic pharmacological screening so far.

In the present study, the anti-inflammatory, analgesic and anti-pyretic activity of the Methanol extract of *p.zeylanica* has been established. The test extract at two different doses (200-400 mg/kg) were found to significantly inhibit the Carrageenan-induced rat paw oedema, a test, which has significant predictive value for anti-inflammatory agents acting by inhibiting the

mediators of acute inflammation[15]. Odema formation due to carrageenan in the rat paw is the biphasic event[16]. The initial phase is attributed to the release of histamine and serotonin. The second phase of oedema is due to release of prostaglandins, protease and lysosome [17]. The second phase is sensitive to most clinically effective anti-inflammatory drugs [18]. Besides in the carrageenan-induced rat paw oedema model the production of protanoides has been through the serum expression of COX-2 by a positive feedback mechanism [19]. Therefore, it is suggested that the mechanism of action of test extract may be related to prostaglandin synthesis inhibition, as described for the anti-inflammatory mechanism of non-steroidal anti-inflammatory drugs in the inhibition of inflammatory process induced by carrageenan.

Like wise, the granulomatous tissue formation is related to the chronic inflammatory process, which is characterized by several phases [20]. In this regard, the oral treatment with 200, 400 mg/kg of the extract of *p.zeylanica* and 100 mg/kg of phenyl butazone lead to 54.78 and 68.69% reduction of the granulomatous tissue formation, respectively ($P < 0.001$).

In addition, the classification of antinociceptive drugs is usually based on their mechanism of action either on the central nervous system or on the peripheral nervous system [21]. With respect to the writhing test the research group of Deraedt et al(1980)[22], described the quantification of prostaglandins by radio immuno assay in the peritoneal exudates of rats, obtained after intra peritoneal injection of acetic acid. They found high levels of prostaglandins, PGE2 and PGF2 alpha during the first 30 min after acetic acid injection. Nevertheless, it was found that the intra peritoneal administration of acetic acid-induces the liberation not only of prostaglandins, but also of the sympathetic nervous system mediators [23, 24]. Thus, the results obtained for the writhing test using acetic acid are similar to those obtained for the odematogenic test using carrageenan. Therefore anti-inflammatory substances may also be involved in the peripheral analgesic activity.

Indeed, non-steroidal anti-inflammatory drugs (NSAIDS), like paracetamol, exert their antipyretic action by largely inhibiting prostaglandin (E-type) production in the hypothalamus [25]. Consequently, elevated plasma prostaglandin level, as observed in fever, is suppressed. Acetyl salicylic acid, another reference anti-pyretic drug (not used in this study), also brings about the same effect by a selective action on a specific cyclo-oxygenase (COX) isoenzyme in the CNS. The Methanol extract of *p.zeylanica* demonstrated effective anti pyretic activity as evident in the inhibition of the temperature elevation in the yeast model.

The antipyretic action of the extract may possibly be through inhibition of prostaglandin production, leading to suppression of elevated plasma level, especially since the extract had been shown to possess analgesic and anti-inflammatory activities.

Conclusions

From these investigations, it may be concluded that the Methanol extract of *p.zeylanica* showed analgesic, anti-inflammatory and antipyretic effects, similar to those observed for non-steroidal drugs such as, phenyl butazone and paracetamol. It is important to point out that the phytochemical analysis showed the presence of flavonoids and this might be responsible for anti-inflammatory and analgesic activity. Further investigations are under process in our laboratory to isolate and characterize the specific active components of the plant extract which is responsible for observed pharmacological actions.

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