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Ethnopharmacological Evaluation of *Peristrophe bicalyculata* Nees. for Anti-inflammatory and Analgesic Activity

Anshu Rathi, Ch.V. Rao*, S. Khatoon, and S. Mehrotra

Pharmacognosy & Ethnopharmacology Division
National Botanical Research Institute, (Council of Scientific Industrial Research)
Rana Pratap Marg, Post Box No. 436, Lucknow-226001 (Uttar Pradesh), India

Abstract – The ethanolic (50% v/v) extracts of *Peristrophe bicalyculata* Nees. (Acanthaceae) were examined for anti-inflammatory and analgesic activities in experimental animals. *P. bicalyculata* in doses of 50, 100 and 200 mg/kg caused a dose dependent inhibition of swelling caused by carrageenin equivalent to 12.25-24.49% protection and 16.62-39.44% in cotton pellet induced granuloma in rats. There was a significant increase in the tail-flick reaction time in mice (18.05-76.43% protection) and analgesy-meter induced pain in rats (14.49-56.85% protection). The extract of *P. bicalyculata* resulted in an inhibition of stretching episodes and the protection range of 14.49-56.85% respectively in acetic acid induced writhing.
Key words – *Peristrophe bicalyculata*, pain, inflammation.

Introduction

Peristrophe bicalyculata Nees. (Acanthaceae) is an erect hispid herb or under shrub 60-180 cm high, found throughout in India in forest as undergrowth, hedges and wasteland (Anonymous, 1991). It is locally known as Kakajangha in Hindi and Kakatikta in Sanskrit. Indian tribes have been using the *P. bicalyculata* in treatment of liver disorders, rheumatism, gout, antidote for snakebite, antinematode and pesticide (Chopra *et al.*, 1956, Bapalala, 1999, Jain, 1991, Kirtikar and Basu, 1975). The chemical composition of the dried aerial parts of *P. bicalyculata* reveals that is comprised of 14-methyltriacont-14-en-15-ol and 35-hydroxynonatriacontanal (Singh *et al.*, 2000). The essential oil of *P. bicalyculata* shows tuberculostatic activity in vitro against the growth of various strains of *Mycobacterium tuberculosis* (Chopra and Chopra, 1959). The ethnic tribal communities and vaidyas of Indian system of medicine have long been using *P. bicalyculata* in treatment of inflammation and pain and the scientific information remains primarily anecdotal. However, in continuation of screening of various plants for analgesic and anti-inflammatory activity, it finds worthful for detailed investigation of *P. bicalyculata* for the scientific validation of the claims of physicians.

*Author for correspondence
Fax: +91-522-205836, E-mail: chvrao72yahoo.com

Materials and methods

Plant materials – The plant material (leaves, stem, flowers, seeds/whole plant) of *P. bicalyculata* was collected in the Muzaffar Nagar district, Uttar Pradesh, India during October 2002. The plant material was identified and authenticated by Dr. R. L. S. Sikarwar, taxonomist of our institute and a voucher specimen was deposited in the institutional herbarium and departmental laboratory for future reference.

Preparation of 50% ethanolic extract – The shade dried plant material was crushed, powdered and exhaustively extracted by overnight maceration with 10 volumes of 50% ethanol. The extracts were filtered, pooled and concentrated on Rotavapour (Büchi, USA) and dried in lyophilizer (Laboconco, USA) under reduced pressure to obtain 10% of solid residue.

Preliminary phytochemical analysis – The chemical constituents of the plant extract were identified by qualitative analysis (Trease and Evans, 1983), which indicates the presence of steroids, terpenoids and alkaloids.

Finger print profile by HPTLC – The high performance thin layer chromatography (HPTLC) studies of the 50% ethanolic extract of *P. bicalyculata*, were carried out on precoated silica gel plate (Merck 60F 254) on the stationary phase and ethyl acetate : methanol : water : ammonia (6.5 :2.5:0.9:0.1) as mobile phase. The extract was spotted using a Camage Linomat IV applicator. The plate was observed at UV 220 nm and was scanned on TLC scanner III using

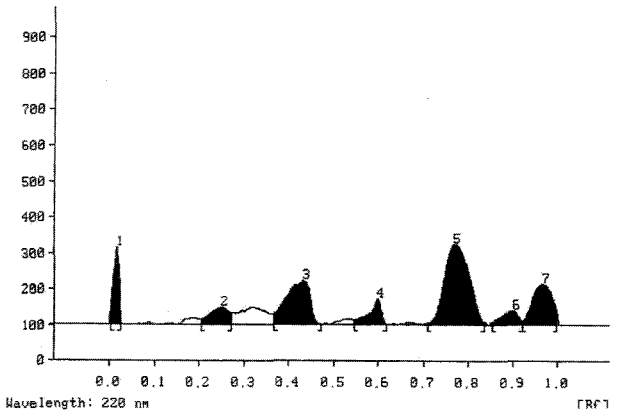
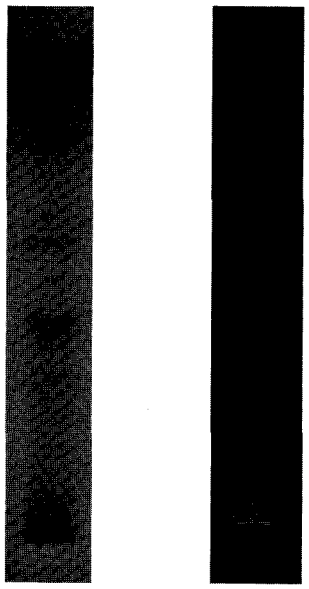


Fig. 1. HPTLC densitometric scan at 220 nm of 50% ethanolic extract of *P. bicalyculata* (whole plant).



A.Under UV 254 B.Under UV 366

Plate 1. HPTLC profile of 50% ethnolic extract of *P. bicalyculata* (whole plant).

CAT software. The HPTLC profile (Fig. 1) and the fingerprint profile of the extract was illustrated in plates 1 (A&B).

Test animals – Sprague-Dawley rats (110-150 g) and albino mice (15-20 g) of either sex were purchased and were kept in departmental animal house in well cross-ventilated room at 26±2°C, relative humidity 44-56%, with light and dark cycles of 10 and 14 h, respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet (amrut, India) and the food was withdrawn 18-24 h before the experiment commenced, water was allowed ad-libitum. All experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmerman, 1983).

Drug treatment – *P. bicalyculata* (suspended in 0.5% carboxy methyl cellulose in distilled water) was administered once daily in dose of 50-200 mg/kg for 3 consecutive days. Phenylbutazone (Sigma, USA) in the dose of 100 mg/kg p.o, was used as the standard anti-inflammatory and analgesic agent. The reference drug was administered 30 minutes before the experiment. Control group of animals received suspension of 0.5% carboxy methyl cellulose in distilled water. Experiments were conducted on day 3, one hour after last drug or vehicle administration.

Carrageenin induced paw edema – Rats were injected with 0.1 ml of 1% λ carrageenin into the subplantar side of the left hind paw (Winter *et al.*, 1962). The paw was marked with ink at the level of lateral malleolus and dipped in perspex cell up to this mark. The paw volume was measured immediately with an Ugo Basile Plethysmometer (No: 61402, 7140 Comerio-varese, Italy) and 3 h after injecting the λ carrageenin suspension. The 50% ethanolic extract of *P. bicalyculata* was administered orally at the doses of 50-200 mg/kg respectively by gavage, 1 h before the λ carrageenin injection. Significant reductions in the paw volume compared to vehicle treated control animals were considered as anti-inflammatory response. Percentage inhibition of edema was calculated as follows:

% Inhibition = $(1 - V_T/ V_C) \times 100$

V_T = Paw volume in drug treated rats.

V_C = Paw volume in control group of rats.

Cotton pellet induced granuloma formation – The rats were anesthetized with ether and incision was made on the lumbar region (Winter and Porter, 1957). By a blunted forceps subcutaneous tunnel was formed and a cotton (100 mg±1 mg) was inserted in the groin area. The animals were treated for seven days at the doses of 50, 100 and 200 mg/kg body weight by oral route. The animals were sacrificed and the pellets were removed and dried until the weight remained constant according to the procedure described (Sheth *et al.*, 1972) and the net dry weight was calculated.

Acetic acid induced writhing – Acetic acid solution at dose of 10 ml/kg (0.6%) was injected i.p. and the number of writhing during the following 15 min period was observed (Witkin *et al.*, 1961). *P. bicalyculata* administration (50, 100 and 200 mg/kg) or the reference standard, the animals received 10 ml/kg acetic acid (0.6%, i.p.). The number of abdominal contractions (writhings) and stretching with a jerk of the hind limb were counted for 15 min after administering acetic acid and percent inhibition was calculated.

Tail flick latent period – The technique described by (Davies *et al.*, 1946) was adopted using a techo analgesio

meter. The rat was placed in a rat holder with its tail coming out through a slot in the lid. The tail was kept on the bridge of the analgesio meter, called jacket with an electrically heated nichrome wire under neath. The tail received radiant heat from the wire, heated by passing a current of 6 mA. The time taken for the with drawl of the tail after switching of the current, was taken as the latent period, in second of "tail flicking response" and was considered as the index of nociception. The cut off time for determination of latent period was taken at 30 sec skin injury was avoided (Bhattacharya *et al.,* 1971). Three tail flick latencies were measured per rat at each time interval and the means of the tail flick latencies were used for statistical analysis.

Analgesy-meter induced pain – The analgesic effect of the 50% ethanolic extract of *P. bicalyculata* was tested in mice of either sex, using an Ugo Basile Analgesy meter (No. 32725, 21025 Comerio-varese, Italy) (Rodriguez Alia, 1990). This method involves the application of force to the paw of the mice using the Analgesy-meter, which exerts a force that increases at a constant rate. The mouse was gently placed between the plinth and plunger. The instrument was switched on and a constant motor rate was used to drive the plunger on to the paw of the mice. When the mice struggled, the instrument was switched off and the force at which the animal felt pain was read on a scale calibrated in grams x 10 by a pointer. The pre and the post treatment weight causing pain was determined for each mice. The last dose of extract was administered 60 minutes before testing at doses of 50, 100 and 200 mg/kg respectively.

Gross behavior and acute toxicity studies – Different doses (50-2000 mg/kg, p.o.) of *P. bicalyculata* were administered to groups of 10 mice of each dose, while one group of the same number of mice served as control. The animals were observed continuously for 1 h and then at half-hourly intervals for 4 h, for any gross behavior changes, inducing general motor activity, writhing, convulsions, response to tail pinching, piloerection, pupil size, fecal output and feeding behavior and further upto 72 h and 15 days for any mortality. (Miller and Tainter, 1994).

Statistical analysis – All the data were presented as mean S.E.M. and analysed by Wilcoxon sum rank test (Padmanabha Pillai *et al.,* 1982) followed by unpaired students t test for the possible significant identification between the various groups. A value of $P<0.05$ was considered statistically significant.

Results

Carrageenin induced paw edema in rats – Oral

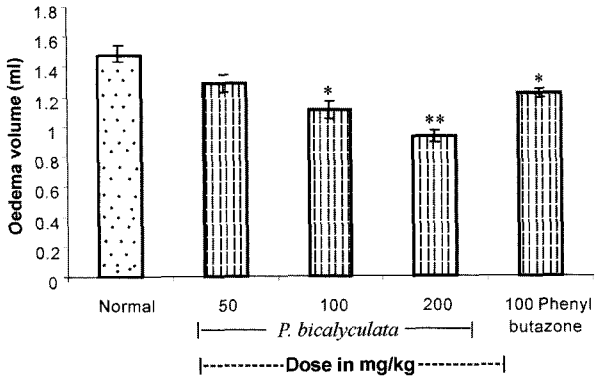


Fig. 2. Effect of *P. bicalyculata* on λ-carrageenin induced paw oedema in rats. Each bar represents mean ± S.E.M. for six rats per group. *P < 0.01, **P < 0.001 compared with the value in normal group.

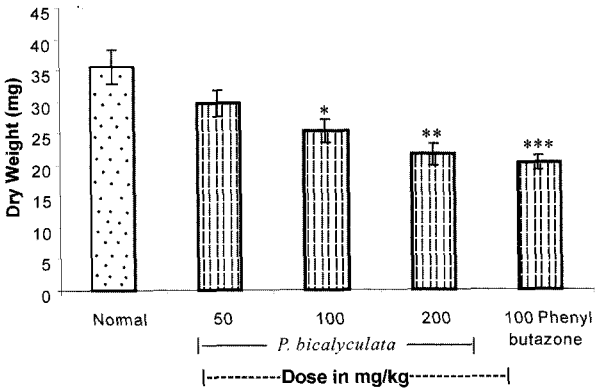


Fig. 3. Effect of *P. bicalyculata* on cotton pellet induced granuloma in rats. Each bar represents mean ± S.E.M. for six rats per group. *P < 0.05, **P < 0.01, ***P< 0.001 compared with the value in normal group.

administration of *P. bicalyculata* dose dependently inhibited the oedema caused by carrageenin in rats. The extract of *P. bicalyculata* at the doses of 50, 100 and 200 mg/kg reduced the oedema and it was 1.29±0.06, 1.11±0.06 and 0.93±0.04 respectively as compared to control 1.47±0.07. Phenyl butazone at the dose of 100 mg/kg significantly decreased the oedema to 1.22±0.03 (Fig. 2).

Cotton pellet induced granuloma in rats – *P. bicalyculata* (50-200 mg/kg, p.o.) significantly decreased the granuloma dry weight from 29.6±2.1 21.5±1.7 and the results are compared to the reference compound phenyl butazone (Fig. 3).

Acetic acid induced writhing – The *P. bicalyculata* extract on writhing response in mice is shown in Fig. 4. It was found that the ethanolic extract caused an inhibition on the writhing reponse induced by acetic acid (0.06%) and increase in the doses of plant extract resulted in the greater inhibition of stretching episodes and the protection

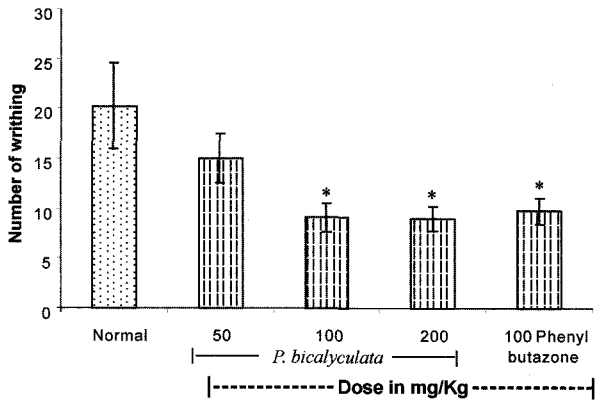


Fig. 4. Effect of *P. bicalyculata* on acetic acid induced writhing in mice. Each bar represents mean ± S.E.M. for six rats per group. *P< 0.05 compared with normal group.

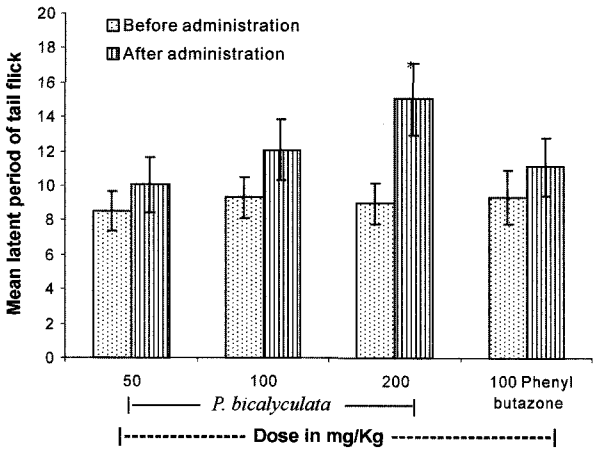


Fig. 5. Effect of *P. bicalyculata* on tail flick latent period in rats. Each bar represents mean ± S.E.M. for six rats per group. *P<0.05 compared with the value in the initial treatment of the same group.

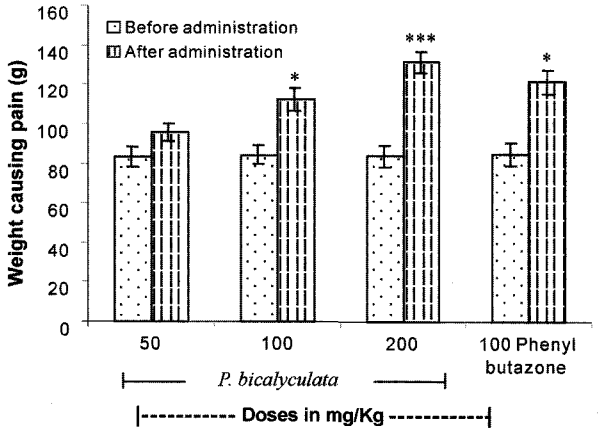


Fig. 6. Effect of *P. bicalyculata* on force induced pain in mice. Each bar represents mean ± S.E.M. for six rats per group. **P<0.01, ***P<0.001 compared with the before administration group.

ranged 14.492 56.85% respectively and phenyl butazone could block the writhing response by 43.38%.

Effect on tail flick latency—Inhibitory effect of the extract of *P. bicalyculata* and reference drug on the tail-flick reflex in rats is represented in Fig. 5. *P. bicalyculata* significantly inhibited the tail-flick response at the dose of 200 mg/kg and protection was 76.43% .

Force induced pain—*P. bicalyculata* in the dose range of 50-200 mg/kg, p.o. showed significant increase in the pain threshold from 95.6±4.6-131.6±5.3 and protection range 14.49-15.89% respectively (Fig. 6).

General gross behavior and acute toxicity studies P. bicalycutata upto 2000 mg/kg body weight, orally showed no gross avoidance of any abnormalities or mortality in mice upto end of the observation period.

Discussion

The present study demonstrated that *P. bicalyculata* extract was effective in animal models of acute inflammation and analgesic activity. Among the many methods used for screening of anti-inflammatory drugs, one of the most commonly employed technique is based upon the ability of such agent to inhibit the oedema produced in the hind paw of the rat after injection of phlogogenic agents. The time course of edema development in carrageenin induced paw oedema model in rats is generally represented by a biphasic curve (Winter *et al.*, 1962, Vinegar *et al.*, 1969). The first phase occurs within an hour of injection and is partly due to the trauma of injection and also to the serotonin components (Crunkhorn and Meacock, 1971). Prostaglandins (PGs) play a major role in development of second phase of reaction, which is measured around three hours (Di Rosa, 1972). Carrageenin induced for edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of nonsteroidal anti-inflammatory agents that is primarily inhibit the enzyme cyclooxygenase in prostaglandin synthesis (Phadke and Anderson, 1988). Based on these reports it can be inferred that the inhibitory effect of *P. bicalyculata* extract on carregeenan induced inflammation in rats could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis.

In cotton pellet induced granuloma model of sub-acute inflammation, *P. bicalyculata* extract significantly reduced the weight of granulation tissue. This method was first described by Meier *et al.* (1950), shown that foreign body granulomas were provoked in rats by subcutaneous implantation of pellets of compressed cotton. This method has been useful for evaluation of steroidal and non-steriodal anti-inflammatory drugs (Vogel and Vogel, 1998). Acetic acid causes an increase in peritoneal fluids of PGE_2 and

$PGF_{2\alpha}$ involving in part, peritoneal receptors (Deraedt *et al.,* 1980; Bentley *et al.,* 1983), and is very sensitive method of screening anti-nociceptive effect of compounds (Collier *et al.,* 1968). The ability of the extract of *P. bicalyculata* in analgesic activity may be due to the involvement of endogenous prostaglandins. The tail flick is widely used to investigate the centrally acting analgesic activity. The tail flick response appears to be a spinal reflex, which is modulated by a supraspinal inhibitory mechanism. The result obtained from tail-flick test suggested that the extract of *P. bicalyculata* found to possess an intensity of analgesic effect that is mostly mediated by a peripheral mechanism by inhibition of prostaglandin mediated potential and analgesic action of bradykinin (Nakamura *et al.*, 1986). However, *P. bicalyculata* alleviated the pain threshold on analgesy-meter induced pain. This offers new perspective in treatment of pain, as there is an evidence that symptoms of vital pain varies in intensity of the pain threshold.

Based on the results of the present study it can be concluded that *P. bicalyculata* has potential anti-inflammatory activity against both exudative-proliferative and sub-chronic phases of inflammation. The extract also has analgesic activity, which is both centrally and peripherally mediated.

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