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Investigations of Pet Ether Extract of *Caesalpinia Pulcherrima* (L.) Swartz Leaves Extract on Analgesic, Anti-inflammatory, and Antipyretic Properties

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Abstract – The pet ether extract of *Caesalpinia pulcherrima*, leaves was studied for its antinociceptive, anti-inflammatory and antipyretic property. The extract at doses of 50 and 200 mg/kg, p.o., significantly ($p<0.05$) reduced the number of writhing induced by acetic acid and inhibited the late phase (20-30 min) in formalin test in mice. The extract failed to increase the pain threshold level in tail immersion test in mice. In carrageenan induced paw edema in rats and in acetic acid induced increase in vascular permeability test in mice, the extract (50-600 mg/kg, p.o.) failed to produce any significant activity. While in cotton pellet granuloma test, the extract at doses of 200 and 600 mg/kg (p.o.) significantly ($p<0.05$) reduced the granuloma formation and was comparable to reference drug, dexamethasone. In ethylphenylpropiolate ear edema test 0.5 mg and 1 mg/ear application of extract significantly ($p<0.05$) inhibited ear edema. In yeast induced hyperthermia in rats, the extract did not produce any reduction in temperature. The results suggest that the extract acts peripherally to produce analgesic action and anti-inflammatory activity through steroidal mechanism.

Key words – *Caesalpinia pulcherrima,* analgesic, anti-inflammatory activity.

Introduction

Caesalpinia pulcherrima (L.) Swartz (Caesalpiniaceae) is a shrub popularly known as peacock flower or locally Mayuram in southern part of India. The leaves, flowers and seeds of *C. pulcherrima* are largely used in Indian medicine (Kirtikar and Basu, 1991) for treatment of fever, ulcers and tumors. One of the folklore claims of the leaves of *C. pulcherrima* is reduction of inflammation and pain in chronic inflammatory conditions like rheumatoid arthritis. Phytochemical analysis of this species has demonstrated the presence of phenolic and related compounds (Praba Choudary and Choudary, 1985). The preliminary qualitative phytochemical screening of this plant in our lab revealed the presence of glycosides, tannins, alkaloids, sterols and phenols. The present study is an evaluation of pet ether extract of *C. pulcherrima* (PECP) leaves in mice and rats using various analgesic, anti-inflammatory and antipyretic models which has not been made so far to find the pharmacological activities of this plant.

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Experimental

Plant material – The leaves of *Caesalpinia pulcherrima* (L.) Swartz (Caesalpiniaceae) was authenticated by Dr. Sasikala, Botanist, Captain Srinivasamurthi Drug Research Institute for Ayurveda, Chennai, India. The leaves were collected locally during the month of December 2001 and the voucher specimen (Pharm. No. 66/01) was deposited in Department of Pharmacognosy and Phytochemistry, C.L. Baid Metha College of Pharmacy, Chennai, India.

Extraction – The dried powdered leaves of *C. pulcherrima* (1000 g) was extracted with pet ether (40-60°C) using Soxhlet apparatus. Solvent was removed under reduced pressure using a rotary evaporator to obtain a dry extract (11.2% w/w). The extract was suspended in sesame oil for oral administration to animals.

Animals – Wistar rats (150-180 g) and Swiss albino mice (20-25 g) of either sex were obtained from the animal house of C.L. Baid Metha College of Pharmacy, Chennai, India. The animals were maintained in standard environmental conditions of temperature, humidity, and light with free access to food and water during the experiments.

Acetic acid-induced writhing test – According to Koesters method (Koster *et al.*, 1959), 0.6% v/v of acetic acid (0.1

ml/10g, i.p.) was administered to mice one hour after the oral administration of vehicle (sesame oil, 0.1 ml/10g), aspirin (25 mg/kg) and PECP (5,50 and 200 mg/kg). The number of abdominal contractions and stretching produced during the following 15 min period were counted. A significant reduction in the number of writhing compared to control was considered as antinociceptive response.

Tail immersion test – The anti-nociceptive effect of PECP was measured using warm-water tail withdrawal procedure (Burke *et al.*, 1994). Before treatment, the terminal 3 cm of each animal tail was immersed in the water bath (55±0.5°C) to determine the time period in seconds from the tail was dipped in hot water until the animal jerked or withdrew its tail (nociceptive end point). Only mice which had reaction time less than 5 sec were used for the experiment and no animal was exposed for a period of greater than 10 sec in order to minimize the tail tissue injury. At 60 and 120 min after the treatment, the reaction time was measured again. PECP (5, 50, and 200 mg/kg) and reference drug morphine (5 mg/kg) were administered orally to groups of 5-7 mice. Control animals received 0.1 ml/10 g of sesame oil.

Formalin test – PECP (5, 50, 200 mg/kg), vehicle (sesame oil 0.1 ml/10 g), morphine (5 mg/kg) and indomethacin (5 mg/kg) were orally administered before the injection of 20 µl of 1% formalin into the mice dorsal hind paw (Hunskaar and Hole, 1987). The time (sec) mice spent on biting or licking the injected paw was recorded for the early phase (0-5 min) and late phase (20-30 min) after formalin injection.

Carrageenan induced rat paw edema (Winter *et al.*, 1962) - Edema was induced in the right hind foot of the rat by sub plantar injection of 10 µl of 1% carrageenan, after one hour oral administration of extract (50, 200, and 600 mg/kg), indomethacin (20 mg/kg), and solvent (sesame oil, 0.1 ml/100 g). Before any treatment, the volume of each rat's right hind paw was determined (vo) using a plethysmometer (Ugo Basile 7140). After the injection of the inflammatory agent, the rat paw volume (vt) was measured at ½, 1, 2, 3, and 5 h. Measure of edema was calculated by subtracting the back paw volume after treatment (vt) with back paw volume before treatment (vo).

Acetic acid-induced vascular permeability in mice – Male mice were injected with acetic acid (0.6% v/v, 0.1 ml/10 g, i.p.) one hour after the oral administration of PECP (50, 200 and 600 mg/kg), sesame oil (0.1 ml/10 g), and indomethacin (20 mg/kg). Immediately after treatment with acetic acid, Evans blue 10% w/v (0.1 ml/10 g) was injected through tail vein (Olajide and Alada, 2001). Thirty min latter. the mice were killed and the extravasation of dye into the peritoneal cavity was measured at 621 nm.

Cotton pellet granuloma test in rats – Four pellets of sterile surgical cotton weighing 20 mg each were implanted subcutaneously in 4 symmetric positions, 2 under the armpits and 2 in the groin region of the rats, under light ether anesthesia (Winter *et al.*, 1957). PECP (50, 200 and 600 mg/kg, p.o.) was given once daily for 6 days. On the day 7, rats were killed. The pellets and the surrounding granulation tissue were removed and dried at 60°C for 24 h and weighed, which was considered as dry granuloma weight. Standard anti-inflammatory agent, dexamethasone (0.5 mg/kg, p.o.) was used and the control animals received 0.1 ml/100g, p.o. of sesame oil.

Ethyl phenylpropiolate (EPP)-induced mouse ear edema – The pet ether extract of *C.pulcherrima* (0.5 and 1.0 mg/ear) and dexamethasone (0.5 mg/ear) were dissolved in acetone, and applied topically (20 µl), 16 h before induction of the ear edema to the right ear. Ear edema was induced by topical application of EPP (dissolved in acetone at a concentration of 50mg/ml) in a volume of 10 µl/ear side. The left ear served as control, received vehicle 20 µl acetone. One hour later the animals were sacrificed and central portion of both the ears were punched and weighed (Bustos *et al.*, 1992). The swelling was measured as the difference in weight between the punches from the left and right ears.

Antipyretic activity – Rat rectal temperature was recorded with multichannel electronic thermometer (Century, India). Animals presenting a temperature between 31-32°C were selected for the test. Hyperthermia was induced by s.c. injection of 20% (w/v) aqueous suspension of brewers yeast in a volume of 0.1 ml/100 g (Adams *et al.*, 1968). At 6 h after yeast injection, PECP (50, 200 and 600 mg/kg), solvent (sesame oil 0.1 ml/100 g), and paracetamol (100 mg/kg) were administered orally to different groups. Rectal temperatures were taken immediately and 1, 2, and 4 h after administration of products. The difference in increase in rectal temperature at different times with respect to the values before yeast administration was calculated for each animal.

Statistical analysis – Data are expressed as mean± s.e.m. Differences between control and treatment groups are tested for significance ($p<0.05$) using unpaired two tailed Students' *t* test and one way of analysis of variance (ANOVA) followed by Dunnets multiple comparison test, wherever applicable.

Results and Discussion

It was observed that PECP in a dose dependent manner

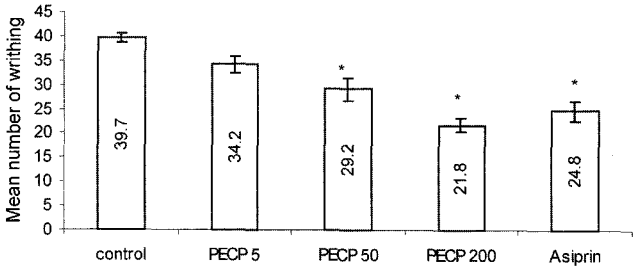

Fig. 1. Effect of pet ether extract of *C. pul*cherrima (PECP) at various doses on the writhes induced by acetic acid. Values are Mean±S.E.M. *p<0.05 vs. control; ANOVA followed by Dunnett's multiple comparison test.

reduced the number of abdominal writhings induced by acetic acid. PECP at 50 and 200 mg/kg showed significant (p<0.05) reduction in writhing 29.2±1.6 and 21.5±0.9 respectively (Fig. 1) when compared to control 39.5±0.9. While in tail immersion test PECP did not significantly alter the pain threshold level when compared to solvent control (results not shown). But in formalin test, PECP in a dose dependant manner reduced the pain response i.e. number of lickings in late phase in a significant manner (p<0.01), while there was no significant reduction in early phase of formalin test (Fig. 2).

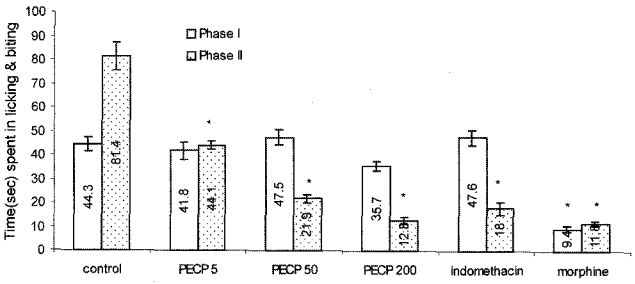

Fig. 2. Antinociceptive effect of p.o. administration of pet ether extract of *C. pulcherrima* (PECP) in formalin test. Noicceptive behavior in early (0-5 min) and late phase (20-30 min) after the injection of formalin was scored as the amount of time in sec the mice spent in licking or biting the injected paw (Mean±S.E.M.), n=6. *p<0.01 vs. control; ANOVA followed by Dunnet's multiple comparison test.

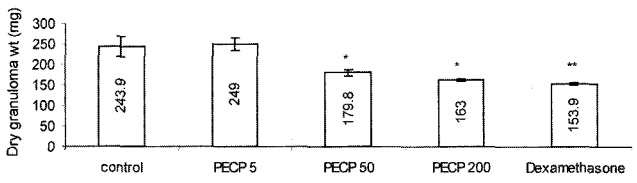

Fig. 3. Effect of pet ether extract of *C. pulcherrima* (PECP) in cotton granuloma test in rats. Each bar represents Mean ± S.E.M. of 6 animals.*p<0.05,**p<0.01 vs. control; ANOVA followed by Dunnett's multiple comparison test.

The results from acetic acid writhing, tail immersion, and formalin test reveals that the plant possess active ingredients which involves analgesic activity through peripheral mechanism (Gradmark *et al.*, 1998; Hunskaar and Hole, 1987)

From Table 1, it can be noticed that PECP at various dose levels inhibited the early phase of inflammation (i.e.) at 0.5 h in a significant manner (p<0.02) except 200 mg/kg. But the extract failed to reduce the inflammation produced by carrageenan significantly at other time intervals. Also in acetic acid induced vascular permeability test (Table 2), PECP at all dose levels did not significantly prevent the dye leakage in the peritoneal cavity when compared to control. In cotton pellet granuloma test, PECP at doses of 200 and 600 mg/kg produced significant (p<0.05) reduction in granuloma tissue formation 179.7±6.5 and 163.0±2.6 respectively when compared to the control 243.9±24.3

Table 2. Effect of pet ether extract of *C. pulcherrima* (PECP) on increased vascular permeability induced by 0.6%v/v acetic acid in mice

Treatment	Dose mg/kg; p.o.	n	Dye leakage (μg/ml)
Control	–	6	59.5 ± 2.6
PECP	50	6	61.3 ± 3.3
	200	6	51.7 ± 2.4
	600	6	50.5 ± 5.1
Indomethacin	20	6	33.5 ± 3.3*

Values are Mean ± S.E.M.
n = number of animals.
*p<0.001 vs.control; unpaired two tailed Student's '*t*' test.

Table 1. Effect of pet ether extract of *Caesalpinia pulcherrima* (PECP) on carrageenan induced paw edema in rats

Treatment	Dose mg/kg; p.o.	n	Mean increase in paw edema				
			0.5 h	1 h	2 h	3 h	5 h
Control		7	1.39 ± 0.13	1.59 ± 0.15	3.05 ± 0.32	4.02 ± 0.48	3.61 ± 0.24
PECP	50	6	0.67 ± 0.21**	1.33 ± 0.21	2.45 ± 0.31	3.62 ± 0.27	4.33 ± 0.31
	200	7	0.93 ± 0.2	1.21 ± 0.31	2.79 ± 0.44	3.53 ± 0.34	3.89 ± 0.37
	600	5	0.94 ± 0.06*	1.18 ± 0.29	3.4 ± 0.42	3.9 ± 0.34	3.92 ± 0.48
Indomethacin	20	6	0.55 ± 0.11***	0.68 ± 0.1***	1.32 ± 0.32*	1.72 ± 0.33**	2.6 ± 0.37**

Values are Mean ± S.E.M.
n= number of animals.
*p<0.01,**p<0.02,***p<0.001 vs. control; unpaired two tailed Student's '*t*' test.

Table 3. Effect of pet ether extract of C. pulcherrima (PECP) on mouse ear edema swelling induced by application of ethyl phenyl propiolate (EPP).

Treatment (N=6)	Dose mg/ear	Edema (mg) Mean±S.E.M.	Edema inhibitio (%)
Control	–	3.6±0.2	–
PECP	0.5	1.8±0.1*	50
	1.0	1.2±0.2*	67
Dexamethasone	0.5	1.8±0.2*	50

N=number of animals per group.
*p<0.01 vs. control; ANOVA followed by Dunnett's multiple comparison test.

(Fig. 3). When the extract (0.5 mg and 1 mg/ear) was subjected to ethyl phenylpropiolate (EPP) ear edema test in mice, PECP significantly (p<0. 01) reduced the edema formation (Table 3). It is to be noted that EPP model, the inflammation caused is inhibited only by corticoids like agents (Brattsand *et al.*, 1982). The results of EPP ear edema test along with cotton pellet granuloma test therefore strongly indicate that the extract has an anti-inflammatory activity dependent of glucocorticoid mechanism. PECP at all dose levels assayed against yeast induced hyperthermia did not show any significant reduction in hyperthermia (results not shown) revealing that the extract does not possess any antipyretic activity, though the plant is used as by local traditional healers to reduce fever.

It can be concluded from the present study that the pet ether extract of *Caesalpinia pulcherrima* has antinociceptive effect in chemical pain test through peripheral mechanism and has anti-inflammatory action through steroidal mechanism. These activities may be due to their content of flavanoids, tannins, and sterols. However the active phytochemical constituents and the mechanism (s) responsible for the pharmacological activities remains to be investigated. An activity-guided fractionation of this extract is being carried out in the attempt to isolate the active components.

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