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Research Article

ACUTE AND CHRONIC ANTI-INFLAMMATORY ACTIVITY OF *PERGULARIA DAEMIA* WHOLE PLANT IN VARIOUS EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

Pergularia daemia (Asclepiadaceae) has been used widely to treat inflammation and pain in folklore medicine. In the present investigation, acute and chronic anti-inflammatory activity of petroleum ether extract (PDPE), ethanolic extract(PDEE) and aqueous extract(PDAE) of whole plant of *Pergularia daemia* (100, 200 and 400 mg/kg) have been investigated in carrageenan- and formalin- induced paw edema and turpentine oil-induced granuloma pouch in rats. Oral administration of PDEE (200 and 400 mg/kg) significantly reduced the paw volume ($P < 0.001$) at 3h in carrageenan model. The treatment of PDEE (200 and 400 mg/kg) significantly reduced the volume of exudates ($P < 0.001$) in turpentine oil-induced granuloma pouch dose dependently. Chronic inflammation induced by formalin injection was significantly ($P < 0.001$) inhibited by PDEE (400 mg/kg) as compared to the control rats. Diclofenac (10 mg/kg) was used as reference drug. Thus, the present study shows that whole plant of *Pergularia daemia* possess significant anti-inflammatory activity and supports the claim in traditional medicine for the treatment of inflammatory conditions.

Key words: *Pergularia daemia,* Anti-inflammatory, Paw edema, Granuloma pouch.

INTRODUCTION

Inflammation is body's response to disturbed homeostasis caused by infection, injury or trauma resulting in systemic and local effects. It is a complex process involving various mediators, such as prostaglandins, leukotrienes and platelet activating factors[1]. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can aggravate many diseases[2].

The current management of inflammatory diseases is limited to the use of anti-inflammatory drugs whose chronic administration is associated with several adverse effects. Therefore, development of newer and more anti-inflammatory drugs with lesser side effects is necessary.

Pergularia daemia (Forsk.) Chiov. (Asclepiadaceae) is a foetid smelling laticiferous twiner found in the plains throughout the hot parts of India, ascending to an altitude of 1000 m in the Himalayas. *Pergularia daemia* is known as "Veliparuthi" in Tamil, "Uttaravaruni" in Sanskrit and "Utranajutuka" in Hindi[3]. In different folk and Ayurvedic system of medicine the plant has been documented for antifertility[4], wound healing[5], antidiabetic[6], hepatoprotective[7], cardiovascular effect[8], antibacterial activity[9]. Traditionally the plant *Pergularia daemia* is used as anthelmintic, laxative, antipyretic and expectorant, and is also used to treat infantile diarrhoea and malarial intermittent fevers. Latex of this plant is used for toothache. Stem bark of this plant is remedy for cold and fever. Ethanol extract of aerial parts of *Pergularia daemia* reported for anti-inflammatory, antipyretic, analgesic activity[10]. Phytochemically the plant has been investigated for cardenolides, alkaloids, triterpenes and saponins[10]. Roots of *Pergularia daemia* (Asclepiadaceae) have been used to treat inflammation and pain and to reduce fever by the folklore people of Salem, Dharmapuri and Coimbatore district, Tamil Nadu state, India. Whether these claims are valid is a subject of great interest and should be probed scientifically. Therefore, the present study was undertaken with the objective to investigate the anti-inflammatory activity of ethanolic extract of whole plant of *Pergularia daemia* using acute and chronic pharmacological experimental models.

MATERIALS AND METHODS

Collection, Identification and extraction of plant material

The plant material was collected from hilly forest of Pal, Faizpur, Tal: Yawal, Dist: Jalgaon (M.S.), India in the month of August 2008. The area falls within the latitude 21° 2' 54" N and longitude 76° 32' 3" E. The plant was taxonomically authenticated by the Botanical Survey of India, Pune (Voucher No. VIMSCGI1).

Plants of *Pergularia daemia* were air dried under shade, powdered with a mechanical grinder and passed through sieve no 40. The sieved powder was stored in airtight container and kept at room temperature for further study. Coarse dried powder of plant of the *Pergularia daemia* was taken in the soxhlet apparatus and extracted successively using different solvents according to their increasing order of polarity, for the present investigation (i. e. Petroleum ether→ Ethanol→ Aqueous). The extraction for each solvent was carried out for 18 to 24 hours. The extracts were collected by evaporating the solvent using rotary evaporator.

Petroleum Ether extract (PDPE), Ethanolic extract (PDEE) and Aqueous extract (PDAE) were stored in refrigerator until used. The preliminary phytochemical analysis was carried out[11, 12].

Animals

Wistar albino rats of either sex, weighing 200-280 g were obtained from National Institute of Bioscience, Pune. Rats were placed separately in polypropylene cages (five per cage) randomly with paddy husk as bedding. The animals were maintained under standard laboratory conditions at temperature 23 ± 2^{0}C, relative humidity 55 ± 10 % and 12 h light and dark cycle throughout all the experiments. Animals had free access of water and standard laboratory feed *ad libitum*. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the institute. Ethical guidelines were strictly followed during all the experiments

Acute toxicity study

Acute toxicity studies were carried out using acute toxic class method as per OECD guideline 423[13]. A starting dose of 2000 mg/kg body weight of PDPE, PDEE and PDAE extracts of *Pergularia daemia* were administered orally to three male rats. The animals were observed for mortality and behavioral changes during 48 h.

Evaluation of anti-inflammatory activity

Carrageenan-induced pedal edema in rat

Animals were divided into five groups comprising six animals in each group. In all groups, acute inflammation was produced by sub-plantar injection of 0.1 ml of freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of the rats and paw volume was measured plethysmometrically at 0 and 3 h after

carrageenan injection. Animals were pretreated either with vehicle (5% gum acacia), or PDPE, PDEE and PDAE (100, 200 and 400 mg/kg) or Diclofenac (10 mg/kg) orally[14] one hour before injection. Mean increase in paw volume was measured and percentage inhibition was calculated.

Turpentine oil-induced granuloma pouch in rat

Subcutaneous dorsal granuloma pouch was made in ether anaesthetized rats by injecting 2 ml of air, followed by injection of 0.5 ml of turpentine oil into it[15, 16].

All drugs were administered orally one hour prior to turpentine oil injection and continued for seven consecutive days. On day 7, the pouch was opened under anesthesia, the amount of exudate was taken out with a syringe, and later on the volume was measured and compared with those of the control and standard group.

Formalin-induced edema in rat hind paw

0.1 ml of 2% formalin was injected into the sub-plantar area of right hind paw of ether anesthetized rat[17]. All drugs were given orally one hour prior to formalin injection and continued for 7 consecutive days. Degree of inflammation was measured plethysmometrically on days 1 and 7.

Statistical analysis

The results of the study were expressed as mean ± SEM. ANOVA was used to analyze and compare the data, followed by Dunnet's test for multiple comparisons. P < 0.05 was considered significant in all experiments.

RESULTS

Phytochemical screening

Preliminary phytochemical evaluation of different extracts showed the presence of alkaloids, glycosides, steroids, flavonoids, cardenolides, saponin, tannin, phenolic compounds, terpenoids, carbohydrates, gums and mucilages (Table 1).

Acute toxicity study

The results of acute toxicity studies showed no clinical signs of toxicity and mortality in the PDPE, PDEE and PDAE treated animals.

Carrageenan-induced pedal edema in rat

Administration of PDEE showed anti-inflammatory effect in carrageenan-induced inflammation as shown in Table 2. PDEE (200 and 400 mg/kg) dose dependently significantly reduced the paw volume (P <0.001) as compared to the control rats. Diclofenac also showed similar reduction (P <0.001) of inflammation in rats.

Turpentine oil-induced granuloma pouch

Table 3 shows the effect of PDEE in turpentine oil-induced granuloma pouch. The treatment of PDEE (200 and 400 mg/kg) significantly reduced the volume of exudates (P <0.001) in turpentine oil-induced granuloma pouch dose dependently, which was comparable with the effect of diclofenac (P <0.001).

Formalin-induced edema

Formalin-induced pedal edema was significantly (P <0.001) inhibited by PDEE (400 mg/kg) as compared to the control rats. Similarly, diclofenac showed inhibitory action on edema formation. Table 4 shows that PDEE was effective in chronic inflammation.

Table 1: Preliminary phytochemical evaluation of different extracts of *Pergularia daemia*

Sr. No.	Phytoconstituents	Pet. ether (60-80 ^{o}C)	Ethanol (95%)	Aqueous
1	Test for Tannins and Phenolic compds.	-	-	+
2	Test for Amino acid	-	-	-
3	Test for Glycosides	+	+	+
4	Test for Phytosterol	-	+	+
5	Test for Flavanoids	+	+	+
7	Test for Terpenoids	-	+	+
8	Test for Alkaloids	-	+	+
9	Test for Carbohydrates	-	+	+
10	Test for Saponins	+	+	+
11	Test for fixed oils and fats	+	-	-

+: Presence; -: Absence

Table 2: Effect of *Pergularia daemia* on carrageenan induced rat paw edema

Drug (100mg/kg)	Paw volume increase after 3 h (ml)	% of inhibition
Control	1.61±0.12	--
PDPE(100)	1.54±0.14	4.34
PDPE(200)	1.49±0.09	7.45
PDPE(400)	1.47±0.08	8.69
PDEE(100)	1.13±0.09*	29.81
PDEE(200)	0.92±0.08**	42.85
PDEE(400)	0.78±0.06**	51.55
PDAE(100)	1.47±0.14	8.69
PDAE(200)	1.45±0.08	9.93
PDAE(400)	1.43±0.07	11.18
Diclofenac(10)	0.52±0.04**	67.70

n = 5 in each group, values are mean ± SEM

*P< 0.05, **P< 0.001 compared to control group (ANOVA followed by Dunnett's test)

Table 3: Effect of *Pergularia daemia* on turpentine oil- induced granuloma pouch in rat

Treatment (mg/kg, p. o.)	Volume of exudates(ml)	% of inhibition
Control	2.68±0.08	--
PDPE(100)	2.55±0.14	4.85
PDPE(200)	2.52±0.08	5.97
PDPE(400)	2.49±0.09	7.08
PDEE(100)	2.01±0.12*	25.00
PDEE(200)	1.79±0.09**	33.20
PDEE(400)	1.33±0.07**	50.37
PDAE(100)	2.62±0.12	2.23
PDAE(200)	2.58±0.09	3.73
PDAE(400)	2.56±0.07	4.47
Diclofenac(10)	0.89±0.08**	66.70

n = 5 in each group, values are mean ± SEM

*P< 0.05, **P< 0.001 compared to control group (ANOVA followed by Dunnett's test)

Table 4: Effect of *Pergularia daemia* on formalin- induced rat hind paw edema

Treatment (mg/kg, p. o.)	Paw volume increase on day 7 (ml)	% of inhibition
Control	1.49±0.17	--
PDPE(100)	1.41±0.15	5.36
PDPE(200)	1.39±0.09	6.71
PDPE(400)	1.36±0.08	8.72
PDEE(100)	0.93±0.14**	37.58
PDEE(200)	0.76±0.19**	48.99
PDEE(400)	0.53±0.22**	64.42
PDAE(100)	1.41±0.12	5.36
PDAE(200)	1.38±0.09	7.38
PDAE(400)	1.35±0.08	9.39
Diclofenac(10)	0.37±0.18**	75.16

n = 5 in each group, values are mean ± SEM

*P< 0.05, **P< 0.001 compared to control group (ANOVA followed by Dunnett's test)

DISCUSSION

The inhibition of carrageenan-induced inflammation in rats is an established model for evaluating anti-inflammatory agents. The development of carrageenan- induced edema is bi-phasic[18], the first phase is attributed to the release of cytoplasmic enzymes, histamine and serotonin from the mast cells, while second phase is mediated by an increased release of prostaglandins in the inflammatory area and continuity between the two phases is provided by kinins. Since PDEE significantly inhibited paw edema induced by carrageenan in the second phase, the present study suggests a possible inhibition of cyclooxygenase synthesis by PDEE, because the carrageenan inflammatory model basically reflects the actions of prostaglandins[19, 20]. Where as PDPE and PDAE could not show significant anti-inflammatory activity in acute and chronic models of inflammation.

Granuloma pouch technique was modified[16] using turpentine oil as irritant. An aseptic inflammation resulting in large volume of haemorrhage exudate is elicited which resembles the sub-acute type of inflammation. Turpentine oil-induced granuloma pouch offer a model for exudative type of inflammation. Though, the chemical mediators of this type of response are unknown, protein synthesis is necessary for the formation of granuloma[21]. PDEE has shown potential inhibitory action on exudates formation. Kinin is said to be the main mediator of granuloma, as it not only vasodilate but also increase the vascular permeability in the early stages of inflammation. Thus, PDEE may possess anti-kinin like activity.

The formalin-induced paw edema assay is one of the most suitable methods to screen antiarthritic and anti-inflammatory agents, as it closely resembles human arthritis. Also, formalin-induced arthritis is a model used for the evaluation of an agent with probable anti-proliferative activity. This experiment is associated with the proliferative phase of inflammation. Result indicates that PDEE appears to be effective against formalin-induced edema and may be useful for chronic inflammatory disease like arthritis.

The anti-inflammatory activities of many plants have been attributed to their saponin[22], terpenoids, flavonoids and steroids contents[23]. We consider that the responsibility for anti-inflammatory effect could be saponin, terpenoids, flavonoids and steroids which are the major components. Thus, the present study shows that whole plant of *Pergularia daemia* possess significant anti-inflammatory activity and supports the claim in traditional medicine for the treatment of inflammatory conditions.

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