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Analgesic and anti-inflammatory activities of *Passiflora foetida* L.

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ABSTRACT

Objective: To investigate the analgesic and anti-inflammatory activities of ethanol extract of *Passiflora foetida* (*P. foetida*) leaves. **Methods:** Ethanol extract of *P. foetida* leaf was evaluated for analgesic action by acetic acid-induced writhing and hot plate method in albino mice. The anti-inflammatory property of ethanolic leaf extract was tested by carrageenan induced acute paw edema and histamine induced acute paw edema in rats. **Results:** The dose 200 mg/kg of *P. foetida* leaf extract exhibited highest significant analgesic activity [(13.50±0.43) min] at a reaction time of 20 min in hot plate method in mice. The ethanol extract of leaf dose 100 mg/kg produced a highly significant anti inflammatory effect [(1.302±0.079) mL] in rats. **Conclusions:** It is very clear that *P. foetida* also has analgesic and anti-inflammatory activities for the pharmaceuticals.

1. Introduction

India is a rich source of medicinal plants and a number of plant derived extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Siddha. Only few of them have been scientifically explored. Plant derived natural products such as flavonoids, terpenes and alkaloids[1,2] have received considerable attention recent years due to their diverse pharmacological properties including inflammatory, antipyretic and analgesic activities. *Passiflora* is the largest genus in the Passifloraceae family and comprises nearly 500 species[3]. *Passiflora foetida* L. (*P. foetida*) (Stinking passion flower) is South American in origin, which has been spread to many tropical areas. It is found in riverbeds, dry forest floors, and wayside thickets, covering the top thorny shrubs and also growing near hamlets. The mostly available wild species are *Passiflora edulis*, *Passiflora leschenaultia*, *Passiflora mollissima* and *Passiflora subpeltata*. Traditional medicines are used to cure many diseases like diarrhea, intestinal tract, throat, ear infections, fever and skin diseases. The ethanobotanical views of *P. foetida*, suggest that decoction of leaves and fruits is used for the treatment of asthma and biliousness, leaves and root decoction is emmenagogue, used for hysteria and leaf paste is applied on the head for giddiness and headache[4]. The plant is said to be used for curing itches[5]. The major phytoconstituents of this plant are alkaloids, phenols, glycosides, flavonoids and cyanogenic compounds[6] and passifloricins, polypeptides and alpha-pyrones in *P. foetida*[7] Several years ago a flavonoid content in the resin of leaves of *P. foetida* was screened which exhibited antifeedant activity in *in vitro* assays against the phytophagous larvae *Dione juno*[8]. Most of pharmacological studies are demonstrated in the central nervous system effects, such as anxiolytic, sedative action and anticonvulsant properties. Some authors describe the use of *Passiflora* species in the popular medicine for the inflammatory diseases. About 294 volatile compounds have been identified in several passion fruit extracts[9]. The purpose of the present study is to investigate the analgesic and anti-inflammatory properties of ethanol extract of *P. foetida* leaves against several experimental models in mice and rats for the purpose of validating its ethnomedicinal uses.

2. Materials and methods

2.1. Plant materials

The fresh plant parts of *P. foetida* L. were collected from Maruthamalai forest hills, Coimbatore. The collected plant material was identified and their authenticity was confirmed by comparing the voucher specimen at the herbarium of Botanical survey of India, Southern circle Coimbatore, Tamilnadu. Freshly collected plant materials were cleaned

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to remove adhering dust and then dried under shade. The dried sample were powdered and used for further studies.

2.2. Preparation of extracts

The air dried, powdered plant material was extracted with petroleum ether and ethanol using soxhlet apparatus. Each time before extracting with the next solvent, the material was dried in hot air oven below 40 ℃. Finally, the material was macerated using hot water with occasional stirring for 24 hr and the water extract filtered. The different solvent was evaporated using a rotary vacuum-evaporator (Yamato RE300, Japan) at 50 ℃ and the remaining water was removed by lyophilization (VirTis Benchtop K, USA). The dried extract obtained was used for assessing the analgesic and anti-inflammatory properties.

2.3. Animals

Swiss Albino mice (20-25 g) and wistar rats (120-140 g) were purchased from Small Animal Breeding Center, Mannuthy, Trissur, Kerala. The animals were housed under standard conditions of temperature[(23±1)℃], relative humidity (55±1)%, 12/12 h light/dark cycles and fed with standard pellet. All the animal experiments were conducted with the permission from Institutional Animal Ethical Committee(KMCH College of Pharmacy, Coimbatore, Tamilnadu, Proposal no. KMC-RET/M.Phil/1/2009).

2.4. Chemicals and drugs

Carrageenan, acetic acid, acetyl salicylic acid, the standard drugs morphine and histamine were purchased from Sigma-Aldrich Chemical Company, Steinheim, Germany. All chemicals and drugs used were of the highest purity and analytical grade.

2.5. Acute toxicity

Acute oral toxicity studies were performed according to organization for Economic Co-operation and Department(OECD)[10]. Swiss albino male mice (n=6/each dose) were selected by random sampling technique. Animals were fasted for 12 h with free access to water only. Ethanol extract of *P. foetida* (dissolved in distilled water) were administered orally at a dose of 5 mg/kg and mortality was observed for 3 days. If mortality was observed in 4/6 or 6/6 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one mice out of six animals, then the same dose was repeated with higher doses such as 50, 300, 500, 1 000 and 2 000 mg/kg. General behaviors such as motor activity, tremors, convulsions, straub reaction, aggressiveness, pilo erection, loss of lighting reflex, sedation, muscle relaxation, hypnosis, analgesia, ptosis, lacrimation, diarrhea and skin colour were observed for the first one hour and after 24 h of drug administration.

2.6. Analgesic activity

The analgesic activity of ethanol extract of *P. foetida* was investigated using the following models.

2.6.1. Hot plate test in mice

The analgesic effect of *P. foetida* extract was studied in mice using hot plate method by Eddy *et al*[11]. Four groups (each group comprising six animals) of Swiss albino mice (20-25 g) were placed on a plate and maintained 15-20 min at room temperature for environmental adaptation. Food was withdrawn on the prior night of the experiment and water was allowed *ad libitum*. The hot plate was maintained at (56±1) ℃. Negative ccontrol group received the vehicle (distilled water, 10 mL/kg body weight, *po.*), positive control group received the morphine (5 mg/kg), whereas groups 3 and 4 were administered with ethanol extract of *P. foetida* (100 and 200 mg/kg, *po.* respectively). Each animal was then individually placed gently on Eddy's hot plate at 55 ℃. The responses of reaction time were recorded at 30, 60, 90 and 120 minutes after administration.

2.6.2. Writhing test in mice

The analgesic effect of plant extract against acetic acid-induced writhing in mice was carried out according to the method suggested by Seigmund *et al*[12]. This method was used to preferentially evaluate possible peripheral effects of ethanol extract of *P. foetida* as analgesic substance. The peripheral analgesic drug, acetyl salicylic acid was used as a positive control. Group 1 received the vehicle distilled water (10 mL/kg, *p.o.*), group 2 with acetyl salicylic acid (10 mg/kg). Whereas group 3 and 4 were orally administered with ethanol extract of *P. foetida* at dose of 100 and 200 mg/kg, *p.o.* respectively. Thirty minutes after treatment, mice were injected with 0.1 mL of 1% acetic acid solution to induce the characteristic writhings. After 5 min, the mice were placed in an observation box, and the number of writhes in a 20 min period was counted. The responses of the extract and acetyl salicylic acid treated groups were compared with those of animals in the control group.

2.7. Anti-inflammatory activity

The anti-inflammatory property of ethanolic leaf extract against carrageenan induced acute paw edema in rats by Winter *et al* and Kulkarni *et al*[13, 14]. Male albino rats (125-150 g) were divided in three groups (each groups comprising six animals). They were fasted overnight prior to the start of the experiment, and water was allowed *ad libitum*. A mark was made on both the hind paws just beyond tibio-tarsal junction, so that every time the paw is dipped in the mercury column up to the fixed mark to ensure constant paw volume. Initial paw volume of each rat was noted by mercury displacement method. The first control group received distilled water (10 mL/kg, *p.o.*), while the second group was treated with indomethacin (8 mg/kg, *p.o.*). The third groups were administered with the ethanol extract of *P. foetida* leaf (100 mg/kg). Acute inflammation was produced by the subplantar administration of 0.1 mL of 1% carrageenan or histamine (in 1% CMC w/v) in the left hind paw of the rats. The right paw served as reference non-inflammed paw for comparison. The paw volume of both legs of control and plant extract treated rats was noted at 0, 60, 120 and 180 min after carrageenan injection, and at 15, 30, 60, and 120 min after histamine injection. Reduction in the paw volume was also noted.

2.8. Statistical analysis

Values were expressed as mean of triplicate analysis of the samples (n=3) standard deviation(SD). Analysis of variance and significant differences (P>0.05) among means were tested by one-way ANOVA and Dunnet multiple range test.

3. Results

3.1. Acute toxicity

The ethanol extract of leaves of *P. foetida* was evaluated for its acute toxicity in mice. The extract did not alter the general behavior and failed to produce any mortality even at the highest dose of 2 000 mg/kg and even after 3 days. Mice were found to be safe.

3.2. Analgesic activity

3.2.1. Hot plate test in mice

The analgesic effect of ethanol extract of *P. foetida* leaf using hot plate test in mice is presented in Table 1. It indicated that oral administration of the extract (200 mg/kg) significantly attenuated the hot-plate thermal stimulation. Analgesic activity of the extract was comparable to the standard drug morphine sulphate (5 mg/kg). Among the two doses tested, 200 mg/kg showed highest analgesic activity[(13.50±0.43) min] at a reaction time of 120 min.

3.2.2. Writhing test in mice

The analgesic effect of ethanol extract of *P. foetida* leaf using writhing test in mice is presented in Table 2. It indicated that oral administration of the extract (200 mg/kg) showed highest analgesic activity (37.50±0.65) with a reaction time of 20 min.

3.3. Anti-inflammatory activity

3.3.1. Carrageenan induced acute paw edema in rats

Carrageenan induced paw edema model was used for the evaluation of anti-inflammatory activity of the ethanol extract of *P. foetida* leaves. Carragenan- induced rat paw edema was markedly inhibited by plant extract (100 mg/kg) and indomethacin (10 mg/kg). The ethanol extract of leaves produced a highly significant acute anti-inflammatory effect[(1.302±0.079) mL] in mice (Table 3).

3.3.2. Histamine induced acute paw edema in rat

The ethanol extract of leaves demonstrated a significant anti-inflammatory effect against histamine-induced inflammation at a dose of 100 mg/kg. The anti-inflammatory effect of the extract was significant[(1.523±0.052) mL] but was less than that of indomethacin[(1.576± 0.055) mL](Table 4).

4. Discussion

Analgesic activities were found in plants possessing some sterols in models of pain induced by acetyl salicylic acid.

Table 1
Analgesic activity of ethanol extract of *P. foetida* leaves using hot plate method(Mean±SD).

Treatment	Dose(mg/kg)	Reaction time in seconds			
		30 min	60 min	90 min	120 min
Negative control	–	5.00±0.37	8.50±0.92	9.00±1.15	8.16±1.10
Morphine sulphate	5	5.67±0.33	14.50±0.34*	14.67±0.21*	14.50±0.34*
Extract	100	5.00±0.37	13.00±0.58*	13.67±0.82**	13.33±0.56*
Extract	200	4.83±0.48	13.00±0.93*	13.83±0.48*	13.50±0.43*

Degrees of freedom 2, 15; *P<0.01, **P<0.001 of ANOVA followed by Dunnett test compared with control.

Table 2
Analgesic activity of ethanol extract of *P. foetida* leaves by writhing test in mice(Mean±SD).

Treatment	Dose (mg/kg)	No. of writhing
Control	–	90.33 ± 3.38
Acetyl salicylic acid	10	41.33 ± 1.62*
Extract	100	39.50 ± 0.65*
Extract	200	37.50 ± 0.65*

Degrees of freedom 2, 15; *P<0.01 of ANOVA followed by Dunnett test compared with control.

Table 3
Anti-inflammatory activity of ethanol extract of *P. foetida* leaves on carrageenan induced paw edema in rats(Mean±SD) (mL).

Treatment	Dose (mg/kg)	Rat paw edema volume at different time interval			
		0 h	1 h	2 h	3 h
Control	–	1.151±0.026	1.288±0.033	1.493±0.04439	1.629±0.035
Indomethacin	10	1.146±0.050	1.271±0.041	1.301±0.034*	1.413±0.057*
Extract	100	1.063±0.039	1.158±0.032 *	1.273±0.060**	1.302±0.079**

Degrees of freedom 2, 15; *P<0.05, **P<0.01 of ANOVA followed by Dunnett test compared with control.

Table 4
Anti-inflammatory activity of ethanol extract of *P. foetida* leaves on histamine induced paw edema in rats(Mean±SD) (mL).

Treatment	Dose (mg/kg)	Rat paw edema volume at different time interval		
		0 h	1 h	3 h
Control	–	1.330±0.056	1.485±0.060	1.819±0.060
Indomethacin	10	1.314±0.032	1.483±0.030	1.576± 0.055**
Extract	100	1.258±0.041	1.430±0.026	1.523±0.052**

Degrees of freedom 2, 15; *P<0.05, **P<0.01 of ANOVA followed by Dunnett test compared with control.

Significant analgesic activities were also found in plants possessing some organic acid and flavonoid[15,16]. Acetyl salicylic acid induced abdominal constriction in mice is another widely used method for evaluation of peripheral analgesic effects. It is found that the ethanol leaf extract significantly inhibited the acetic acid induced writhing response in a dose dependent manner. Even a 200 mg/kg dose of plant extract produced 37.50±0.65 writhing as compared to standard dose of acetyl salicylic acid (41.33 ±1.62). The writhing response of the mouse to an intra peritoneal injection of noxious chemical is used to screen for both peripherally and centrally acting analgesic activity. Acetic acid causes analgesia by liberating endogenous substances and many others that excite pain nerve endings[17]. Anti-inflammatory activity of many plants has been attributed to their high sterol and triterpene[18] or flavonoids contents[19]. Ethanol extract of *P. foetida* leaves produced highly significant acute anti-inflammatory effect. Other studies have demonstrated that various flavonoids such as rutin, quercetin, luteolin, hesperidin and biflavonoids produced significant antinociceptive and/or anti-inflammatory activities[20]. Increased body temperature and pain are known as main symptoms of the body against an inflammatory stimulation. Hence, a drug possessing anti-inflammatory activity may also exhibit anti-pyretic and analgesic properties. Carrageenan induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis[21]. Results of the present study clearly indicate significant histamine induced anti-inflammatory properties of the ethanol extract of *P. foetida*. The effect is however, lower than that obtained with indomethacin (10 mg/kg), a standard non-steroidal anti-inflammatory drug. Significant effect was observed in the histamine-induced edema in rats. In the literature, there is one report concerning the anti-inflammatory activity of the *Passiflora incarnata*[22] in addition to previous report[23].

In conclusion, results of these experiments suggest that *P. foetida* may be used as an alternative or supplementary herbal remedy for the treatment of analgesic and inflammatory diseases. *P. foetida* ethanol extract may have beneficial effects together with drugs known for a strong analgesic as well as anti-inflammatory effects. Thus the present study warrants further investigation involving components of ethanol extracts of *P. foetida* for possible development of new class of analgesic and anti-inflammatory drugs.

Conflict of interest statement

We declare that we have no conflict of interest.

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