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Prostaglandin mediated anti-inflammatory and analgesic activity of *Cissampelos pareira*

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Abstract

50% ethanolic extract of the aerial part of *Cissampelos pareira* Linn. var. Hirsuta (*Menispermaceae*) was tested for anti-inflammatory (paw edema induced by carrageenin and arachidonic acid) and analgesic activity (abdominal writhes and hot plate) in rats and mice, respectively. Oral administration of extract exhibited significant and dose dependent anti-inflammatory activity in the carrageenin test, which was based on interference with prostaglandin synthesis, as confirmed by the arachidonic acid test. In the abdominal writhing test induced by acetic acid, higher dose of the plant extract had the highest analgesic activity, whereas in the hot-plate test the best dose was 100 mg/kg ($P < 0.05$). The LD_{50} showed that *Cissampelos pareira* (2000 mg/kg) presented low toxicity.

Keywords: *Cissampelos pareira*, anti-inflammatory, analgesic, prostaglandin

Introduction

Cissampelos pareira Linn. var. hirsuta is a very variable, lofty, slender, dioecious, perennial, climber commonly distributed throughout topical and sub topical India, ascending up to an altitude of *c* 2,000m, traditionally known as Laghupatha in Ayurveda, an Indian traditional system of medicine (Annonomus, 1992; Asolkar *et al.,* 1992). It is thought to be an excellent remedy to alleviate and help with symptoms associated with menstruation and balances hormones in women (Kirtikar *et al.,* 2001). Members of the Palikur tribe in Guyana use a poultice of *Cissampelos pareira* as a topical pain-reliever, and the Wayãpi Indians use a decoction of the leaf and stem as an oral analgesic (Amresh *et al.,* 2003; Gogte, 2000).

The presence of two crystalline alkaloids, hayatin and hayatinin were reported along with the other constituents as quercitol and a sterol. Plant alkaloid has shown inhibitory activity against human carcinoma cells of the naso-pharynx in cell culture (Morita *et al.,* 1993a; Morita *et al.,* 1993b). Effects of hayatin methochloride and (+)-tubocurarine chloride have been studied

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on autonomic ganglia of cats (Patnaik *et al.*, 1973). Bisbenzylisoquinoline alkaloids which are anti-inflammatory constituents of plants were tested for suppressive effect on *in vitro* nitric oxide (NO) production by lipopolysaccharide-stimulated peritoneal macrophages which were induced with thioglycollate or Bacillus Calmette-Guerin in mice (Kondo *et al.*, 1993).

Plant has been documented for potent diuretic (Caceres *et al.*, 1987), neuromuscular blocking, antihypertensive (Patnaik *et al.*, 1973), anti-tumor (Kupchan *et al.*, 1965; Kupchan *et al.*, 1960), antibacterial against Gram-positive bacteria (Adesina, 1982), antimalarial (Gessler *et al.*, 1994), antibiotic (George *et al.*, 1949), antidiarrhoeal (Amresh *et al.*, 2004), antioxidant (Amresh *et al.*, 2007a), antimicrobial and β-glucosidase inhabitation (Sanchez-Medina *et al.*, 2001), immunomodulatory (Bafna *et al.*, 2005), anti-inflammatory activities of root (Amresh *et al.*, 2006), cytotoxic and anti-cancerous (Bork *et al.*, 1997) properties. On this basis, the objective of the present investigation was to study the anti-inflammatory and analgesic effects of aerial parts of 50% ethanolic extract of *Cissampelos pareira*.

Materials and methods

Plant material

The aerial parts of *C. pareira* were collected in the botanical garden of National Botanical Research Institute, India in September 2004. The plant material was identified and authenticated taxonomically at National Botanical Research Institute, Lucknow. A voucher specimen (NAB 68004) of the collected sample was deposited in the institutional herbarium for future reference.

Preparation of extracts

Aerial parts of *C. pareira* were washed with distilled water to remove dirt and soil, and shade dried. Routine pharmacognostic studies including organoleptic tests, macroscopic and microscopic observations were carried out to confirm the identity of the materials. The dried materials were powdered and passed through a 10-mesh sieve. The coarsely powdered material (2 Kg) was extracted thrice with ethanol (50% v/v). The extracts were filtered, pooled and concentrated at reduced temperature (-5 ^{0}C) on a rotary evaporator (Buchi, USA) and then freeze-dried (Freezone® 4.5, Labconco, USA) at high vacuum ($133X10^{-3}$ m Bar) and at temperature –40 ± 2 ^{0}C. For the pharmacological tests the extract was suspended in double distilled water containing carboxy methyl cellulose (1% w/v, CMC).

Animals

Sprague-Dawley rats and Swiss albino mice of either sex were procured from the National Laboratory Animal Centre (NLAC), Central Drug Research Institute, Lucknow, India. They were kept in departmental animal house in well cross ventilated room at 27 ± 2 ^{0}C, and relative humidity of 44 - 56%, light and dark cycles of 10 and 14h 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 - 24h before the experiment through water was allowed *ad-libitum*. All studies were conducted after obtaining prior approval from the institutional ethical committee in accordance with the National Institute of Health "Guide for the Care and Use of Laboratory Animals" (NIH publication no. 86-23, 1985).

Anti-inflammatory activity

Carrageenin-induced rat paw edema

The test was used to determine the anti-inflammatory action of the extract by the method of Rao *et al.* (2003). Groups of 6 animals received by the intragastric route either 10 mg/kg indomethacin (reference drug) or the crude 50% ethanolic extract of *Cissampelos pareira* (100, 200, and 400 mg/kg), one hour before they had a subplantar injection of 0.1 ml/paw of carrageenin solution (200 g/kg) suspended in distilled water into the right hind paw. Paw volume was measured by dislocation of the water column in a plethysmograph (model 7140, Ugo Basile) immediately after carrageenin application (time zero) and 1, 2, 3, and 4 h after the stimulus.

Arachidonic acid-induced rat paw edema

Rat paw edema was induced in six animals by subplantar injection into the right hind paw of 0.1 ml 0.5% arachidonic acid dissolved in carbonate buffer, pH 8.5. Norhydroguaiaretic acid (NDGA, 100 mg/kg) as reference and *Cissampelos pareira* 50% ethanolic extract (400 mg/kg) were administered intraperitonally 30 min before arachidonic acid injection. Edema volume was measured by a plethysmography immediately after arachidonic acid injection and at 15 min intervals thereafter for a period of 2 h (Di-Martino *et al.*, 1987).

Analgesic activity

Analgesic activity was assessed by abdominal writhing test (n = 10) using acetic acid (Koster *et al.*, 1959; Amresh *et al.*, 2007b) and by hot-plate test (n = 8) applied to mice of both sexes. In the writhing test, 0.6% acetic acid was injected intraperitoneally and the number of writhes was counted starting 10 min after injection for a period of 20 min. Indomethacin (10 mg/kg) and morphine (2.5 mg/kg), were used as reference drugs, and the *Cissampelos pareira* extracts (100, 200, and 400 mg/kg) were administered by the intragastric route 1 h before acetic acid injection.

The hot-plate test was performed at a fixed temperature of 55±0.5 ^{0}C, with a maximum duration estimated at 30 s. Reaction time (paw licking, jumping, etc.) was measured 30, 45, 60, and 90 min after intraperitoneal injection of 10 mg/kg morphine as a reference drug and of *Cissampelos pareira* extracts (100, 200, and 400 mg/kg).

Toxicity (LD_{50})

Acute toxicity was tested in Swiss mice (n = 20) of both sexes according to the method of Brito (1994). The animals received a *Cissampelos pareira* extract (2000 mg/kg) and vehicle (water) by the intragastric route and the mortality rate was observed for 48 h, followed by daily weight monitoring for 14 days.

Statistical analysis

Values were represented as mean ± S.E.M. and data were analyzed by paired-t-test using SPSS software for the Windows 11.0 package. A value of $P < 0.05$ was considered significant.

Results and discussion

The 50% ethanolic extract of aerial parts of *Cissampelos pareira* presented a dose dependent anti-inflammatory activity at all concentrations tested, with no significant difference at the concentration of 100 mg/kg 4 h after carrageenin injection (Table 1).

The paw edema induced by carrageenin has been extensively studied in the assessment of the anti-inflammatory action of steroidal and non-steroidal drugs involving several chemical mediators such as histamine, serotonin, bradykinin and prostaglandins (Amresh *et al.*, 2006). The fact that the *Cissampelos pareira* extract inhibited edema starting from the first hour and during all phases of inflammation suggests that it probably inhibited different aspects and chemical mediators of inflammation.

Table 1. Effect of *Cissampelos pareira* 50% ethanolic extract (CPE) on carrageenin- induced rat paw edema (n = 6).

Treatment (mg/kg)	Mean ± S.E.M. (ml)			
	1 h	2 h	3 h	4 h
Control	0.28 ± 0.04	0.45 ± 0.03	0.53 ± 0.02	0.39 ± 0.02
Indomethacin (10)	0.13 ± 0.02**	0.18 ± 0.02**	0.22 ± 0.02**	0.16 ± 0.02**
CPE (100)	0.17 ± 0.02**	0.37 ± 0.03*	0.45 ± 0.03**	0.36 ± 0.04
CPE (200)	0.13 ± 0.03**	0.20 ± 0.03**	0.26 ± 0.03**	0.25 ± 0.05**
CPE (400)	0.09 ± 0.03**	0.24 ± 0.07**	0.27 ± 0.07**	0.24 ± 0.06**

*$P < 0.05$ vs. control.

**$P < 0.01$ vs. control.

The paw edema induced by arachidonic acid is a widely used method for distinguishing between 5-lipooxygenase and cyclooxygenase inhibitors (Griswold *et al.*, 1987). Subplantar injection of arachidonic acid produced significant edema as early as after 15 min and reached a peak at 75 min. The 50% ethanolic extract of aerial parts of *Cissampelos pareira* did not block edema formation when administered intraperitoneally, but edema was inhibited by NDGA (Table 2). The rat paw edema induced by arachidonic acid is perceptibly reduced by inhibitors of arachidonic acid metabolism and by corticosteroids and is insensitive to selective cyclooxygenase inhibitors (Di-Martino *et al.*, 1987). On the basis of the present results, we may propose that the 50% ethanolic extract of *Cissampelos pareira* has an anti-inflammatory action interfering with prostaglandin synthesis.

Table 2. Effect of *Cissampelos pareira* 50% ethanolic extract (CPE) on arachidonic acid-induced rat paw edema (n = 6).

Time (min)	Mean ± S.E.M. (ml)		
	Control	NDGA (100 mg/kg)	CPE (400 mg/kg)
15	0.30 ± 0.03	0.05 ± 0.01**	0.18 ± 0.02*
30	0.48 ± 0.03	0.09 ± 0.02**	0.50 ± 0.04
45	0.51 ± 0.04	0.17 ± 0.01**	0.52 ± 0.04
60	0.54 ± 0.04	0.21 ± 0.03**	0.55 ± 0.04
75	0.55 ± 0.04	0.21 ± 0.02**	0.53 ± 0.04
90	0.52 ± 0.04	0.21 ± 0.22**	0.48 ± 0.02
105	0.52 ± 0.04	0.21 ± 0.02**	0.48 ± 0.04
120	0.52 ± 0.04	0.21 ± 0.02**	0.48 ± 0.02

*P < 0.05 vs. control.

**P < 0.01 vs. control.

In the acetic acid-induced writhing test, the *Cissampelos pareira* extract demonstrated a significant analgesic effect at 400 mg/kg dose, inhibiting pain by 50.07% compared to control, while at lower doses, 100 and 200 mg/kg, the inhibition was not significant, 22.15 and 27.85%, respectively (Table 3).

Table 3. Analgesic effect of the 50% ethanolic extract (CPE) of *Cissampelos pareira* on abdominal acetic acid (0.6%)-induced writhes in mice (n = 6).

Treatment (mg/kg)	Number of writhes (mean ± S.E.M.)	Percent inhibition of writhes
Control	29.8 ± 4.2	–
Indomethacin (10)	12.9 ± 1.9**	56.7
Morphine (2.5)	06.8 ± 3.2**	77.2
CPE (100)	23.2 ± 3.2	22.1
CPE (200)	21.5 ± 2.3	27.8
CPE (400)	14.9 ± 1.1**	50.1

**P < 0.01 vs. control.

This result suggests that the analgesic effect of *Cissampelos pareira* related to the mechanism of prostaglandin synthesis as is the case for the anti-inflammatory process induced by carrageenin, indicating the presence of an inflammatory pain process (Duarte *et al.*, 1988). In the hot-plate test, an analgesic effect was observed at concentrations of 100 and 200 mg/kg (Table 4), indicating that the extract possesses an activity related to both inflammatory and non-inflammatory pain.

Table 4. Analgesic effect of 50% ethanolic extract (CPE) of *Cissampelos pareira* on the latency of paw withdrawal in the hot-plate test in mice (n= 6).

Treatment (mg/kg)	Latency time (Sec.) (mean S.E.M.)			
	30 min	45 min	60 min	90 min
Control	7.06 ± 1.16	7.06 ± 1.54	6.49 ± 1.28	5.55 ± 0.66
Morphine (10)	12.66 ± 1.12*	11.32 ± 1.17*	11.88 ± 1.25*	11.32 ± 0.79*
CPE (100)	10.85 ± 0.86*	10.71 ± 0.55*	9.01 ± 0.91	9.56 ± 0.99*
CPE (200)	9.82 ± 0.87	9.80 ± 1.24	9.15 ± 1.23	9.30 ± 0.49*
CPE (400)	9.34 ± 1.18	7.34 ± 1.54	8.30 ± 0.95	8.60 ± 0.87*

* $P < 0.05$ vs. control.

Animals treated with 2000 mg/kg of the 50% ethanolic extract of *Cissampelos pareira* were observed and weighed daily for 14 days and showed no changes in behavior or weight, a fact indicating low toxicity of the extract. According to Lorke (1983), substances are considered to be of low toxicity when the LD_{50} reaches levels above 2000 mg/kg.

Conclusion

The 50% ethanolic extract of *Cissampelos pareira* showed an anti-inflammatory and analgesic activity by the models used in the present study. This fact suggests that the 50% ethanolic extract has an anti-inflammatory action interfering with prostaglandin synthesis.

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