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Antinociceptive and antiarthritic activity of *Cissampelos pareira* roots

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Abstract

In the present study, 50% aqueous ethanolic extract of *Cissampelos pareira* (Menispermaceae) roots (*C. pareira*) at the dose levels of 100–400 mg/kg, once daily for 3 days exhibited significant ($P < 0.001$) resistance against mechanical pain after 30 min in analgesymeter induced pain in mice. In acetic acid (0.6%; i.p.) inducing writhing, *Cissampelos pareira* significantly ($P < 0.05$) decreased the writhing episodes; the degree of percent protection at 200 and 400 mg/kg was 22.73 and 51.63. The hot plate reaction time was increased by 2.07 ($P < 0.05$) and 2.70 ($P < 0.001$) folds. respectively. Further *Cissampelos pareira* showed the dose dependent significant protective effect against complete Freund's adjuvant induced arthritis. The percentage protection on the 18th day was 40.54 ($P < 0.01$) and 71.52 ($P < 0.001$) at 200 and 400 mg/kg respectively. Lysosomal enzymes (acid phosphatase and *N*-acetyl glucosaminidase) were decreased by 50% ($P < 0.01$) and 26.26% ($P < 0.05$) by using *Cissampelos pareira*, dextramethasone decreased them 56.56% ($P < 0.01$) and 31.82% ($P < 0.01$) and the glycoprotein contents (total hexose and sialic acid) were increased by 1.55-folds ($P < 0.01$) and 1.51-folds ($P < 0.05$) by using *Cissampelos pareira* while dextramethasone increases them by 1.51-folds ($P < 0.001$) and 1.60-folds ($P < 0.01$) respectively in stomach homogenate with respect to arthritic group. The increased pain threshold and protective effect against CFE by *Cissampelos pareira* vindicated its medicinal value in treatment of pain and arthritis.
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Keywords: Arthritis; Antinociceptive activity; *Cissampelos pareira*; Complete Freund's adjuvant; Inflammation; Menispermaceae

1. Introduction

Cissampelos pareira (L.) Hirsuta (Menispermaceae), roots are having the property of wound healing and antidote, paste of roots is used in fistula, purities, skin disorders and snake poison externally (Amresh et al., 2003). Internally roots are useful in anorexia, indigestion, abdominal pain, gastric disorders, diarrhea and dysentery (Amresh et al., 2004). The roots show significant antibacterial activity against Gram-positive organisms than against Gram-negative strains (Adesina, 1982). Traditionally the plant is reported for its blood purifier and anti-inflammatory properties in India (Gogte, 2000). Astringent, mild tonic, diuretic, stomachic, analgesic, antipyretic and emmenagogue properties are also reported in roots (Vaidya, 1988; Feng et al., 1962). It is also used in cough and as it purifies breast milk, it is used in various disorders of breast milk secretion (Jain, 1991). It is a potent diuretic (Caceres et al., 1987). Plant is frequently prescribed for cough, dyspepsia, dropsy, urino-genital troubles such as prolapsed uteri, cystitis, hemorrhage and menorrhagia, and calcular nephritis (Kirtikar and Basu, 2001). The antibiotic activities of the plant are also documented by George and Pandalai (1949).

Rheumatoid arthritis (RA) is a kind of chronic inflammatory autoimmune disease (Arend and Dayer, 1990). Although a number of drugs (non-steroidal or steroidal anti-inflammatory agents and immunosuppressants) used in the treatment of RA have been developed over the past few decades, there is still an urgent need for more effective drugs with lower side effects (Badger and Lee, 1997). *Cissampelos pareira* has been recently reported for immunomodulatory (Bafna and Mishra, 2005) and anti-inflammatory activity (Amresh et al., 2007) while *Cissampelos sympodialis* inhibits the neutrophils degranulation by cAMP-dependent mechanism (Thomas et al., 1999). Keeping this in mind and in continuation of our earlier studies, we proceeded to evaluate the antinociceptive and anti arthritic activity of *Cissampelos pareira* roots.

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2. Materials and methods

2.1. Plant material

The roots of *Cissampelos 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.

2.2. Animals

Sprague-Dawley rats and Wistar strain mice were obtained from the institutional animal house and they were kept in the departmental animal house at 25 ± 2 °C and relative humidity 45–51.5%, light and dark cycles of 10 and 14 h, respectively for 1 week before and during the experiments for acclimatization. The animals were provided with standard rodent pellet diet (*Amrut*, *India*) and water was allowed ad labium. Rearing up of animals in the experimental period and there upkeep during the entire experimental span conformed to the norms of Institutional Animal Ethical Committee (IAEC) of NBRI, India and ethical guidelines for investigations of experimental pain in conscious animals (Zimmerman, 1983). The standard orogastric cannula was used for oral drug administration in experimental animals.

2.3. Preparation of extracts

Roots of *Cissampelos 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 processed as per our earlier described process that is powdered and passed through a 10-mesh sieve. The coarsely powdered material (1 kg) was extracted thrice with aqueous ethanol (50%, v/v). The extracts were filtered, pooled and concentrated at reduced temperature (−5 °C) on a rotary evaporator (Buchi, USA) and then freeze-dried (Freezone® 4.5, Labconco, USA) at high vacuum (133×10^{-3} mbar) and at temperature −40 ± 2 °C (yield 3.4%, w/w) (Rao et al., 2003a).

2.4. Drug treatment

For the pharmacological tests, the obtained extract was suspended in double distilled water containing carboxy methyl cellulose (1%, w/v, CMC) in doses of 100, 200 and 400 mg/kg. The doses were fixed based on our earlier studies on the 50% aqueous ethanolic extract of *Cissampelos pareira* (Bafna and Mishra, 2005; Amresh et al., 2007). The prepared extract was administered once daily for 3 consecutive days. Experiments were conducted on day 3, 30 min after last dose drug or vehicle administration. Aspirin 300 mg/kg p.o. for analgesic and dexamethasone 5 mg/kg p.o. for anti arthritic activities were used as standard drugs. Control group of animals received suspension of 1% (w/v) CMC in double distilled water.

2.5. Analgesymetre induced pain

The analgesic effect of *Cissampelos pareira* was tested in male mice using an Ugo Basile analgesymetre. This method involves the application of force to the paw of the mice using the analgesymetre, which exerts a force that increases at a constant rate. The mice were gently placed between the plinth and plunger. The instrument was switched on and constant motor rate was used to drive the plunger on to the paw of mice. When the mice struggles the instrument was switched off and force at which the animal felt pain was read on a scale calibrated in gram × 10 by pointer (Rodriguezalia, 1990).

2.6. Acetic acid induced writhing

Acetic acid solution at a dose of 10 ml/kg (0.6%) was injected i.p. and the number of writhes during the following 15 min period was observed (Witkin et al., 1961). Significant reductions in number of writhes by drug treatment as compared to vehicle treatment (control) animals were considered as a positive analgesic response. The percent inhibition of writhing was calculated as follows:

$$\%\ \text{Inhibition} = \left(1 - \frac{VT}{VC}\right) 100$$

VT, number of writhes in drug treated mice. VC, number of writhes in control group of mice

2.7. Hot plate reaction time in mice

Mice were screened by placing them on a hot plate maintained at 55 ± 1 °C and the reaction time was recorded in seconds for fore paw licking or jumping. Only mice which reacted with in 15 s and which did not show large variation when tested on four separate occasions, each 15 min apart, were taken for the test. The time for fore paw licking or jumping on the heated plate of the analgesia meter was taken as a reaction time (Woolfe and MacDonald, 1944).

2.8. Freund's adjuvant induced arthritis

Arthritis was induced by the intradermal injection of 0.1 ml of complete Freund's adjuvant (CFA, Sigma) in the right hind paw (Newbould, 1963). The adjuvant contained 10 mg heat killed *Mycobacterium tuberculosis* in 1 ml paraffin oil. Two different groups of six male rats were administered orally with *Cissampelos pareira* 200 and 400 mg/kg body weight once a day for 3 days and then treated with CFA as in control arthritis group. Further treatment with the *Cissampelos pareira* was continued for the 18 days. Another group of rats was administered with dexamethasone (5 mg/kg, p.o.) as a standard reference. The paw sizes did not change before and after extract administration. Slide calipers was used to measure the

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