# Phytochemical and Pharmacological Investigation of Ethanol Extract of *Cissampelos pareira*

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**Reza, *et al.*: Phytochemical and Pharmacological Study of *C. pareira***

**In this study, the ethanol extract of *Cissampelos pareira* has been evaluated. The extract was tested for analgesic properties using both hot plate and acetic acid-induced writhing methods. Antiinflammatory effect was investigated using two different doses of 250 and 500 mg/kg body weight on Evans rats by carrageenan-induced paw edema test. The antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in Wistar rats. The phytochemical screening of the extract of *Cissampelos pareira* exhibited the presence of several phytochemical compounds including saponins, gums and carbohydrates, reducing sugars, alkaloids and terpenoids. Ethanol extract of *Cissampelos pareira* exhibited significant analgesic, antiinflammatory and antipyretic activity in a dose-dependent manner. The results obtained from these studies confirm its therapeutic value against diseases caused by various pain and fever.**

**Key words: *Cissampelos pareira*, analgesic, antioxidant, antiinflammatory, antipyretic, carrageenan-induced paw edema, yeast**

*Cissampelos pareira* is a woody climbing vine with leaves up to one foot long and it belongs to the family Menispermaceae and genus *Cissampelos*. It is found throughout the tropical region of India and Bangladesh. The parts of the plant used for medicinal effect are whole vine, seed, bark and leaf[1].The leaves of the plant contain alkaloids like tetrandrine, which has analgesic effect and has recently been shown to have antitumor and antileukemic properties as well. The roots and stem contain the bisbenzylisoquinoline alkaloids that have been demonstrated as antiinflammatory agent[1]. Traditionally *C. pareira* has been used in numerous

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medical conditions like pain, fever, diarrhea and infection. *C. pareira* is thought to be responsible for many of its properties like antioxidant and anti-inflammatory[1,2]. The present study was undertaken aiming to find out the detailed phtyochemicals as well as analgesic and antiinflammatory activity of ethanol extract of *C. pareira* using *in vivo* model.

The whole plant of *C. pareira* was collected, washed and cut into small pieces and dried under shade. Dried pieces were powdered and extracted by maceration with 50% aqueous alcohol for 72 h at room temperature. The whole extract was collected in a conical flask, filtered, pooled and concentrated at reduced temperature on a rotary evaporator and then freeze-dried at high vacuum and at low temperature.

Swiss albino mice weighing 20-25 g and Long Evans rats weighing 100-120 g of either sex were used for the study. They were kept in departmental animal house which has well cross ventilation system, a temperature of 27±2°, and relative humidity of 44-56%, and light and dark cycles of 10 and 14 h, respectively. Animals were provided with standard rodent pellet diet and maintained them without food for 12-16 h before the experiment was performed. All the experiments were performed in the morning following current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals[3]. For oral drug administration, the standard orogastric cannula was used.

The reagents and drugs used were: ethanol, acetic acid, diclofenac sodium, paracetamol, carrageenan, ferric chloride, α-napthol, hydrochloric acid and sulfuric acid. Phytochemical screening was carried out on the ethanol extract of *C. pareira* according to the method described by Antonisamy *et al.*[4]

Swiss albino mice were divided into four groups with five mice in each group, pretreatment reaction time for each mouse was determined[5]. Group-I animals received distilled water (10 mg/kg body weight) and group-II received diclofenac sodium (standard) at 10 mg/kg body weight. Group-III and group-IV were treated with 250 and 500 mg/kg orally, respectively of the ethanol extract of *C. pareira*. Mice were placed on the hot plate (Analgesiometer, Ugobasile, Italy) and the time until either licking or jumping occurs was recorded by a stop-watch. A posttreatment cut off period of 30 s was maintained to avoid damage to the paw. The drugs or vehicle were administered orally and the reaction time was observed again at 0, 30, 60, 120, 180 and 240 min after drug administration. % inhibition=(post treatment latency−pretreatment latency)/(cut-off time−pretreatment latency)×100. Acetic acid was administered intraperitoneally (ip) to the mice to create painful sensation[6]. In this study diclofenac sodium was used to serve as standard drug. Albino mice of either sex were used for the study. The ethanol extract of *C. pareira* was administered at two different doses (250 and 500 mg/kg) to the mice after an over-night fast. Test animals were administered orally with the drugs half an hour prior to acetic acid (0.6% v/v in water, 0.1 ml/10 g, ip) administration. The mice were placed individually in glass beakers 5 min after acetic acid injection and were then observed for 15 min and the number of writhing was recorded for each animal. The number of writhes in each treated group was compared to that of a control group (distilled water), while diclofenac (10 mg/kg) was used as a reference substance. % inhibition=(reaction time control−reaction time treated/reaction time control)×100. For antiinflammatory test, Long Evans rats were randomly divided into four groups, each consisting of five animals, of which group-I was kept as control giving only distilled water. Group-II was given ketorolac (10 mg/kg) as standard. Group-III and group-IV were given the test sample at the dose of 250 and 500 mg/kg, respectively. Half an hour after the oral administration of the test materials, 1% carrageenan was injected to the left hind paw of each animal. The volume of paw edema was measured at 0.5, 1, 2, 3 and 6 h using plethysmometer after administration of carrageenan. The right hind paw served as a reference of non-inflamed paw for comparison[7]. The average percent increase in paw volume with time was calculated and compared against the control group. Percent inhibition was calculated using the formula: % Inhibition of paw edema=$(V_c - V_t/V_c) \times 100$, where $V_c$ and $V_t$ represent average paw volume of control and treated animal, respectively.

The antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in rats[8]. Adult Wistar rats were selected, weighed and divided in three groups of five animals each and they were fasted 18 h prior to commencement of experiment but water was provided *ad libitum*. Fever was induced by injecting 20 ml/kg of 20% aqueous suspension of Brewer's yeast in normal saline below the nape of the neck and rectal temperature

**TABLE 1: EVALUATION OF ANALGESIC ACTIVITY BY HOT PLATE METHOD**

| Sample | Response time in seconds at different time intervals (mean±SEM) (% inhibition) | | | | | |
|---|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 120 min | 180 min | 240 min |
| Control (10 mg/kg) | 10.7±0.84 | 9.66±0.94 (-) | 8.00±0.81 (-) | 6.58±0.64 (-) | 5.52±0.54 (-) | 5.00±0.4 (-) |
| Standard (10 mg/kg) | 9.14±0.52 | 11.0±1.00 (20.6) | 12.6±0.94* (37.9) | 14.2±1.07* (54.9) | 15.9±0.67* (74.6) | 12.5±0.69* (36.5) |
| CP (250 mg) | 8.36±0.29 | 10.3±0.63 (23.2) | 11.7±0.84* (39.5) | 13.0±0.62* (55.5) | 14.8±0.35* (77.5) | 11.1±0.28* (32.5) |
| CP (500 mg) | 8.26±0.41 | 11.0±1.02 (33.2) | 12.4±0.70* (50.4) | 13.9±0.67* (68.0) | 15.2±0.20* (84.5) | 11.2±0.34* (36.1) |

All values are mean±SEM, *n*=5. One way Analysis of Variance (ANOVA) followed by Dunnett's test was performed as the test of significance. The minimum value of *P*<0.05 was considered significant as compared with control group , CP: ethanol extract of *C. Pariera*

**TABLE 2: EVALUATION OF ANALGESIC ACTIVITY BY ACETIC ACID-INDUCED WRITHING TEST**

| Treatment | Dose (mg/kg ip) | No. of writhing (15 min) | % inhibition |
|---|---|---|---|
| Control (distilled water) | 10 ml/kg | 16.60±1.16 | - |
| Standard (diclofenac sodium) | 10 mg/kg | 7.40±0.927* | 55 |
| *Cissampelos pareira* (ethanol extract) | 250 mg/kg | 9.60±0.927* | 42.2 |
| | 500 mg/kg | 9.40±0.509* | 43.4 |

All values are mean±SEM, *n*=5. One way Analysis of Variance (ANOVA) followed by Dunnett's test was performed as the test of significance. The minimum value of *P*<0.05 was considered significant as compared with control group

**TABLE 3: ANTIINFLAMMATORY ACTIVITY STUDY USING CARRAGEENAN-INDUCED RAT PAW EDEMA METHOD**

| Sample (mg/kg) | Paw volume at different time interval (ml) | | | | |
|---|---|---|---|---|---|
| | 0h | 1h | 2h | 3h | 6h |
| Control (10 mg/kg) | 0.68±0.048 | 0.87±0.06 (-) | 1.08±0.052 (-) | 1.16±0.01 (-) | 1.212±0.037 (-) |
| Standard (10 mg/kg) | 0.666±0.044 | 0.85±0.02 (27.63) | 0.992±0.035 (48.94) | 1.150±0.029 (72.67) | 0.978±0.056* (46.85) |
| CP (250 mg/kg) | 0.612±0.033 | 0.83±0.04 (35.9) | 1.078±0.043 (76.1) | 1.176±0.087 (92.1) | 0.898±0.039* (46.7) |
| CP (500 mg/kg) | 0.654±0.027 | 0.85±0.04 (30.6) | 1.096±0.056 (67.6) | 1.1980±0.0.45 (83.2) | 0.922±0.029* (40.9) |

All values are mean±SEM, *n*=5. One way Analysis of Variance (ANOVA) followed by Dunnett's test was performed as the test of significance. The minimum value of *P*<0.05 was considered significant as compared with control group , CP: ethanol extract of *C. Pariera*

**TABLE 4: ANTIPYRETIC ACTIVITY**

| Treatment | Normal (A) | After 18 h (B) | Rectal temperature (°F) after administration of drug | | |
|---|---|---|---|---|---|
| | | | 1 h ($C_1$) | 2 h ($C_2$) | 3 h ($C_3$) |
| Control (10 mg/kg) | 92.0±0.44 | 96.9±0.46 | 99.2±0.44 (-) | 99.9±0.5 (-) | 98.7±0.6 (-) |
| Standard (100 mg/kg) | 91.9±0.42 | 95.6±0.76 | 94.6±0.68 (4.57) | 93.5±0.6* (6.32) | 91.9±0.6* (6.8) |
| CP (500 mg) | 94.6±0.69 | 98.4±0.65 | 95.3±0.68 (3.87) | 95.4±0.7* (4.44) | 93.8±0.73* (4.96) |

All values are mean±SEM, *n*=5. One way Analysis of Variance (ANOVA) followed by Dunnett's test was performed as the test of significance. The minimum value of *P*<0.05 was considered significant as compared with control group, CP: ethanol extract of *C. Pariera*

was recorded by clinical thermometer immediately before (-18 h) and 18 h after (0 h) Brewer's yeast injection. Prior to the experiment, the rats were maintained in separate cages for 7 days and the animals with approximately constant rectal temperature were selected for the study. Paracetamol (100 mg/kg) was used as standard drug for comparing the antipyretic action of extract. The extract at the doses of 500 mg/kg was administered ip, one group was administered with paracetamol (100 mg/kg) ip control group was given 0.5 ml normal saline. The rectal temperature was measured at 1, 2 and 3 h after drug administration by using digital thermometer. Percent reduction in rectal temperature was calculated by considering the total fall in temperature to normal level. The institutional guidelines for the care and use of laboratory animals were followed. All values are expressed as mean±SEM (standard error of mean). Comparisons were made using one-way ANOVA with Dunnett post hoc test. Statistical significance of $P<0.05$ was used in all cases.

The ethanol extract of *C. pareira* was found to have gum and carbohydrates, alkaloids, reducing sugars and terpenoids. Result of hot plate test is shown in Table 1. Both doses of the extract produced the dose dependent increase in latency time compared to control. The percent inhibition at the dose of 250 mg/kg was 39.47% and at the dose of 500 mg/kg was 50.36% at the end of 1 h. The results were found to be statistically significant ($P<0.05$).

In the writhing test, intraperitoneal injection of acetic acid evidently resulted in writhing reflexes in mice. Pretreatment of mice with ethanol extract (250 and

500 mg/kg) of *C. pareira* significantly inhibited the acetic acid induced writhing episodes in a dose dependent manner with an inhibition of 42.2 and 43.4%, respectively when compared to control group that received distilled water only (Table 2).

Again ethanol extract of *C. pareira* at 250 and 500 mg/kg dose exhibited antiinflammatory activity in a dose-dependent manner with the percent inhibition of 46.7 and 40.9%, respectively, as compared with the control group (Table 3).

Furthermore, the ethanol extract of *C. pareira* at a dose of 500 mg/kg body weight exhibited significant ($P$<0.05) antipyretic activity. The body temperature of the experimental animals started to fall in 2 h following administration of the extract and lasted for 3 h (Table 4). However, the response was not comparable to that of antipyretic activity of paracetamol.

So, even though *C. pareira* has not been evaluated in depth for pharmacological properties, but in our study its ethanol extract has been shown to have significant analgesic, antiinflammatory and antipyretic properties. Further investigations are required to find the active components of the extract and to confirm the mechanism of action in the development of potent analgesic, antiinflammatory and antipyretic agents.

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