Comparative antipyretic and analgesic activities of Cissampelos pareira Linn. and Cyclea peltata (Lam.) Hook. F. & Thomas

Suman G. Singh, K. Nishteswar, Bhupesh R. Patel, Mukesh Nariya
Department of Dravyaguna, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

Abstract

Background: Cissampelos pareira Linn. is considered as an established source of Patha, whereas Cyclea peltata (Lam.) Hook. F. & Thomas is used as a source plant of Patha in the southern part of India. In classical texts, two different varieties of Patha, i.e. Rajpatha (C. peltata) and Laghupatha (C. pareira), are mentioned which possess almost similar properties. Objective: To compare antipyretic and analgesic activities of C. pareira and C. peltata in suitable experimental model. Materials and Methods: Powder (540 mg/kg) and ethanolic extract (200 mg/kg) of both the test drugs (C. pareira and C. peltata) were evaluated for antipyretic activity in Brewer’s yeast-induced pyrexia model in rats. Analgesic activity was evaluated by radiant heat model in rats and acetic acid-induced writhing syndrome in mice. Results and Discussion: Result of the present study had shown that powder of C. pareira (540 mg/kg) has moderate antipyretic activity as compared to the powder of C. peltata and extract of both test drugs. C. pareira powder showed better analgesic effect than ethanolic extract (200 mg/kg) of both the test drugs in radiant heat model in rats, whereas in acetic acid-induced writhing syndrome, ethanolic extract (280 mg/kg) of both drugs showed pronounced effect as compared to powder form (780 mg/kg) in mice. Conclusion: Both C. pareira and C. peltata exhibited analgesic effects in experimental animals. The effect is more significant in C. peltata treated group compared to C. pareira. Antipyretic effect was observed with the pretreatment of C. pareira.

Keywords: Analgesic, antipyretic, Cissampelos pareira, Cyclea peltata, Patha

Introduction

Patha is extensively used as a medicine from Vedic Period[1] and Samhita Kala. Patha is indicated in treating various ailments such as Jwara (pyrexia), Atisara (diarrhea), Arsha (hemorrhoids), Frameha (diabetes), Grahani (gastrointestinal disorders), Shoola (pain), Kasa (cough), and Shwasa (bronchitis).[2] Patha is mainly indicated in Kwatha (decoction) and Churna (powder) dosage forms in various Chikitsagranthas. Acharya Charaka has mentioned Patha Dwaya in Kasachikitsa context without much details,[3] Acharya Sushruta and Acharya Vagbhata did not mention any such types. Shodhala Nighantu was the first book to classify Patha into two types, i.e., Laghupatha and Rajpatha.[4] Priya Nighantu quoted that both Laghupatha and Rajpatha possess similar properties.[5] Cissampelos pareira Linn. is known as Laghupatha; however, in southern part of India, Cyclea peltata (Lam.) Hook. F. and Thomas is widely used as Patha, which is considered as Rajpatha. Both these species belong to Menispermaceae family. In tribal communities, Patha (Laghupatha and Rajpatha) is used widely in the management of pain and fever.[6,7] Various research activities were reported on different dosage forms of C. pareira and C. peltata such as anti-inflammatory,[8,9] anti-arthritis, antiinociceptive,[10] hepatoprotective,[11] antioxidant,[12,13] anti-diabetic,[14] and antibacterial.[15] Till date, no work has been carried out to evaluate the comparative antipyretic and analgesic activities of C. pareira and C. peltata. Hence, keeping these facts in view, the pharmacological study was planned on suitable...
animal models to compare the activity profile of the different dosage forms (powder and alcoholic extract) of *C. pareira* and *C. peltata*.

**Materials and methods**

**Drugs**

Roots of *C. pareira* were collected from Una area situated on the Western coast of Gujarat in October 2012, and roots of *C. peltata* were collected from forest area situated near Udupi district of Karnataka in November 2012. The authenticity of these samples was confirmed by comparing their morphological characters of various floras and standard herbarium sample available at the Pharmacognosy Laboratory, IPGT & RA, Gujarat Ayurved University, Jamnagar, with the help of subject experts. Roots of the both test drugs were washed, shade dried, pulverized to prepare powder, sieved through #80 mesh, and preserved in an air-tight glass bottle.

**Method of preparation of ethanolic extract**

An accurately weighed 20 g root sample of each of *C. pareira* and *C. peltata* was macerated with 400 ml of ethyl alcohol procured from Udyog Sahakari Mandal, Surat, of specified strength (99%) in a closed flask for 24 h, shaken frequently during the first 6 h, and then allowed to stand for 18 h. Taking precaution against the loss of solvent, it was filtered and filtrate was evaporated to a dried form.

**Animals**

Wistar albino rats (*Rattus norvegicus*) of both sexes weighing between 200 ± 20 g were taken for tail flick method and antipyretic activity, whereas Swiss albino mice weighing between 30 ± 5 g were used for acetic acid-induced writhing method. The animals were obtained from the animal house attached to the Pharmacology Laboratory of Institute. The rats were exposed to natural day and night cycles under ideal ambient laboratory conditions (temperature 22°C ± 2°C and humidity 50%–60%). They were fed with Amrut brand rat pellet feed procured from Pranav Agro Industries Ltd. and drinking water was supplied ad libitum. The experimental protocols were approved by the Institutional Animal Ethics Committee (M.D./IAEC/14/2013/20) in accordance with the guidelines formulated by CPCSEA, India.

**Dose calculation**

The human therapeutic dose mentioned in Ayurvedic Pharmacopoeia of India for the powder is 3–6 g.[16] The dose for experimental study was calculated by extrapolating the human dose to animal dose based on the body surface area ratio using the table of Paget and Barnes.[17] The dose of extract of *C. pareira* and *C. peltata* was fixed on the basis of results of acute toxicity study and efficacy studies reported by previous authors.[13,18,19]

**Antipyretic activity**

This experiment was carried out as explained by Gujaral and Khanna with modification as per the experimental need.[20] Wistar albino rats of either sex weighing between 200 ± 20 g were divided into five groups containing six rats in each group. The groups were Group I - normal control, Group II - *C. pareira* powder (540 mg/kg, po) (CP1), Group III - *C. peltata* powder (540 mg/kg, po) (CP2), Group IV - *C. peltata* extract (200 mg/kg, po) (CPE1), and Group V - *C. peltata* extract (200 mg/kg, po) (CPE2). Animals were kept on fasting overnight but were provided with drinking water. Next morning, before drug administration, initial rectal temperatures of all rats were recorded with digital telethermometer procured from EIE Instruments, Ahmedabad. The respective test dosage forms of drug were administered to the respective groups and distilled water was given to the control group. Then, after 1 h of test drug administration and vehicle to the control group, pyrexia was induced by injecting suspension of 12.5% w/v dried Brewer’s yeast in normal saline subcutaneously in a dose of 1 ml/100 g body weight. The rectal temperature was again recorded after 3, 6, and 9 h. The difference between actual rectal temperature and initial rectal temperature was recorded for each time interval. The maximum reduction in rectal temperature in comparison to control group was also recorded.

**Analgesic activity**

**Tail flick method**

The tail flick response was measured with the help of anTail Flick Analgesiometer (INSIF-Ambala).[21] Wistar albino rats of either sex weighing between 200 ± 20 g were divided into six groups containing six rats in each group. The groups were Group I - normal control, Group II - *C. pareira* powder (540 mg/kg, po) (CP1), Group III - *C. peltata* powder (540 mg/kg, po) (CP2), Group IV - *C. pareira* extract (200 mg/kg, po) (CPE1), Group V - *C. peltata* extract (200 mg/kg, po) (CPE2), and Group VI - standard reference, pentazocine sodium (20 mg/kg, IP). Tail flick response was evoked by nichrome wire heated electrically. The intensity of heat produced by nichrome wire was adjusted so that the baseline tail flick latency average was 3–4 s in all the animals. Cutoff period of 15 s was observed to prevent the damage to the tail. Tail flick response was measured three times in each animal initially to obtain base value. After initial reading, the test drugs and standard drug were administered to respective groups. Tail flick response was again recorded after 30, 60, 120, 180, and 240 min of drug administration. The difference between actual values and initial values were registered for each time interval. The changes in tail flick response were calculated and results were compared with control group.

**Acetic acid-induced writhing syndrome**

Albino mice of either sex weighing between 30 ± 5 g were divided into six groups containing six mice in each group. The groups were Group I - normal control, Group II - CP1 (780 mg/kg, po), Group III - CP2 (780 mg/kg, po), Group IV - CPE1 (280 mg/kg, po), Group V - CPE2 (280 mg/kg, po), Group VI - standard reference, pentazocine sodium (20 mg/kg, IP). The test drugs were administered to respective groups and
Singh, et al.: Comparative antipyretic and analgesic activities of two plants source of Patha

Statistical analysis
The obtained data have been presented as mean ± standard error of mean difference between the groups, statistically determined by ANOVA followed by Dunnet t-test to assess the statistical significance between the groups. The value $P < 0.05$ is considered as statistically significant.

Results

Antipyretic activity
Powder of *C. pareira* showed nonsignificant decrease in yeast-induced pyrexia after 6 h (12.58%) and after 9 h (30.26%) in comparison to control group. CP2, CPE1, and CPE2 did not produce decrease in rectal temperature after 3 and 6 h but produced marked decrease in pyrexia after 9 h in comparison to control group [Table 1].

Analgesic activity
The standard drug showed significant increase of the tail flick response after 30 and 60 min as compared to initial reading and in the control group. *C. pareira* powder treated group showed nonsignificant increase in latency of tail flick response at all-time intervals in comparison to control group. *C. peltata* root powder showed statistically significant increase in latency of tail flick response after 60 min and statistically nonsignificant increase in tail flick response after 30, 120, and 180 min compared to the control group. Extract of *C. pareira* and *C. peltata* extract produced nonsignificant increase in tail flick response after 30 min and afterward did not show any significant increase in tail flick response compared to initial reading and in the control group [Table 2].

Acetic acid-induced writhing syndrome
All the treated groups had shown increased latency of onset in abdominal writhing; among them, CP1 treated group showed marked increase in latency of onset of abdominal writhing response compared to other treated groups. CP1 showed statistically nonsignificant decrease in abdominal writhing at 0–10 min (44.21%) interval and 0–30 min (45.96%) interval while CP2 showed statistically nonsignificant decrease in abdominal writhing at 0–10 min (23.22%) and at 0–30 min (30.48%) interval in comparison to control group. CPE1 showed statistically significant decrease in abdominal writhing at 0–10 min (69.56%) and nonsignificant decrease at 0–30 min (57.28%) interval while CPE2 showed statistically significant decrease in abdominal writhing at 0–10 min (73.28%) interval and between 0–30 min (70.87%) interval when compared with control group [Table 3].

| Groups          | After 3 h | Percentage | After 6 h | Percentage | After 9 h | Percentage |
|-----------------|-----------|------------|-----------|------------|-----------|------------|
| Control         | 0.98±0.38 | -          | 1.43±0.37 | -          | 1.52±0.45 | -          |
| CP1             | 1.33±0.33*| 35.71↑     | 1.25±0.35 | 12.58↓     | 1.06±0.40 | 30.26↓     |
| CP2             | 1.13±0.14 | 15.30↑     | 1.78±0.24 | 24.47↑     | 0.48±0.33 | 68.42↓     |
| CPE1            | 1.35±0.22*| 37.75↑     | 1.45±0.73 | 1.39↑      | 0.78±0.59 | 48.68↓     |
| CPE2            | 1.18±0.20*| 20.40↑     | 1.73±0.23 | 20.97↑     | 0.90±0.33 | 40.79↓     |

*P=0.05 compared with control group (ANOVA followed by Dunnett multiple t-test). Data: Mean±SEM, ↑: Increase, ↓: Decrease. SEM: Standard error of mean, CP1: *Cissampelos pareira* powder, CP2: *Cyclea peltata* powder, CPE1: *Cissampelos pareira* extract, CPE2: *Cyclea peltata* extract

Discussion
In the present study, powder and alcoholic extract of *C. pareira* and *C. peltata* were evaluated for comparative antipyretic and analgesic activities in suitable animal models. Brewer’s yeast is a fungus containing lipo-polysaccharide, which is a cell wall component of Gram-negative bacteria. It binds with macrophages, releasing cytokines, interleukin-1, etc., into the blood circulation, leading to antigen-antibody reaction. It reduces blood–brain barrier and releases arachidonic acid mediated by the enzymes phospholipase, prostaglandin E2 (PGE2) synthase, and cyclo-oxygenase. Finally, synthesis and release of PGE2 into anterior hypothalamus results in inducing pyrexia. Study indicates that powder form of *C. pareira* produced moderate antipyretic activity after 6 h while alcoholic extract of both drugs and powder of *C. peltata* did not produce any effect in early phase while nonsignificant activity was seen after 9 h. The observed activity is likely due to inhibition of synthesis and/or release of local PGE2 into the preoptic area of anterior hypothalamus.[22,23]

Considering the relationship between anti-inflammatory and analgesic effect, another objective of the present work was to study the anti-nociceptive activity of test drugs. The models investigating anti-nociception were selected on the basis on their capacity to investigate both centrally and peripherally mediated effects. The tail flick method investigates the central activity while acetic acid-induced writhing model investigates peripheral activity. Tail flick model, which is thermal induced nociception, indicates narcotic involvement, which is sensitive to opioid µ receptors.[24] The increase in pain threshold produced by powder of *C. pareira* and *C. peltata* tests drugs in tail flick model suggests involvement of central

Table 1: Effects of test drugs on brewer's yeast induced pyrexia in rats
Singh, et al.: Comparative antipyretic and analgesic activities of two plants source of Patha

Table 2: Effects of test drugs on radiant heat-induced pain in rats

| Groups     | Duration of latency of tail flick response (s) recorded at different time intervals |
|------------|-------------------------------------------------------------------------------------|
|            | Initial 30 min | 60 min | 120 min | 180 min | 240 min | Percentage |
| Control    | 2.11±0.07      | 2.50±0.22 | -       | 2.33±0.21 | -       | 2.50±0.34 | -       | 2.17±0.17 | -       | 2.33±0.21 | -       |
| Reference  | 2.11±0.21      | 8.50±2.09** | 240.0†  | 3.83±0.79  | 64.38†  | 2.83±0.31  | 13.20†  | 2.67±0.33  | 23.04†  | 2.33±0.33  | -       |
| CP1        | 5.37±0.58      | 2.91±0.43  | 16.40†  | 2.71±0.41  | 16.30†  | 2.86±0.45  | 14.40†  | 2.49±0.40  | 14.74†  | 2.33±0.31  | -       |
| CP2        | 3.20±0.40      | 4.40±0.62  | 77.20†  | 4.36±0.67* | 87.12†  | 3.05±0.50  | 22.00†  | 2.77±0.44  | 27.64†  | 2.25±0.09  | 3.43↓  |
| CPE1       | 3.53±0.48      | 3.37±0.75  | 34.80†  | 2.26±0.40  | 3.00†  | 2.64±0.38  | 5.6†  | 1.81±0.25  | 41.47↓  | 1.94±0.24  | 16.74↓  |
| CPE2       | 2.51±0.25      | 2.91±0.28  | 14.00†  | 2.59±0.37  | 11.16†  | 2.59±0.36  | 3.6†  | 2.10±0.33  | 3.22↓  | 1.50±0.10  | 35.62↓  |

*CPE2: Cissampelos pareira powder, CPE1: Cyclea peltata powder, CPE1: Cissampelos pareira extract, CPE2: Cyclea peltata extract

Table 3: Effect of test drugs on acetic acid-induced writhing syndrome in mice

| Groups     | Latency of onset (s) | Percentage |
|------------|---------------------|------------|
| Control    | 34.33±7.13          | -          |
| CP1        | 59.67±17.83         | 73.81†     |
| CP2        | 42.50±11.91         | 23.80†     |
| CPE1       | 52.80±23.84         | 53.80†     |
| CPE2       | 53.00±12.66         | 54.38†     |

*P<0.01 when compared with control group (ANOVA followed by Dunnett multiple t-test). Data: Mean±SEM, †: Increase, ↓: Decrease. SEM: Standard error of mean, CP1: Cissampelos pareira powder, CP2: Cyclea peltata powder, CPE1: Cissampelos pareira extract, CPE2: Cyclea peltata extract

Pain pathways. Extract of C. pareira and C. peltata had mild effect on this parameter.

Writhing test is a chemical method used to induce pain of peripheral origin by injection of irritant principles such as phenylquinone and acetic acid in mice. Analgesic activity of the test compound is inferred from decrease in the frequency of writhing. The writhing response is considered as a reflexive test.[25] In general, acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, PGs, bradykinins and substance P, endings. Local peritoneal receptors are postulated to be involved in the abdominal constriction response.[26] The method has also been associated with prostanoids in general, i.e., increased levels of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products. Pain is centrally modulated through a number of complex processes including opiate, dopaminergic descending noradrenergic, and serotonergic systems.[27,28] Powder of C. pareira and C. peltata produced nonsignificant peripheral analgesic activity while extract of both plants produced significant analgesic activity in acetic acid-induced pain in mice. The analgesic effect produced by the tests and standards may be acting through central mechanisms involving opioid receptor systems or through peripheral mechanisms involved in the inhibition of PGs, leukotrienes, and other endogenous substances that are key components in pain.

Conclusion

The present study suggests that powder form of C. pareira has moderate antipyretic activity in rats may be due to inhibition of the synthesis and/or release of local PGE2 into the preoptic area of anterior hypothalamus. Further, powder form of test drugs has shown better central analgesic effect in rats and extract of test drugs have shown significant effect through peripheral mechanism in mice. The results of the present study corroborate with the classical uses and traditional claims made on Patha, i.e. C. pareira and C. peltata for the management of fever and painful conditions.

Financial support and sponsorship

IPGT & RA, Gujarat Ayurved University, Jamnagar, Gujarat.

Conflicts of interest

There are no conflicts of interest.

References

1. Shastri JL. Dravyaguna Vijnana (Knowledge on Vedic Herbs, Controversial Herbs and Ignored Medicinal Plants). Vol. 5. Varanasi: Chaukhambha Orientalia; 2008. p. 60-61.
2. Mishra BS, editor. Bhavaprakash Nighantu of Bhavnishra, Vidyosthan Hindi Commentary. 11th ed. Varanasi: Chaukhambha Sanskrit Sansthan; 2007. p. 394.
3. Acharya YT, editor. Charaka Samhita of Agnivesha, Chikitsasthana. Ch. 18, Ver. 39. Reprint Edition. Varanasi: Chaukhambha Surabharati Prakashan; 2011. p. 541.
4. Sharma PV, editor. Sodhala Nighantu of Acharya Sodhala. Baroda: Oriental Institute; 1978. p. 14, 103.
5. Sharma PV, editor. Priya Nighantu. Varanasi: Chaukhambha Surabharati Prakashan; 2004. p. 61.
6. Raveendra K, Martin P. Ethnomedicinal Plants. Jodhpur: Agrobias; 2006. p. 11.
7. Kingston C, Nisha BS, Kiruba S, Jeeda S. Ethno medicinal plants used by indigenous community in traditional healthcare system. Ethnobotanical Leaflets 2007;11:32-37.
Singh, et al.: Comparative antipyretic and analgesic activities of two plants source of Patha

8. Amresh G, Reddy GD, Rao CH, Singh PN. Evaluation of anti-inflammatory activity of Cyclea peltata root in rats. J Ethnopharmacol 2007;110:526-31.
9. Saha G, Senapati P, Sahu N. Anti-inflammatory activity of methanolic extract of root of Cyclea peltata on carrageenan-induced rat paw edema. Pharmacutur art 1324
10. Amresh G, Singh PN, Rao CV. Antinoceptive and antiarthritic activity of Cyclea peltata roots. J Ethnopharmacol 2007;111:531-36.
11. Sangameswaran B, Singh BK. Hepatoprotective effect of hydroalcoholic extract of Cyclea peltata against rifampicin and isoniazid induced hepatotoxicity. Cont J Pharm Sci 2012;6:30-35.
12. Amresh G, Rao CV, Singh PN. Antioxidant activity of Cyclea peltata on benzo (a) pyrene-induced mucosal injury in mice. Nutr Res 2007;27:625-32.
13. Kumar KA, Satyanarayana K, Mathwes A, Srinivasa Rao Y, Kiran K. Antihyperglycemic activity of methanolic extract of Cissampelos pareira Linn roots on blood glucose levels of Streptozotocin-induced diabetic rats. J Pharm Res 2011;4:3399-401.
14. Christina A, Christopher V, Packialakshmi M, Tobin GC, Preethi J, John C, et al. Effect of ethanolic extract of Cyclea peltata Lam. on a hypercholesterolemic rat model. Phcog Mag 2005;1:59-62.
15. Jyothi A, Thomas D. Antibacterial activity of medicinal plant Cyclea peltata (Lam) Hooks & Thoms. Asian Pac J Trop Dis 2012;5:280-84.
16. The Ayurvedic Pharmacopoeia of India. Part 1. Vol. 1. New Delhi: Ministry of Health and Family Welfare, Govt. of India; 1999. p. 123.
17. Paget GE, Barnes JM. Evaluation of drug activities. In: Laurence DR, Bacharach AL, editors. Pharmacometrics. Vol. 1. New York: Academic Press; 1964. p. 161.
18. Yadav MS, Kumar A, Singh A, Sharma US, Sutar N. Phytochemical investigation and hepatoprotective activity of Cissampelos pareira against carbon-tetrachloride induced hepatotoxicity. Asian J Pharm Sci 2011;1:106-10.
19. Kirana H, Srinivasan BP. Effect of Cyclea peltata Lam. roots aqueous extract on glucose levels, lipid profile, insulin, TNF-alpha and skeletal muscle glycogen in type 2 diabetic rats. Indian J Exp Biol 2010;48:499-502.
20. Gujaral MC, Khanna BK. Comparative evaluation of some narcotic analgesies. J Sci Ind Res 1956;16:11-13.
21. Kulkarni SK. Handbook of Experimental Pharmacology. Delhi: Vallabh Prakashan; 1999. p. 117.
22. Dalal S, Zhukovsky DS. Pathophysiology and management of fever. J Support Oncol 2006;4:9-16.
23. Li S, Ballou LR, Morham SG, Blatteis CM. Cyclooxygenase-2 mediates the febrile response of mice to interleukin-1 beta. Brain Res 2001;910:163-73.
24. Abbott FV, Young SN. Effect of 5-hydroxytryptamine precursors on morphine analgesia in the formalin test. Pharmaco Biochem Behav 1988;31:855-60.
25. Gawade SP. Acetic acid induced painful endogenous inflation in writhing test on mice. J Pharmacol Pharmacother 2012;3:348.
26. Bentley GA, Newton SH, Starr J. Studies on the antinociceptive action of alpha-agonist drugs and their interactions with opioid mechanisms. Br J Pharmacol 1983;79:125-34.
27. Bensreti MM, Sewell RD. Selective effects of dopaminergic modifiers on antinociception produced by different opioid receptor agonists. Pro Br Pharmacol 1983;70.
28. Headley PM, Shaughnessy CT. Evidence for opiate and dopamine interaction in striatum. Br J Pharmacol 1985;86:700.

हिंदी सारांश
सिसम्पेल्लोस परा लिन. को एवं साइक्लिया पेल्टाटा (लेम) हुक.एफ. एंड थॉम्स के एंटीपाइरेटिक और एनॉलजेसिक गतिविधियों का तुलनात्मक अध्ययन

सुमन जी सिह, के. , निश्चेत्वर, भृंगश आर पेटेल, मुकेश भी मारिया