

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

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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, while 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), *Prameha* (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 *Chikitsasagranthas*. *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, antinociceptive,^[10] hepatoprotective,^[11] antioxidant,^[12] anti-diabetic,^[13] antihyperlipidemic,^[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

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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 $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ 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. pareira* 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 an Tail 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

distilled water was given to the control group. One hour after drug administration, 1% v/v of acetic acid in dose of 1 ml/100 g body weight of mice was injected intraperitoneal. The mice were placed individually under glass jar for observation. Onset of writhing was noted for each mouse. Number of abdominal contractions, trunk twist response, and extension of hind limb were noted.^[21]

Statistical analysis

The obtained data have been presented as mean \pm standard error of mean difference between the groups, statistically determined by ANOVA followed by Dunnett 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 of 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].

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 the 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

Groups	Increase in pyrexia (°C) recorded at different time intervals (h.)					
	After 3 h	Percentage	After 6 h	Percentage	After 9 h	Percentage
Control	0.98 \pm 0.38	-	1.43 \pm 0.37	-	1.52 \pm 0.45	-
CP1	1.33 \pm 0.33*	35.71 \uparrow	1.25 \pm 0.35	12.58 \downarrow	1.06 \pm 0.40	30.26 \downarrow
CP2	1.13 \pm 0.14	15.30 \uparrow	1.78 \pm 0.24	24.47 \uparrow	0.48 \pm 0.33	68.42 \downarrow
CPE1	1.35 \pm 0.22*	37.75 \uparrow	1.45 \pm 0.73	1.39 \uparrow	0.78 \pm 0.59	48.68 \downarrow
CPE2	1.18 \pm 0.20*	20.40 \uparrow	1.73 \pm 0.23	20.97 \uparrow	0.90 \pm 0.33	40.79 \downarrow

* $P < 0.05$ compared with control group (ANOVA followed by Dunnett multiple t -test). Data: Mean \pm SEM, \uparrow : Increase, \downarrow : Decrease. SEM: Standard error of mean, CP1: *Cissampelos pareira* powder, CP2: *Cyclea peltata* powder, CPE1: *Cissampelos pareira* extract, CPE2: *Cyclea peltata* extract

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	Percentage	60 min	Percentage	120 min	Percentage	180 min	Percentage	240 min
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	4.43±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
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
CPE2	2.51±0.25	2.81±0.28	12.40↑	2.59±0.37	11.16↑	2.59±0.36	3.6↑	2.10±0.33	3.22↓	1.50±0.10

* $P<0.05$, ** $P<0.01$ when compared with control group (ANOVA followed by Dunnett multiple t -test). Data: Mean±SEM, ↓: Decrease, ↑: Increase. SEM: Standard error of mean, CP1: *Cissampelos pareira* powder, CP2: *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	Number of writhing at time intervals			
			0-10 min	Percentage	0-30 min	Percentage
Control	34.33±7.13	-	23.00±5.53	-	51.50±13.48	-
CP1	59.67±17.83	73.81↑	12.83±2.00	44.21↓	27.83±5.96	45.96↓
CP2	42.50±11.91	23.80↑	17.66±3.12	23.22↓	35.80±6.54	30.48↓
CPE1	52.80±23.84	53.80↑	7.00±2.16*	69.56↓	22.00±6.54	57.28↓
CPE2	53.00±12.66	54.38↑	6.16±1.97*	73.28↓	15.00±5.09*	70.87↓

* $P<0.01$ 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 PGF2 α in peritoneal fluids as well as lipoxigenase 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.

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Conflicts of interest

There are no conflicts of interest.

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हिन्दी सारांश

सिसम्पेलॉस परेरा लिन. को एवं साइक्लिया पेल्टाटा (लेम) हुक.एफ. एंड थॉमस के एंटीपाइरेटीक

और एनॉलजेसिक गतिविधियों का तुलनात्मक अध्ययन

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सिसम्पेलॉस परेरा लिन. को पाठा के एक स्थापित स्रोत के रूप में माना जाता है और साइक्लिया पेल्टाटा (लेम) हुक. एफ. एंड थॉमस को भारत के दक्षिण हिस्से में पाठा के स्रोत के रूप में उपयोग किया जाता है। शास्त्रीय ग्रन्थों में पाठा की दो विभिन्न किस्में जैसे राजपाठा (सिसम्पेलॉस परेरा लिन.) एवं लघु पाठा (साइक्लिया पेल्टाटा (लेम) हुक. एफ. एंड थॉमस) का उल्लेख किया गया है। प्रस्तुत शोध में सिसम्पेलॉस परेरा लिन. एवं साइक्लिया पेल्टाटा (लेम) हुक. एफ. एंड थॉमस की एंटीपाइरेटीक और एनॉलजेसिक गतिविधियों का तुलनात्मक प्रयोगिक अध्ययन चूहों में किया गया है। इन दोनों परीक्षणों के लिये औषधियों के चूर्ण एवं इथेनॉलिक सार का प्रयोग किया गया। एंटीपाइरेटिक गतिविधि का विश्लेषण चूहों में ब्रीवर यीस्ट प्रेरित पाइरेक्सिया से किया गया तथा एनॉलजेसिक गतिविधि का मूल्यांकन रेडिएंट हीट मॉडल और एसीटिक एसिड प्रेरित ब्रिथिंग सिंड्रोम द्वारा चूहों में किया गया। परिणाम से ज्ञात हुआ कि सिसम्पेलॉस परेरा चूर्ण की एंटीपाइरेटिक गतिविधि साइक्लिया पेल्टाटा के चूर्ण की तथा दोनों औषधों के इथेनॉलिक सार की अपेक्षा मध्यम हैं। सिसम्पेलॉस परेरा के चूर्ण ने दोनों औषधियों के इथेनॉलिक सार की अपेक्षा अच्छा एनॉलजेसिक परिणाम रेडिएंट हीट मॉडल में दिया। इसके विपरीत दोनों औषधियों के इथेनॉलिक सार ने एसीटिक एसिड प्रेरित ब्रिथिंग सिंड्रोम में दोनों औषधियों के चूर्ण की अपेक्षा अधिक प्रभाव दिखाया। इस शोध से यह परिणाम प्राप्त हुये कि दोनों ही औषधियों सिसम्पेलॉस परेरा एवं साइक्लिया पेल्टाटा में एनॉलजेसिक प्रभाव है परंतु यह साइक्लिया पेल्टाटा में तुलनात्मक दृष्टि में अधिक है।