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Pharmacological study

Comparative anti-inflammatory and analgesic activities of leaf powder and decoction of *Chirabilva* [*Holoptelea integrifolia* (Roxb.) Planch]

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Abstract

Background: Ethno-medical claims indicate that leaf of *Holoptelea integrifolia* (Roxb.) Planch is being used in pain, inflammatory conditions by the Koya tribes. **Aim:** To evaluate and compare the anti-inflammatory and analgesic activity of leaves of *H. integrifolia* in powder and decoction forms**. Materials and Methods:** The leaves of *H. integrifolia* were made into powder and decoction form using guidelines mentioned in Ayurvedic Pharmacopeia of India. The anti-inflammatory activity of test drug was evaluated against carrageenan and formalin induced paw edema and analgesic activity with formalin induced paw licking and tail flick response using Wistar albino rats. **Results:** Administration of leaf powder showed insignificant inhibition of carrageenan induced paw edema at 1 h (21.62%) compared to the control group. Administration of decoction of leaves showed insignificant inhibition of carrageenan induced paw edema at 1 h (18.12%) and 3 h (9.78%). Administration of leaf powder decreased the paw edema at 24 h (37.65%) and 48 h (66.30%) while treatment with leaf decoction showed apparent decrease in paw edema at 24 h (13.68%) and 48 h (52.42%) but failed to reach at significant level of formalin induced paw edema in rats. The test drugs did not produce any effect on radiant heat induced pain in rats and formalin induced paw licking response. **Conclusion:** Leaf decoction of *H. integrifolia* has better anti-inflammatory activity than leaf powder while they have not shown significant analgesic effects in both the experimental models.

Key words: Analgesic, anti-inflammatory, *Chirabilva*, *Holoptelea integrifolia*

Introduction

Holoptelea integrifolia (Roxb.) Planch. is of Ulmaceae family, a large and deciduous tree. It is distributed throughout the greater part of India.[1] Leaves are elliptic-ovate, acuminate, base rounded or sub-cordate. Flowers greenish yellow, in short racemes or fascicles on the leafless branches. Fruit is sub-orbicular samara with membranous wing. Seed is solitary and flat.[2] In Ayurveda, *Chirabilva* is having *Tikta* (bitter), *Katu* (pungent), *Kashaya* (astringent) *Rasa* (taste), *Katu Vipaka* and *Ushna Veerya*, possess *Laghu* (light), and *Tikshna* properties.[3] Ethno-medically, the leaves and stem bark of this plant are being used by tribal people for skin diseases, puerperal disease and facial paralysis, arthritis, body pain in the form of paste. Mainly leaves are used for treating edema, diabetes, leprosy and other skin diseases, intestinal disorders, piles and sprue by tribal people.[4] Some recent researches have reported analgesic, anti-inflammatory, antiviral, antioxidant, antimicrobial and wound healing activities of this plant.[5] The scientific evidence is already established in case of aqueous and alcoholic extract of leaf of *H. integrifolia*, still no work is reported in case of powder and decoction form of *H. integrifolia*. As in tribal claims, leaves are used in a *Kwatha* (decoction) as well as *Kalka* (paste) form therefore present study was carried out to evaluate the analgesic and anti-inflammatory activity of *Chirabilva* leaves in different dosage forms in animal models.

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Materials and Methods

Collection of plant

The leaves were collected just prior to flowering in the month of February 2012 in the campus of Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar by the scholar herself. The identification

and confirmation of the sample was carried out by botanist and a copy has been preserved for the future reference at the herbarium of the institute Pharmacognosy Laboratory, Institute for Postgraduate Teaching and Research in Ayurveda, Jamnagar (Phm: 6014/2012).

Animals

Wistar albino rats (*Rattus norvegicus*) of both sexes weighing between 180 and 220 g were used for the experimentation. The rats were obtained from animal house. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC/12/2012/03) in accordance with the guideline formulated by Committee for Purpose of Control and Supervision of Experiments on Animals, India.

Six rats were housed in each cage made of poly-propylene with stainless steel top grill. The animals were exposed to 12 h light and 12 h dark cycle with the relative humidity of 50 to 70% and the ambient temperature was 22°C ± 3°C. All animals were kept on same environmental conditions. The rats were given food and water *ad libitum*.

Drug derivation

- Powder: The collected drug was shade-dried and pulverized to fine powder (mesh no. 120) and stored in air tight container. The powder was administered by making stock solution in distilled water (1 ml/100 g body weight of the rat)
- Decoction: 10 g coarse powder of leaves of *H. integrifolia* was taken and 40 ml of water was added (1:4 ratio) and boiled till it reduced to one-fourth of the total quantity and filtered.[6]

Dose fixation

Dose of the drugs was fixed by extrapolating the human dose to laboratory animals on the basis of body surface area ratio.[7] The calculated doses for dosage forms of *H. integrifolia* are -

Leaf powder

Human Dose	–	10g[8]
Rat Dose	–	900 mg/kg p.o.

Leaf decoction

Human Dose	–	50ml[9]
Rat Dose	–	4.5 ml/kg p.o.

Anti-inflammatory activity

Carrageenan induced hind paw edema

Wistar strain albino rats of either sex were weighed and randomly divided into four groups of six animals in each group. First group received distilled water and served as a control group. The second group was kept as a standard reference and treated orally with phenylbutazone with water (100 mg/kg). Third and fourth group received stock solution of powder of *H. integrifolia* (900 mg/kg, p.o.) and decoction of *H. integrifolia* (4.5 ml/kg, p.o.) respectively. The vehicle and test drugs were administered to the respective groups for 5 consecutive days. On 5th day, 1 h after drug administration, initially left hind paw volumes up to the tibio-tarsal articulation were recorded prior to carrageenan injection using digital plethysmograph (Model 520, IITC - Life Science Inc.)[10] and then edema was produced by injecting 0.1 ml freshly prepared 1% w/v carrageenan in sterile saline solution to the sub-plantar aponeurosis of the left hind limb. The rats were administered distilled water in the dose of 2 ml/100 g body weight to ensure uniform hydration and hence to minimize variations in edema formation. Paw volume was recorded at the interval of 3 h and 6 h after carrageenan injection. Results were expressed as a percentage change in paw volume in comparison to the initial paw volumes.

Formaldehyde induced paw edema

The test conditions and groupings were similar to carrageenan induced paw edema as mentioned above. The drugs were administered once daily for 5 consecutive days. On 5th day, initial left hind paw volumes were recorded with the help of digital plethysmometer, (Model 520, IITC - Life Science Inc.). One hour after the drug administration, 0.1 ml of 1% v/v formaldehyde solution was injected to sub-plantar aponeurosis of the left hind limb. Paw volumes were measured at 24 h and 48 h after the formaldehyde injection as described earlier. Results were expressed as a percentage change in paw volume at various time intervals in comparison to the initial values.[11]

Analgesic activity

Formaldehyde induced paw licking

The effect of test drugs on formaldehyde induced paw licking was studied in Wistar strain albino rats of either sex. The selected animals were grouped into four groups of 6 rats each. First group received distilled water and served as a control group. The second group kept as a positive control group and received diclofenac sodium (5 mg/kg, p.o.). Third and fourth group received powder of *H. integrifolia* (900 mg/kg, p.o.) and decoction of *H. integrifolia* (4.5 ml/kg, p.o.) respectively. The test drugs were administered to the respective groups for 5 consecutive days. On 5th day, pain response was induced by injecting 0.1 ml of 1% v/v formalin in distilled water in subplantar region of left hind paw. The number of paw licking was noted as an index of nociception at different time intervals periods of 0–10 min (early phase), 11–20 min and 21–30 min (late phase).[12]

Radiant heat tail flick test

Wistar albino rats of either sex were placed on the tail flick unit so that constant heat intensity was applied to the lower third of the animal's tail. When the animal flicked its tail in response to the noxious stimulus both the heat source and timer were stopped. A cut off time of 10 s was set to avoid tail damage. The basal reaction time of each rat to radiant heat was recorded, and those having tail flick latency (TFL) less than 10 s were selected. Selected rats were randomly divided into four groups of six each. First group received distilled water and served as a control group. The second group kept as positive control group received pentazocine sodium (20 mg/kg, p.o.). Third and the fourth group received, powder of *H. integrifolia* (900 mg/kg, p.o.) and decoction of *H. integrifolia* (4.5 ml/kg, p.o.) respectively. The TFL was recorded at the intervals of 30, 60, 120, 180 and 240 min after drug administration.[13]

Statistical analysis

The obtained data have been presented as mean ± standard error of mean, difference between the groups, statistically determined by one-way "ANOVA" test followed by Dunnette's *t*-test to assess the statistical significance between the groups. The value $P < 0.05$ is considered as statistically significant.

Results

Administration of leaf powder showed insignificant inhibition of carrageenan induced paw edema at 1 h (20.41%) compared to the control group. Administration of decoction of leaves showed non-significant inhibition of carrageenan induced paw edema at 1 h (16.88%) and at 3 h intervals (20.45%). The decoction shows prolonged effect as compared to leaf powder. Both drugs did not produce any significant effects after 6 h. Phenyl butazone treated rats showed a significant decrease in paw edema after 3 h and effects sustained even up to 6 h [Table 1].

Administration of leaf powder decreased the paw edema at 24 h (36.35%) and 48 h (64.19%), however the observed inhibition was found to be statistically non-significant. Treatment with decoction of the leaf showed apparent decrease in paws edema at 24 h (13.68%) and 48 h (50.33%) but failed to reach a significant level. Diclofenac sodium treated rats showed decreased in paw edema at 24 h (36.34%) and 48 h (58.21%). In this model, leaf powder has shown better result compare to leaf decoction [Table 2].

The standard drug shows a significant increase in the tail flick response after 30 min and a insignificant increase in TFL various time intervals compared to the control group. The test drugs did not produce any effects on radiant heat induced pain in rats [Table 3].

The standard drug shows a significant decrease in numbers of paw licking response in 0–10 min time interval and also showed remarkable nonsignificant decrease in paw licking response in 21–30 min intervals. Administration of leaf powder and decoction did not produce any effects on formalin induced paw licking response in both phases that is, early and late phase in rats compared to the control group [Table 4].

Discussion

Subcutaneous injection of carrageenan into the rat paw produces acute inflammation characterized by increased tissue water and plasma protein exudation with neutrophil extravasation and metabolism of arachidonic acid by both cyclooxygenase and lipoxygenase enzyme pathways. Besides, carrageenan induced acute inflammation involves the synthesis and release of mediators at the injured site. These mediators include prostaglandin, e-series, histamine, bradykinin, leukotriene and serotonin that cause pain and fever.[14] Inhibition of these mediators from reaching the injured site or from bringing out their pharmacological effect will normally ameliorate the inflammation and other symptoms.

In the present study, the standard drug phenylbutazone has suppressed the biphasic response of carrageenan induced inflammation in rats. Leaf powder and leaf decoction also decreased the edema in a insignificant manner after 1 h and the decoction showed the effect after 3 h that suggest that decoction has prolonged the effect as compared to leaf powder. Inflammation induced by formaldehyde is biphasic, an early neurogenic component is mediated by substance *P* and bradykinin followed by a tissue mediated response where histamine, 5-hydroxytryptamine, prostaglandin and bradykinin are known to be involved.[15] The initial phase of the edema is due to the release of histamine and serotonin and the edema is maintained during the plateau phase by kinin like substance and the second accelerating phase of swelling due to release of prostaglandin like substances. Hence, it is speculated that apart from inhibition of chemical mediators of inflammation, test drug may also modulate the pain response in central nervous system. In the present study, both leaf powder and decoction produced insignificant decrease of formaldehyde induced paw edema in rats. Among these groups, the effect in leaf powder treated group is found to be better. This indicates that it has some inhibitory effect on proliferation of fibroblast.

Considering the relationship between anti-inflammatory and analgesic effect, another objective of the present work was to study the anti-nociceptive activity of *H. integrifolia*. The models investigating anti-nociception were selected based on their capacity to investigate both centrally and peripherally mediated effects.[16] The tail flick method investigates the central activity while formalin based study investigates both central as well as peripheral effects.

A tail flick model is thermal induced nociception, indicates narcotic involvement, which is sensitive to opioid μ receptors.[17]

Table 2: Effect of *H. integrifolia* on formaldehyde induced paw edema in rats

Groups	Dose	% increase in paw volume at different time intervals after formaldehyde injection	
		24 h	48 h
Control	-	18.712±3.374	26.735±7.673
Diclofenac sodium	5.0 mg/kg	11.91±1.46	11.17±2.18
Leaf powder	900.0 mg/kg	11.667±1.597	9.010±2.300
Leaf decoction	4.5ml/kg	16.152±3.584	12.718±1.355

Values are given in mean±SEM, *n*=6 in each group. SEM: Standard error of mean, *H. integrifolia: Holoptelea integrifolia*

Table 1: Effect of *H. integrifolia* on carrageenan induced paw edema in rats

Groups	Dose	% increase in paw volume at different time interval after carrageenan injection		
		1 h	3 h	6 h
Control	-	23.81±4.30	64.79±4.15	58.28±4.86
Phenylbutazone	100.0 mg/kg	21.04±0.87	40.07±5.06*	45.36±3.91
Leaf powder	900.0 mg/kg	18.95±3.54	60.12±9.47	67.68±7.64
Leaf decoction	4.5 ml/kg	19.79±3.35	51.54±5.60	57.35±5.35

Values are given in mean±SEM, *n*=6 in each group. *P<0.05 compared with the control group. SEM: Standard error of mean, *H. integrifolia: Holoptelea integrifolia*

Table 3: Effect of *H. integrifolia* on tail flick response at different intervals in rats

Group	Dose	Duration of latency of tail flick response (s) recorded at different time intervals					
		Initial	30 min	60 min	120 min	180 min	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
Pentazocine sodium	20.0 mg/kg	2.11±0.21	8.50±2.09*	3.83±0.79	2.83±0.31	2.67±0.33	2.33±0.33
Leaf powder	900.0 mg/kg	3.82±0.57	2.15±0.39	2.15±0.34	2.13±0.29	2.11±0.42	1.95±0.32
Leaf decoction	4.5 ml/kg	3.53±0.48	2.50±0.21	2.35±0.23	2.43±0.24	2.13±0.44	1.78±0.40

Data: Mean±SEM. *$P<0.001$ compared with control group. SEM: Standard error of mean, *H. integrifolia: Holoptelea integrifolia*

Table 4: Effect of *H. integrifolia* on formalin induce paw licking at different intervals in rats

Groups	Dose	Numbers of paw licking		
		0-10 min	11-20 min	21-30 min
Control	-	13.83±1.08	1.83±0.94	6.16±1.81
Diclofenac sodium	100.0 mg/kg	08.00±1.86*	0.66±0.333	2.66±0.803
Leaf powder	900.0 mg/kg	14.17±1.35	1.66±0.667	2.83±0.833
Leaf decoction	4.5 ml/kg	15.50±3.32	1.83±0.703	8.33±2.231

Values are given in mean+SEM, $n=6$ in each group. *$P<0.05$ when compared with the control group (unpaired t-test) ($F=2.53$). SEM: Standard error of mean, *H. integrifolia: Holoptelea integrifolia*

The drugs that prolong the reaction latency to thermally induced pain in albino rats have a central analgesic activity. In formalin induced paw licking, animals present two distinct nociceptive behavior phases, which probably involves different stimuli. The first phase initiates immediately after formalin injection and lasts for 3–10 min, representing neurogenic pain. The second phase initiates within 15–20 min after formalin injection, lasts for 20–30 min and represents inflammatory pain. The second phase (phase II) is proposed to result from activity-dependent sensitization of CNS neurons within the dorsal horn. Therefore analgesics inhibit only phase II responses, but not in phase I.[18] In the present study, *H. integrifolia* leaf powder and leaf decoction did not produce analgesic activity against radiant heat induced pain and formalin induced paw licking in rats.

Conclusion

Holoptelea integrifolia of leaf powder and decoction have anti-inflammatory activity against carrageenan induced acute inflammation and formalin induced sub-acute inflammation in rats. Leaf decoction had shown better effect in carrageenan induced paw edema while leaf powder had more effect in formalin induced paw edema. Both the dosage forms have not shown significant effects against radiant heat induced pain and formalin induced paw licking in rats which suggests that both the dosage form have not shown the analgesic activity at the studied dose levels.

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हिन्दी सारांश

चिरबिल्व (होलोप्टेलिया इटीग्रिफोलिया प्लांच) के पत्र चूर्ण एवं पत्र क्वाथ के शोथहर और शूलहर कर्म का तुलनात्मक परीक्षण

सुष्मा भुवाड, मुकेश बी. नारिया, के. निष्ठेश्वर

चिरबिल्व (होलोप्टेलिया इंटीगिफोलिया प्लांच) ये वनस्पति अल्मेसी वर्ग की है। सामान्य लोगों में इसके पत्र और त्वक का कल्क या क्वाथ के स्वरुप में सूतिका काल के शूल, संधिशूल, त्वचारोग, व्रणरोपण के लिए उपयोग में लाया जाता है। इसके आधार पर चिरबिल्व के शोथहर और शूलहर कर्म का परिक्षण करने का प्रयोजन किया। इस परीक्षण में चिरबिल्व के पत्र चूर्ण एवं पत्र क्वाथ का उपयोग करने का प्रयोजन किया क्योंकि उसके पत्रसत्व का इसी कर्म के तौर पर परीक्षण हो चुका है। शोथहर कर्म के परीक्षण के लिये केराजीन और फॉर्मलीन से प्रेरित पॉ लिकींग और टैल फ्लीक इन दो मॉडल का उपयोग किया। चिरबिल्व पत्र चूर्ण ९०० मि.ग्रा./कि.ग्रा. और क्वाथ ४.५ मि.लि./कि.ग्रा. मात्रा में चूहों को मुखमार्ग से दिये गये। प्रयोग के बाद परीक्षण में प्राप्त संख्याओं का विश्लेषण करने के लिए स्टूडेन्ट टी टेस्ट का उपयोग किया। इस परीक्षण में चिरबिल्व के पत्र चूर्ण एवं पत्र क्वाथ का शोथहर कर्म नियंत्रित वर्ग से सांख्यिकी तौर पर प्रभावी सिद्ध हुआ। परंतु दोनों कल्पनाओं का शूलहर कर्म सांख्यिकी के तौर पर सिद्ध नहिं हुआ।