

Original Research Article

Evaluation of local anesthetic and antipyretic activities of Cinchona alkaloids in some animal models

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Abstract

Purpose: To evaluate the local anesthetic and antipyretic activities of an aqueous extract of *Cinchona officinalis* (*C. officinalis*) in experimental animal models.

Methods: Various doses of the aqueous extract was tested for its local anesthetic activity in guinea pigs and frogs using intracutaneous and plexus anesthesia, respectively. For comparison, 2 % xylocaine was used as a reference drug. The anti-pyretic activity of the aqueous extract was determined by Brewer's yeast-induced pyrexia in rats, using aspirin (300 mg/kg) as reference.

Results: *C. officinalis* extract, at concentrations of 10 and 20 %, produced significant anesthetic effects, of 72.12 and 88.08 %, respectively, compared with 96.86 % anesthetic effect of 2 % standard xylocaine ($p < 0.001$). In the plexus model, the mean onset of anesthetic effect was recorded at 6.44 ± 0.68 min versus 3.86 ± 0.42 min ($p < 0.001$) for the standard drug. Single administration of the extract (100, 200 and 400 mg/kg) showed significant dose-dependent anti-pyretic activity throughout the observation period, which was comparable to the standard aspirin group.

Conclusions: The findings suggest that the aqueous extract of *C. officinalis* has significant local anesthetic and anti-pyretic activities in rats.

Keywords: *Cinchona officinalis*, Antipyretic, Aspirin, Local anesthesia, *Cinchona* alkaloids, Xylocaine

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INTRODUCTION

The use of medicinal plants in treating ailments has been an integral part of traditional medicine for centuries. Importantly, herbal medicines play a vital role in developed, as well as developing, countries in improving primary healthcare because of their effective biological and medicinal properties with high safety margins and low costs.

C. officinalis is one traditional medicinal plant, belonging to the family Rubiaceae. Its extracts are the main ingredient in Indian Ayurvedic formulations to treat various fever types, including malaria [1]. Apart from this, various

peoples have used cinchona barks for different purposes as medicinal agents. In Brazilian medicine, it has been used as a digestive tract stimulant and fever reducer, while in South America generally, it is used to treat cancers, in Europe, cinchona extract has been used as an anti-protozoal and anti-spasmodic, and in the USA, it has been used as a digestive tonic, and as a treatment for cardiac disorders.

Basically, *Cinchona* alkaloids are abundantly present as organic molecules that have been isolated from the bark of about 40 species of cinchona trees around globe. The major chemical constituents of these alkaloids are quinine and quinidine; cinchonine and

cinchonidine can also comprise up to 16 % by mass of total tree bark. Nearly, most of these extracts have been utilized in the food and beverage industry as bitter additives. They have also been used as an important anti-malarial drug; as a muscle spasm relaxant agent; and as a cardiac depressant (anti-arrhythmic). Because of their increasing commercial importance, several cinchona species are now widely cultivated in various parts of the world [2,3].

In the present study, we evaluated the local anesthetic and anti-pyretic potential of an aqueous extract of *C. officinalis*. We used some animal models (guinea pig, frog and albino rat) in the experiments.

EXPERIMENTAL

Materials

Xylocaine, sodium chloride, Brewer's yeast, and acetyl salicylic acid were obtained from Sigma Aldrich and were used without further processing.

Preparation of *C. officinalis* extract

Fresh stem bark of *Cinchona* was collected from Western Ghats in Tamil Nadu, India, and authenticated in the Pharmacognosy and Phytochemistry Department of Agricultural University, Tamil Nadu, India with a voucher specimen (no. PEAR-PAA-06/14) preserved in the department's herbarium. The shade-dried stems were ground to a fine powder with a mechanical grinder, and 300 g of the pulverized powder material was extracted with double-distilled water using a Soxhlet apparatus at 60 °C for approximately 48 h. The yield of the aqueous extract was 14.1 % (w/w) in the air-dried powder form, and it was stored in an air-tight container at 4 °C until used.

Phytochemical studies

The powdered aqueous extract was subjected to phytochemical screening analysis using fluorescence, thalleoquin, and Rosequin tests [4].

Experimental animals

Adult male guinea pigs weighing 300–400 g, frogs, and albino rats weighing 2.5–3 kg, were procured from the central animal house of the Xinxiang Medical Institute. The animals were acclimatized to the laboratory atmosphere for 7 days prior to the experiment, at a controlled room temperature (24 ± 2 °C; relative humidity 60 – 70 %) under a 12/12-h light-dark cycle. They were

given a standard laboratory diet and water ad libitum. The experimental protocol was conducted after approval of the Institutional Animal Ethics Committee of Xinxiang Medical University (approval ref no. 2013/514B-21). The studies were performed in compliance with Directive 2010/63/EU on careful handling of animals for scientific purposes [5].

Acute oral toxicity study

An acute oral toxicity study was carried out using the aqueous extract (0–2000 mg/kg) and the acute toxicity method prescribed by the Organization of Economic Co-operation and Development (OECD) Guidelines (No. 423) [6].

Evaluation of local anesthetic activity

Intradermal wheal guinea pig model

Guinea pigs were shaved (four different areas of 4 cm² each) and divided into four groups (Table 1). The normal response of the animals to a pin prick was tested with 0.2 ml extract of *C. officinalis* (50 mg) injected intradermally on the left side of the animal, with 0.2 ml normal saline injected on the right. Six pin pricks were applied uniformly 5 min at an interval of 3 – 4 s on the wheal areas for up to 30 min. A skin twitch, usually accompanied by a squeak, was considered as a normal response to the pin prick. Animals that failed to respond or produced no squeak response to all pin pricks were considered to show a positive response to the anesthetic effect [7,8].

Table 1: Local anesthetic activity of aqueous extract on intracutaneous wheal in guinea pigs

Group	Drug	No. of negative responses	Mean failure of response (%)
A (Control)	0.9% saline	1.7 ± 0.84	5.26
B (Test)	10% AQ	27.46 ± 4.16*	72.12*
C (Test)	20% AQ	33.48 ± 2.04*	88.08*
D (Standard)	2% xylocaine	37.12 ± 0.68*	96.86*

AQ = Aqueous extract; *P* < 0.001, vs. control. *n* = 6 in each group

Plexus anesthesia in frog model

Frogs were divided into three groups (Table 2). They were pitched and the upper part of the spinal cord was destroyed. Four limbs were nailed on the frog board and a transverse incision was made on the abdominal wall just below the sternum. The viscera were removed carefully through this opening and the lumbar plexus was exposed carefully without damaging

it. Then, the abdominal pouch was filled with 20 % of the extract as a test drug. Reflex activity was noted by immersing the left and right limbs of the frog, every 2 min for up to 10 s, into 0.01 N HCl and normal saline, respectively. The time taken for the disappearance of reflex activity in the feet was recorded and the time of onset of local anesthetic action was calculated [8-12].

Table 2: Local anesthetic activity of aqueous extract on plexus of frog

Group	Drug	Onset of anesthetic action (mean \pm SEM) (min)
A (Control)	0.9% saline	25.27 \pm 1.65
B (Test)	20% AQ	6.44 \pm 0.68*
C (Standard)	2% xylocaine	3.86 \pm 0.42*

AQ = aqueous extract; * $P < 0.001$, vs. control. $n = 6$ in each group

Evaluation of antipyretic activity

Rats were divided into five groups. Fever was induced by subcutaneous injection of 20 mg/kg of a 20 % normal saline suspension of Brewer's yeast below the nape of the neck. Initial rectal temperature was recorded and, after 18 h, animals showing a temperature difference of 0.3–0.5 °C were selected. The control group (A) received vehicle (1 % Tween 80), the standard group (E) received aspirin 150 mg/kg, and groups B, C, and D received 100, 250, and 500 mg/kg of the aqueous extract (po), respectively. Variation in rectal temperature was recorded at different time intervals [13-15].

Statistical analysis

Statistical analysis was carried out by one-way ANOVA followed by Dunnett's multiple t test. $P < 0.05$ was considered to indicate statistical significance. GraphPad Prism5 software (GraphPad Software, Inc, La Jolla, CA, USA) was used to perform the analysis.

RESULTS

The aqueous extract of *C. officinalis* showed blue fluorescence and unique colors in each test, suggesting the presence of alkaloids, tannins, flavonoids, phenolic compounds, and triterpenes [16]. The aqueous extract, tested at up to 2000 mg/kg, was found to be non-toxic (category 5 according to the OECD guidelines).

The local anesthetic activity results of the samples in the guinea pig wheel test are presented in Table 1, which shows that the

extract at 20 % concentration produced 88.08 %, and that at 10 % concentration produced 72.12 % anesthetic activity; in contrast, the standard xylocaine showed 96.86 % activity. The extract of *C. officinalis* was significant versus the control group [17,18].

Table 2 shows the activity of the aqueous extract on plexus anesthesia in the frog model. The mean onset of anesthetic action for 20 % aqueous extract was 6.44 \pm 0.68 min versus 3.86 \pm 0.42 min for the standard drug xylocaine. Compared with the control group (saline), the 20 % aqueous extract showed a significant difference in anesthetic action.

The results of the anti-pyretic effects of the control, standard drug (aspirin), and aqueous extract (at doses of 100, 250, and 500 mg/kg) are shown in Figure 1. Aspirin and the extract at 250 and 500 mg/kg started to show significant reduction in body temperature at 1 h post-dosing compared with the control group. The anti-pyretic action was sustained for up to 3 h after aspirin and aqueous extract administration [19,20].

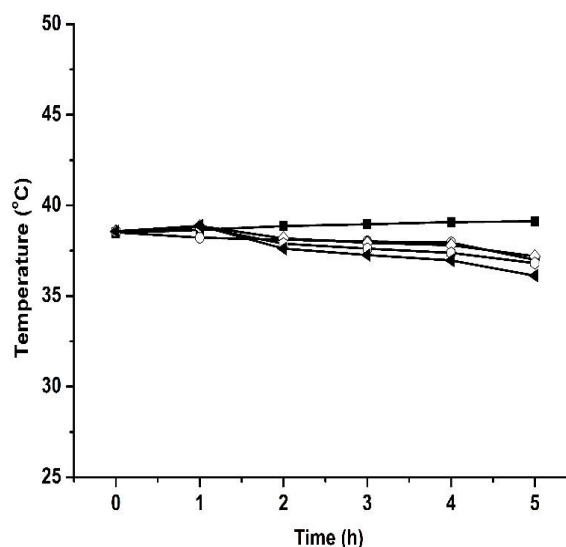


Figure 1: Anti-pyretic activity of *C. officinalis* extract in albino rats. Closed square, Group A; open circle, Group B; open square, Group C; open circle, Group D; and closed rectangle, Group E

DISCUSSION

A comparative study of the local anesthetic activity of a natural plant extract was made in two animal species, guinea pigs and frogs. The aqueous extract of *C. officinalis* showed local anesthetic activity following intradermal injection in guinea pigs. Plant extract produced rapid dose-dependent local anesthesia in the wheel model. The results suggest that the extract was

able to inhibit nerve impulse conduction in the skin of guinea pigs. The *C. officinalis* extract also produced statistically significant rapid plexus anesthesia in the frog model. The onset of the local anesthetic effect with the aqueous extract was almost equal to that of the standard drug. This was consistent with previous reports.

Basically, most of the anti-inflammatory drugs possess anti-pyretic effect. Here, the tested *C. officinalis* extract markedly reduced the rectal temperature of pyretic rats. This indicated anti-pyretic activity of the extract in the yeast-induced rats, possibly because the production of prostaglandins was suppressed by the chemical constituents of the plant extract, which is responsible for fever induction in the central nervous system (CNS). Indeed, the flavonoid content in *C. officinalis* may be responsible for its anti-pyretic activity, by inhibiting prostaglandin synthesis in the hypothalamus.

CONCLUSION

In conclusion, the present study showed that an aqueous extract of plant *C. officinalis* had local anesthetic and anti-pyretic activity in guinea pigs, frogs, and rats. Because this is a preliminary report, further studies are needed to analyze the possible mechanisms of action.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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