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Original Research Article

Mechanistic insights into the analgesic and anti-inflammatory effects of alcoholic extracts from *Curcuma longa*

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ABSTRACT

Background: Aim of the study was to scientifically validate the traditional Indian claims of *Curcuma longa*'s (turmeric) antinociceptive (pain-relieving) and anti-inflammatory effects.
Methods: The alcoholic extract of C. longa was tested in three rodent nociceptive models: acetic acid-induced writhing: examines visceral pain, formalin test: evaluates both acute and chronic neurogenic and inflammatory pain and tail immersion test to assess thermal pain. The extract's effects were compared to a control group and morphine (reference drug).
Results: *C. longa* extract significantly reduced abdominal constrictions in the acetic acid test (59.36% inhibition). In the formalin test, the extract significantly decreased paw licking response time in both early (54.12% inhibition) and late phases (78.59% inhibition). *C. longa* extract significantly increased the tail flick reaction time in the immersion test, indicating pain relief.
Conclusions: This study confirms the antinociceptive and anti-inflammatory activities of *C. longa*, providing scientific evidence for its traditional use in pain management.

Keywords: *Curcuma longa*, Anti-inflammatory, Antinociceptive, Morphine, Alcoholic extract

INTRODUCTION

In India, *Curcuma longa* (*C. longa*) has been in use as a culinary ingredient since 3000 BC. It is used as a food colouring for curry and as a preservative for food. As a medicine it is used to treat a wide variety of ailments including abdominal pain, skin problems, muscular problems and arthritis. *C. longa* has also been used as a clothing dye and as a cosmetic. Indians are thought to consume between 80 and 200 mg per day of *C. longa* extract. India as a whole consumes 480,000 tons of turmeric annually. In China it has been used as a topical analgesic, and for colic, hepatitis, ringworm infection and chest pain. In Europe it is used in many foods, as a colouring in mustard, cheese, margarine, beverages and cakes. In the recent past it has been used for dyspepsia, chronic anterior uveitis and *Helicobacter pylori* bacteria. It is generally recognized as safe by the Food and Drug Administration (FDA) of the United States.[1]

Evidence suggests the benefits of turmeric in relieving acne, inflammation, joint pain, asthma, eczema, and tonic and acute allergies; in wound healing; in maintaining a balanced mood and blood sugar levels; and in immunomodulation *C. longa* contains carbohydrates, fiber, certain proteins and lipids (no cholesterol), vitamin C, pyridoxine, magnesium, phosphorus, potassium, and calcium, which makes it a nutritionally rich natural food ingredient. Curcumin is available in several forms including capsules, tablets, ointments, energy drinks,

soaps, and cosmetics and is used in daily activities, albeit in many forms. curcumin offers a favourable and encouraging potential as it is categorized as a generally recognized as safe (GRAS) material having a stable metabolism and low toxicity among humans.[2] *C. longa* is highly regarded as universal panacea in the herbal medicine with a wide spectrum of pharmacological acidities.[3] Curcumin, the yellow colour pigment of turmeric, is produced industrially from turmeric oleoresin. *C. longa* of India is particularly popular when compared with those from other countries due to its high curcumin concentration, which is the most essential and active biological ingredient responsible for its therapeutic potential.[4]

C. longa has shown properties which prevent hepatic toxicity. So, it can also be used with common analgesics like paracetamol, which in high doses are fatal for the liver.[5] Based on the above traditional claims and research principles the present study was undertaken to scientifically validate the traditional claims of *C. longa* with particular reference to its antinociceptive and anti-inflammatory effects.

METHODS

Assessment of antinociceptive activity

Three methods were employed to assess the anti-nociceptive activity: acetic acid induced writhing method, formalin test, and tail immersion test.

Acetic acid induced writhing method

Male Swiss albino mice were used for the study. All the animals were fasted overnight and grouped as mentioned below. Control group received 1 ml/100 gm of 1% v/v tween 80 orally, sixty minutes before acetic acid challenge. The test group received orally alcoholic extract of *C. longa* (200 mg/kg/b.wt). The extract was administered sixty minutes prior to acetic acid challenge. Morphine (1 mg/kg s.c) was included as a reference drug for comparison and administered 30 minutes before acetic acid challenge. The abdominal constriction induced by acetic acid after treatment with extract was compared with that of the vehicle treatment. The percent inhibition of abdominal constrictions produced by different groups was calculated using the formula, where C=number of abdominal constriction in vehicle treated group, and T=number of abdominal constriction in treatment group.[6]

$$Percent\ inhibition = C{-}T/C \times 100$$

Formalin test

Twenty µl of 1% formalin in saline was injected s.c into the plantar surface of the left hind paw of the mice. Mice react with a licking or biting response of injected paw. The time spent in paw licking or biting the injected paw was recorded every 5 minute for a period of 30 minutes. The summation of responses of first ten minutes was taken as acute phase and ten to thirty minutes was counted as chronic phase. Male Swiss albino mice were used for the study. All the animals were fasted overnight and grouped as mentioned below. Control group received 1 ml/100 gm of 1% v/v tween 80 orally, sixty minutes before formalin treatment. The test group received orally alcoholic extract of *C. longa* (200 mg/kg/b.wt). The extract was administered sixty minutes prior to formalin treatment. Morphine (10 mg/kg s.c) was included as a reference drug for comparison and was administered 30 minutes before formalin treatment.[7] The percentage inhibition of paw licking time compared with vehicle treatment was also calculated using the formula, where C=biting/paw licking response time (seconds) in vehicle treated group, and T=biting/paw licking response time (seconds) in treatment group.

$$Percent\ inhibition = C{-}T/C \times 100$$

Tail immersion test

The tail of the mice was immersed in a water bath maintained at 55±0.50 °C and the time taken to flick the tail was taken as reaction time. A cut off period of 10sec was maintained to prevent thermal injury to the tail. The reaction time was measured just before the administration of test substances (0 min) then at an interval of 30 min up to a period of 90 min. Male Swiss albino mice were used for the study. All the animals were fasted overnight and grouped as mentioned below. Control group received 1 ml/100 gm of 1% v/v tween 80 orally. The test group received orally alcoholic extract of *C. longa* (200 mg/kg/b.wt). Morphine (10 mg/kg s.c) was included as a reference drug for comparison of the reaction time and the tail flick was recorded at 0 min, 30 min, 60 min, 90 min.[8]

Statistical analysis

The results were expressed as mean±S.E.M. The statistical comparison was performed using one-way analysis of variance (ANOVA). The study was approved by institutional animal ethics committee-MMCRI-Kanchipuraam-Tamil Nadu and the study was conducted during the period April 2010-2013.

RESULTS

Acetic acid induced abdominal constrictions

The mean number of abdominal constrictions in vehicle treated control animals was 38.16±0.60 (Table 1). A significant reduction in the number of abdominal constrictions was recorded for morphine treated mice with the mean value being 2.33±0.49 and the percentage inhibition of nociception was 93.89%. Reduction in the number of abdominal constrictions was noticed after the administration of alcoholic extract of *C. longa*. The reduction was significant with 200 mg/kg (17.00±0.57) of

the extract. In the above dose, the percentage inhibition of nociception was 59.36%.

Table 1: Effect of *C. longa* extract on acetic acid induced abdominal constrictions in mice.

Treatment (n=6)	Mean number of writhes±SEM	Inhibition (%)
Vehicle (Tween 80 1% v/v), p.o	38.16±0.60	-
Morphine 1 mg/kg s.c	2.333±0.49*	93.89
***Curcuma longa* alcoholic extract 200 mg/kg p.o**	17.±0.57	55.45

P<0.05; p<0.01 (compared with control, one-way ANOVA), *the value in parenthesis indicates the percentage inhibition of nociception, n-number of animals.

Formalin-induced nociception

In vehicle treated control animals the paw licking response time was 51.25±0.32 sec in early phase (0-10 min) and 90.06±0.38 sec in the late phase (10-30 min). In morphine treated animals the paw licking response time was significantly reduced both in the early (10.48±0.46 sec) and late phase (6.17±0.34 sec). A significant reduction in the paw licking response time was evident in the early phase (23.51±0.21 sec) and late phase (19.27±0.33 sec) after treatment with alcoholic extract of C. longa. The extract produced 54.12% and 78.59 % inhibition of nociceptive response in the early and late phases respectively (Table 2).

Tail immersion test

The mean reaction time in the vehicle treated mice during the observation periods of 30, 60 and 90 min were 2.80±0.21, 2.90±0.21 and 2.60±0.15 sec. respectively (Table 3). Morphine treatment significantly increased the reaction time in all the observation periods. The reaction time with 200 mg/kg alcoholic extract of *C. longa* was also significantly increased when compared with the vehicle treated mice. It showed maximum latency period (3.80±0.58 sec) at 90 minutes.

Table 2: Effect of *C. longa* extract on formalin induced nociception in mice.

Treatment (n=6)	Dose (mg/kg)	Early phase 0-10 min	% inhibition at 0-10 min	Late phase 10-30 min	% inhibition at 0-10 min
Vehicle (tween 80, 1% v/v)	0.1 ml/10 g, p.o	51.25±0.32	-	90.06±0.38	-
Morphine	10 mg, sc	10±0.46* (79.55%)	79.55	6.74±0.34* (92.50%)	92.50
***C. longa alcoholic* extract**	200 mg, p.o	23.51±0.21* (54.12%)	54.12	19.27±0.33* (78.59%)	78.59

P<0.05; p<0.01 (compared with control, one-way ANOVA), *the value in parenthesis indicates the percentage inhibition of nociception, n-number of animals.

Table 3: Effect of *C. longa* extract on thermal nociception in mice.

Groups (n=6)	0 min	30 min	60 min	90 min
Vehicle (tween 80, 1% v/v, p.o)	2.50±0.33	2.80±0.21	2.90±0.21	2.60±0.15
Morphine 10 mg, sc	2.80±0.23	5.50±0.39*	6.93±0.49*	8.25±0.59*
***Curcuma longa alcoholic* extract 200 mg, p.o**	2.40±0.25	5.00±0.23*	6.20±0.43*	7.60±0.58*

P<0.05; p<0.01 (compared with control, one-way ANOVA), n-number of animals.

DISCUSSION

Turmeric has been used in India for over 2500 years and is a major part of the Ayurvedic system of medicine. It was first used as a dye and then later for its medicinal properties. Ancient Indian medicine, Ayurveda, has recommended *C. longa* use in food for its medicinal value, much of which is now being researched in the modern day. The present study is unique in nature as it is designed to investigate the anti-nociceptive activity of the alcoholic extract of *C. longa* in three different types of rodent nociceptive models viz., visceral (acetic acid induced writhing method), neurogenic and inflammatory (formalin test) and thermal pain (tail immersion test).

The purpose and rationale for choosing the formalin test in rodents is because, it has been proposed as a chronic pain model which is sensitive to centrally active analgesic agents by Dubuisson et al.[9] The purpose and rationale for choosing tail immersion tests in the present study is for the following reasons. This method has been developed to be selective for morphine-like compounds. The procedure is based on the observation that morphine-like drugs are selectively capable of prolonging the reaction time of the typical tail-withdrawal reflex in mice induced by immersing the end of the tail in warm water of 55 °C. The test is useful to differentiate central opioid like analgesics from peripheral analgesics. In the present study the purpose and rationale for choosing the writhing test in mice is because the pain is induced by injection of irritants into the peritoneal cavity of mice. The animals react with

a characteristic stretching behavior which is called writhing which is easily observable and the stretching reaction is evaluated. The nociceptive models for evaluation is designed to analyse visceral nociception, thermal nociception, neurogenic and inflammatory nociception, by acetic acid induced abdominal constriction model, tail immersion test and formalin induced paw licking respectively.[10] The analysis of visceral nociception by acetic acid induced abdominal constrictions exhibited a statistically significant 59.36 percentage inhibition of nociception when compared to vehicle treatment group. The thermal nociception analysis performed using tail immersion test revealed significantly increased reaction time to thermal stimulus when compared with the vehicle treated mice. It showed maximum latency period (4.50±0.43 sec) at 90 minutes. The *C. longa* extract produced statistically significant 54.12% and 78.59% inhibition of the neurogenic and inflammatory nociception by formalin induced paw licking response in the early and late phases respectively compared to vehicle treatment.

Limitations related to the study were conducted on mice, which may not perfectly represent human pain responses. Differences in physiology and pain pathways could lead to variations in the effectiveness of the extract in humans. While the study used three different pain models, there are many other types of pain not covered. It is unclear if the extract would be effective for other types of pain. The study does not explore the specific mechanisms by which the extract might be exerting its pain-relieving effects. Understanding the mechanism would be valuable for further development and potential clinical applications. Further research addressing these limitations is necessary to fully understand its potential as a therapeutic agent for pain management in humans.

CONCLUSION

The present study revealed antinociceptive activity of *C. longa* in thermal, visceral and neurogenic models and scientifically validating the benefits shown by traditional claims and use of *C. longa* in traditional medicine.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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