Antinociceptive effects of purified Curcuma longa in mice

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ABSTRACT

Background: Since ancient times Curcuma longa is said to have antinociceptive effects in literature.
Methods: It was a quantitative experimental study done in the laboratory setting of the department of Clinical Pharmacology and Therapeutics, BP Koirala Institute of Medical Sciences, Dharan, Nepal (BPKIHS). Hot plate test, Tail flick test and Writhing test were used for evaluating the antinociceptive effects. Animals were divided into five groups of six each, group I as control, group II as standard control whereas groups III, IV and V as test groups (three doses). Control and the three test drug doses were given for 21 days. Data were presented as mean ± Standard Error of Mean. Statistical differences between the test drug and control groups as well as within the test drug groups were calculated using Mann-Whitney U test. A probability level less than 0.05 (p < 0.05) was considered significant.
Results: Significant effects in comparison to vehicle were observed in all the three anti nociceptive test models at 200mg /kg test dose of aqueous extract of purified Curcuma longa (CL). No significant effect as compared to vehicle was observed at 50mg/kg and 100 mg/kg test doses of CL.
Conclusions: This study showed that CL possesses antinociceptive effect. The mechanism(s) and active principle(s) behind the effects of CL could not be established.

Keywords: Curcuma longa, Antinociceptive, Aqueous extract, Soxhlet

INTRODUCTION

Turmeric (Curcuma longa) is a rhizomatous herbaceous perennial plant of the ginger family Zingiberaceae.¹ Plants are gathered annually for their rhizomes, and propagated from some of those rhizomes in the following season. When not used fresh, the rhizomes are boiled for several hours and then dried in hot ovens, after which they are ground into a deep orange-yellow powder commonly used as a spice in curries and other South Asian and Middle Eastern cuisine, for dyeing, and to impart colour to mustard condiment.

More than 100 components have been isolated from root of Curcuma longa. The main component of the root is a volatile oil, containing turmerone, and there are other coloring agents called curcuminoids also. Curcuminoids consist of curcumin demethoxycurcumin, 5′-methoxycurcumin, and dihydrocurcumin, which are found to be natural antioxidants.²³ In a standard form, dried powdered rhizome of Curcuma longa (turmeric) contains moisture (>9%), curcumin (5-6.6%), extraneous matter (<0.5% by weight), mould (<3%), and volatile oils (<3.5%). Volatile oils include d-α-phellandrene, d-sabinene, cedrol, borneol, zingeribene, and sesquiterpenes.⁴ The rhizomes are also reported to contain four new polysaccharides-ukonans along with stigmasterol, β-sitostereol, cholesterol, and 2-hydroxymethyl anthraquinone.⁵⁶ Nutritional analysis showed that 100 g of turmeric contains 390 kcal, 10 g total fat, 3 g saturated fat, 0 mg cholesterol, 0.2 g calcium, 0.26 g phosphorous, 10 mg sodium, 2500 mg potassium, 47.5 mg iron, 0.9 mg thiamine, 0.19 mg riboflavin, 4.8 mg niacin, 50 mg ascorbic acid, 69.9 g
total carbohydrates, 21 g dietary fiber, 3 g sugars, and 8 g protein. Turmeric is also a good source of the ω-3 fatty acid and α-linolenic acid (2.5%).

Anti-inflammatory effects of Curcuma longa and its constituents

Gujral et al. reported a wound healing property of turmeric powder. Turmeric powder was applied over septic as well as aseptic wounds in rats and rabbits and it was found that the healing process was accelerated to an extent of 23-24% in both cases which was comparable to the effect of scarlet red. Sulfanilamide powder, copper sulfate solution (0.1%), and Silver nitrate solution (0.1%) were less effective.

Ghatak and Basu reported high anti-inflammatory activity of curcumin as well as of sodium curcuminate in the carrageenin-induced edema test in rats with an ED50 of 2.1 and 0.36 mg/kg i.p., respectively. Mukhopadhyay, et al found that 3 mg of curcumin was effective in rats against carrageenan edema when injected locally in the paw. Naturally occurring analogues of curcumin, i.e. feruloyl-(4-hydroxycinnamoyl)-methane (FHM) and bis-4-hydroxycinnamoyll-methane (BHMM), were screened for anti-inflammatory activity. The most potent among the 3 curcumin analogues studied was FHM. Curcumin analogues revealed a dose-dependent effect up to the dose of 30mg/kg.

Sharma, et al reported curcumin to be an effective agent inhibiting lipid peroxide formation in liver during inflammation and attributed this effect to an antioxidant property to explain the anti-inflammatory activity. A similar effect was obtained with phenylbutazone. Curcumin also inhibited the lipid peroxide formation in vitro.

Chandra and Gupta found that the volatile oil (orally 0.1 ml/kg per day) of Curcuma longa suppressed acute edema. The activity of the essential oil has been attributed to its ability to stimulate the adrenohypophyseal axis as it was not effective in adrenalectomized animals. Tripathi, et al reported that the volatile oil of the Curcuma longa plant inhibited trypsin and hyaluronidase enzymes extracts. Arora, et al reported anti-inflammatory activity of the petroleum ether extract of Curcuma longa.

**METHODS**

**Design of the study**

Quantitative experimental study in mice.

**Setting**

Laboratory of Department of Clinical Pharmacology and Therapeutics, BP Koirala Institute of Medical Sciences, Dharan, Nepal (BP KIHS). The ethical guidelines for investigations were followed in accordance with Indian National Science Academy (INSA).

**Duration of the study**

One year

**Drugs and chemicals**

1. Purified Curcuma longa (The Himalaya Drug Company, India)
2. Indomethacin (Ranbaxy laboratories Ltd, India);
3. Morphine (Martindale Pharmaceuticals, UK);
4. Acetic acid (Qualigens fine chemical, India)

**Plant material**

Purified Curcuma longa was obtained from The Himalaya Drug Company, India.

**Extract preparation of the plant**

The purified Curcuma longa were obtained from the Himalaya drug company in the form of coarse powder. Then 25g of this powder was subjected to Soxhlet extraction in 150 ml distilled water for 12 hours at 100°C. The crude extract thus obtained was first subjected to filtration with Whatman filter paper no 1 and then concentrated to dryness at room temperature to yield 257.3mg brown/black viscous residue, this is the aqueous extract of purified Curcuma longa (CL). The above procedure was repeated several times to yield 5.10g of CL. CL thus obtained was then utilized for the experiments by suspending in distilled water.

**Animals**

The experiments were performed on adult albino mice of either sex weighing 20-35g. The animals were bred in the animal house of the Department of Clinical Pharmacology and Therapeutics. They were maintained under controlled room temperature (25±2°C), and light and dark (12:12 hour) conditions. The animals were given food pellets and water ad libitum but fasted overnight before the experiment. Before conducting the experiment, ethical clearance was obtained from the Local Ethical Committee on Animal Research, BP Koirala Institute of Medical Sciences, Dharan, Nepal (BP KIHS). The ethical guidelines for investigations were followed in accordance with Indian National Science Academy (INSA).

**Experimental design**

All animals were randomly divided into five groups. Each group consisted of six animals. Group I was vehicle control animals used to estimate the baseline values of the parameters studied. Group II were standard control animals which were given standard drugs. Group III, IV and IV animals were given three different doses of the test drug, i.e CL. The test drugs, and vehicle (distilled water) were given through oral route with the help of orogastric tube. Either per oral (p.o.) or intra peritoneal
(i.p.) route was used for standard control drugs. The test drug was administered in doses of 50, 100 and 200 mg/kg body weight (b. w.) to the groups III, IV and V respectively once daily for 21 consecutive days in the morning. The vehicle (distilled water) was administered to the group I in a dose of 10 ml/kg body weight, daily for 21 days. The doses of the test drug were chosen according to the study done by, Kumar, et al (mice) and Volume Guidelines for Compound Administration. All the oral drugs were administered 60 minutes prior to the experiment and all the intraperitoneal drugs were administered 30 minutes prior to the experiment. The experiments in test drug and vehicle treated groups were conducted on day 21, 60 minutes after the last dose administration. CL, Indomethacin and Morphine were dissolved in distilled water. Only the freshly prepared drug solutions were used.

Distilled water (10 ml/kg p.o.) was used as vehicle control in all the experiments.

The standard control for the analgesic effect was Morphine 5mg/kg i.p. in hotplate and Tail-flick test and Indomethacin 10 mg/kg p.o. in Acetic acid-induced writhing test.

The different groups received drugs and vehicles as follows:

Group 1 (vehicle control 10 mg/kg b. w.);
Group 2 (standard control);
Group 3 (CL50 mg/kg b. w.);
Group 4 (CL100 mg/kg b. w.) and
Group 5 (CL200mg/kg b. w.).

Experimental models

I. Antinociceptive effect

1. Hot plate test:

Central analgesic activity was evaluated using hot plate test as described by Woolfe and MacDonald. The test was carried out using a hot plate apparatus (UGO Basile, Italy). The thermal noxious stimulus was induced to mice by placing them individually in the hot-plate maintained at 53±1°C. 10 minutes prior to the experiment. The mice that showed initial nociceptive response within 30 seconds were selected for the experiment. The latency of nociceptive response such as licking, flicking of a hind limb or jumping was noted. The experiment was terminated 30 second after their placement on the hot plate to avoid damage to the paws. Morphine 5mg/kg i.p. was used as the standard control. The standard control group mice were used for the experiment 30 minutes after the drug administration.

II. Tail flick test:

The central antinociceptive effect was determined using the Tail Flick Test. For the tail-flick method, pain was induced by giving infrared heat on the tail of the mice (Tail Flick Unit, UGO Basile, Italy) 1-2 cm away from the tip of the tail. The tail withdrawal from the heat (flicking response) was taken as the end point. The intensity of the thermal stimulus was adjusted to produce 3-4 second latency in tail-flick response. The animals which showed a flicking response within 3-5 sec were selected for the study. Reaction time (tail-flick latency) was noted by observing the interval between placement of the tail on the infrared heat source and the withdrawal of the tail. A maximum radiation exposure period of 15 seconds was taken as the cut off time to avoid damage to the tail. Morphine 5mg/kg i.p was used as standard control, administered 30 minutes prior to the test.

III. Acetic acid induced writhing test

Peripheral analgesic activity was evaluated using acetic acid-induced writhing test. This procedure is considered as very sensitive with minimal noxious stimulus. The method described by Koster, et al was employed to assess the analgesic activity. After 60 minutes post dosing with the test drugs, the number of abdominal constrictions (writhings) in mice for a period of 10 minutes was counted following intraperitoneal injection of 0.6% acetic acid in a dose of 10ml/kg b. w. For scoring purposes, a writh is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. Any significant reduction in the number of abdominal constrictions when compared with vehicle treated animal was considered as antinociceptive response. Standard control for test was Indomethacin 10 mg/kg p.o. administered 1 hour before the experiment.

Statistical analysis

All data were presented as mean ± Standard Error of Mean (SEM). Statistical differences between the test drug and control groups as well as within the test drug groups were calculated using Mann-Whitney U test.

A probability (p-value) level less than 0.05 were considered significant.

RESULTS

Effect of CL on nociception in hot plate test

CL at 200mg/kg significantly (p<0.05) increased the hot plate latency when compared with the vehicle. Hot plate latency of mice was also increased significantly (p<0.05) with morphine pre-treatment when compared with the vehicle. The difference between the morphine and CL 200mg/kg group was not significant (p>0.05). CL at 50 and 100 mg /kg also increased the latency time but this
increase was not statistically significant (p>0.05) (Table 1, Figure 1).

![Figure 1: Hot plate test (antinociceptive test).](image1)

**Figure 1: Hot plate test (antinociceptive test).**

![Figure 2: Tail flick test (antinociceptive test).](image2)

**Figure 2: Tail flick test (antinociceptive test).**

**Table 1: Effect of CL on nociception in hot plate test.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Time in seconds (Mean ± SEM)</th>
<th>Median</th>
<th>Standard deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Distilled water</td>
<td>9.833 ± 1.327</td>
<td>10</td>
<td>3.521</td>
<td>IV 0.780</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V 0.025*</td>
</tr>
<tr>
<td>II. Morphine</td>
<td>20.667±2.348</td>
<td>22</td>
<td>5.75</td>
<td>IV 0.01*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>III 0.078*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IV 0.261</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V 0.149</td>
</tr>
<tr>
<td>III.CL50mg/kg</td>
<td>13.533±3.062</td>
<td>10.9</td>
<td>7.501</td>
<td>IV 0.200</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V 0.229</td>
</tr>
</tbody>
</table>

* Significant (p < 0.05); SEM: Standard error of mean.

**Table 2: Effect of CL on nociception in tail flick test.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Tail flick latency time in seconds (Mean ± SEM)</th>
<th>Median</th>
<th>Standard deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Distilled water</td>
<td>3.2 ± 0.370</td>
<td>3.3</td>
<td>0.894</td>
<td>IV 0.416</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V 0.005*</td>
</tr>
<tr>
<td>II. Morphine</td>
<td>9.333±0.422</td>
<td>10</td>
<td>1.033</td>
<td>IV 0.003*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>III 0.003*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IV 0.003*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V 0.009*</td>
</tr>
<tr>
<td>III.CL50mg/kg</td>
<td>3.333±0.169</td>
<td>3.2</td>
<td>0.413</td>
<td>IV 0.419</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>V 0.004*</td>
</tr>
<tr>
<td>IV.CL 100mg/kg</td>
<td>3.8±0.482</td>
<td>4</td>
<td>1.18</td>
<td>V 0.100</td>
</tr>
<tr>
<td>V.CL200mg/kg</td>
<td>6.533±0.543</td>
<td>6.7</td>
<td>1.331</td>
<td></td>
</tr>
</tbody>
</table>

* Significant (p < 0.05); SEM: Standard error of mean.

**Effect of CL on nociception in tail flick test**

In this test, there was dose dependent increase in tail flick latency (TFL) in the mice pretreated with CL at all tested doses as compared to vehicle treated mice although the result was statistically significant (p<0.05) only at 200mg/kg dose. There was significant (p<0.05) difference between CL 200mg/kg and CL 50mg/kg groups. Morphine caused maximal increase in the tail flick latency time among all the study groups and these differences were statistically significant (p<0.05) (Table 2, Figure 2).

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Table 3: Effect of CL on Nociception in Acetic acid induced Writhing test.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number of writhes (Mean ± SEM)</th>
<th>Median</th>
<th>Standard deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Distilled water</td>
<td>16.833 ± 1.447</td>
<td>15</td>
<td>3.545</td>
<td></td>
</tr>
<tr>
<td>II. Indomethacin</td>
<td>1.667 ± 0.843</td>
<td>1</td>
<td>2.066</td>
<td></td>
</tr>
<tr>
<td>III.CL50mg/kg</td>
<td>12.5±0.885</td>
<td>12</td>
<td>2.168</td>
<td></td>
</tr>
<tr>
<td>IV.CL 100mg/kg</td>
<td>13.5±1.260</td>
<td>14.5</td>
<td>3.082</td>
<td></td>
</tr>
<tr>
<td>V.CL200mg/kg</td>
<td>3.833±0.543</td>
<td>4</td>
<td>1.329</td>
<td></td>
</tr>
</tbody>
</table>

* Significant (p < 0.05); SEM: Standard error of mean.

Effect of CL on nociception in acetic acid induced writhing test

In acetic acid induced writhing test, CL 200mg/kg significantly (p<0.05) decreased the number of writhes in mice when compared with the CL 50mg/kg, CL 100mg/kg and vehicle treated groups. The decrease in the number of writhes was also significant (p<0.05) in Indomethacin treated group as compared to the vehicle, CL 50mg/kg as well as CL 100mg/kg groups. In comparison to CL 200mg/kg group the decrease in the number of writhes was more in indomethacin treated group but this difference was not significant(p>0.05) statistically (Table 3, Figure 3).

![Figure 3: Writing test (antinociceptive test).](image)

DISCUSSION

CL was given daily for twenty one days in three graded doses of 50, 100 and 200mg/kg in mice. The experiments were performed on the twenty first day.

Although alcoholic extract of Curcuma longa has been studied in great detail by a vast number of workers, very less work has been done to evaluate the CNS effect of its aqueous extract. The present study indicates that the CL possesses analgesic property.

CL was evaluated for its antinociceptive activity in peripheral as well as central analgesic models. The antinociceptive tests used in the present work were chosen in order to test different nociceptive stimuli namely thermal (hot plate test), radiant (tail flick test) and chemical visceral nociceptive stimuli (acetic acid). It was essential to employ more than one test to confirm the antinociceptive action, as it has been shown that some ‘false positive’ activity can be observed with agents that are not normally considered as analgesic.27

In hot plate test, CL showed significant analgesic property only at 200mg/kg.

Morphine also showed analgesic effect in the hot plate test.

Tail flick model showed similar results, 200mg/kg dose of CL was found most effective in decreasing pain sensations among all the doses of CL although its effect was quite less than that of Morphine.

In writhing test again, CL at 200mg /kg was found most effective in decreasing the number of writhes in mice and thus pain among all the doses of CL. Although Indomethacin was more effective in reducing pain in mice.

Hence, from the above experiments it is evident that CL at 200mg/kg has analgesic properties although its effect is less than that of Morphine. Similar results were obtained by Bagad, et al as well as John, et al Bagad, et al showed the anti-inflammatory activity of aqueous extract of Curcuma longa in xylene-induced ear edema, cotton pellet granuloma models in albino Swiss mice and albino Wistar rats, respectively. 28,29 Aqueous extract of C. longa at three dose levels (90,180 and 360 mg/kg b.w.) significantly inhibited inflammation in both models, as evidenced by reduction in ear weight and decrease in wet as well as dry weights of cotton pellets, when compared to the vehicle control. John, et al showed significant
analgesic activity in Swiss albino mice in the tail withdrawal test and writhing test.²⁹

Pain is distinguished as two types, peripheral and neurogenic pain. ³⁰ The hot plate and tail flick tests are considered to be selective for opioid like compounds, which are centrally acting analgesics in several animal species.³¹ The increase in the reaction time of the mice on the hot plate following administration of the CL suggests that the extract possess central analgesic activity. The suppression of the acetic acid-induced writhing suggests, however, that CL may act via local peritoneal receptors.³²

The i.p. injection of acetic acid produces pain through the activation of chemosensitive nociceptor or irritation of the visceral surface, thereby leading to liberation of bradykinin, histamine, prostaglandin and serotonin. Acetic acid causes inflammatory pain by inducing capillary permeability and liberating endogenous substances that excite pain nerve endings.³³,³⁴ NSAIDs can inhibit COX in peripheral tissues and, therefore, interfere with the mechanism of transduction of primary afferent nociceptors.³⁵ CL has inhibited the pain induced by acetic acid which indicates that it acts through peripheral mechanisms. Therefore, it can be inferred that the CL may have produced analgesic effects via both central and peripheral mechanisms. However, the mechanism of this action has not been investigated here. It is not known whether this action is opioid like in nature and/or involves acetylcholine or other receptors. The use of selective antagonists (e.g. naloxone or atropine) might delineate this. The mechanism of analgesic effect of CL in acetic acid induced writhing could probably be due to blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of indomethacin and other NSAIDs.³⁶,³⁷

CONCLUSION

Evaluation of antinociceptive activities of purified Curcuma longa was done using its aqueous extract at 50, 100, 200 mg/kg doses in respective animal models. This study showed that aqueous extract of purified Curcuma longa possesses antinociceptive effect. Since not much work has been done on the aqueous extract of purified Curcuma longa, there is a need for more precise studies to isolate the active constituents and to elucidate the mechanism of action. Hence a lot of work is needed to know the mechanism(s) behind the antinociceptive effects.

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

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