

## LSP76

**Antinociceptive Activity of *Curcuma longa* Essential Oil**

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**Introduction.** Pain is a major symptom in various medical conditions, and can significantly obstruct a person's quality of life and general functioning. The current treatment of pains remains highly reliant upon non-steroidal anti-inflammatory drugs (NSAIDs), steroidal anti-inflammatory drugs (SAIDs) and opioids, which are very effective in the treatment of acute nociceptive pain, but are poor therapies for other several types of pain. Chronic dosing of the drugs usually may lead to serious side effects such as kidney failure, gastropathy and liver damage. Meanwhile, back far for centuries, there are numerous herbs and spices used by old folks as a treatment for pain which they believe to have medicinal properties with less or no side effect.

**Literature review.** *Curcuma longa* L. (Zingiberaceae) or turmeric is a perennial herb being used as a common household medicine and as a spice in India and Southeast Asia. Turmeric contains essential oil, curcuminoids, starch and oleoresin. Its rhizomes and oils have great importance in medicine. Traditional Indian medicine claims the use of turmeric powder against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis [1]. Turmeric is also used in drugs against cancer, dermatitis, AIDS and high cholesterol level [2-4]. The essential oil extracted from turmeric possesses anti-inflammatory, anti-platelet, anti-hepatotoxic and anti-bacterial activities [5-8]. Previous study also reported that essential oil from fresh rhizomes of *Curcuma longa* has very high antioxidant properties [9]. Despite the widespread use of this herb in folk's medicine, yet there is no scientific investigation reported to evaluate the antinociceptive property of *Curcuma longa* essential oil.

**Materials and Methods.** The following reagents and drugs were used: Tween 20, absolute ethanol (100%), formalin, acetic acid, morphine hydrochloride, acetylsalicylic acid (ASA) and naloxone (Sigma Chemicals, USA).

**Preparation of extract.** The turmeric powder was purchased from Chow Kit's wet market, Kuala Lumpur, Malaysia and its essential oil was identified by Dr. Nadeem Akhtar, a chemist from Institute of Bioscience, Universiti Putra Malaysia. The turmeric powder (1 kg) was extracted in ethyl acetate (EA) and concentrated on rotary evaporator to obtained 62.5 gm of extract. The extract was then dissolved by flash silica gel column chromatography using EA and hexane to obtain 8.7 gm of hexane extract as yellow oil.

**Animals.** Male ICR mice (20–30 g) were used throughout the study. They were housed at the animal house facility, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia under a 12 h light/12 h dark cycle and provided with standard pellet and water *ad libitum*. The experimental procedures were carried out in strict compliance with the ethical guidelines for investigations of

experimental pain in conscious animals [10], approved by Animal Care Unit Committee, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

**Acetic acid-induced writhing test.** The acetic acid-induced abdominal constriction test was carried out as described previously [11], with minor modifications. In order to induce pain in mouse peritoneal cavity, 0.6% of acetic acid (10 ml/kg) was injected intraperitoneally, 30 min after the administration of *Curcuma longa* essential oil (CLEO; 0.1, 0.5, 1 and 5 mg/kg, i.p.). The number of abdominal constrictions was counted cumulatively over a period of 30 min, 5 min after acetic acid administration. Antinociception of CLEO was indicated by the reduction in the mean of the number of abdominal constrictions in the test groups compared to the control group. Acetylsalicylic acid (ASA, 100 mg/kg; i.p.) was used as reference drug while control group received vehicle that being used to dissolve CLEO.

**Formalin-induced paw licking test.** The procedures use in the formalin-induced paw licking test was similar to that described previously [12]. Animals were pretreated with CLEO (0.1, 0.5, 1 and 5 mg/kg, i.p.) 30 min before the formalin injection. Control animals were received only the vehicle (10 ml/kg, i.p.) while ASA (100 mg/kg, i.p.) and morphine (5 mg/kg, s.c.) were used as reference drugs. After 30 min, the intra plantar area of the right hind paw of the mice was injected with 20  $\mu$ l of 2.5% of formalin. The animals were then immediately placed individually in an observation chamber made of transparent acrylic and the amount of time that the animals spend licking the injected paw was recorded for 30 min following formalin injection. The first 5 min after formalin injection (early phase) and 15–30 min after formalin injection (late phase) were represented as neurogenic and inflammatory pain, respectively [13].

**Hot plate test.** The hot plate test was performed to assess the central antinociceptive properties of CLEO according to the method described previously [14] with minor modifications. In this test, the hot plate (Ugo Basile, Model 7280, Italy) was maintained at  $55 \pm 0.2$  °C. Animals were placed in the perspex cylinder on the heated surface, and the latency to a discomfort reaction (licking paws and jumping) was determined before and after CLEO or drug administration. The CLEO (0.1, 0.5, 1 and 5 mg/kg, i.p.) and morphine (5 mg/kg, s.c.) were administered 30 min before the beginning of the experiment. Animals were observed before and at 30, 60, 90, 120, 150, 180 and 210 min after CLEO or morphine administration. The cut-off time is 20 s to avoid tissue injury.

**Involvement of opioid receptors.** The possible involvement of the opioid system in the antinociceptive effect caused by CLEO was investigated using a method similar to the acetic acid-induced abdominal writhing test and hot plate test. The animals were pretreated with CLEO (1 mg/kg, i.p.), morphine (5 mg/kg, s.c.) or vehicle (10 ml/kg, i.p.) 30 min before acetic acid (0.6%, i.p.) injection or before they were placed on the hot plate. A non-selective opioid receptor antagonist, naloxone (5 mg/kg, i.p.) was injected 15 min before hand, against the antinociceptive effect caused by acetylsalicylic acid, morphine and CLEO [15].

**Statistical test.** The results were expressed as means  $\pm$  S.E.M. of 6 mice. The differences were estimated by one-way ANOVA followed by Dunnett's multiple comparison test, unless stated otherwise. Differences between means were considered as statistically significant at  $p < 0.05$ .

## Results

**Acetic acid-induced abdominal constriction test.** CLEO (0.1, 0.5, 1 and 5 mg/kg, i.p.) exerted significant dose dependent reduction in the number of abdominal constriction with 17.38%, 41.84%, 88.48% and 99.65% of inhibition, respectively. ASA (100 mg/kg, i.p.) with 38.12% of inhibition as compared to control group, produced moderate inhibitory effect in this test (refer with: Fig.1).

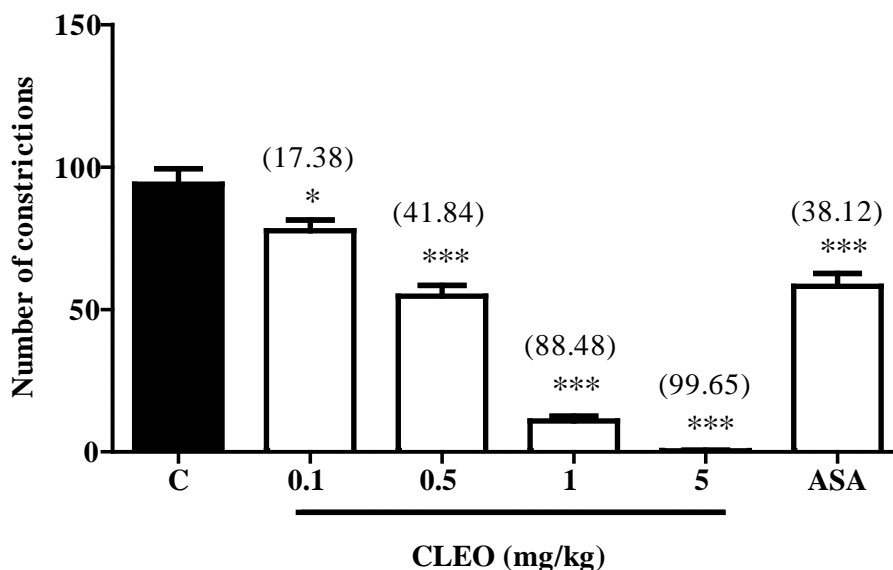


Fig.1. Effect of CLEO in the acetic acid-induced abdominal constriction test in mice. Each column represents the mean ± S.E.M. of 6 mice. The mice were pretreated with vehicle (C, control), acetylsalicylic acid (ASA, 100 mg/kg, i.p.) or CLEO (0.1, 0.5, 1 and 5 mg/kg, i.p.), 30 min before 0.6% of acetic acid (10ml/kg, i.p.). The asterisks denote significance levels as compared to control, \*\*\*p < 0.001 by one-way ANOVA followed by Dunnett’s post hoc test. Values in parentheses are percentage of inhibition.

Pretreatment with naloxone (5 mg/kg, i.p.) do not reversed the antinociceptive activity of CLEO (1 mg/kg, i.p.) which suggests that opioid system does not involved in antinociceptive effect of CLEO (refer with: Fig.2).

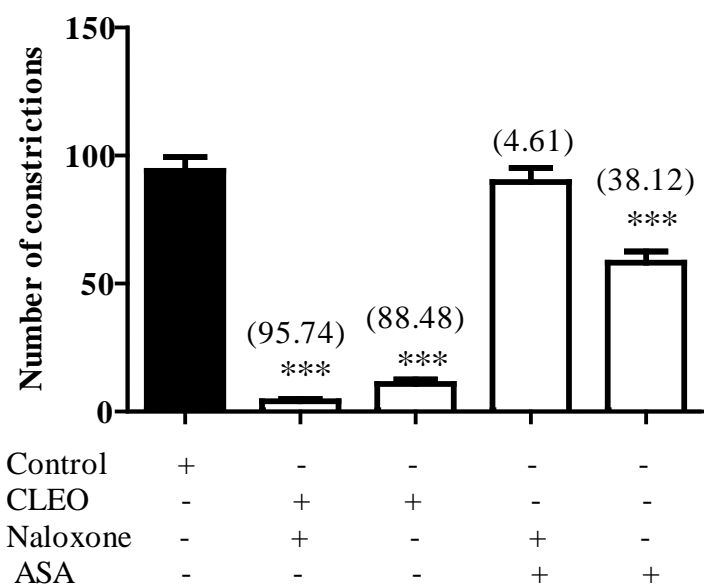


Fig.2. Effect of naloxone (5 mg/kg, i.p.) on antinociception caused by CLEO (1 mg/kg, i.p.) in the acetic acid-induced abdominal constriction test. Each column represents the mean ± S.E.M. of 6 mice. The asterisks denote significance levels as compared to control, \*\*\*p < 0.001 by one-way ANOVA followed by Dunnett’s post hoc test. Values in parentheses are percentage of inhibition.

**Formalin-induced paw licking test.** CLEO had significant analgesic effects on both early (refer with: Fig. 3a) and late phases (refer with Fig. 3b) in formalin-induced pain, in which all doses of CLEO elicit greater inhibition against inflammatory pain rather than neurogenic pain.

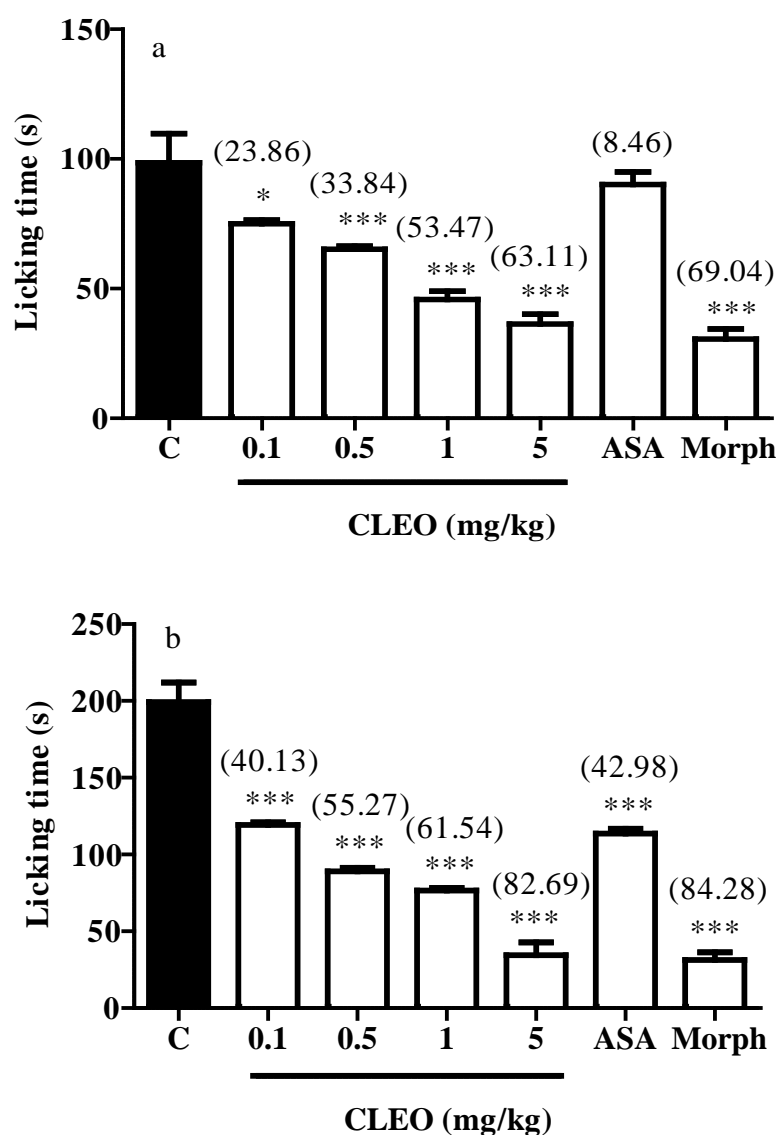


Fig. 3. Effect of CLEO in formalin induced paw licking test (early phase, panel a, and late phase, panel b) in mice. Each column represents the mean  $\pm$  S.E.M. of 6 mice. The mice were pretreated with vehicle (C, control), acetylsalicylic acid (ASA, 100 mg/kg, i.p.), morphine (5 mg/kg, s.c.) or CLEO (0.1, 0.5, 1 and 5 mg/kg, i.p.), 30 min before i.pl. injection of formalin. The asterisks denote significance levels as compared to control, \*\*\* $p < 0.001$  by one-way ANOVA followed by Dunnett's post hoc test. Values in parentheses are percentage of inhibition.

**Hot plate test.** Significant increase in baseline was observed after administration of CLEO (1 and 5 mg/kg; i.p.) as compared to control group. On the contrary, the animals treated with morphine (5 mg/kg; s.c.) showed most powerful inhibitory activity. Pretreatment with naloxone (5 mg/kg; i.p.) did not reversed the antinociception produced by CLEO (1 mg/kg; i.p.) (refer with: Table 1).

Table 1. Effect of CLEO on hot plate test in mice. Results are expressed in mean  $\pm$  S.E.M. of reaction time (s) of 6 mice. Statistical significance was determined by two-way ANOVA followed by Bonferroni post hoc test.

Treatments	Dose (mg/kg)	Latency time (s)							
		0 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min
Control (i.p.)		6.17 $\pm$ 0.17	6.83 $\pm$ 0.31	6.67 $\pm$ 0.33	6.67 $\pm$ 0.21	6.83 $\pm$ 0.31	6.67 $\pm$ 0.21	6.83 $\pm$ 0.31	6.33 $\pm$ 0.21
CLEO (i.p.)	0.1	6.50 $\pm$ 0.34	7.83 $\pm$ 0.54	7.83 $\pm$ 0.31	9.67 $\pm$ 0.71*	9.33 $\pm$ 0.49	8.83 $\pm$ 0.54	10.00 $\pm$ 0.26*	7.50 $\pm$ 0.56
	0.5	7.00 $\pm$ 0.26	7.00 $\pm$ 0.86	8.67 $\pm$ 0.88	9.00 $\pm$ 0.52	10.00 $\pm$ 0.73*	9.33 $\pm$ 0.49	10.17 $\pm$ 0.70**	9.83 $\pm$ 0.79**
	1	7.00 $\pm$ 0.37	8.50 $\pm$ 0.62	10.67 $\pm$ 0.99***	10.67 $\pm$ 0.56***	12.33 $\pm$ 0.71***	10.83 $\pm$ 0.40***	10.00 $\pm$ 1.00*	9.83 $\pm$ 0.65**
CLEO (i.p.) + naloxone (i.p.)	5	7.33 $\pm$ 0.21	8.67 $\pm$ 0.33	9.50 $\pm$ 0.43*	11.50 $\pm$ 0.50***	12.50 $\pm$ 1.15***	12.00 $\pm$ 1.15***	11.00 $\pm$ 1.24***	11.50 $\pm$ 0.76***
	1 +	7.17 $\pm$ 0.31	8.33 $\pm$ 0.67	10.00 $\pm$ 0.82	10.17 $\pm$ 0.87	10.33 $\pm$ 0.67	9.83 $\pm$ 0.98	12.00 $\pm$ 1.13	11.50 $\pm$ 0.76
	5	7.50 $\pm$ 0.34	18.33 $\pm$ 0.67***	17.00 $\pm$ 0.82***	16.33 $\pm$ 0.42***	16.33 $\pm$ 0.99***	15.33 $\pm$ 0.61***	15.17 $\pm$ 0.40***	14.83 $\pm$ 0.40***
Morph (s.c.)	5	6.33 $\pm$ 0.21	6.67 $\pm$ 0.33#	6.67 $\pm$ 0.33#	6.50 $\pm$ 0.22#	7.00 $\pm$ 0.37#	6.50 $\pm$ 0.22#	6.83 $\pm$ 0.17#	6.50 $\pm$ 0.22#
Morph (s.c.) + naloxone (i.p.)	5 +	6.33 $\pm$ 0.21	6.67 $\pm$ 0.33#	6.67 $\pm$ 0.33#	6.50 $\pm$ 0.22#	7.00 $\pm$ 0.37#	6.50 $\pm$ 0.22#	6.83 $\pm$ 0.17#	6.50 $\pm$ 0.22#

\*  $p < 0.05$  as compared to control.

\*\*  $p < 0.01$  as compared to control.

\*\*\*  $p < 0.001$  as compared to control.

#  $p < 0.05$  compared to the group receiving appropriate drug/extract at the same dose without naloxone.

**Discussion.** Both chemical- and thermal-induced nociception models were used to investigate the potential antinociceptive activity of *Curcuma longa* essential oil (CLEO). The acetic acid-induced abdominal constriction test served as a screening tool for the assessment of new analgesic agents [16]. Injection of acetic acid into peritoneal cavity caused the increased of cyclooxygenase (COX) and lipooxygenase (LOX) products in peritoneal fluids and stimulate the release of various inflammatory mediators such as bradykinin and substance P, which then activates primary afferent nociceptors to send nerve impulse into dorsal horn neurons of central nervous system [17]. Therefore, the present study strongly suggests that inhibition of COX and/or LOX and other inflammatory mediators maybe partially involved in the mechanism of CLEO. The poor specificity of this test that is due to muscle relaxants caused data misinterpretation [18], thus further investigations were conducted to evaluate the analgesic effect of CLEO. The formalin-induced paw licking test is characterized by two distinct phases: first phase corresponds to neurogenic pain due to direct activation on sensory nerve fibers while second phase or inflammatory pain caused by the release of chemical mediators [13, 19]. Present study showed that time spent in paw licking was significantly reduced in both neurogenic and inflammatory phases. Central-acting drugs are well known to inhibit both phases equally, while peripherally acting drugs inhibit only the inflammatory phase [20], which in our study was being indicated by morphine and acetylsalicylic acid respectively. The central antinociception effect of CLEO was observed in hot plate test, a sensitive and specific test to show the involvement of central mechanism [21, 22]. Our results demonstrated that CLEO (1 and 5 mg/kg; i.p.) exerts significant prolongation in the response latency to heat stimuli. Thus, the increased nociceptive threshold of mice in this test together with the reduction of nociception in the formalin-induced paw licking test (early phase) have further supported the evidence of centrally mediated antinociceptive activity of CLEO. In addition, the present study also showed that pretreatment with a non-selective opioid receptor antagonist, naloxone did not reversed the antinociceptive effect of CLEO in acetic acid-induced abdominal constriction test and hot plate

test, which suggest that opioid system is not likely to participate in the antinociceptive activity of CLEO at both peripheral and central level.

**Conclusion.** Based on the present study, it can be concluded that CLEO possessed pronounced antinociception activity, both centrally and peripherally which justify its folkloric use to relieve some pain conditions.

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