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The Effect of Curcumin (Active Substance of Turmeric) on the Acetic Acid-Induced Visceral Nociception in Rats

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Abstract: In the present study, the effect of chronic oral administration of curcumin in the presence or absence of morphine and noloxone was investigated on the visceral nociception induced by acetic acid in rats. Intraperitoneal injection of acetic acid (1 mL, 2%) produced contractions in the abdominal musculature (writhes). The latency time to the beginning of the first writhe was measured and the total number of writhes in the 1 h after acetic acid injection was counted. The latency time to the beginning of the first writhe was significantly ($p<0.05$) increased and the number of writhes was significantly ($p<0.05$) decreased by curcumin (20 and 40 mg kg^{-1} body weight). The same results were obtained after subcutaneous injection of morphine (1 mg kg^{-1} b.wt.). Naloxone at the dose of 1 mg kg^{-1} body weight had no effect on pain intensity. Curcumin significantly ($p<0.05$) enhanced the effect of morphine on the visceral pain responses, however did not reverse the effect of naloxone. Present data suggest that in the acetic acid-induced visceral nociception of rats, curcumin may produce an antinociceptive effect and the endogenous analgesic opioid system is involved in the curcumin-induced antinociception.

Key words: Curcumin, turmeric, morphine, naloxone, visceral nociception, rats

INTRODUCTION

Turmeric, *Curcuma longa* L. rhizomes, has been widely used for centuries in the indigenous medicine for the treatment of inflammatory conditions (Ammon and Wahl, 1991). Curcumin, the major component of turmeric, has wide range of biological and pharmacological activities including anti-inflammatory, anti-oxidant, anti-cancer, anti-microbial, anti-fungal, anti-viral and anti-coagulant activities (Araujo and Leon, 2001; Maheshwari *et al*., 2006).

On the antinociceptive effect of curcumin it was reported that curcumin-produced antinociception using tail immersion and hot plate assays of pain in a diabetic mouse model of neuropathic pain (Sharma *et al*., 2006). In addition, Tajik *et al*. (2007) reported an antinociceptive effect of curcumin in the formalin test of rats. JCICM-6 is an extract of an anti-arthritic herbal formula and *Curcuma longa* is one of its components. It was found that JCICM-6 produced antinociception in both tail flick of rats and writhing reflex of mice (Zhou *et al*., 2006).

The intraperitoneal administration of agents such as acetic acid that irritate serous membranes provokes a very stereotyped behavior in the mouse and the rat which characterized by abdominal contractions, movements of body as a whole, twisting of dorso-abdominal muscles and a reduction in motor activity. The test is sometimes called the abdominal contortion test, the abdominal constriction response, or the stretching test, but more recently it is known as the writhing test (Le Bars *et al*., 2001).

To our knowledge, studies examining the effect of curcumin on the visceral pain are lacking. Therefore, the present study was designed to investigate the effect of curcumin on the acetic acid-induced visceral nociception in rats. In addition, to identify the mechanism that possibly mediating the effect of curcumin on pain, we assessed the contribution of the endogenous analgesic opioid system using morphine (an opioid agonist) and naloxone (an opioid antagonist) with curcumin.

MATERIALS AND METHODS

Animals: Healthy adult male albino Wistar rats weighing 220-250 g were obtained from the Laboratory Animals Care and Use Center of Urmia University. Rats were maintained in polypropylene cages with four rats in each cage with food and water available *ad libitum*, in a laboratory with controlled ambient temperature (20-23°C) and under a 12 h light-dark cycle (lights on 07:00 h; lights

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on 19:00 h). Eight rats were used in each treatment. The experimental protocol was approved by the Laboratory Animals Care and Use Center of Urmia University.

Drugs and treatments: Drugs used in the present study were curcumin (Merck, Darmstadt, Germany), morphine sulphate and naloxone hydrochloride (Temad, Tehran, Iran). Curcumin suspension was freshly prepared in 0.15 M NaCl (normal saline) and was administered orally at the doses of 10, 20 and 40 mg kg^{-1} b.wt. once daily for eight days. Oral administration of curcumin was made in a constant volume of 0.2 mL rat^{-1} over a period of 1-2 min. Morphine and naloxone were dissolved in normal saline and were injected subcutaneously at the same dose of 1 mg kg^{-1} body weight 30 min before induction of pain. In combined treatments, morphine and naloxone were injected 30 min after the latest oral administration of curcumin. It was reported that the acute oral administration of curcumin at the dose of 50 mg kg^{-1} had no effect on the formalin pain (Tajik *et al*., 2007), therefore, in the present study, the acute effect of curcumin at the doses of 10, 20 and 40 mg kg^{-1} b.wt., on the visceral pain was not performed.

Writhing test: Each rat was placed inside a plexiglass observation chamber (40×30×20 cm) for an acclimation period of 30 min. At the end of this period, drug treatment was performed according to the time schedule for each treatment and then 1 mL of 2% acetic acid was intraperitoneally injected using a 25-gauge injection needle. Immediately after injection of acetic acid, the latency time to the beginning of the first contraction of the abdominal musculature (writhe) was measured and the number of writhes was counted during a 60 min observation period. A writhe was defined as a wave of the contraction of the abdominal musculature followed by extension of the hind limbs (Fukui *et al*., 2006). Control rats which received appropriate amount of normal saline did not show any spontaneously occurring body writhes.

Statistical analysis: Data were expressed as mean±SEM. Differences among treated groups were statistically evaluated using the one way analysis of variance (ANOVA) followed by Duncan's test. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

After intraperitoneal injection of acetic acid, the latency time to the beginning of the first writhe and the number of writhes were obtained 5.1±1 min and 36.5±2.9, respectively. Curcumin at the dose of 10 mg kg^{-1} b.wt. did not affect the latency time and number of writhes, whereas at the doses of 20 and 40 mg kg^{-1} b.wt., curcumin significantly ($p<0.05$) increased the latency time to the beginning of the first writhe. The number of writhes was significantly ($p<0.05$) decreased by curcumin at the doses of 20 and 40 mg kg^{-1}. The effect of curcumin (40 mg kg^{-1}) on the pain response was greater than that of curcumin (20 mg kg^{-1}) (Table 1).

The latency time to the beginning of the first writhe was increased and the number of writhes was decreased after subcutaneous injection of morphine (1 mg kg^{-1} b.wt.). However, noloxone alone had no effect. Curcumin, with no effect on the action of naloxone, significantly ($p<0.05$) enhanced the effect of morphine on the latency time to the beginning of the first writhe. The decrease in the number of writhes induced by morphine was also significantly ($p<0.05$) enhanced by morphine (Table 2).

In the present study, it was found that intraperitoneal injection of acetic acid produced contraction in the abdominal wall musculature. The acetic acid-induced writhing is a standard test for visceral pain, sensitive to opiates as well as non-opiates analgesics (Steranka *et al*., 1987). The associated antinociceptive response is believed to involve the release of endogenous substances, such as bradykinin and prostanoids among others that stimulate nociceptive endings (Berkerkopf and Weichman, 1988).

Table 1: Effect of chronic oral administration of curcumin on the visceral nociception induced by acetic acid in rats

Treatments	Latency time[a] (min)	No. of writhes[b]
Normal saline (p.o., 8 days)	5.1±1.0	36.5±2.9
Curcumin (p.o., 8 days, 10 mg kg^{-1})	6.1±1.4	31.7±4.8
Curcumin (p.o., 8 days, 20 mg kg^{-1})	9.6±1.3*	24.0±2.6*
Curcumin (p.o., 8 days, 40 mg kg^{-1})	17.7±3.1*†	11.7±1.4*†

[a]: Measured after acetic acid administration (1 mL, 2%), [b]: Counted in an 1 h observation period after acetic acid administration. Values are mean±SEM, n = 6 in each group, *: $p<0.05$ vs. normal saline group, †: $p<0.05$ vs. curcumin (20 mg kg^{-1}), one way ANOVA followed by Duncan's Test, p.o.: Per oral

Table 2: Effect of chronic oral administration of curcumin on the visceral nociceptive changes induced by morphine and naloxone in the writhing test of rats

Treatments	Latency time[a] (min)	No. of writhes[b]
Normal saline (p.o., 8 days)	5.1±1.0	36.5±2.9
Curcumin (p.o., 8 days, 20 mg kg^{-1})	9.6±1.3*	24.0±2.6*
Morphine (s.c., 1 mg kg^{-1})	10.4±1.2*	18.2±2.4*
Naloxone (s.c., 1 mg kg^{-1})	6.4±1.1	41.7±3.8
Curcumin (p.o., 8 days, 20 mg kg^{-1}) + Morphine (s.c., 1 mg kg^{-1})	15.3±0.9*†	6.0±1.4*†
Curcumin (p.o., 8 days, 20 mg kg^{-1}) + Naloxone (s.c., 1 mg kg^{-1})	7.9±1.2	39.3±4.3

[a]: Measured after acetic acid administration (1 mL, 2%), [b]: Counted in an 1 h observation period after acetic acid administration. Values are mean±SEM, n = 6 in each group, *: $p<0.05$ vs. normal saline group, †: $p<0.05$ vs. curcumin and morphine groups, one way ANOVA followed by Duncan's Test, p.o.: Per oral, s.c.: Subcutaneous

In this study, chronic oral administration of curcumin suppressed visceral nociception induced by acetic acid. It seems that anti-inflammatory property of curcumin may contribute to its antinociceptive effect. In the formalin test of rats, it has been shown that acute oral administration of curcumin at the high doses (100 and 200 mg kg^{-1} b.wt.) suppresses the second phase of pain (Tajik *et al*., 2007). The second phase of formalin-induced pain is well known as an inflammatory pain (Tjolsen *et al*., 1992). In addition, it has been reported that curcumin has ability to inhibit the activation of inflammatory mediators such as cyclooxygenase-2, lipooxygenase, inducible nitric oxide synthase and nuclear factor kappa B (Bengmark, 2006). On the other hand, it has been found that after intraperitoneal injection of acetic acid, inflammatory reactions develop in the peritoneum (Clementi *et al*., 1999).

In current study, morphine but not naloxone suppressed the acetic acid-induced visceral pain. This finding is in accordance with previous reports that intraperitoneal injection of morphine reduces the number of acetic acid induced abdominal contractions in mice and pretreatment with naloxone has been shown to inhibit the suppressive effect of morphine (Reichert *et al*., 2001).

In this study, curcumin, without any effect on the naloxone action, potentiated the morphine-induced antinociception. This indicates that the antinociceptive effect of curcumin may be associated on the activation of the opioid system. The sensation of pain is well known to be modified by endogenous opioid system. Morphine (an opioid agonist) and naloxone (an opioid antagonist) are used to explore the involvement of the endogenous opioid analgesic system activated by novel analgesics (Ananthan, 2006).

In conclusion, it seems that several mechanisms are involved in the antinociceptive effect of curcumin. Further studies are needed to identify the antinocieptive activity of curcumin in pain mechanisms.

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