Effects of curcumin, morphine and naloxone on the experimentally-induced paw edema in rats

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Abstract: **In this study, effects of curcumin (active substance of turmeric), morphine (an opioid receptor agonist) and naloxone (an opioid receptor antagonist) in separate and combined treatments have been investigated on the paw edema induced by formalin in rats. Subcutaneous injection of formalin (50µl, 2.5%) in the ventral surface of the hind paw induced a considerable edema in the paw that lasted up to 24h after formalin injection. Chronic oral administration of curcumin (30 and 60mg/kg, 15 days) and subcutaneous injection of morphine (1mg/kg) significantly ($p<0.05$) decreased the paw edema induced by formalin. Naloxone (1mg/kg) used alone was without effect, whereas pretreatment with naloxone (1mg/kg) before morphine (1mg/kg) significantly ($p<0.05$) prevented the suppressive effect of morphine on the paw edema. The reducing effect of curcumin on the paw edema was potentiated by morphine, whereas naloxone did not change the anti-edematous effect of curcumin. Curcumin (30mg/kg, 15 days) in the presence of naloxone (1mg/kg) plus morphine (1mg/kg) treatment, produced an anti-edematous effect, which resembled the one following administration of curcumin (30 mg/kg, 15 days) alone. Present findings indicate that curcumin may produce an anti-edematous effect. The endogenous opioid system may involve in modulation of local edema. In addition, morphine may potentiate the anti-edematous effect of curcumin.**

Key words: curcumin, morphine, naloxone, paw edema, rats.

Introduction

Renewed interest has been observed in recent years on the multiple activities of natural flavonoids. Curcumin is a major yellow-orange pigment extracted from turmeric, a commonly used spice, derived from the rhizome of the herb *Curcuma longa* (Maheshwari *et al.,* 2006). It is well known that curcumin has a wide range of biological and pharmacological effects, including antioxidant, anti-carcinogenic, anti-mutagenic, anti-diabetic, anti-bacterial, anti-fungal and anti-viral effects (Araujo and Leon, 2001; Chattapadhyay *et al.,* 2004; Maheshwari *et al.,* 2006). There are some evidences that suggest that curcumin may have an anti-inflammatory property. It was reported that curcumin prevented the paw edema in carrageenan and cotton pellet models of inflammation in rats (Mukhophadhyay *et al.,* 1982). Moreover, the anti-rheumatic activity of curcumin has also been established in patients who showed significant improvement of symptoms after administration of curcumin (Deodhar *et al.,* 1980).

The endogenous opioid system, besides its well recognized effect on pain modulation, also affects the inflammatory responses (Walker, 2003). It was reported that morphine attenuated the paw edema induced by intraplantar injection of carrageenan in rats (Amann *et al.,* 2002). Moreover, using the yeast-induced paw inflammation model, Sacerdote *et al*., (1996) reported an inhibitory effect of beta-

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endorphin on the paw edema in rats. On the other hand, it was found that the intraperitoneal injection of naloxone enhanced the edema induced by intraplantar injection of carrageenan in the hind paw of rats (Planas *et al.,* 1995).

Paw and ear edemas have been induced by local peripheral injection of inflammatory mediators, such as histamine, serotonin, bradykinin, and arachidonic acid in rats and mice (Zhou *et al.,* 2006). Different concentration of formalin injected intraplantarly also produced paw edema in rats (Lee and Jeong, 2002). The pain elicited by formalin is only one component of a local inflammatory reaction induced by this irritant. It has been reported that formalin injection produces an edema and an increase of vascular permeability (Damas and Liejeois, 1999; Taylor *et al.,* 2000).

The present study was designed to investigate the effects of curcumin on the formalin-induced paw edema in rats. In addition, to identify the mechanism that possibly mediating the effects of curcumin on edema, the association of the endogenous opioid system using morphine (an opioid receptor agonist) and naloxone (an opioid receptor antagonist) with curcumin has been assessed.

Materials and Methods

Seventy-six healthy adult male Wistar rats weighing between 220-250g were obtained from the Laboratory Animal Care and Use Center of the College of Veterinary Medicine of Urmia University. They were maintained in polypropylene cages in groups of four with food and water available ad libitum, with controlled ambient temperature (23±0.5° C) and under a light-dark cycle (lights on at 07:00 - 19:00). Eight rats were used in each experiment.

Drugs used in the present study were curcumin (Merck, Darmstadt, Germany), morphine sulfate 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 7.5, 15, 30 and 60 mg/kg once daily for 15 days. Oral administration of curcumin was made in a constant volume of 0.2 ml per rat over a period of 1-2 min using a needle free 1 ml syringe (through licking). The selected doses of curcumin and the time period schedule used in this study were close to other studies performed in rats and mice (Sharma *et al.,* 2006; Gautam *et al.,* 2007; Tajik *et al.,* 2008; Tamaddonfard *et al.,* 2008). Morphine and naloxone were dissolved in normal saline and were subcutaneously injected in the back of neck at the same doses of 1mg/kg using 29-gauge injection needle.

For induction of paw edema, 60 min after the last oral administration of curcumin and 40 and 30 min after subcutaneous injections of naloxone and morphine, respectively, rats were subcutaneously injected with 50µl of 2.5% formalin solution into the ventral surface of the right hind paw using a 29-gauge injection needle. All rats were then returned to their cages. According to Fu *et al*., (2001), the magnitude of paw edema was assessed by measuring the dorsal-plantar paw thickness with a fine caliper at 1, 4, and 24h after formalin injection. The thickness of both hind paws (injected and non-injected ones) was measured simultaneously. Percent change in paw thickness was then calculated using the following formula (Fu *et al.,* 2001):

[(thickness of the injected paw - thickness of the non-injected paw)/ thickness of the non-injected paw] × 100

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

Results

Intraplantar injection of normal saline produced no edema in the paw. Therefore, the results (0.0±0.0) obtained from normal saline injection are not shown in the figures. Subcutaneous injection of formalin into the ventral surface of hind paw induced a local edema, which lasted up to 24h after formalin injection. Twenty four hours post-formalin paw edema was significantly ($p<0.05$) lower than that of 1 and 4h after formalin injection. Chronic oral administration of curcumin at the doses of 7.5 and 15

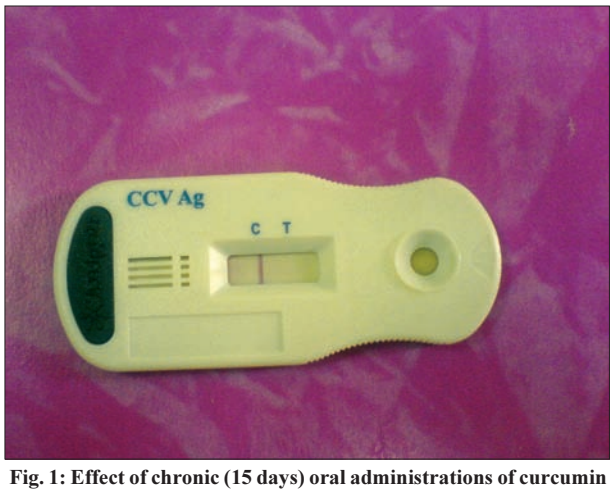

Fig. 1: Effect of chronic (15 days) oral administrations of curcumin on the paw edema induced by intraplantar injection of formalin in rats. *p<0.05 compared with other groups in each mentioned time, n: 8 rats in each group, po: per oral, ipl: intraplantar.

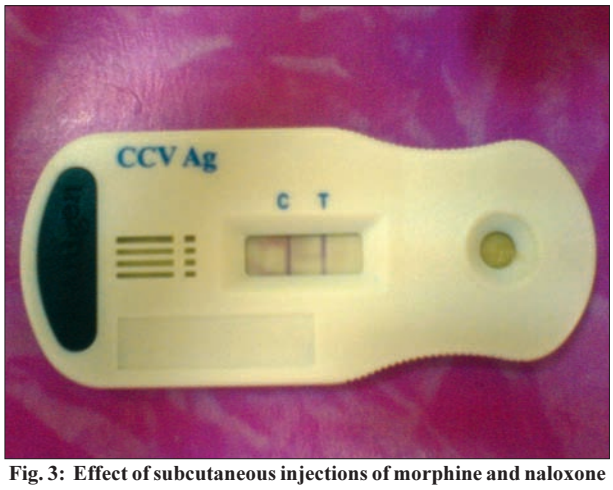

Fig. 3: Effect of subcutaneous injections of morphine and naloxone on the suppressive action of curcumin in the formalin- induced paw edema in rats. * p<0.05 compared with normal saline plus formalin in each mentioned time, n: 8 rats in each group, po: per oral, ipl: intraplantar, sc: subcutaneous.

mg/kg did not produce any significant effect, whereas at the doses of 30 and 60 mg/kg, curcumin significantly ($p<0.05$) decreased formalin-induced paw thickness at 1, 4, and 24h (Fig. 1).

Morphine (1mg/kg, sc) significantly ($p<0.05$) decreased formalin-induced paw thickness at 1, 4, and 24h. Naloxone (1mg/kg, sc) used alone produced no significant effect on the paw edema induced by formalin at 1, 4, and 24h. Pretreatment with naloxone before morphine significantly ($p<0.05$) prevented the anti- edematous effect of morphine at 1 and 24h, whereas the anti-edematous effect of morphine was not completely inhibited by naloxone pretreatment at 4h (Fig. 2).

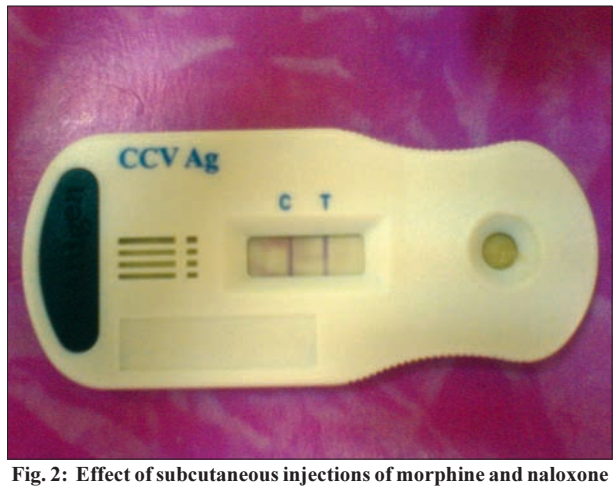

Fig. 2: Effect of subcutaneous injections of morphine and naloxone on the paw edema induced by intraplantar injection of formalin in rats. *p<0.05 compared with other groups in each mentioned time, †p<0.05 compared with normal saline plus formalin group at 4h, n: 8 rats in each group, po: per oral, ipl: intraplantar, sc: subcutaneous.

Chronic treatment with curcumin (30 mg/kg., p.o.) significantly ($p<0.05$) decreased formalin-induced paw thickness at 1, 4, and 24h. Morphine (1mg/kg, sc) after chronic oral administration of curcumin (30mg/kg) significantly ($p<0.05$) decreased paw thickness compared with both morphine (1mg/kg) and curcumin (30mg/kg) used alone. Naloxone after curcumin did not change the suppressive effect of curcumin on the paw edema induced by formalin. In the presence of naloxone (1mg/kg) plus morphine (1 mg/kg) treatment, the curcumin (30 mg/kg, p.o., 15 days)-induced anti-edematous effect was not changed (Fig. 3).

Discussion

In this study, it was found that intraplantar injection of formalin produced paw edema which lasted up to 24h after formalin injection. Several studies have shown that, after an injection of formalin, paw edema develops rapidly, reaches its peak at 4 to 5 hours and lasts several days after injection (Fu *et al.,* 2001; Lee and Jeong, 2002). It has been reported that inflammatory mediators, such as bradykinin, histamine, serotonin, and prostanoids, contribute to the paw edema and paw vascular permeability induced by intraplantar injection of formalin (Damas and Liegeois, 1999).

In the present study, chronic administration of curcumin at the doses of 30 and 60 mg/kg attenuated

the formalin-induced paw edema. It has been reported that chronic (5 weeks) dietary application of curcumin at the doses of 1 and 20 mg/kg produces no effect on the immune responses, but at the dose of 40 mg/kg curcumin stimulates the production of IgG that was induced by sheep red blood cells in rats (South *et al.,* 1997). Sharma *et al*., (2006) reported antihyperalgesic effects of chronic (14 days) administration of curcumin used at the doses of 30 and 60mg/kg in the neuropathic pain of rats. Moreover, pain suppressive effect of chronic administration of curcumin (20 and 40 mg/kg, 8 days) was reported in the writhing test of rats (Tajik *et al.,* 2008).

On the anti-inflammatory effect of curcumin, it has been reported that curcumin lowers the carrageenan-induced paw edema in the foot pads of rats (Reddy and Lokesh, 1994). In addition, Huang *et al*., (1991) reported an anti-edematous effect of curcumin in the arachidonic acid-induced ear edema in rats. It seems that the anti-edematous effect of curcumin observed by Kim *et al*., (2005) may be related to antihistaminic and anti-serotonin activities of curcumin. Moreover, the involvements of histamine and serotonin in the formalin-induced edema have been reported (Damas and Liegeois, 1999). JCICM-6 is an extract of an anti-arthritic herbal formula, in which Curcuma longa is one of its components. It was found that JCICM-6 reduced paw edema induced by intraplantar injection of histamine, serotonin, bradykinin, and prostaglandin E2 in rats and suppressed ear inflammation induced by subcutaneous injection of arachidonic acid in the ear of mice (Zhou *et al.,* 2006). In addition to these effects of curcumin, it has been reported that curcumin has the ability to inhibit the activation of other inflammatory mediators, such as nuclear factor kappa B and cyclooxygenase 2, lipooxygenase and inducible nitric oxide synthase products (Bengmark, 2006).

In the present study, morphine attenuated, but naloxone did not change the formalin-induce paw edema. Pre-treatment with naloxone before morphine significantly, but not completely, prevented the anti-edematous effect of morphine, because naloxone did not block the morphine-induced anti-edematous effect at 4h observed in the present study. It has been reported that the highest level of formalin-induced paw edema occurs at 4h after intraplantar injection of formalin (Lee and Jeong, 2002). Morphine acts through mu-opioid receptors (Pasternak, 2001), and naloxone is a competitive antagonist of mu, kappa and sigma receptors with higher affinity for the mu receptors (Helm II *et al.,* 2008). Morphine and naloxone have been frequently used to explore the role of endogenous opioid system in inflammation (Planas *et al.,* 1995; Sacerdote *et al.,* 1996; Taylor *et al.,* 2000). These studies suggested that opioid agonists via naloxone-sensitive and -insensitive receptors were involved in the modulation of inflammatory reactions. Besides opioid mu-receptors, it has been reported that delta- and kappa-opioid receptors participate in the regulation of inflammation by endogenous opioid system (Romero *et al.,* 2005). However, other mechanisms, such as inhibitory effect of morphine on the activation of nuclear kappa factor B (Welters *et al.,* 2000) and antioxidant activity of morphine (Gulcin *et al.,* 2004), in the anti-edematous effect of morphine have been reported.

In this study, morphine potentiated the suppressive effect of curcumin on the paw edema. There is not any report identifying the interaction between curcumin and opioid system in the paw inflammation. In one report, it has been found that curcumin potentiates the effect of morphine on the acetic acid-induced visceral pain (Tajik *et al.,* 2008). In the present study, naloxone did not influence the suppressive effect of curcumin on the paw edema, and in the presence of naloxone plus morphine, the anti-edematous effect of curcumin was not changed. These indicate that curcumin may make use of non-opioid dependent mechanisms to produce an anti-edematous effect. It has been reported that curcumin is able to inhibit the activation of nuclear factor kappa B (Weber *et al.,* 2006). Nuclear factor kappa B positively regulates the production of pro-inflammatory enzymes, such as inducible nitric oxide synthase and cyclooxygenase 2 (Pahl, 1999). Moreover, the antioxidant properties of curcumin

were reported (Gulcin *et al.,* 2004; Sandur *et al.,* 2007; Tamaddonfard *et al.,* 2008). It has been found that reactive oxygen species, such as peroxide, superoxide anion, hydroxyl radical, and singlet oxygen, are involve in the paw edema induced by Ferund's complete adjuant (Symons *et al.,* 2003).

Finally, it seems that several mechanisms may be involved in the anti-edematous activity of curcumin in the formalin-induced paw edema. In this study, it was shown that anti-edematous effect of curcumin was potentiated by morphine whereas no change was observed with naloxone, and further studies are needed to identify the mechanisms involved.

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مجله بین المللی تحقیقات دامپزشکی، ۱۳۸۸، دوره ۳، شماره ۱، ۳۰-۲۵

اثر کورکومین، مرفین و نالوکسان بر ادم تجربی پنجه پا در موش های صحرایی

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چکیده

در این مطالعه، اثرات کورکومین (ماده فعال زردچوبه)، مرفین (آگونیست گیرنده های اپیوئیدی) و نالوکسان (آنتاگونیست گیرنده های اپیوئیدی) به صورت جداگانه و ترکیبی بر ادم پنجه پای ناشی از فرمالین در موش های صحرایی بررسی شده است. تزریق زیر جلدی فرمالین (۵۰ میکرولیتر، ۲/۵ درصد) در کف پنجه پا یک ادم قابل توجهی در پنجه پا ایجاد کرد که تا ۲۴ ساعت پس از تزریق فرمالین قابل مشاهده بود. خوراندن مزمن (۱۵ روز) کورکومین در مقادیر ۳۰ و ۶۰ میلی گرم به ازای یک کیلوگرم وزن بدن و تزریق زیر جلدی مرفین در مقدار ۱ میلی گرم به ازای یک کیلوگرم وزن بدن به طور معنی دار ($p<0/05$) ضخامت پنجه پای ناشی از فرمالین را کاهش دادند. نالوکسان در مقدار ۱ میلی گرم به ازای یک کیلوگرم وزن بدن به تنهایی اثر نداشت، در حالی که پیش تزریق نالوکسان قبل از مرفین به طور معنی دار ($p<0/05$) از اثر تضعیف کننده مرفین بر ادم پنجه پا جلوگیری کرد. اثر کاهش دهنده کورکومین بر ادم پنجه پا به وسیله مرفین تقویت شد، در حالیکه نالوکسان اثر ضد ادم کورکومین را تغییر نداد. کورکومین در حضور درمان با نالوکسان به علاوه مرفین، یک اثر ضد ادم ایجاد کرد که مشابه اثر ضد ادم ایجاد شده پس از تجویز به تنهایی کورکومین بود. نتایج حاضر بیان می کنند که کورکومین ممکن است اثر ضد ادم ایجاد کند. سیستم اپیوئیدی داخلی ممکن است در تعدیل آدم موضعی دخالت نماید. به علاوه مرفین اثر ضد ادم ناشی از کورکومین را تقویت می نماید.

واژه‌های کلیدی: کورکومین، مرفین، نالوکسان، ادم پنجه پا، موش صحرایی.

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