Original Article

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# Investigation on the Analgesic and Anti-inflammatory Effects of Curcumin in Mice

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## ABSTRACT

**BACKGROUND AND OBJECTIVE:** Curcumin is the main ingredient of turmeric which has been used in traditional medicine for pain relief. This study was done to investigate the mechanism of possible analgesic and anti-inflammatory effect of curcumin using hot-plate and formalin method on mice.

**METHODS:** This experimental study was done on white mice weighing 20-25 g were used. Two approaches including analgesic effects of curcumin at a dose of 10, 20 and 30 mg/kg using Hot-plate method and analgesic with probable anti-inflammatory effects of curcumin at a dose of 5, 10 and 20 mg/kg using formalin test were examined. The data gathered from the experiments was analyzed using statistical methods.

**FINDINGS:** Curcumin alone on the hot-plate, showed no significant difference in mice latency time compared to saline group. Significant increases in latency time were seen after treatment of curcumin in doses of 10, 20, and 30 mg/kg in combination with morphine (20 mg/kg) at 45 min of hot plate test (22.7±9.8, 25.7±11.2 and 31.1±9.4) and 60 min (22.2±8.8, 25.3±9.7 and 29.1±10.7, respectively) compared to control group ($p<0.05$). There was a significant difference between curcumin in combination with morphine and saline with morphine ($p=0.025$ and 0.041, respectively). In formalin test, curcumin alone had a considerable effect in reducing the pain following formalin injection. The formalin test latency at 5 min in curcumin groups (5, 10 and 20 mg/kg) was significant ($p<0.05$).

**CONCLUSION:** According to the results, it can be proposed that curcumin acts centrally and its effects on opioid system appear to facilitate the opioids effects. Its effect is dose dependent and can be blocked with naloxone.

**KEY WORDS:** *Curcumin, Analgesic, Anti-inflammatory, Formalin test, Hot-plate, Morphine, Naloxone.*

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## Introduction

The use of herbal medicine had been considered as the oldest form of therapy to relieve pain and inflammation. Turmeric is a plant of the ginger family and is yellow or gray-brown. This plant has healing properties for some diseases and problems such as back pain, chest pain, diarrhea, dysentery and jaundice (1, 2). The yellow color of the plant is related to main active ingredient called curcumin, which is said to have anti-inflammatory effects than steroids in the treatment of acute inflammation (3).

Curcumin stops pain through similar mechanisms with drugs that inhibit inflammation (4). Curcumin can improve morning stiffness of the joints and relieve pain. In addition, it can stop about 46 to 69 percent of inflammation in the body and in the laboratory (5). It has healing properties for inflammatory bowel disease, colitis, pancreatitis, osteoarthritis and rheumatoid arthritis (6).

Curcumin is a substance insoluble in water and only in very small quantities and is hardly soluble in water. But in Gastric acid is stable. After absorption, curcumin is metabolized by the liver and excessive amounts are excreted in the feces. Therefore, due to limited absorption, dose should be increased for more effects (7).

In the process of inflammation, several enzymes such as lipoxygenase, phospholipase, cyclooxygenase-2, collagenase, hyaluronidase and protein kinase c (8) and substances such as leukotrienes, thromboxanes, prostaglandins, nitric oxide, interleukins (IL12, IL8, IL6, IL2, IL1) and TNF are implicated and studies have shown that curcumin has anti-inflammatory effect by inhibiting this pathways(9).

Curcumin also has effects on the L and D isomers of phenylalanine that may improve nervous system function, and inhibits the transmission of pain and suppresses its pathway (10). Phenylalanine is an amino acid that has two forms, L and D-alanine. D-alanine sequestrates enkephalin (a neurotransmitter that suppresses pain messages), prevents and improves the general condition of the patient and reduces fatigue and impatience (11).

This substance prevents from sudden muscle pain that may arise in sports or other factors. L-Phenylalanine could be converted to tyrosine form, brain chemical intermediates such as epinephrine and dopamine are released that actually reduce pain (12). The frequent use of curcumin can cause gastrointestinal irritation and in sensitive person may cause rash and heartburn (13). According to various studies on the effects of curcumin and the likely effectiveness of the various systems of pain and inflammation, this study was designed to evaluate its analgesic and anti-inflammatory potential in mice.

## Methods

**Laboratory animals and storage conditions:** white laboratory rats weighing approximately 24-18 gr were kept in a peaceful environment, separated cages away from the stress (12 hours light, 12 hours dark) and with easy access to food and water before the test. During experiments in laboratory temperature was kept constant at 2±23 ° C.

**Chemical materials:** Curcumin, ethanol (95 degrees), Tween80, ethyl acetate (Merck, Germany) and morphine (in powder form manufacturing of pharmaceuticals, Iran) and 6.0% formalin were used.

**Methods: Hot-plate experiments:** In these experiments 7 Series including 6 mice were selected and divided into two categories. In the first three groups, mice were selected and curcumin at doses of 20 mg/kg and 10 mg/kg as well as saline 10 ml/kg was injected. In order to solve the curcumin, Tween 80, 10% in normal saline solution was used. In the second category five groups of mice curcumin at doses 10, 20, 30, 40 mg/kg along with 20 mg/kg morphine (5 minutes after the curcumin) were injected.

In addition, saline was injected as a control group. Injection of morphine was done subcutaneously and other drugs were injected intraperitoneally. At least six mice in each group were used. The base of tolerance in each animal was measured. The temperature used in Hot-plate device was considered 55 ° C. Start time (zero) was specified and the base of animal tolerance was recorded as soon as sstarting licking the hands or specific changes in the steps of the rats on the hot plate. After that saline and drug were injected based on the related group. Then, 15 minutes after the injection, followed by 30, 45, 60, 75, 90 minutes later, the tolerance rate was measured and compared with the base tolerance. The maximum time (cut-off time) to stay on the hot plate and tolerance rate of mice was determined 40 seconds.

**Formalin series of experiments:** In this study, five groups including four groups of curcumin receiving doses of 5, 10, 20 and 30 mg/kg and the control group receiving saline with dose 10 ml/kg were used. Injection of drug in formalin test was performed 10

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minutes before the injection of formalin. In this test, mice after injection were kept in the funnel on a high level for 15 minutes. After 15 minutes, the animals removed from the funnel and about 20 -15 ml of formalin (6.0%) was injected in the right foot or left subcutaneously (in the same series). At this stage the animal is placed under the funnel (for 30 minutes), then respond to the pain was recorded within 30 minutes (5 minutes each) per second. Formalin test is a standard test for measuring response to pain. Pain is defined as the set of time (in seconds) with the mere shaking, licking or biting of injected screw. These times are measured every 5 minutes and numerical value that represents the amount of pain after the injection of formalin in hind paw was considered (14).

**Statistical analysis:** After performing experiments and collecting data, results were analyzed using ANOVA repeated measurs, Kruskalvalis test and p <0.05 was considered significant.

## Results

**A) Hot-plate test:** The results showed that curcumin alone can exert a significant effect on the animal tolerance on the hot plate. Compared with saline in any group received curcumin alone in none of the doses of curcumin and in no time, no significant difference was observed in animals for pain tolerance (Fig 1).

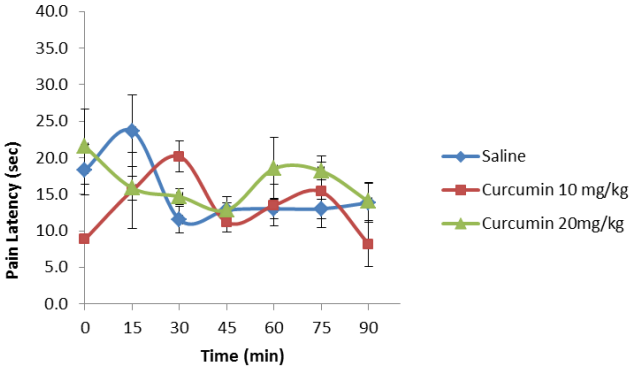


**Figure 1. Comparison of average times of pain in both saline and curcumin groups after placing on a hot plate at doses of 20 and 10 mg per kilogram body weight of the mice**

**Results of curcumin and morphine:** In the first group that presents data related to saline group with morphine, there was no much progress over the time period of testing. Other groups receiving different doses of curcumin along with morphine have shown significant effects. So that curcumin significantly strengthened the effects of morphine. Because morphine alone at a dose mg/kg 20 can have a significant effect compared to saline and curcumin at a dose of 40 mg/kg with morphine. Curcumin at dose of 30 mg/kg, especially in the fifteenth minutes showed a statistically significant difference ($p<0.05$) (Fig 2).

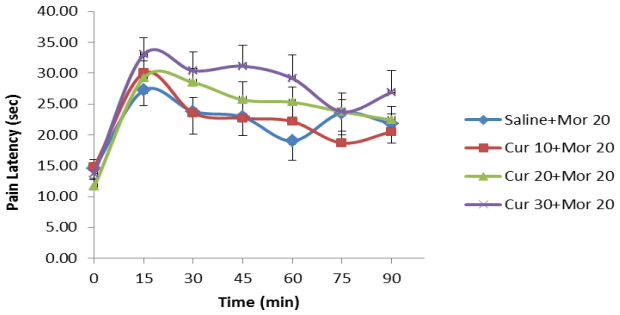


**Figure 2. Comparison of the average time of pain after placement on a hot plate in both the saline and curcumin groups at doses of 10, 20 and 30 milligrams per kilogram of mice body weight along with morphine**

Curcumin at dose of 40 mg/kg with morphine indicated a lower effect than morphine alone and the analgesic effect was decreased (Fig 3). Curcumin used at all doses demonstrated significant differences from the results of saline (in 45 minutes difference in time with $p=0.025$ and in 60 minutes difference with the $p=0.041$). In addition, in 45 minutes between curcumin 30 along with morphine and curcumin 20 along with morphine was significantly different ($p=0.019$). Interestingly, the effects of curcumin decreased by increasing the dose of curcumin compared its effects with lower doses in combination with morphine (Fig3). Comparing the groups showed significant differences within the group at all times ($p<0.0001$). Considering saline group, the differences between the groups will be significant with $p=0.017$.

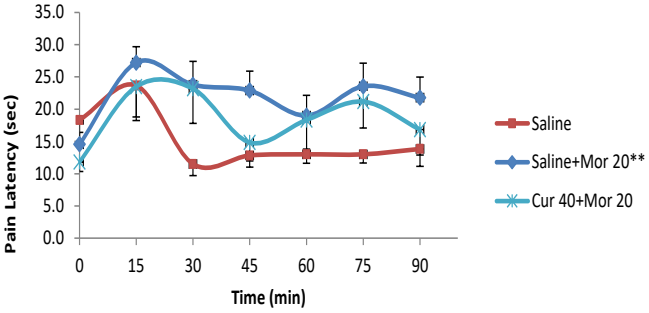


**Figure 3. Comparison of average times of pain after placement on a hot plate in mice into three groups: saline, saline - morphine (20 mg/kg) and curcumin (40 mg /kg) of body weight in mice**

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**B) Formalin test:** The findings of this study suggest that curcumin alone could have a significant effect in reducing the effects of formalin inflammation in the animal. Apart from the effect of curcumin at a dose 20 mg/kg, the rest of the doses in the time of 0-5, which shows sensitivity to pain was increased after the injection of formalin, in other cases, significant effects of curcumin in different doses is evident at the time of tolerance to the effects of formalin stimulation. In other words, even without the presence of morphine curcumin has been able to reduce the response time to a painful stimulus in the formalin received animals. Despite nearly 30 minutes after testing, animals are still sensitive and reactive to stimuli of early formalin. In other words, the effects of formalin pain starts in the second phase that different doses of curcumin in this case are comparable with each other, indicating the effectiveness of curcumin alone.

The difference between saline and curcumin 20 mg/kg at the time of 5 minutes ($p<0.0001$), curcumin 5 mg/kg and curcumin 20 mg/kg ($p<0.001$) and between curcumin 10 mg/kg and curcumin 20 mg/kg at the time of 5 minutes ($p=0.008$) was significant. But the difference between groups was statistically significant only at the time of 5 minutes ($p<0.05$). In fact, in the short term curcumin has analgesic effect but in long-term, there is no significant anti-inflammatory effects, although its effect is significant (Fig 4).

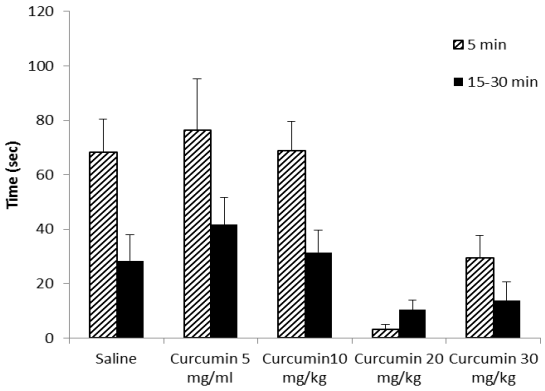


**Figure 4. Comparison of average times of acute and chronic pain (s) against injected formalin in seconds in both saline and curcumin groups of mice at doses of 5, 10, 20, 30 mg/ kg in the timeline after formalin injection**

## Discussion

According to the findings of this research, in Hot-plate method curcumin alone despite the apparent increase in tolerance of animal on the hot plate, significant superiority compared to saline injection was not evident. In addition, curcumin (at all doses 10, 20 and 30 mg/kg) with morphine (20 mg/kg) resulted in a substantial and significant increase in time of animal tolerance compared to saline injection with morphine. In addition, curcumin 40 mg/kg with morphine led also to a significant increase and this indicates that curcumin has the dual effect. On the other hands, curcumin at certain doses has analgesic effect and at higher doses, the analgesic effect of morphine decreases.

This finding is consistent with the findings of a previous study (15). In high dosages curcumin could suppress the transmission of pain and morphine performance can be improved. Effect of different doses is also significant, so that by increasing the dose of curcumin to a certain range can increase the effects of morphine reinforcement. Therefore, it can be said that the effect of increasing curcumin should be done in a controlled manner. Search the mechanism of this material shows that curcumin can reduce pain (16). But cannot completely remove it and therefore can only strengthen the effect of morphine. In fact, many of these analgesic effects is due to chemical mediators such as serotonin, dopamine and noradrenaline that curcumin can boost their release and relieve pain. In fact, curcumin increases the number of presynaptic ends related to neurotransmitter and increase of opening time of these terminals will increase the level of relevant chemical intermediates (17).

Curcumin increases the level of 5-Hydroxy tryptophan (a precursor of serotonin) and postsynaptic cell's sensitivity to this matter and a high serotonin levels are increased (18). Curcumin inhibits the enzyme monoamine neurotransmitter oxidase A, B (enzymes that break down dopamine and serotonin in the synaptic space) the sustainability of the neurotransmitters and increases the effects of them (19). Curcumin increases the noradrenaline levels in the frontal lobe and the hippocampus and is effective in reducing pain (13).

It can be useful on the positions of the body that are painful. Various studies show that curcumin can affect the opioid receptors and its effects on pain and pain relief is through opioid system (20). But the results of the formalin test is interesting. So that curcumin alone even have a significant effect in reducing inflammation induced by formalin in the animals. Especially in times of 30-15 minutes this effect is more pronounced. It has been found that curcumin inhibits the inflammatory

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chemicals such as cyclooxygenase 2. Cyclooxygenase-2 results in the synthesis of prostaglandins (mediators of inflammation and fever) and curcumin reduces inflammation by inhibiting this pathway (21). PKC (protein kinase C) is a substance that increases the expression of cyclooxygenase-2. Curcumin prevents from cyclooxygenase 2 expression by inhibiting PKC and reduces inflammation (22). Curcumin inhibits cyclooxygenase-2 and nitric oxide by the action of nuclear factor kappa B (NF- | B). In fact, these factors inhibits the inflammatory process by decreasing the expression of cyclooxygenase-2 and nitric oxide (23). Curcumin also inhibits the arachidonic acid metabolism that leads to production of lipoxygenase and cyclooxygenase (24). The effects of curcumin on the formalin test was also dose-dependent. According to the results of the analgesic, it can be concluded that curcumin appears as a reinforcement factor of opioid effect through the central act and its effects on opioid system. These effects were dose-dependent and can be removed by opioid antagonist.

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