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ANTI-NOCICEPTIVE EFFECT OF CURCUMIN IN MICE

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ABSTRACT

Hot plate, Formalin induced Paw licking and Tail immersion analgesic models in mice were used to investigate the analgesic activities of the curcumin. Curcumin was used at the doses of 50, 100 and 200mg/kg while aspirin (25mg/kg) was used as the standard reference drug. In hot plate test curcumin showed dose dependant analgesic activity upto 120 min and thereafter, the activity was decreased. In formalin induced paw - licking in mice curcumin caused dose-dependent reduction of licking time at early as well as at late phase. Curcumin at 200 mg/ kg exhibited high reduction in paw licking in late phase than the standard drug aspirin. Curcumin at 200 mg/ kg exhibited high reduction in paw licking in late phase than the standard drug aspirin. For evaluation of peripheral analgesic activity in tail immersion method curcumin at 100 and 200 mg/kg showed dose dependent and significant ($p<0.01$) increase in the reaction time upto 120 min thereafter the response was decreased but it was significantly differ compared to control. The antinociceptive action of curcumin may be due to both peripheral and central mechanisms. In conclusion, the present study demonstrates that curcumin has analgesic actions with considerable margin of safety.

INTRODUCTION

Pain is defined as neuralgia, an unpleasant sensory experience associated with tissue damage. The nerves in our body send a response to brain which allows the body to feel pain. and is a very common sensation experienced by everyone. Drug derived from natural product, particularly from medicinal plants, are believed to be a vital source of chemical substances that have good potential therapeutic efficacy with fewer side effects. When analgesic herb used properly, it can be a powerful alternatives to conventional NSAIDs for pain management (Gautam *et al*., 2013). Turmeric has been used traditionally for many ailments because of its wide spectrum of Pharmacological activities. Turmeric is an Indian spice derived from the rhizomes of *Curcuma longa* is a perennial member of the Zingiberaceae family and is cultivated in India and other parts of Southeast Asia. Turmeric has been traditionally used in prevention and treatment of several conditions and diseases. The primary active constituent of turmeric is curcumin. Curcumin exerts hepato and nephro-protective, thrombosis suppressing, myocardial infarction-protective properties. Additionally, its strong antioxidant, antimicrobial, anticarcinogenic and anti-inflammatory activities were also reported (Aggarwal and Harikumar, 2009). Based on early research curcumin may have potential as a therapeutic agent in diseases such as inflammatory bowel disease, pancreatitis, arthritis, and chronic anterior uveitis, as well as certain types of cancer. Numerous clinical trials are currently in progress that, over the next few years, will provide an even deeper understanding of the therapeutic potential of curcumin (Jurenka, 2009). As per the reported literature no substantial work has been reported on analgesic properties of curcumin, therefore, in present study we investigated whether curcumin possess anti-nociceptive activity.

MATERIALS AND METHODS

Acute Toxicity Study

The study was carried out according to OECD (Organization of Economic Co-operation and Development) 420 guidelines. The curcumin at different doses up to 2000 mg/kg were administered to the mice and the animal were observed for behavioral changes, toxicity and mortality up to 24 hours. Based on acute toxicity studies three doses were selected and used for evaluation of analgesic activity.

Experimental Animals:

The adult mice were procured from the recognized CPCSEA authorized laboratory animal breeding resource station. All the experimental animals were acclimatized for one week with local environment before the start of experiment under hygienic and standard managemental condition in laboratory animal house of Department of Veterinary Pharmacology and Toxicology, PGIVAS Akola. All animals were given standard pelleted feed and *ad-lib* clean drinking water. The experimental protocol was approved by IAEC, PGIVAS, Akola before start of experiments.

Central Analgesic Activity

Hot Plate Method :

The technique of Eddy and Leimbach (1953) was be used in this study with some modifications. The temperature of hot plate was maintained at $55^{o}\pm1^{o}C$. The time between placement of the animal on the hot plate and the occurrence of discomfort, indicated by either licking of the paws or jumping off the surface, was recorded as response latency. Mice with baseline latencies of more than 10 s were eliminated from the study, and the cut-off time for hot plate latency was set at 15 s. The latency of discomfort was measured at 0, 30, 60, 120, and 180 min after test solution administration. The per cent increase in latency against the control group was calculated. Per cent increase in reaction time = $[(R_t / R_c) -1] \times 10$. Where, R_t – Reaction time in treated group. R_c – Reaction time in control group.

Formalin test

In formalin test, thirty mice were divided randomly and equally into five groups (6 each). At least one hour before performing formalin test, animals of all group receive oral treatment. Each mouse was placed in transparent plastic cage and left 5 min before formalin injection to allow adaptation of the new environment. 20 µl of 0.2% v/v formalin in normal saline was injected into the planter region of the right paw of mice using microsyring. The injection of diluted formalin produced two pain related behavioral components that can be measured in terms of nociceptive response namely flinching (is one pain related behaviors of formalin model characterized by spontaneous, rapid and brief shaking or lifting of the paw) and licking of the injected paw. The nociceptive response was recorded from 0 min to 5 min in 1st phase and later on again recorded 20 to 25 min in

2nd phase (Hunskaar *et al.,* 1985). Amount of aqueous solution of treatment materials were individually adjusted according to body weight of animals and given orally. The time spent in licking a hind paw was evaluated for analgesia which was expressed as per cent (%) inhibition of the time spent in licking. The per cent inhibition was determined for each experimental group as: Per cent inhibition (%) = [1-Licking time (Control) – Licking time (Treated) / Licking time (Control)] × 100

Peripheral Analgesic Activity

Tail Immersion Technique

The tail immersion method used to evaluate peripheral analgesic activity (Aydin *et al.,*1999; Kaushik *et al.,* 2012). Here, the painful thermal stimulus in mice generated by thermal stimulus by dipping the tip of the tail in hot water. The Swiss albino mice were divided equally into five groups. After 30 min, of drug administration each mouse restrained in horizontal cylinders. The lower 5 cm portion of tail was marked and immersed in a beaker of freshly filled water bath maintained at 55±1.0 °C. Within a few second mice reacted by withdrawing a tail. The time taken for the mouse to remove its tail out of the water recorded. The cut of time of immersion was kept 15 sec and measurement was then stopped to avoid injury to mice. The withdrawal time of untreated animals was taken between 1 and 5.5 sec. Latency period was calculated as per the formula given in the hot plate test.

Statistical analysis:

The data was analyzed by one way ANOVA followed by Student's T-test for comparison between test and control using standard statistical method (Snedecor and Cochran, 1967). The t-value at *p < 0.05 and **p < 0.01 were considered for analysis of significance.

OBSERVATIONS AND RESULTS

Table 1: Effect of Curcumin on reaction time in Hot plate test in mice[n]

Treatment	Dose (mg/kg)	0 Min	30 Min	60 Min	120 Min	180 Min
CMC(Control)	**0.5%**	4.04 ± 0.36	4.20 ± 0.27	4.03 ± 0.34	4.20 ± 0.30	4.36 ± 0.26
Curcumin	**50**	4.13 ± 0.26	4.91 ± 0.22	5.13 ± 0.24*	6.57 ± 0.43**	6.03 ± 0.33
	100	4.11 ± 0.11	5.22 ± 0.19*	6.58 ± 0.33**	7.13 ± 0.32**	6.04 ± 0.31*
	200	4.08 ± 0.24	5.88 ± 0.13*	7.46 ± 0.26**	7.68 ± 0.34**	6.68 ± 0.22**
Aspirin	**25**	4.16 ± 0.32	7.50 ± 0.30**	7.66 ± 0.48**	7.28 ± 0.63**	7.50 ± 0.32**
One way Anova df = 4, 25, **F = 0.029 36.011 23.377 12.064 10.898**						

Values expressed as mean ± S. E. (n = 6), *P < 0.05, **P < 0.01; CMC represent Carboxy methyl cellulose.

Table 2: Effect of Curcumin on formalin induced paw licking in mice

Treatment	Dose	Early phase (0-5 min)	% inhibition	Late phase (20-25 min)	% inhibition
CMC(Control)	**0.5% w/v**	60.21 ± 1.85	-	33.11 ± 4.89	--
Curcumin (mg/kg)	**50**	58.11 ± 2.29	3.47	27.84 ± 3.82	15.91
	100	50.06 ± 2.36*	16.85	9.05 ± 0.37**	72.67
	200	44.29 ± 1.75**	26.44	8.53 ± 0.79**	74.24
Aspirin (mg/kg)	**25**	41.30 ± 1.99**	31.39	15.31 ± 1.65**	53.77
One way Anova:	**Df=**4, 25	**F =**	**19.142**		**14.862**

Values expressed as mean ± S. E. (n = 6), *P < 0.05, **P < 0.01; CMC represent Carboxy methyl cellulose.

Table 3: Effect of curcumin and aspirin on latency period on thermal noxious stimuli in tail immersion test in mice

Treatment	Dose (mg/kg)	Before Treatment	After treatment				
		0	30	60	90	120	180
Control (CMC)	**0.5% w/v**	2.41 ± 0.10	2.61 ± 0.30	2.52 ± 0.20	2.58 ± 0.19	2.37 ± 0.11	2.59 ± 0.13
Curcumin	**50**	2.43 ± 0.14 (0.83)	3.19 ± 0.16* (22.3)	3.30 ± 0.26 (30.9)	3.78 ± 0.25** (46.5)	2.99 ± 0.27 (26.2)	2.97 ± 0.22 (14.6)
	100	2.50 ± 0.24 (3.74)	3.19 ± 0.24* (22.3)	3.53 ± 0.22* (40.1)	4.21 ± 0.18** (63.1)	4.55 ± 0.25** (91.9)	3.54 ± 0.22** (36.7)
	200	2.63 ± 0.18 (9.13)	3.25 ± 0.16* (24.5)	4.20 ± 0.24** (66.7)	4.89 ± 0.12** (89.5)	5.24 ± 0.14** (121.1)	3.58 ± 0.22** (38.2)
Aspirin	**25**	2.46 ± 0.16 (2.07)	4.26 ± 0.28** (63.2)	4.33 ± 0. 37** (71.8)	5.07 ± 0.19** (96.5)	5.95 ± 0.25** (151.1)	5.86 ± 0.30** (126.3)
One way Anova,	**Df=**4, 25	**F = 0.273**	**6.274**	**7.835**	**27.042**	**47.908**	**33.040**

Values are expressed as Mean ± SEM. Student's *t*-test (*N* = 3).) *P< 0.05;**P< 0.01 Vs vehicle Controls
Figures in parenthesis indicate per cent increase in reaction time.

Hot Plate Method:

Curcumin 50 mg/kg showed significant (P<0.05) increase in reaction time at 60 min and significant (P<0.01) increase also noted at 120 min of drug administration. Curcumin at 100 mg/kg showed significant (P<0.05) increased in thermal latency period at 30 and 180 min however, more significant (P<0.01) increase in reaction time is observed at 60 and 120 min when compared to control group. Curcumin at 200 mg/kg showed maximum significant increase (P<0.01) in reaction time at 120 min. Thus, curcumin showed dose dependant analgesic activity upto 120 min and thereafter, the activity was decreased. The analgesic effect shown by curcumin at 200 mg/kg found to be comparable to reference drug aspirin at 120 and 180 min of drug administration (Table 1).

Formalin Induced Paw Licking in Mice

Curcumin caused dose-dependent reduction in licking time at early as well as at late phase. Curcumin at 100 and 200 mg/ kg in early phase showed significant reduction in the paw licking time whereas in late phase significant reduction (P< 0.01) was observed. The reduction in licking time shown by curcumin at 100 and 200 mg/kg was much lesser than the standard drug aspirin in early phase. Curcumin at 200 mg/ kg exhibited high reduction in paw licking time in late phase than the standard drug aspirin indicating better antinociceptive effect than aspirin during late phase (Table 2).

Tail immersion method

Curcumin at 50 mg/kg showed significant (p<0.01) analgesic activity at 90min compared to control animals. Curcumin exhibited potent and significant analgesic activity at 100, and 200 mg/kg from 1hr onwards upto experimental period of 3hrs. The duration as well as the intensity of analgesia induced by curcumin was dose dependent. The analgesic effect of curcumin at 200 mg/kg dose level was highest at 120 minutes and thereafter the activity found to be decreased. The effect of curcumin against thermal noxious stimuli was found comparable to that of standard drug aspirin at 60 and at 90 min after treatment. Overall effect of curcumin was found lesser compare to standard drug aspirin (Table 3).

DISCUSSION

Hot plate test is used for evaluation of central pain at the supraspinal level (Wong, 1994). Tail immersion method and Hot plate method in mice were employed to assess the central

mechanism of compound in producing analgesia. These two methods were differing from each other in their tendency to respond to nociceptive stimuli conducted through the neuronal pathway. Hot plate method involves higher brain functions and is considered supraspinally organized response whereas tail immersion method mediates spinal reflex to painful stimuli. μ, k_3 and d_2 are the opioid receptor sub-type primarily responsible for supraspinally mediated analgesic action i.e., Hot plate method whereas spinal analgesia appears to be mediated through μ_2, k_1 and d_2 receptor (Vani *et al.*,2012). Curcumin showed dose dependent analgesic effect upto 2 hrs. Curcumin at 200 mg/kg showed lesser analgesic effect than standard drug aspirin upto 1 hr but thereafter curcumin showed higher analgesic activity upto 3 hrs. These results indicate that analgesic effect of curcumin may be due to central mechanisms which might occur through μ opioid receptors. However, initial significant response at 30 and 60 min may be occurred due to peripheral mechanism through inhibition of prostaglandin synthesis. The direct stimulation of nerve ending caused substance P release with co-operation with bradykinnin, while in the late phase (Tail immersion) inflammatory pain is mediated by the peripheral effect via the release of transmitters such as histamine, serotonin, bradykinin and prostaglandins, which to some degree can cause the sensitization of central nociceptive neuron (Verma *et al.*, 2007). The curcumin showed an inhibitory effect in both early and late phase, so it was indicated that like in hot plate test results curcumin had central and peripheral analgesic properties. Thus, it might be conclude that analgesic effect of curcumin on the early phase of the curcumin was due to the direct effect on the nociceptor via blockade of the nociceptor or the inhibition of releasing the substance P or bradykinnin.

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