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Anti-Nociceptive Effect of the Fruit Essential Oil of *Cuminum cyminum L.* in Rat

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ABSTRACT

***Cuminum cyminum* Linn. (Umbelliferae) is a plant, which has been used as a toothache remedy in folk medicine of Iran. In this study, the potential anti-nociceptive and anti-inflammatory activities of the fruit essential oil of *C. cyminum* has been evaluated in chemical (formalin test) and thermal (tail-flick test) models of nociception and formalin model of acute inflammation in rats and mice. The essential oil at the doses ranging between 0.0125 and 0.20 ml/kg exhibited a significant and dose-dependent analgesic effect in the model of chronic and inflammatory pain. However, the essential oil was devoid of anti-inflammatory activity. Moreover, the essential oil had no analgesic effect in tail flick test as a model of acute pain. The LD_{50} value of 0.59 ml/kg was obtained for the essential oil. This low toxicity of the essential oil makes it worthy for further studies.** *Iran. Biomed. J. 6 (4): 141-145, 2002*

Keywords: Anti-nociceptive effect, Essential oil, *Cuminum cyminum* Linn.

INTRODUCTION

Cuminum cyminum Linn., Umbelliferae, is a wild grassy plant with 15-50 cm height, growing in many parts of Iran [1]. In Iranian folk medicine, the fruits of this plant have been used to treat diarrhea, toothache and epilepsy [1]. In recent years, there are some reports regarding the anti-diabetic and estrogenic activities of this plant [2, 3]. We recently reported the anticonvulsant activity of the fruit essential oil of *C. cyminum* [4], which is in agreement with the traditional belief on the anticonvulsant effect of the plant. On the other hand, there are some similarities between the pathophysiological phenomena observed in some epilepsy models and in neuropathic pain models [5]. The role of anticonvulsant drugs in the treatment of neuropathic pain is evolving and has been clearly demonstrated with gabapentin and carbamazepine [5]. Therefore, it is possible that an essential oil of *C. cyminum* with anticonvulsant activity may have analgesic effect. Traditional application of *C. cyminum,* as a toothache remedy, supports this hypothesis. Furthermore, it has been reported that the ether extract of *C. cyminum* inhibits eicosanoid biosynthesis [6], which is a pain mediator.

Chemical studies have demonstrated the presence of cuminaldehyde (18.7%), alpha-pinene (1.2%), beta-pinene (19.9%), para-cymene (25.2%), gama-terpinene (29.1%), perrialdehyde (2.4%) and myrcene (1.5%) as the major compounds of the fruit essential oil of *C. cyminum* [7]. Some of the constituents of the essential oil such as alpha-pinene and beta-pinene have been reported to possess anti-inflammatory activity [8]. Moreover, myrcene has peripheral analgesic effect acting by the stimulation of nitric oxide pathway [9, 10].

Here, we have examined the putative anti-nociceptive and anti-inflammatory effects of the fruit essential oil of *C. cyminum* in formalin and tail-flick models of nociception and in formalin-induced edema.

MATERIALS AND METHODS

Plant material and isolation of the essential oil. Fruits of *C. cyminum* were obtained from a local market. The plant was authenticated and voucher specimen (C-1456) was deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran. The fruits were subjected to hydrodistillation for 4 h using a Clevenger apparatus and produced 3% (v/w) yield.

Drugs. Piroxicam and morphine were purchased from Sigma (Poole, UK). Tween 80 and

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formaldehyde were from Merck (Darmstadt, Germany). All drugs were dissolved in saline. The essential oil was diluted with 5% v/v solution of Tween 80 in distilled water. All intraperitoneal (i.p.) injections were administered in volumes not higher than 10 ml/kg of body weight of animals.

Animals. Male NMRI mice weighing 20-28 g and male Wistar rats weighing 220-260 g (the Pasteur Institute of Iran) were used throughout this study. They were fed ad libitum with rodent's chow and had free access to drinking water. The animals were kept in a room with controlled 12 h light /dark cycle (6:00-18:00) and temperature (22 ± 3°C). All experiments were performed between 9.00 and 18.00.

Tail-flick test. Tail-flick to radiant heat (Tail-flick apparatus type 812, Hugo Sachs Elektronik, Germany) was used to measure acute nociceptive responses in mice [11]. The intensity of the thermal stimulus was adjusted to produce 3 to 4 s latency in tail-flick response. The latency was measured just before, 30, 60 and 120 min after injections. The trial was automatically terminated at 10 s if a response did not occur (cut off time). Five groups of mice were pretreated i.p. with saline (10 ml/kg, control), Tween preparation (10 ml/kg, control), morphine (10 ml/kg, positive control) and the essential oil (0.1 and 0.2 ml/kg).

Formalin test. The test was performed in accordance with the method of Dubuisson and Dennis [12]. The essential oil, piroxicam (5 mg/kg as a positive control) and vehicles were injected i.p. to rats. Fifty microliters of formaldehyde 2.5% (v/v in distilled water) was injected subcutaneously into the plantar surface of the left hind paw of the animals at 30, 60 and 180 min after pretreatment. Then, behavioral responses to nociception including biting, licking and scratching of the injected paw were noted and time was recorded till 1 h. The first 5 min was considered as the early phase and the period of 15 to 60 min as the late phase of the nociceptive response.

Anti-inflammatory activity. Formaldehyde 2.5% was used as inflammagen and 50 µl was injected into the subplantar region of the left hind paw of the rats. The rats were divided into five groups. Thirty min before the injection of formalin, the groups were treated i.p. as follows: group I: piroxicam (5 mg/kg, as positive control); groups II and III: the essential oil (0.1 and 0.2 ml/kg); group IV: saline (1 ml/kg, as control) and group V: Tween preparation (1 ml/kg, as control). Paw volume (ml) was measured at 0, 1, 3 and 5 h after formalin injection using volume-differential meter (model S-79, Electronic Industry Development, Iran).

Lethality assessment. Doses of 0.75, 1.0, 1.25, 1.5 and 2 ml/kg of the essential oil were administrated i.p. to mice and the incidence of mortality was noted up to 24 h after injection.

Data analysis. The one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple-comparisons test was used to analyze data obtained from tail-flick, formalin test and anti-inflammatory activity. $P<0.05$ was the critical criterion for statistical significance. The dose of the essential oil needed to produce lethal effect in 50% of the animals (LD_{50}) and its associated 95% confidence limits was calculated by the method of Litchfield and Wilcoxon [13] using a commercial computer program (PHARM/PCS version 4.2).

RESULTS

Tail-flick test. There was no effect on tail-flick response after pretreatment with the essential oil up to 0.2 ml/kg. However, this response was significantly altered by morphine (10 ml/kg) pretreatment (latency time from 3.07 ± 0.14 s in control group reached to 9.15 ± 0.56 s in morphine pretreated group, $P<00.1$). The essential oil in higher doses produced sedation, therefore evaluation of the analgesic activity in doses higher than 0.2 ml/kg was of no value.

Formalin test. The results presented in Figure 1 show that the essential oil at doses ranging between 0.0125 and 0.2 ml/kg, significantly reduced formalin-induced nociception for 1 h. This effect was more pronounced in the late phase. Piroxicam (5 mg/kg) also exerted a significant analgesic effect at least for 3 h (Fig. 2).

Anti-inflammatory activity. In control animals, subplantar injection of formaldehyde 2.5%, produced a local edema reaching its maximum at least 5 h after injection (Table 1). Piroxicam significantly inhibited the progressive increase in paw edema. However, the essential oil had no effect on inflammation.

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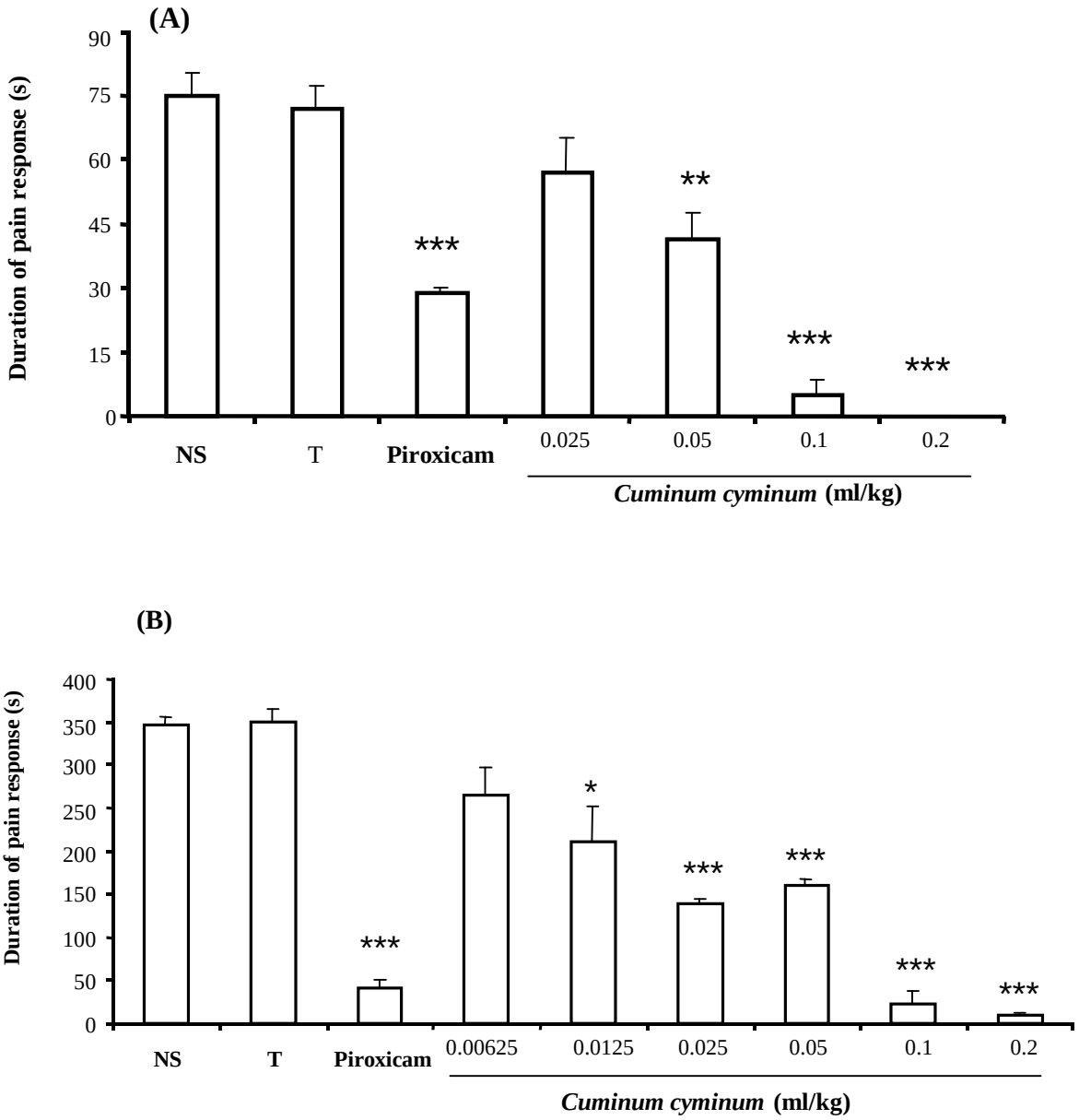

Fig. 1. Effect of the essential oil of *Cuminum cyminum* on the early (A) and the late (B) phases of formalin test. Histograms are mean ± S.E.M. for 6 animals. **p*< 0.05; ***p*< 0.01 and ****p*< 0.001 compared to control value. All injections were i.p. Piroxicam was administered by the dose of 5 mg/kg. NS, Normal saline; T, Tween 80, 5% v/v.

Table 1. Effect of the essential oil of *Cuminum cyminum* on paw edema induced by formalin.

Treatment	Dose	Paw edema (ml)		
		1 h	3 h	5 h
Saline	10 ml/kg	0.28 ± 0.03	0.32 ± 0.03	0.34 ± 0.04
Piroxicam	5 mg/kg	0.07 ± 0.01***	0.15 ± 0.03**	0.21 ± 0.03*
Tween 80, 5%	10 ml/kg	0.18 ± 0.03	0.24 ± 0.02	0.28 ± 0.02
C. cyminum	0.1 ml/kg	0.13 ± 0.01	0.12 ± 0.01	0.15 ± 0.02
C. cyminum	0.2 ml/kg	0.25 ± 0.03	0.24 ± 0.03	0.20 ± 0.04

Data represent mean ± S.E.M. for 7 rats. **p*<0.05; ***p*<0.01 and ****p*<0.001 compared to control group.

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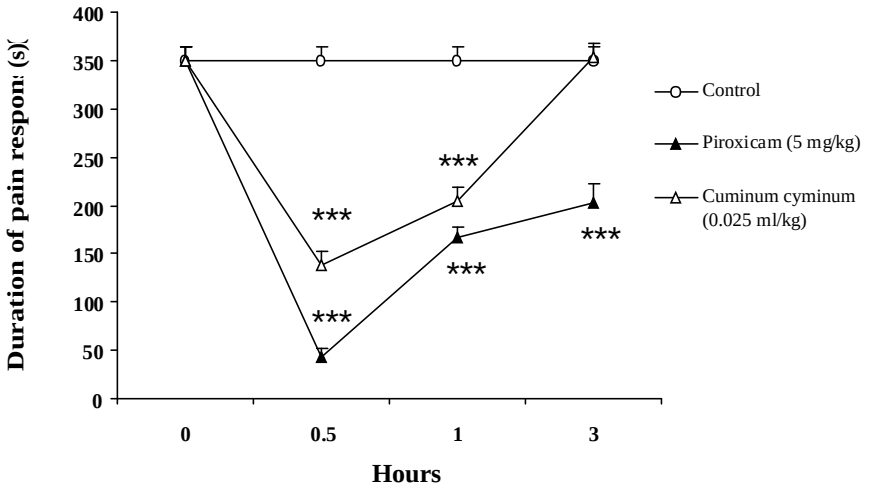

Fig. 2. Time course of analgesic activity of the essential oil of *Cuminum cyminum* in the late phase of formalin test. All injections were i.p. Each point represents mean ± S.E.M. for 7 animals. ****p*< 0.001 compared to control group.

Lethality. The LD_{50} value of 0.59 (0.52-0.68) ml/kg was obtained for the essential oil.

DISCUSSION

We have evaluated the analgesic and anti-inflammatory activities of the fruit essential oil of *Cuminum cyminum* to clarify the traditional belief in the painkiller effects of this plant in Iranian folk medicine. The essential oil demonstrated significant analgesic effect in formalin test. However, it had neither analgesic activity in tail-flick test, nor anti-inflammatory effect against formalin-induced edema.

Thermal painful stimuli are known to be selective to centrally-, but not peripherally-acting analgesic drugs [14]. In the present study, morphine, a centrally-acting analgesic drug, produced an inhibitory effect on the nociceptive response in tail-flick test, while the essential oil of *C. cyminum* failed to affect the response. Therefore, it seems that the apparent anti-nociceptive action of *C. cyminum* in formalin test is not mediated through central mechanism (s).

Our results show that the essential oil exerts significant inhibitory effect on nociceptive response of the late phase of the chemical pain model, formalin test. It has been stated that part of the manifestation of the second phase of formalin test results from an inflammatory reaction in the peripheral tissue [15]. Therefore, a substance that affects the pain in the late phase of formalin test is expected to attenuate the associated inflammation as well. However, the essential oil did not decrease the localized inflammation of the rat paw caused by formalin. This paradoxical behavior of the essential oil has also been observed for other typical analgesics such as indomethacin. Indomethacin has been shown to reduce the response in the second phase of the formalin test, but it is not particularly effective in inhibiting the formalin-induced edema [15]. It seems that to elicit the nociceptive behavior in the second phase of formalin test, inflammation alone is not involved [15]. Thus, the present results suggest that the analgesic action of the essential oil in the formalin test is not exerted through inhibition of the inflammatory processes.

Experimental results have indicated that substance P participates in the early phase, while histamine, serotonin, excitatory amino acids and prostaglandins are involved in the late phase of formalin test with bradykinin affecting both phases [16-19]. So, it seems that there is possible interaction of the active constituent (s) of the essential oil with mediators involved in the peripheral pain. In support of this hypothesis, Srivastava [6] has shown that the ether extract of *C. cyminum* inhibits eicosanoid synthesis with a simultaneous increase in the formation of lipoxygenase-derived products. Moreover, it has been reported that one of the constituents of the essential oil of *C. cyminum*, myrcene, possesses peripheral analgesic effect on the hyperalgesia

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induced by prostaglandin in the rat paw test [9]. Thus, it can contribute to the analgesic effect of the essential oil.

Preliminary acute toxicity assessment of the *C. cyminum* essential oil performed in this study shows that the LD_{50} value obtained for the essential oil (0.59 ml/kg) is greater (more than 47 times) than the minimal analgesic dose (0.0125 ml/kg). This makes the essential oil worthy of further studies.

In conclusion, present results indicate that the essential oil of *C. cyminum* has significant analgesic action when assessed in chemical (but not thermal) model of nociception in rats. The essential oil did not inhibit the local inflammation induced by formalin. However, further studies on the effect of the essential oil in weak inflammatory models such as cotton pellet granuloma and acetic acid-induced vascular permeability are needed before any precise conclusions can be drawn.

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