

Anti-nociceptive effect of safranal, a constituent of *Crocus sativus* (saffron), in mice

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ABSTRACT

Safranal is one of the main constituents of saffron. In view of previous reports of anti-nociceptive activity of saffron, we examined the anti-nociceptive property of safranal. Antinociceptive activity was determined using hot-plate, writhing and formalin tests in mice. Safranal at doses 0.1, 0.3 and 0.5 ml/kg/ip inhibited the abdominal constrictions induced by acetic acid and also at 0.5 ml/kg/ip increased the pain threshold of mice against the thermal source only at 30 min after treatment. In formalin test, safranal at doses 0.05 ml/kg/ip significantly decreased pain-related behaviors in phase I and with lower dose (0.05 and 0.025 ml/kg/ip) phase II. Generally, naloxone (2 mg/kg, subcutaneously) did not abolish the anti-nociceptive effects of safranal completely. Our results showed that safranal have anti-nociceptive activity in chemical (formalin and acid acetic tests) methods and this effect may be mediated more peripherally.

Key words: Safranal, Saffron, Anti-nociceptive, Writhing test, Hot plate test, Formalin test

INTRODUCTION

Natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy. Taking into account that the most important analgesic prototypes (e.g. salicylic acid and morphine) were originally derived from the plant sources, the study of plant species traditionally used as pain killers should still be seen as a fruitful research strategy in the search of new analgesic and anti-inflammatory drugs [1]. *Crocus sativus* L. (Iridaceae), commonly known as saffron is used in folk medicine for various purposes such as aphrodisiac, antispasmodic and expectorant [2]. Modern pharmacological studies have demonstrated that saffron extracts have antitumour [3–4], anticonvulsant effects [5] and improve activity on learning and memory [6-7]. Chemical studies on *C. sativus* have shown the presence of constituents such as crocin, crocetin, safranal and picrocrocin [8–9].

As saffron stigma and petal aqueous and ethanolic maceration extracts have

antinociceptive effect in chemical pain test and have acute and/or chronic anti-inflammatory activity [10], we evaluated the anti-nociceptive activity of safranal, one constituent of saffron stigma, in mice.

MATERIALS AND METHODS

Animals

Male albino mice 20-25 g was obtained from a random bred colony in the animal house of Mashhad University of Medical Sciences. Animals were housed in colony room 12/12 hr light/dark cycle at $21 \pm 2^\circ\text{C}$ and had free access to water and food. All animal experiments were carried out in accordance with Mashhad University of Medical Sciences, Ethical Committee Acts.

Materials

The following reagents were used: morphine (Daru Pakhsh, I.R. Iran), naloxone hydrochloride (Tolid Daru, I.R. Iran), safranal (Fluka), acetic acid, formalin and paraffin (Merck).

Anti-nociceptive activity

Two models, acetic acid induced writhing response (chemical method); hot plate reaction time assay (thermal methods) using albino mice were employed to study the anti-nociceptive effect [11]. The animals were divided into twelve groups that containing eight animals. Group I and II served as controls and received normal saline or paraffin (10 ml/kg ip), respectively, group III served as a reference group and received morphine (5 mg/kg/ip), other groups served as treatment groups received different doses of safranal alone and accompanying with naloxone.

Chemical method

Writhing test

Groups of 8 mice were used for controls and test mice. Half hour after the administration of drug the mice were given an interaperitoneal injection of 0.7% v/v acetic acid solution (volume of injection 10ml/kg). The mice were placed individually into glass beakers and five min were allowed to elapse. The number of writhes produced in these animals was counted for 30 min. For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb.

Negative controls received normal saline and paraffin (10 ml/kg, i.p.). Morphine (5 mg/kg, i.p.) was used as reference drug. In groups that received naloxone (2 mg/kg, s.c.), it was administered 10 min after of the safranal or morphine injections [11].

Thermal method

Hot-plate test

The hot-plate test was assessed on groups of 8 mice. The temperature of a metal surface was maintained at $55 \pm 0.2^\circ\text{C}$. Latency to a discomfort reaction (licking paws or jumping) was determined before and after drug administration. The cut-off time was 20 s. The latency was recorded before and 30, 60, 120, 150 and 180 min following interaperitoneal administration of the agents. The prolongation of the latency times compared with the values of the control was used for statistical

comparison. Negative controls received normal saline and paraffin (10 ml/kg, i.p.). Morphine (5 mg/kg, i.p.) was used as reference drug [11].

Anti-nociceptive and anti-inflammatory method

Formalin test

The formalin test in mice is a valid and reliable model of nociception and is sensitive for various classes of analgesic drugs [12]. Two distinct periods of high licking activity can be identified, an early phase lasting the first 5 min and a late phase 15-30 min after the injection of formalin. Half hours before testing, mice were given an interaperitoneal injection of drugs and were individually placed in transparent observation chambers (32 cm high, 24 cm diameter) for adaptation. Then, each of the animals were taken out of the chamber, and 50 μl of 2.5% formalin solution was injected into the dorsal surface of the right hind paw with a syringe with a fine needle. Immediately after formalin injection, each mouse was returned to the observation chamber, and number of displaying pain-related behaviors was counted for 5-min block for 30 min after formalin injection. The nociceptive behaviors consisted number of licking, of the injected paw. The cumulative responses countered during 0–5 min and during 15-30 min were regarded as the first-phase (I phase) and second-phase (II phase) responses, respectively. In groups that received naloxone (2 mg/kg, S.C.), it was administered 10 min after of the safranal or morphine injections [12].

Statistical analysis

The data were expressed as mean values \pm S.E.M. and tested with analysis of variance followed by the multiple comparison test of Tukey-Kramer.

RESULTS

Safranal at doses 0.1, 0.3, 0.5 ml/kg and morphine (5 mg/kg) significantly ($P < 0.001$) reduced the number of abdominal constrictions and stretching of hind limbs induced by the

injection of acetic acid in a dose-dependent manner (Figures 1-2). Naloxone, (2 mg/kg, s.c.)

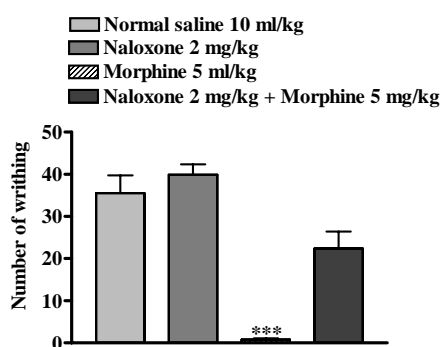


Figure 1- Effect of a subcutaneous injection of naloxone on the antinociceptive effect of intraperitoneally administered, morphine on acetic acid-induced writhing test in mice. Values are the mean ± S.E.M. of writhes number for 8 mice, ***P<0.001, compared to control (normal saline); Tukey-Kramer test.

pretreatment after i.p. injection of safranal did not inhibit the antinociceptive activity of safranal (Figures 1).

Morphine significantly showed antinociceptive activity in hot-plate test (Figure 3). Safranal with low and high dose (0.5ml/kg) practically did not decrease the reaction time of animals against the thermal source, except in 30

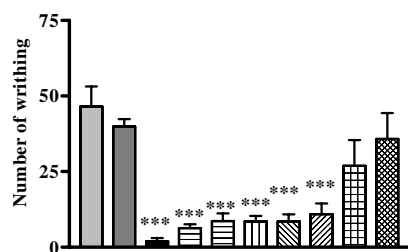
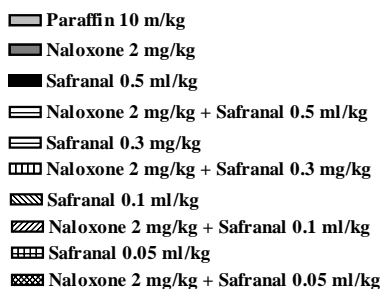


Figure 2- Effect of a subcutaneous injection of naloxone on the antinociceptive effect of intraperitoneally administered safranal on acetic acid-induced writhing test in mice. Values are the mean ± S.E.M. of writhes number for 8 mice, ***P<0.001, compared to control (paraffin); Tukey-Kramer test.

min after treatment (Figures 4 and 5). Naloxone partially decreased this effect of safranal at a high dose. In formalin test morphine demonstrated antinociceptive activity in both phase I and phase II (Figures 6 and 8). Our data indicated that safranal 0.05 ml/kg in early phase and late phase have analgesic effects and naloxone did not inhibit its antinociceptive activity (Figures 7 and 9). A lower dose (0.025 ml/kg) of safranal significantly decreased pain-related behaviors in only phase II (Figure 9). Naloxone inhibited the antinociceptive effect of this dose of safranal in the phase II (Figures 9).

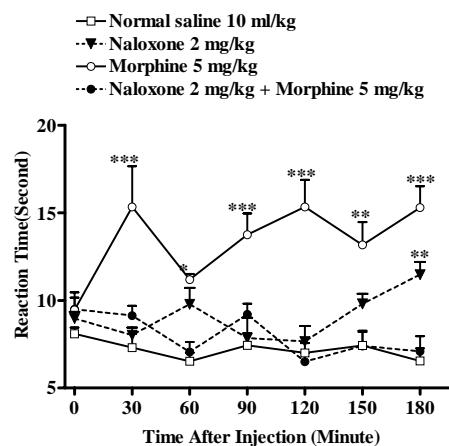


Figure 3- Effect of a subcutaneous injection of naloxone on the antinociceptive effect of intraperitoneally administered of the morphine (i.p.) on pain threshold of mice in the hot-plate test. Each point represents the mean ± S.E.M. of reaction time for n = 8 experiments on mice.*P<0.05, **P<0.01, ***P<0.001, compared to control (normal saline), Tukey-Kramer test.

DISCUSSION

The anti-nociceptive activity of safranal was evaluated using both chemical and thermal methods of nociception in mice.

Antinociceptive activity of opioid agonist, opioid partial agonist, non-steroidal anti-inflammatory drugs (NSAIDs) can be determined by the writhing test [15]. Safranal reduced the number of abdominal constrictions induced by the injection of acetic acid in a dose-dependent manner and naloxone did not inhibit the antinociceptive activity of safranal. It is therefore possible that safranal analgesic

effect probably mediated by inhibiting the

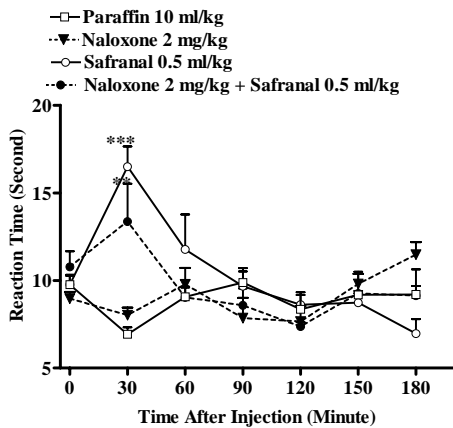


Figure 4- Effect of a subcutaneous injection of naloxone on the antinociceptive effect of intraperitoneally administered of safranal 0.5 ml/kg on pain threshold of mice in the hot-plate test. Each point represents the mean \pm S.E.M. of reaction time for n = 8 experiments on mice. **P<0.01, ***P<0.001, compared to control (paraffin), Tukey-Kramer test.

synthesis or action of prostaglandins and this effect is not mediated via opioid receptors.

Opioid agents exert their analgesic effects via supraspinal (μ_1 , κ_3 , δ_1) and spinal (μ_2 , κ_1 , δ_2) receptors (13). The hot plate test is a specific test for central antinociceptives (14). Safranal practically did not decrease the reaction time of animals against the thermal

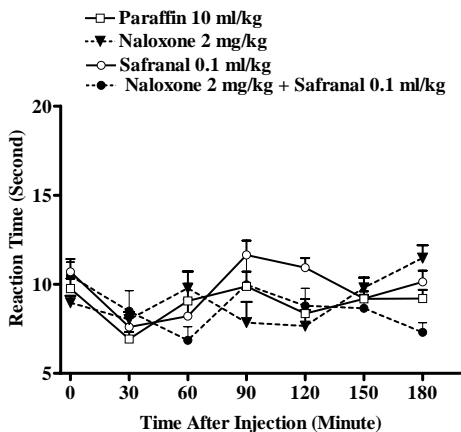


Figure 5- Effect of a subcutaneous injection of naloxone on the antinociceptive effect of intraperitoneally administered of the safranal 0.1 ml/kg on pain threshold of mice in the hot-plate test. Each point represents the mean \pm S.E.M. of reaction time for n = 8 experiments on mice, There is no different compared to control (Paraffin), Tukey-Kramer test.

source, except in 30 min after treatment 0.5 ml/kg of safranal (Figures 4 and 5). Naloxone partially decreased this effect of safranal at a high dose. Overall, it seems safranal did not act its antinociceptive activity via central action.

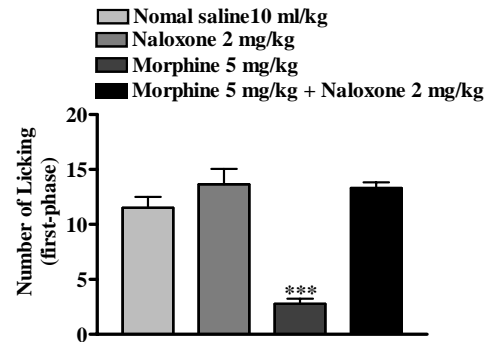


Figure 6- Effect of a subcutaneous injection of naloxone on the antinociceptive effect of morphine (i.p.) in phase I of the formalin test. Values are the mean \pm S.E.M. number of licking 5 min immediately after injection formalin for 8 mice, ***P<0.001, compared to control (normal saline); Tukey-Kramer test.

The formalin test is sensitive to opioid agents, non-steroidal anti-inflammatory drugs and other mild analgesics. The test possesses two distinct phases, possibly reflecting different types of pain.

The earlier phase reflects a direct effect of

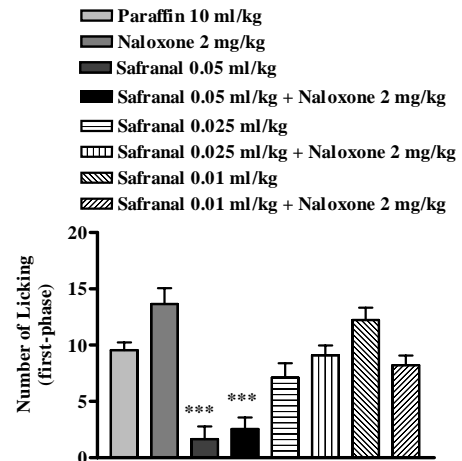


Figure 7- Effect of a subcutaneous injection of naloxone on the antinociceptive effect of safranal (i.p.) in phase I of the formalin test. Values are the mean \pm S.E.M. number of licking in 5 min immediately after injection formalin for 8 mice, ***P<0.001, compared to control (paraffin); Tukey-Kramer test.

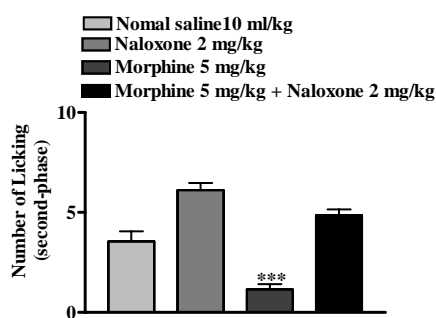


Figure 8- Effect of subcutaneous injection of naloxone on the antinociceptive effect of morphine (i.p.) in phase II of the formalin test. Values are the mean \pm S.E.M. number of licking in 15-30 min after injection formalin for 8 mice, *** P <0.001, compared to control (normal saline); Tukey-Kramer test.

formalin on nociceptors (non-inflammatory pain), where as the late phase reflects inflammatory pain [12]. Our data indicated that safranal with higher dose showed antinociceptive activity in both phase of formalin test. and naloxone only inhibited

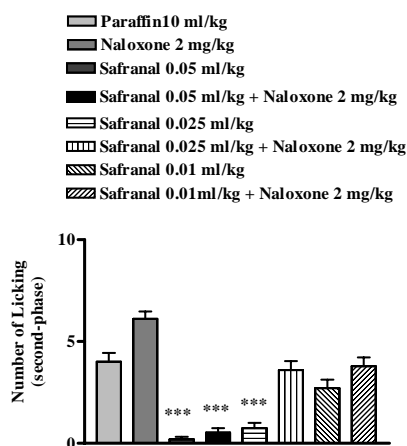


Figure 9- Effect of subcutaneous injection of naloxone on the antinociceptive effect of safranal (i.p.) in phase II of the formalin test. Values are the mean \pm S.E.M. number of licking in 15-30 min after injection formalin for 8 mice, *** P <0.001, compared to control (paraffin); Tukey-Kramer test.

lower dose of safranal (0.025 ml/kg). Naloxone increased licking numbers of mice which received safranal (0.025 ml/kg) (Figures 9) but this effect was almost equal to stimulatory effect of naloxone on this behavior in control naloxone group. As safranal was more

effective on phase II than phase I, it seems this saffron component acts more on inflammatory and peripheral phase.

Our results showed that safranal have anti-nociceptive activity in chemical (formalin and acid acetic tests) methods and this effect may be medicated more peripherally.

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