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Antinociceptive properties of the essential oil of *Ocimum gratissimum* L. (Labiatae) in mice

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Abstract

We have investigated the antinociceptive effects of the essential oil of *Ocimum gratissimum* L. (Labiatae) (EOOG) in two classical models of pain in male Swiss mice (25-35 g), the writhing test and the formalin test. At doses of 30, 100 and 300 mg/kg (*po*), EOOG produced a dose-dependent inhibition (from 58.3 ± 4.4 to 40.7 ± 6.3, 36.4 ± 3.6 and 24.6 ± 3.6, respectively; N = 8-10, P<0.05) of acetic acid-induced writhing, causing up to a ~60% inhibition at the highest dose used, comparable to that obtained with indomethacin (10 mg/kg, *po*). At the same doses, EOOG predominantly inhibited the late (inflammatory) phase of the formalin-induced pain response (from 59.3 ± 8.3 to 40.4 ± 4.8, 23.2 ± 2.8 and 25.3 ± 5.5, respectively; N = 6, P<0.05), with a maximal reduction of ~60% of the control, although a significant reduction of the initial (neurogenic) phase was also observed at 300 mg/kg (from 62.5 ± 6.07 to 37 ± 5.9; P<0.05). On the basis of these data, we conclude that EOOG possesses interesting antinociceptive properties in the writhing and formalin tests. Due to the relatively low toxicity of EOOG, further detailed examination is strongly indicated for a better characterization of its pharmacological properties and its potential therapeutic value.

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In the Northeast of Brazil the plant *Ocimum gratissimum* L. (Labiatae), commonly known as "Alfavaca-cravo", is widely used for medicinal as well as culinary purposes. One of the many uses of the plant is for digestive problems and we have recently shown that the essential oil of *O. gratissimum* (EOOG) has relaxant effects on the isolated guinea-pig ileum (1). However, many other common uses of *O. gratissimum* exist. For example, this plant is used as an antiseptic (2) and for the treatment of cough, fever and conjunctivitis (3). Generally the flowers and leaves of the plant, which are a rich source of essential oils, are utilized to prepare infusions or teas for the treatment of common ailments (2). Despite a multiplicity of applications in popular folk medicine, there has been surprisingly little pharmacological investigation of the effects of this plant. Interestingly, however, a preliminary report has indicated that an aqueous extract of the Nigerian variety of the plant has nociceptive properties (4).

Plants are known to be a rich source of naturally occurring antinociceptive substances (5), and in the present study we decided to evaluate the effects of EOOG in two classical pharmacological tests for nociception: the acetic acid-induced writhing test (6) and the formalin test (7,8).

EOOG was obtained from the Horto de Plantas Medicinais of the Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, Fortaleza, CE, Brazil. The plant is registered in the Herbário Prisco Bezerra of the Universidade Federal do Ceará (EAC 14968).

The essential oil was isolated from the leaves of the plant by steam distillation (2). The composition of EOOG, determined by gas chromatography and mass spectrometry, was: 52.14% eugenol, 29.17% 1,8-cineole, 5.56% ß-selinene, 3.35% trans-caryophyllene, 2.73% ocimene, 1.58% α-selinene, 1.51% ß-pinene, and 3.96% not identified.

In the writhing test, male Swiss mice, 25-35 g, were kept in a temperature-controlled environment (23 ± 2ºC) with a 12-h light-dark cycle. Food and water were freely available. The abdominal constriction resulting from intraperitoneal injection of acetic acid (0.8%), consisting of a contraction of the abdominal muscle together with a stretching of hind limbs, was recorded according to standard procedures (6). Animals were pretreated (*po*) with EOOG (10-300 mg/kg) or indomethacin (10 mg/kg) 60 min prior to acetic acid injection. Control animals received only a similar volume of the solvent (0.1% Tween 80) used to dissolve EOOG. All experiments were carried out at 20-22ºC. After administration of acetic acid, pairs of mice were placed in separate cages and the number of abdominal constrictions was cumulatively counted over a period of 20 min. Antinociceptive activity was expressed as the reduction of the number of abdominal constrictions observed in test mice pretreated with EOOG compared to control animals pretreated with Tween. In separate experiments, indomethacin (10 mg/kg, *po*) was employed as a positive control for comparison.

Male Swiss mice, 25-35 g, were used in the formalin test according to a procedure described previously (7,8). Animals were pretreated with EOOG (10-300 mg/kg, *po*) 60 min prior to the injection of 1.0% aqueous formalin (20 µl) administered by the intraplantar route into the right hindpaw. The amount of time that the animal spent licking the injected paw during the first 5 min (initial phase, neurogenic) and 15-30 min after formalin injection (late phase, inflammatory) was measured. The test was carried out at ambient temperature (20-22ºC) with special care taken to avoid environmental disturbances that might influence the animal's response. Control animals received only the vehicle used to dilute EOOG (0.9% NaCl).

Data are reported as mean ± SEM, with N indicating the number of animals used. The results were analyzed statistically by the Student *t*-test, with the level of significance set at $P<0.05$.

Oral administration of EOOG (30-300 mg/kg) induced a dose-dependent inhibition of the number of writhes elicited by acetic acid, with a reduction of approximately 60% of the control response at the highest dose employed (N = 10, Table 1). The dose of 10 mg/kg also reduced the mean response, but not significantly (N = 10). In separate experiments, indomethacin (10 mg/kg) inhibited the acetic acid-induced response by approximately 54% of the control (N = 10; Table 1).

In the formalin test (Table 2), EOOG (30-300 mg/kg) elicited a dose-dependent inhibition of licking time during the second phase, with a maximal reduction of approximately 60% of the control response (N = 6). In addition, a significant reduction of licking time was observed during the first phase at the highest dose of 300 mg/kg (Table 2).

The present results show for the first time

that the EOOG has a significant antinociceptive activity in traditional pharmacological models. It has been previously shown that an aqueous extract of the plant also produces this effect (4) and the present data suggest that *O. gratissimum* L. possesses several inherent antinociceptive principles. EOOG had inhibitory effects in both the acetic acid-induced writhing test and formalin test at oral doses of 30-300 mg/kg. This route of administration was chosen for the present study because of the preparation of the plant as infusions and teas in popular medicine, and we suggest that the effects observed in the present study justify the therapeutic uses of the plant.

The magnitude of the inhibitory action of EOOG in the acetic acid-induced writhing test was comparable to that obtained with the standard drug, indomethacin, indicating the importance of our current findings. This test is a standard test for pain that is sensitive to opiates as well as non-opiate analgesics (9). The associated nociceptive response is believed to involve the release of endogenous substances that stimulate nociceptive endings, such as bradykinin and prostanoids, amongst others (10). This test, although highly sensitive to weak analgesics, is also affected by a variety of other pharmacological substances including muscle relaxants and neuroleptics (11), and as such limits definitive conclusions about the mechanism of action of EOOG. It is possible, for example, that a sedative action might also produce a positive result in this test, although this seems unlikely since the locomotor activity of the animals did not appear to be impaired by EOOG within the experimental protocols.

In contrast, the formalin test measures the response to a long-lasting nociceptive stimulus, and may thus bear a closer resemblance to clinical pain (8). The formalin test is divided into two phases: neurogenic (first phase) and inflammatory (second phase) pain. The first phase is attributed to direct activation of peripheral nociceptors and sensory afferent fibers by formalin, while the second phase may reflect a combination of low ongoing activity in primary afferents and increased sensitivity of spinal cord neurons (12,13). Our data show that EOOG exerts inhibitory effects predominantly during the late phase of the formalin response, although the highest dose of EOOG (300 mg/kg) also produced a significant inhibition of the first phase, and may thus reflect multiple sites of action.

It was beyond the scope of the present study to investigate which component(s) was (were) responsible for the antinociceptive effects of EOOG; however, several appear to be likely candidates. For example, eugenol, the major compound found in our chemical analysis of EOOG, has been shown to have analgesic effects (14,15), possibly via inhibition of nerve conduction through A

Table 1. Effects of the essential oil of *Ocimum gratissimum* (EOOG) (*po*) on acetic acid-induced writhing in mice.

Group/dose (mg/kg)	Writhes (No.)	Inhibition (%)	N
Control	58.3 ± 4.4	–	10
EOOG (10)	44.7 ± 6.0	23.3	10
EOOG (30)	40.7 ± 6.3*	30.0	8
EOOG (100)	36.4 ± 3.6*	37.6	9
EOOG (300)	24.6 ± 3.6*	57.8	10
Indomethacin (10)	26.5 ± 2.6*	54.5	10

Data are reported as means ± SEM. N indicates the number of animals per group. *P<0.05 *vs* control (Student *t*-test).

Table 2. Effects of the essential oil of *Ocimum gratissimum* (EOOG) (*po*) on the formalin test in mice.

Group/dose (mg/kg)	Licking time first phase (s)	Licking time second phase (s)	Inhibition first phase (%)	Inhibition second phase (%)	N
Control	62.5 ± 6.07	59.3 ± 8.3	–	–	6
EOOG (10)	53.66 ± 14.5	44.3 ± 9.2	14.1	25.3	6
EOOG (30)	61.25 ± 9.0	40.4 ± 4.8*	2.0	31.9	6
EOOG (100)	57.5 ± 9.2	23.2 ± 2.8*	8.0	60.9	6
EOOG (300)	37.0 ± 5.9*	25.3 ± 5.5*	40.8	57.3	6

Data are reported as means ± SEM. N indicates the number of animals per group. *P<0.05 *vs* control (Student *t*-test).

and C fibers (16), and/or inhibition of prostaglandin biosynthesis (17,18), although a stimulatory effect on capsaicin-sensitive afferent sensory neurons has also been reported (19). In addition 1,8-cineole, another major compound present in EOOG, possesses both anti-inflammatory and antinociceptive properties (20). Thus, a combination of pharmacological effects probably underlies the observations of the present study.

EOOG (30-300 mg/kg) has antinociceptive properties in the classical pharmacological tests. Since EOOG is remarkably nontoxic, with an LD_{50} value >2.4 g/kg (Rabelo M and Criddle DN, unpublished observations), greatly in excess of that required to produce an antinociceptive action, the current results indicate the potential of this plant essential oil as a therapeutic agent. Further detailed experiments are thus strongly indicated to clarify the properties of this medicinal plant and to investigate the mechanism of action of its essential oil.

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