

Anti-inflammatory and antinociceptive activities of eugenol essential oil in experimental animal models

Apparecido N. Daniel, Simone M. Sartoretto, Gustavo Schmidt, Silvana M. Caparroz-Assef, Ciomar A. Bersani-Amado, Roberto Kenji N. Cuman*

Departamento de Farmácia e Farmacologia, Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900 Maringá-PR, Brazil

RESUMO: “Atividades antiinflamatória e antinociceptiva do eugenol em modelos experimentais em animais”. *Eugenia caryophyllata*, popularmente conhecida como “cravo-da-índia”, cresce naturalmente na Indonésia e é cultivada em várias partes do mundo, incluindo o Brasil. O cravo-da-índia é utilizado em culinária, em farmácia, perfumaria e cosméticos. O óleo essencial extraído do cravo-da-índia cujo principal componente é o eugenol tem sido utilizado em odontologia como anti-séptico e analgésico. O objetivo deste estudo foi avaliar as atividades antiinflamatória e antinociceptiva do eugenol de uso odontológico, administrado oralmente, em modelos experimentais *in vivo*. A atividade antiinflamatória do eugenol foi avaliada através do volume de exsudato e migração leucocitária no teste de pleurisia e do edema de pata de rato induzido pela carragenina. A atividade antinociceptiva foi avaliada através dos testes de contorções induzidas pelo ácido acético e da placa quente. O eugenol (200 e 400 mg/kg) reduziu o volume de exsudato pleural sem interferir na contagem de leucócitos totais presentes na pleura. Na dose de 200 mg/kg, o eugenol inibiu significativamente o edema de pata, 2-4 h após a injeção do agente flogístico. No teste da placa quente, a administração do eugenol (100 mg/kg) mostrou atividade significativa à reação de desconforto-tempo dependente, avaliada como a latência da resposta, inibida pela meperidina. Eugenol nas doses de 50, 75 e 100 mg/kg apresentou efeito antinociceptivo significativo no teste de contorções abdominais induzidas pelo ácido acético em comparação com o grupo controle. Os dados obtidos indicam que o eugenol apresenta atividade antiinflamatória e antinociceptiva periférica.

Unitermos: *Eugenia caryophyllata*, *Syzygium aromaticum*, Myrtaceae, atividade antiinflamatória, atividade antinociceptiva, óleo essencial, cravo-da-índia.

ABSTRACT: *Eugenia caryophyllata*, popular name “clove”, is grown naturally in Indonesia and cultivated in many parts of the world, including Brazil. Clove is used in cooking, food processing, pharmacy; perfumery, cosmetics and the clove oil (eugenol) have been used in folk medicine for manifold conditions include use in dental care, as an antiseptic and analgesic. The objective of this study was evaluated the anti-inflammatory and antinociceptive activity of eugenol used for dentistry purposes following oral administration in animal models *in vivo*. The anti-inflammatory activity of eugenol was evaluated by inflammatory exudates volume and leukocytes migration in carrageenan-induced pleurisy and carrageenan-induced paw edema tests in rats. The antinociceptive activity was evaluated using the acetic acid-induced writhing and hot-plate tests in mice. Eugenol (200 and 400 mg/kg) reduced the volume of pleural exudates without changing the total blood leukocyte counts. At dose of 200 mg/kg, eugenol significantly inhibited carrageenan-induced edema, 2-4 h after injection of the flogistic agent. In the hot-plate test, eugenol administration (100 mg/kg) showed unremarkable activity against the time-to-discomfort reaction, recorded as response latency, which is blocked by meperidine. Eugenol at doses of 50, 75 and 100 mg/kg had a significant antinociceptive effect in the test of acetic-acid-induced abdominal writhing, compared to the control animals. The data suggest that eugenol possesses anti-inflammatory and peripheral antinociceptive activities.

Keywords: *Eugenia caryophyllata*, *Syzygium aromaticum*, Myrtaceae, anti-inflammatory activity, antinociceptive activity, essential oil, clove.

INTRODUCTION

Eugenia caryophyllata L. Merr. & Perry

(syn.: *Syzygium aromaticum*) (clove) plant belongs to Myrtaceae families. Clove spice is a nail-shaped dried flower but of *Eugenia caryophyllata* Thunb. species. It

is grown naturally in Indonesia and cultivated in many parts of the world, including Brazil (Costa, 1994; Corrêa et al., 1998; Agra et al., 2008). The plants have a strong phenolic smell and sharp acrid taste. Clove is used in cooking, food processing, pharmacy, perfumery and cosmetics. It contains essential oil at 15-20%, tannin 13% and fixed oil 10%. Non-essential ether extract constitutes 6-7%. Essential oil of clove is a colorless or light yellowish fluid extract from dried flower buds by steam distillation. Through GC/MS analysis of clove essential oil, 36 components were identified (Chaieb et al., 2007). The highest concentration was of eugenol (88.58%), eugenyl acetate (5.62%) and β -caryophyllene (1.38%). However, the differences in oil composition are correlated with different regions or countries where the plant is cultivated (Öztürk & Özbek, 2005).

The eugenol is widely used and well known for its medicinal properties. Traditional uses of clove oil include use in dental care, as an antiseptic and analgesic (Oliveira et al., 2007). It is active against oral bacteria associated with dental caries and periodontal disease (Cai & Wu, 1996) and effective against a large number of other bacteria (Burt & Reinders, 2003; Larhsini et al., 2001; Cressy et al., 2003; Friedman et al., 2002) and virus (Kim et al., 2001). Previous studies have reported biological activities of eugenol including antifungal (Gayoso et al., 2005; Manohar et al., 2001; Chami et al., 2005), anticarcinogenic (Zeng et al., 1992), anti-allergic (Kim et al., 1998; Corrêa et al., 2008), antimutagenic activity (Miyazawa & Hisama, 2001), antioxidant (Ogata et al., 2000) and insecticidal (Park et al., 2000) properties. Eugenol has been used topically in dental practice to relieve pain arising from a variety of sources, including pulpitis and dentinal hypersensitivity. In the present study, the anti-inflammatory and antinociceptive activities of eugenol used for dentistry purposes following oral administration in animal models *in vivo* were evaluated.

MATERIAL AND METHODS

Plant material and extraction of the essential oil

Eugenol (ANVISA n° 10041120164) was purchased from S.S. White (Rio de Janeiro, Brazil). For the pharmacological assays, an aqueous suspension of eugenol was used.

Animals

Male Swiss mice weighing 25 ± 5 g were used in the experiments on antinociceptive activity and acute toxicity. Male Wistar rats weighing 200 ± 30 g were used for the evaluation of the anti-inflammatory activity. The animals were obtained from Central Animal House of the Universidade Estadual de Maringá. They were housed at 22 ± 2 °C under a 12 hour light/ 12 hour dark

cycle and had free access to water and food, before experimentation. Prior to the experiments, the animals were fasted overnight, with water provided *ad libitum*. The experimental protocols were approved by and were followed in accordance with the guidelines of the Ethical Committee in Animal Experimentation of the Universidade Estadual de Maringá.

Carrageenan-induced paw edema in rats

The anti-inflammatory test was evaluated by the carrageenan-induced paw edema test in the rat, according to the method of Winter et al. (1962). Male Wistar rats (190-230 g) were briefly anesthetized with ethyl ether and injected subplantarily into the right hind paw with 0.1 mL of suspension of carrageenan (200 μ g/mL) in isotonic saline. The left hind paw was injected with 0.1 mL of saline and used as a control. Paw volume was measured prior and 1, 2 and 4 h after carrageenan administration, using a mercury plethysmograph (Ugo Basile, Italy). Eugenol (100, 200 and 400 mg/kg) was orally administered 30 minutes prior to carrageenan injection. The control group received an equivalent volume of water. Indomethacin (5 mg/kg, *p.o.*) and celecoxib (10 mg/kg, *p.o.*) were used as the reference drugs.

Carrageenan-induced pleurisy in rats

The test was performed according to Vinegar et al. (1973). The groups of rats were pre-treated with eugenol (100, 200 and 400 mg/kg, *p.o.*), indomethacin (5 mg/kg, *p.o.*) and celecoxib (10 mg/kg, *p.o.*) as the standard drugs, or water (0.1 mL, *p.o.*) as the control. Thirty minutes later, all animals received an intrapleural injection of carrageenan (200 μ g/animal). Four hours later the animals were anesthetized with ethyl ether. The pleural exudate was collected, the volume determined, and the pleural cavity was washed with 1.0 mL saline containing heparin (10 IU/mL). The number of migrating leukocytes in the exudate was determined with a Neubauer chamber. Results were expressed as mean \pm S.E.M. of exudate pleural volume and of counts of total leukocytes.

Acetic acid-induced writhing test

The antinociceptive activity of eugenol was assessed using the writhing test (abdominal constriction test), according to the method of Siegmund et al. (1957). Acetic acid solution (10 mL/kg, 0.6%) was injected intraperitoneally, and the constriction of the abdominal muscles together with stretching of the hind limbs was counted over a period of 20 minutes, starting immediately after acetic acid injection. The essential

oil (50, 75 and 100 mg/kg, *p.o.*), indomethacin (10 mg/kg, *p.o.*) and celecoxib (10 mg/kg, *p.o.*) as the standard drug (positive controls), and water (0.3 mL, *p.o.*) as the negative control were administered 30 minutes before the acetic acid injection. Antinociceptive activity was expressed as the percentage of inhibition of abdominal constrictions between the control animals and the mice pre-treated with the oil.

Hot-plate test

The hot-plate test was performed to measure response latencies according to the method previously described (MacDonald et al., 1946). The hot-plate (Model DS 37, Ugo Basile, Italy) was maintained at 54.0 ± 1 °C. The time taken (sec) to cause a discomfort reaction (licking paws or jumping) was recorded as the response latency 0, 15, 30, 60 and 90 min after administration of eugenol (50, 75 and 100 mg/kg, *p.o.*), meperidine (50 mg/kg, *i.p.*) as the standard drug (positive control) or water (0.3 mL, *p.o.*) as the negative control. A latency period of 25 sec was defined as complete analgesia. The experiment was halted if the latency period was exceeded, in order to avoid injury.

Statistical analysis

All data are expressed as mean \pm S.E.M. Results were statistically analyzed using Student's t-test for unpaired data (two-tailed) or by one-way analysis of variance (ANOVA) followed Tukey test. P values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

In this study we evaluated the anti-inflammatory activity of eugenol in experimental models of inflammation (paw edema and pleurisy induced by carrageenan). The oral administration of eugenol at doses of 400 mg/kg, significantly inhibited paw edema, 2-4 h after carrageenan injection and the inhibition rate was comparable to that of indomethacin and celecoxib. The results are presented in Figure 1.

It is related in literature that some essential oil extracted of plants exhibit an inhibitory effect of on pleural exudates formation (Siani et al., 1999; Vendruscolo et al., 2006). Similarly, in the pleurisy model, eugenol administered orally at doses of 200 and 400 mg/kg caused significant inhibition of fluid extravasation, but not on the leukocyte migration when compared with references drugs (Table 1). On the other hand, geranium, lemongrass and spearmint essential oil provoked inhibition of neutrophil accumulation in the peritoneal cavity (Abe et al., 2004).

It is well known that different mechanisms may be involved in the genesis of inflammatory reactions. The development of the inflammatory response induced by

carrageenan (paw edema and pleurisy) is characterized by an initial stage (1-2 h) which is dependent on the release of histamine, serotonin and bradykinin, followed by a later stage (3-4 h) which is maintained principally by the release of prostanoids (Crunkhorn & Meacock, 1971; Niemegeers et al., 1964). It has also been shown that nitric oxide (NO) has an important role as much as in the regulation of vascular permeability as in cell migration induced by proinflammatory agents, including carrageenan (Vinegar et al., 1982; Costa et al., 2008). In macrophages and other cell types, cytokines and lipopolysaccharide induce nitric syntase (iNOS) and cyclooxygenase-2 (COX-2). Both iNOS and COX-2 are responsible for the production of large proinflammatory mediators, nitric oxide and prostaglandins at the inflammatory site (Lee et al., 1992; Nussler & Biliar, 1993). Eugenol showed similar anti-inflammatory effects to COX antagonist (indomethacin) and COX-2 selective antagonist (celecoxib). The COX-2 inhibitory effect of eugenol has been described by Huss et al., (2002) and *in vitro* by Kim et al., (2003) in LPS-stimulated mouse macrophage.

Two different analgesic testing methods were employed in the current investigation with the objective of identifying possible peripheral (acetic acid-induced writhing test) and central (hot-plate test) effects of the essential oil. Acetic acid causes an increase in the peritoneal fluid level of prostaglandins (PGE_2 and $PGF_{2\alpha}$), involving in part peritoneal receptors (Deraedt et al., 1980) and inflammatory pain by inducing capillary permeability (Amico-Roxas et al., 1984). Collier et al. (1968) postulated that acetic acid acts indirectly by inducing the release of endogenous mediators, which stimulate the nociceptive neurons. Indeed, traditional anti-inflammatory (NSAIDs) can inhibit COX in peripheral tissues and interfere with the mechanism of transduction of primary afferent nociceptors. Eugenol (50, 75 and 100 mg/kg) oral administration significantly decreased (61.6, 64.8 and 88.3%, respectively) the number of acetic acid-induced writhes in mice in comparison to the control group, as observed for indomethacin and celecoxib (Table 2). On the other hand, oral administration of eugenol (50, 75 and 100 mg/kg) failed to prolong latency time compared with control animals in mice in the hot-plate test (Figure 2). Meperidine (50 mg/kg, *i.p.*), the positive control used in this study caused significant antinociception effect. Eugenol exhibited significant antinociceptive activity against chemical stimuli (acetic acid tests), but not against thermal stimuli, suggesting that eugenol predominantly inhibits the peripheral pain mechanism. Similar results were observed by Kurian et al. (2006) after intraperitoneal administration of eugenol in mice. The analgesic effect was also demonstrated with others essential oils obtained from *Zingiber officinale* Roscoe (Vendruscolo et al., 2006), *Lavandula angustifolia* Mill L (Hajhashemi et al., 2003), *Hypericum brasiliense*

Table 1. Effect of eugenol on pleurisy induced by intrapleural injection of carrageenan in male Wistar rats.

Group	Exudate volume (mL)	% Inhibition of edema	Leukocyte count (cells/mm ³) x 10 ³
Control	0.90 ± 0.11	-	62.6 ± 4.8
Indomethacin 5 mg/kg	0.50 ± 0.03*	44.5	61.5 ± 6.0
Celecoxib 10 mg/kg	0.49 ± 0.03**	45.6	54.9 ± 4.9
Eugenol 100 mg/kg	0.70 ± 0.08	22.2	53.9 ± 3.4
Eugenol 200 mg/kg	0.54 ± 0.07*	40.0	48.2 ± 5.1
Eugenol 400 mg/kg	0.53 ± 0.04*	41.1	45.7 ± 5.9

Each value represents the mean number of leucocytes ± SEM from 10-13 for each group.

*p < 0.05, ** p < 0.01 compared to the control group (ANOVA, Tukey test).

Table 2. Effects of eugenol on acetic acid-induced writhing in mice.

Group	Number of writhings	Inhibition (%)
Control	70.4 ± 3.2	-
Indomethacin 10 mg/kg	44.4 ± 1.2*	36.9
Celecoxib 10 mg/kg	30.4 ± 4.7*	56.8
Eugenol 50 mg/kg	27.0 ± 6.6*	61.6
Eugenol 75 mg/kg	24.8 ± 4.6*	64.8
Eugenol 100 mg/kg	6.2 ± 1.1*	88.1

Each value represents the mean ± SEM from 7-9 for each group.

*p < 0.05 compared to the control group (ANOVA, Tukey test).

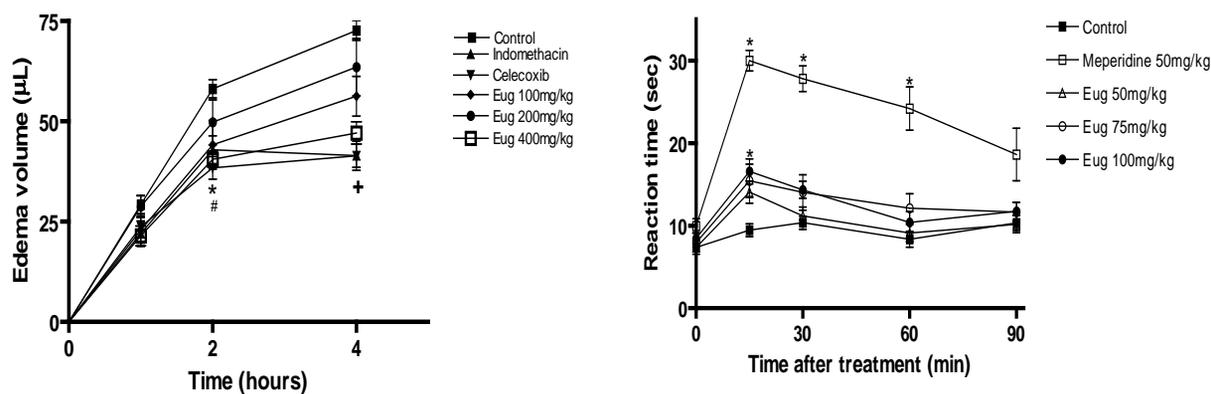


Figure 1. Effect of oral treatment with eugenol on the development of edema induced by intraplantar injection of carrageenan in rats (n = 8-10 animals). Rats were treated orally with eugenol (100, 200 and 400 mg/kg), 1 h prior to the injection of carrageenan (200 µg). Indomethacin (5 mg/kg, *p.o.*) and celecoxib (10 mg/kg, *p.o.*) were used as the reference anti-inflammatory. Each column represents the mean volume of the paw ± S.E.M., at 1, 2 and 4 h after injection of the carrageenan. *p < 0.001; #p < 0.01; *p < 0.01, compared to the control group, treated with saline (ANOVA, Tukey test).

Figure 2. Effect of eugenol on the pain threshold of mice in the hot-plate test. The vehicle (control group), eugenol (50, 75 and 100 mg/kg) and meperidine (50 mg/kg) were administered orally. The reaction time (sec) was measured 15, 30, 60 and 90 min after treatment. Each point represents the mean ± S.E.M. reaction time.*p < 0.001 compared to the control group (ANOVA, Tukey test).

willd (Perazzo et al., 2008) and *Satureja hortensis* L (Hajhashemi et al., 2002).

Our data demonstrated that the anti-inflammatory and antinociceptive activities of eugenol might be partially related to inhibition of prostaglandin synthesis or release of other endogenous mediators. Overall, our data provide support for the popular use of eugenol in folk medicine for some inflammatory and pain ailments.

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