

Effect of eugenol on animal models of nociception

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ABSTRACT

Objective: To investigate the antinociceptive potential of eugenol on different pain models in mice.

Materials and Methods: Eugenol was evaluated (1-100 mg/kg, i.p.) in various experimentally induced pain models like, formalin induced hyperalgesia, acetic acid induced abdominal constrictions, and thermal pain experiment using Eddy's hot plate.

Results: Eugenol significantly inhibited acetic acid induced abdominal constrictions, with the maximal effect (92.73% inhibition) at 100 mg/kg. In formalin induced paw licking pain model, eugenol exhibited more pronounced antinociceptive effect in the inflammatory phase than the neurogenic phase (maximal effect was 70.33% and 42.22%, respectively, at 100 mg/kg, i.p.). A mild reduction in the pain response latency at 100 mg/kg, i.p. dose of eugenol was observed in the hotplate thermal pain studies in mice. In the rotarod motor coordination experiment eugenol reduced the endurance time at the dose of 100 mg/kg, i.p.

Conclusion: The data suggest that eugenol exerts antinociceptive activity in different experimental models of pain in mice.

KEYWORDS: Antinociceptive, clove oil, pain models.

Introduction

Eugenol (4-allyl-2-methoxyphenol), the principal chemical constituent of clove oil has been primarily derived from a variety of plant sources, including *Eugenia caryophyllus* and *Myristica fragrans*. For years eugenol has been used in dental practice to relieve pain arising from a variety of sources, including pulpitis and dentinal hypersensitivity. In the recent past, a wealth of literature has been generated on eugenol's antidepressant, antistress, anticonvulsant, and analgesic activities.^[1-3] Eugenol is also reported to possess antiinflammatory, antioxidant, anaesthetic and muscle relaxant properties.^[4-6]

The objective of the present investigation was to study a range of doses of eugenol (from 1 to 100 mg/kg) towards possible analgesic potential in both peripheral and central experimental pain models.

Materials and Methods

Chemicals

Eugenol and indomethacin were purchased from Sigma Chemical Co. (St. Louis, USA). Pentazocine lactate (Fortwin® Ranbaxy, India) and diazepam (Calmpose® Ranbaxy, India) were obtained as injections from local market. Eugenol and indomethacin were dissolved in 0.5% Tween 80 in saline. Pentazocine and diazepam were diluted with saline.

Animals

Adult male Swiss albino mice (22-26 g) were obtained from the National Toxicology Centre, Pune, India. The animals were randomly allocated to treatment groups (six animals per group, per treatment) in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30 to 70%. A 12:12 light:dark cycle was followed. All animals had free access to water and standard pelleted laboratory animal diet. All the experimental protocols were approved by the Institutional Animal Care and Use Committee of Poona College of Pharmacy, Pune, India and were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Forests and Environment, Government of India. Rules of the CPCSEA are based on ILAR (Institute of Laboratory Animal Resources, USA) guidelines.

Acetic acid induced abdominal constrictions in mice

The i.p. injection of acetic acid (1%), resulted in constriction of abdominal muscle together with a stretching of hind limbs. This procedure was carried out as described by Santos *et al.*^[7] Eugenol (1-100 mg/kg) and positive control indomethacin (20 mg/kg) were administered i.p., 15 min prior to acetic acid injection. The number of writhing movements was counted for 30 min, from the time immediately after acetic acid injection. Antinociception was expressed as the number of abdominal

constrictions between saline treated control and animals pretreated with eugenol or indomethacin.

Formalin induced paw licking in mice

This procedure was essentially similar to that described by Hunskaar and Hole.^[8] Mice were injected intraperitoneally with eugenol (1-100 mg/kg) or 0.5% Tween 80 (10 ml/kg) or indomethacin (20 mg/kg). Fifteen minutes later 20 μ l of 1% formalin was injected subcutaneously under the dorsal surface of the hind paw, and the animals were observed in the chambers. The time spent for licking the paw injected with formalin was counted for 30 min post formalin injection and considered as indicative of pain stimuli. The formalin test had two distinctive phases, possibly reflecting different types of pain. The first phase of the nociceptive response normally peaked at 5 min and the second phase 20 to 30 min after formalin injection. This represented neurogenic and inflammatory responses, respectively.

Hot plate test in mice

The hot plate test was carried out according to the method described by Eddy and Leimbach.^[9] Animals were placed on the hot plate (Ugo Basile, Italy) maintained at $55 \pm 1^\circ\text{C}$ and the time between placement on the hot plate and the occurrence of either licking of the paws, shaking, or jumping off from the plate was recorded as response latency. Mice with basal latency of more than 10 sec were not included in the study. The response latencies was measured before distraction (basal) and after drug treatment [eugenol (1-100 mg/kg, i.p.) or 0.5% Tween 80 (10 ml/kg, i.p.) or pentazocine (10 mg/kg, i.p.)] at 30, 60, 90, 120 and 180 min. The cut off time for hot plate latency was set at 20 sec.

Motor coordination (rotarod test) in mice

A rotarod tread mill device (Techno, India) was used for the evaluation of motor coordination. Mice were placed on a horizontal rotating (16 RPM) rod. These mice had been selected for their ability to remain on the revolving bar for a 2 min period. Fifteen minutes after the administration of either eugenol (30 and 100 mg/kg, i.p.) or diazepam (5 mg/kg, i.p.), each mouse was placed on the rotating rod for 60 sec, at intervals of 30 min for 3 h.^[10] The endurance time for each mouse on the rota-rod was noted.

Statistical analysis

Values are expressed as mean \pm SEM. The statistical significance of difference between the means was analysed by one-way non-parametric ANOVA and Dunnett's test. $P < 0.05$ was considered significant. All the statistical manipulations were carried out using GraphPad® Prism Software (Graphpad Software Inc., USA).

Results

Acetic acid induced abdominal constrictions in mice

The results of the abdominal constriction test are shown in Figure 1. Eugenol elicited a dose-dependent inhibition of abdominal constrictions compared with the control group. Eugenol produced 10.61% inhibition at 1 mg/kg dose, with a maximal of inhibition 92.73% ($P < 0.001$; $F = 8.20$) at 100 mg/kg, which was comparable to indomethacin (93.64% inhibition at 20 mg/kg, i.p.).

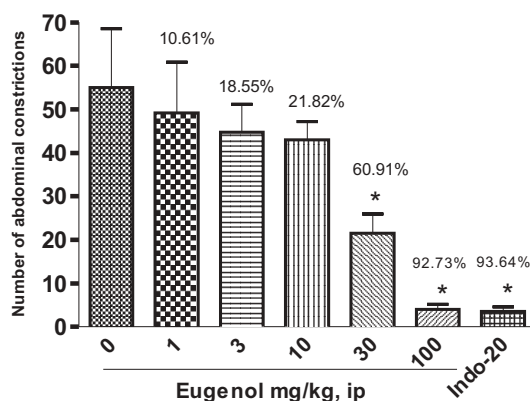
Formalin induced paw licking in mice

Eugenol exhibited no effect during the neurogenic phase

(0-10 min) of formalin induced licking in mice at 1 mg/kg, 11.67% inhibition at 10 mg/kg, i.p., and 42.22% at 100 mg/kg ($P < 0.001$; $F = 5.30$) compared with the vehicle treated animals. The standard, indomethacin (20 mg/kg) caused 63.93% inhibition. [Figure 2]

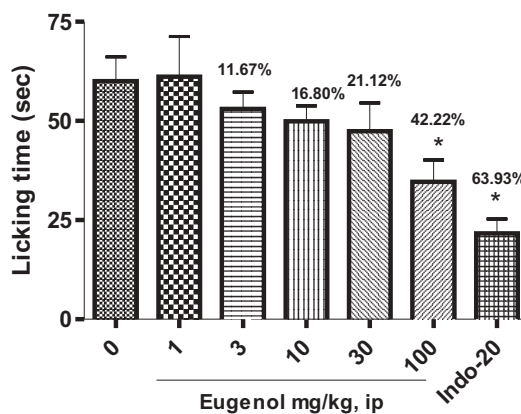
A mild inhibitory effect (3.14%) with eugenol on the inflammatory phase (20 to 30 min) of the formalin induced paw licking in mice was observed at 1 mg/kg, and a statistically significant maximal inhibition (70.33% inhibition) was observed at 100 mg/kg ($P < 0.05$; $F = 5.69$). The standard drug, indomethacin (20 mg/kg), also exhibited a statistically significant inhibition (66.18%) of the inflammatory phase. [Figure 3]

Figure 1. Effect of eugenol on acetic acid induced abdominal constrictions in mice.



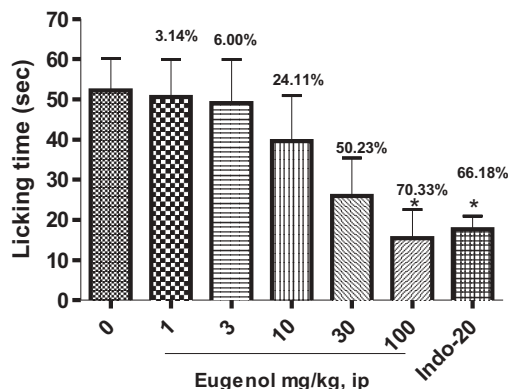
n=6 in each group. The bars represent mean \pm SEM and the percentages indicate %inhibition. * $P < 0.005$ compared with vehicle treatment. Indo: indomethacin.

Figure 2. Effect of eugenol on neurogenic phase of formalin induced licks in mice.



n=6 in each group. The bars represent mean \pm SEM and the percentages indicate %inhibition. * $P < 0.005$ compared with vehicle treatment. Indo: indomethacin.

Figure 3. Effect of eugenol on inflammatory phase of formalin induced licks in mice.



n=6 in each group. The bars represent mean±SEM and the percentages indicate %inhibition. * $P < 0.005$ compared with vehicle treatment. Indo: indomethacin.

Hot plate test in mice

Eugenol (1 to 100 mg/kg) pretreatment increased the response latency in the hot plate test. This, however, was not statistically significant. The centrally acting analgesic pentazocine also increased the response latencies at various time points.

Motor coordination in mice

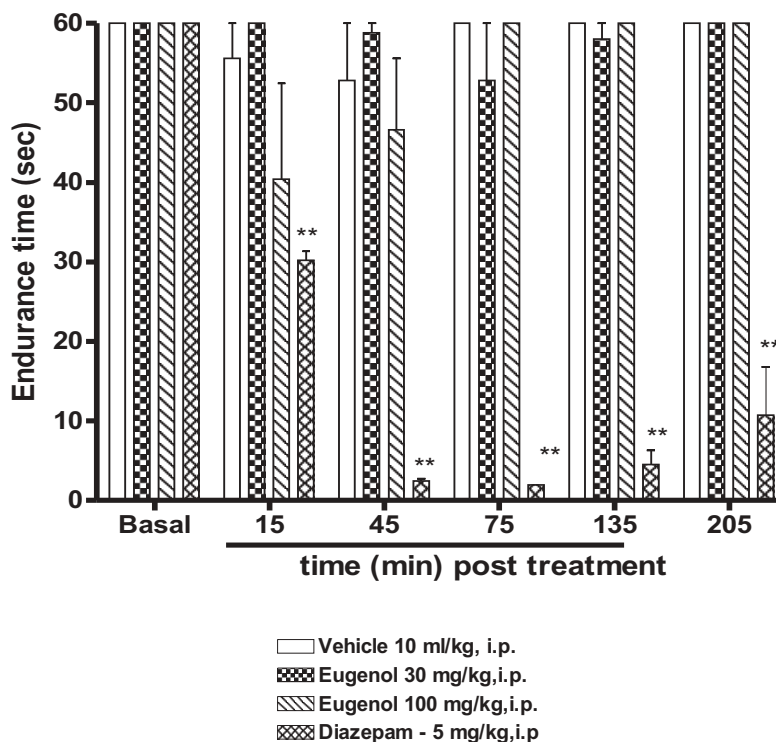
Eugenol administered intraperitoneally at 30 mg/kg did not affect motor coordination. A dose of 100 mg/kg, however, produced a statistically insignificant reduction in the endurance time at 60 minutes. The standard drug, diazepam at 5 mg/kg, i.p. dose exhibited a statistically significant ($P < 0.05$) effect on motor coordination by reducing the endurance time at the various times points. [Figure 4]

Discussion

In the present investigation, eugenol was studied for its nociceptive activity in both peripheral and central algescic models. This study differs from the earlier reports on the analgesic activity of eugenol^[11,12] primarily with respect to the route of administration, the doses employed, and the source of eugenol.

The intensity of analgesic effect of eugenol at 100 mg/kg dose was similar to that of indomethacin (20 mg/kg, i.p.) in acetic acid induced abdominal constrictions in mice. Acetic acid causes inflammatory pain by inducing capillary permeability^[13] and liberating endogenous substances that excite pain nerve endings.^[14] NSAIDs can inhibit COX in peripheral tissues and, therefore, interfere with the mechanism of transduction of primary afferent nociceptors.^[15] The mechanism of analgesic effect of eugenol could probably be due to blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of

Figure 4. Effect of eugenol on motor coordination test in mice.



n=6 in each group. Values are mean±SEM. * $P < 0.001$ compared with vehicle treatment. Each mouse was placed on the rotating rod for 60 sec.

indomethacin and other NSAIDs. Recently, eugenol and its derivatives have been reported to exert inhibitory effect on various mediators of inflammation. This includes inhibition of lipopolysaccharide-stimulated nuclear factor kappa B activation and cyclooxygenase-2 expression in macrophages.^[16,17]

Eugenol exhibited an efficacy comparable to that of indomethacin in inhibiting neurogenic (first phase) and inflammatory (second phase) pain stimuli caused by formalin. The formalin test is used to evaluate the mechanism by which an animal responds to moderate, continuous pain generated by the injured tissue.^[18] This test is characterised by two phases. The early phase (immediately after injection) seems to be caused by C-fibre activation due to the peripheral stimulus. The late phase (starting approximately 20 min after formalin injection) appears to depend on the combination of an inflammatory reaction, activation of NMDA and non-NMDA receptors, and the NO cascade^[19] in the peripheral tissue and functional changes in the dorsal horn of the spinal cord.^[18] Both these functional changes appear to be initiated by the C-fibre barrage during the early phase and to be related to excitatory amino acid (EAA) release in the spinal cord and activation of NMDA receptor subtypes. The formalin test has been used to evaluate the antinociceptive effects of competitive and non-competitive NMDA receptor antagonists administered intrathecally and systemically.^[20] CGP 37849, memantine, ketamine and dextromethorphan were reported to have antinociceptive activity in formalin test^[21]

Although a wealth of literature is available on the inhibitory effect of eugenol on prostaglandin bio-synthesis and or nerve conduction as shown in the rat vagus nerve,^[22] there has been a recent upsurge in the research focus on the role of vanilloid receptors and calcium channels in the antinociceptive action of eugenol.

In a comparative study of β -caryophyllene oxide, eugenol, and nifedipine it was reported that eugenol blocked calcium channels. This was demonstrated in voltage clamp experiments in cardiac myocytes.^[23] In cell lines stably expressing human N-type calcium channels, eugenol reportedly inhibited high-voltage-activated calcium currents.^[24]

The role of vanilloid receptor in the antinociceptive activity of eugenol becomes evident from the studies conducted by Yang *et al.*^[25] In vanilloid receptor 1 (TRPV1 or VR1), expressing human embryonic kidney (HEK) 293 cells and trigeminal ganglion neurons, eugenol activated inward currents while capsazepine, a competitive vanilloid receptor antagonist, completely blocked eugenol induced inward currents. This experiment supports the *in vivo* studies carried out by Ohkubo and Shibata^[26] who demonstrated the inhibitory effect of capsazepine on eugenol induced antinociceptive activity in mice. These studies provide strong evidence that eugenol produces its antinociceptive effects through different mediators and, at least in part, *via* blockade of calcium channels and vanilloid receptor modulation.

Eugenol produced antinociception against thermal induced pain stimuli in mice at various time points post treatment. The effect observed was, however, very mild and not statistically significant. The hot plate test is considered to be selective for opioid-like compounds, which are centrally

acting analgesics in several animal species.^[27] In motor coordination test using rotarod apparatus, eugenol at 100 mg/kg, i.p. exhibited an insignificant sedative effect that was evidenced by reduction in endurance time. This could be the possible explanation for its mild central analgesic activity observed in hot plate test.

Conclusion

Eugenol administered intraperitoneally exhibits antinociceptive activity and possibly exerts its effect through diverse mechanisms that may involve both central and peripheral pathways. Present data support the traditional application of eugenol as a dental analgesic. Further pharmacodynamic investigations are required to understand the precise mechanism of antinociception exhibited by eugenol.

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