

Analgesic effect of clove essential oil in mice

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Abstract

Objective: Results obtained from literature reviews and human studies have shown the analgesic effects of clove plant in toothache. The present work was undertaken in order to investigate the possible analgesic effect of clove oil in mice.

Materials and Methods: Fifty mice were divided into 5 groups: 1) Saline; 2) Essential oil (Ess) 2%, 3) Ess 5%, 4) Ess10% and 5) Ess 20%. The hot plate test (55 ± 0.2 °C; Cut-off 60 sec) was performed as a base record 15 min before injection of drugs (Saline or 2, 5, 10 and 20% concentrations of Essential oil) and consequently repeated every 15 minutes after injection.

Results: Repeated measures ANOVA test showed that maximal percent effect (MPE) in animal groups treated by 5, 10 and 20% essential oil was significantly higher than saline group. Comparison between 4 treated groups showed that MPE in 10% essential group was higher than 2 and 5% groups however; there was no significant difference between 10% and 20% groups.

Conclusion: The result of present study showed that clove essential oil has analgesic effect in mice using hot plate test. More investigations are needed to elucidate the exact mechanism (s).

Keywords: Clove, Essential oil, Analgesia, Mice, Hot plate

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Introduction

The clove (*Eugenia caryophyllata*) is a tree from Myrtaceae family with a height ranging from 10 to 20 meters which is growing in islands of Indonesia, Tanzania, Sri Lanka, Madagascar, India and Malaysia (Arung et al., 2011) (Tyler et al., 1988). Traditionally, several parts of the plant such as leaves and buds are used in cooking, food processing, pharmacy, perfumery and cosmetics (Daniel et al., 2009). It has also been used to treat many diseases such as disorder of digestive systems (Baytop, 1984). It has been shown that some components of clove are useful in bacterial and fungal infections (Zheng et al., 1992; Zhang and Chen, 1997). The cytotoxic and anti-carcinogenic effects of the plant and its components have also been reported (Zheng et al., 1992; Zhang and Chen, 1997; Koudhi et al., 2010). Several antimicrobial agents are present in clove and therefore, the extracts of this plant have been frequently used to treat the oral bacteria which are commonly associated with dental caries and periodontal disease (Cai and Wu, 1996). The antimicrobial and anti-fungal properties of clove oil allow its use for acne, warts, scars and parasites (Saeed and Tariq, 2008). Vaso-relaxant as well as smooth muscle relaxant effects of the essential oil has also been demonstrated (Nishijima et al., 1999; Damiani et al., 2003). Beneficial effects of the plant in asthma as well as various allergic disorders has also been reported (Kim et al., 1998).

Results obtained from human studies also confirmed analgesic effects of the plant in toothache and in patients suffering from anal fissure (Tyler et al., 1988; Elwakeel et al., 2007). In experimental researches it has been reported that essential oil from several parts of this plant has anesthetic effects in fish (Park et al., 2011). Phytochemical analysis of clove

essential oil has shown the presence of eugenol as a main component (Yu and Hungju, 1981; Daniel et al., 2009). The anesthetic effects of eugenol in dental pain as well as the analgesic and anti-inflammatory effects of this component in animal models has been well documented (Diaz and Sembrano, 1985; oztürk and ozbek, 2005; Kurian et al., 2006; Daniel et al., 2009). Pharmacological studies have also demonstrated the anticonvulsant and anti-stress properties of eugenol (Dallmeier and Carlini, 1981) (Sen et al., 1992). In Iranian folk medicine, the buds of this plant have been used as an antiepileptic remedy (Avicenna, 1988). Therefore, the present work was undertaken in order to investigate the possible analgesic effect of clove oil in mice.

Material and Methods

Animals and drugs

50 male mice (27-32 g) were used. All mice were housed in 4-6 per standard cages, at room temperature 2 ± 1 °C) on a 12 h light/dark cycle. Food and water were available ad libitum. Animal handling and all related procedures were carried out in accordance with Mashhad University of Medical Sciences, Ethical Committee Acts. Essential oil was kindly provided by Eksir Gol Sorkh Company, Mashhad, Iran. Different concentrations of Essential oil were prepared in 10 ml saline.

Nociceptive test

To assess nociceptive responses, hot plate method was used. In hot plate method, animals were placed on the hot plate with temperature setting controlled at 55 ± 0.2 °C. Cut-off time was 60 seconds (Hosseini et al., 2011). Nociceptive response was defined as licking forepaws or moving hindpaws. Time duration between placing the animals on hot plate and licking forepaws or moving hind paws

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was considered as reaction time. The hot plate test was performed as a 3 base records (10 min interval) 15 min before injection of essential oil or saline (10 ml/kg; i.p.) and consequently was repeated 5 times, every 10 minutes after injection.

Experimental design

Fifty mice were divided into 5 groups: 1) Saline; 2) Essential oil (Ess) 2%, 3) Ess 5%, 4) Ess 10% and 5) Ess 20%. The animals were treated with saline or different concentrations of essential (2, 5, 10 and 20 %) oil 30 min before testing in hot plate. The volume of injection was 10 ml/ kg;(i.p.).

Statistical analysis

Analgesic effect of essential oil or saline was calculated as maximal possible effect (MPE) [MPE (%) = [(test response time-basal response time)/(cut-off time-basal response time) × 100%] (Sepehri and Shafeiee, 2006). All data were presented as mean ± S.E.M of %MPE. Statistical comparison of base reaction latency time between groups was done with one-way analysis of variance (ANOVA) and post hoc Tukey's HSD test. Repeated measures ANOVA followed by post hoc Tukey's HSD test was used for comparison of %MPE after injection of drugs. Differences were considered statistically significant when $p < 0.05$.

Results

The basal reaction time in saline group was 4.29 ± 0.8 sec. As the Figure 1 shows, basal reaction latency times in Ess 2%, Ess 5%, Ess 10% and Ess 20 % groups were 3.26 ± 0.7 , 3.53 ± 0.8 , 4.93 ± 0.9 and 3.52 ± 0.8 sec respectively. There was no significant difference between 5 groups (Figure 1). Repeated measures ANOVA test showed that MPE in animal groups treated by 5, 10 and 20% essential oil was

significantly higher than saline group ($p < 0.05$ and $p < 0.001$, Figure 2).

Comparison between 4 treated groups showed that MPE in 10% essential oil group was higher than 2 and 5% groups ($p < 0.001$, Figure 2). There was no significant difference between 10 and 20 groups.

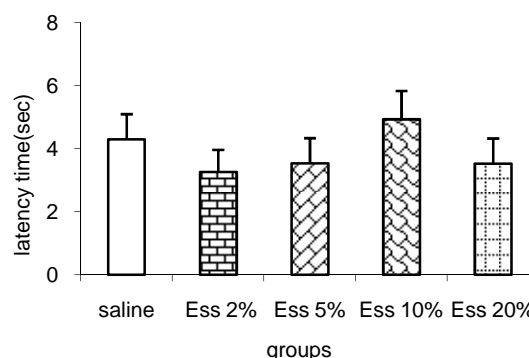


Figure 1. Comparison of basal reaction time between the animals of different groups. Data were shown as mean ± SEM (n=10 in each group). There was no significant difference between groups.

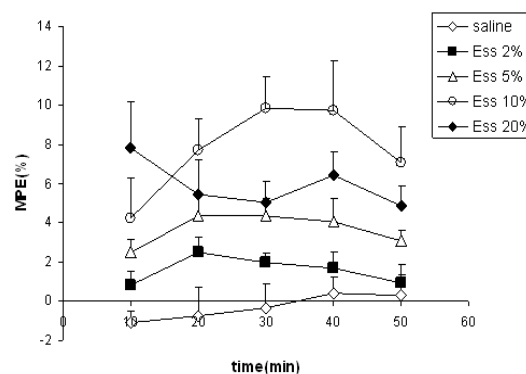


Figure 2. Comparison of MPE between the groups which received different concentrations of essential oil or Saline. Data were shown as mean ± SEM (n=10 in each group). Repeated measures ANOVA test showed that MPE in animal groups treated by 5, 10 and 20% essential oil was significantly higher than saline group ($p < 0.05$ and $p < 0.001$). Comparison between 4 treated groups showed that MPE in 10% essential oil group was higher than 2 and 5% groups ($p < 0.001$). There was no significant difference between 10 and 20 groups.

Discussion

The result of present study showed that essential oil from clove had analgesic effects tested in hot plate. The results also showed that 10% concentration of essential oil was the most effective in comparison with other concentrations. A maximum effect of 10% concentration was seen 70 min after injection. There is evidence that different parts of clove are beneficial in toothache (Yu and Hungju, 1981). The extracts of this plant has been frequently used to anesthetize the fish which was comparable to lidocaine (Anderson et al., 1997; Waterstrat, 1999; Oryzias dancena ; Park et al., 2011). It was also demonstrated that topical application of clove oil cream had a significant beneficial effect in patients suffering from chronic anal fissure (Elwakeel et al., 2007). Essential oil of clove is a colorless or light yellowish fluid extract from dried flower buds by steam distillation. The results obtained by GC/MS analysis showed that clove essential oil contains 36 components. The highest concentration was of eugenol (88.58%), eugenyl acetate (5.62%) and β -caryophyllene (1.38%). (oztürk and ozbek, 2005; Chaieb et al., 2007). The analgesic effects of the essential oil which was seen in the present study may be due to this component. Daniel et al (2009) and kurain et al (2006) also confirmed the antinociceptive activity of eugenol against chemical (acetic acid tests), as well thermal stimuli. They suggested that eugenol predominantly inhibits the peripheral pain mechanism (Kurian et al., 2006; Daniel et al., 2009). In several studies the analgesic effects of eugenol has been attributed to its capability to suppress prostaglandins and other inflammatory mediators such as leukotrienes (Raghavenra et al., 2006). Anti inflammatory, antipyretic and anti allergic effects of this compound may confirm this hypothesis (Feng and Lipton, 1987; Murakami et al., 2003). It has been reported that eugenol reduces paw edema

in carrageenan induced inflammation test and pleural exudates in carrageenan-induced pleurisy in rats .(Daniel et al., 2009) Eugenol is also believed to depress the sensory receptors involved in pain perception (Robbers and Tyler, 1999). The results of present study also showed that the essential olive of clove has analgesic effects with mechanism(s) which are different from previous studies.

Eugenol also inhibits the conduction of action potential in sciatic nerves (Kozam, 1977). Eugenol produces anesthesia in rodents similar to propofol (Sell and Carlini, 1976; Guénette et al., 2007), and alleviates thermal hypersensitivity in an experimental model of neuropathic pain in rats (Guénette et al., 2007). Eugenol inhibits *N*-methyl-D-aspartate (NMDA) receptors but potentiates ionotropic γ -aminobutyric acid (GABA_A) receptors, which are both involved in pain sensitivity (Aoshima and Hamamoto, 1999). Eugenol depresses compound action potentials in both A and C fibers which may explain its analgesic effects (Brodin, 1985). Eugenol is similar in chemical structure to capsaicin and therefore its effect on a vanilloid receptor should not be ignored (Yang et al., 2003). It was also shown that eugenol inhibits Na⁺ currents in rat dorsal root ganglion neurons (Cho et al., 2008). β -Caryophyllene, the other main component of clove oil, showed anti-inflammatory activity in several animal models, including carrageenan- and PGE-induced hind paw edema (Ghelardini et al., 2001). The role of this component in analgesic effects of clove essential oil which was shown in the present study should not be ignored.

It is concluded that different concentrations of the essential oil from clove have analgesic effects however the exact mechanism (s) need to be investigated.

Acknowledgments

Authors would like to thank the Vice Chancellery of Research of Mashhad University of Medical Sciences for financial supports.

References

- Anderson WG, McKinley RS, Colavecchia M. 1997. The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance. *North American Journal of Fisheries Management*, 17:301-307.
- Aoshima H, Hamamoto K. 1999. Potentiation of GABAA receptors expressed in *Xenopus* oocytes by perfume and phytoncid. *Biosci Biotechnol Biochem*, 63:743-748.
- Arung ET, Matsubara E, Kusuma IW, Sukaton E, Shimizu K, Kondo R. 2011. Inhibitory components from the buds of clove (*Syzygium aromaticum*) on melanin formation in B16 melanoma cells. *Fitoterapia*, 82:198-202.
- Avicenna A. 1988. In: Ghanoon dar Teb. Soroosh, Tehran:244-251. Baytop T. 1984. Therapy with medicinal plants in Turkey (Past and Present). Nobel T p Bas mevi. Brodin P. 1985. Differential inhibition of A, B and C fibres in the rat vagus nerve by lidocaine, eugenol and formaldehyde. *Arch Oral Biol*, 30:477-480.
- Cai L, Wu CD. 1996. Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. *J. Nat. Prod*, 59:987- 990.
- Chaieb K, Hajlaoui H, Zmantar T, Kahla Nakbi AB, Rouabhia M, Mahdouani K, Bakhrouf A. 2007. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzygium aromaticum* L. Myrtaceae): a short review. *Phytotherapy research*, 21:501-506.
- Cho JS, Kim TH, Lim JM, Song JH. 2008. Effects of eugenol on Na⁺ currents in rat dorsal root ganglion neurons. *Brain Res*, 1243:53-62.
- Dallmeier K, Carlini EA. 1981. Anesthetic, hypothermic, myorelaxant and anticonvulsant effects of synthetic eugenol derivatives and natural analogues. *Pharmacology*, 22:113.
- Damiani CEN, Rossoni LV, Vassallo DV. 2003. Vasorelaxant effects of eugenol on rat thoracic aorta. *Vascular pharmacology*, 40:59-66.
- Daniel AN, Sartoretto SM, Schmidt G, Caparroz-Assef SM, Bersani-Amado CA, Cuman RKN. 2009. Anti-inflammatory and antinociceptive activities A of eugenol essential oil in experimental animal models. *Revista Brasileira de Farmacognosia*, 19:212- 217.
- Diaz MR, Sembrano JM. 1985. A comparative study of the efficacy of garlic and eugenol as palliative agents against dental pain of pulpal origin. *The Journal of the Philippine Dental Association*, 35:3-10.
- Elwakeel HA, Moneim HA, Farid M, Gohar AA. 2007. Clove oil cream: a new effective treatment for chronic anal fissure. *Colorectal Dis* 9:549-552.
- Feng J, Lipton JM. 1987. Eugenol: Antipyretic activity in rabbits. *Neuropharmacology*, 26:1775-1778.
- Ghelardini C, Galeotti N, Di Cesare Mannelli L, Mazzanti G, Bartolini A. 2001. Local anaesthetic activity of [beta]-caryophyllene 1. *Il Farmaco*, 56:387-389.
- Guénette SA, Ross A, Marier JF, Beaudry F, Vachon P. 2007. Pharmacokinetics of eugenol and its effects on thermal hypersensitivity in rats. *European journal of pharmacology*, 562:60-67.
- Hosseini M, Tairani Z, Hadjzadeh MA, Salehabadi S, Tehranipour M, Alaei HA. 2011. Different responses of nitric oxide synthase inhibition on morphine-induced antinociception in male and female rats. *Pathophysiology*, 18:143- 149.
- Kim HM, Lee EH, Hong SH, Song HJ, Shin MK, Kim SH, Shin TY. 1998. Effect of *Syzygium aromaticum* extract on immediate hypersensitivity in rats. *Journal of ethnopharmacology*, 60:125- 131.
- Kouidhi B, Zmantar T, Bakhrouf A. 2010. Anticariogenic and cytotoxic activity of clove essential oil (*Eugenia caryophyllata*) against a large number of oral pathogens. *Annals of Microbiology*, 60:1-6.
- Kozam G. 1977. The effect of eugenol on nerve transmission. *Oral Surgery, Oral Medicine, Oral Pathology*, 44:799-805.
- Kurian R, Arulmozhi DK, Veeranjanyulu A, Bodhankar SL. 2006. Effect of eugenol on animal models of nociception. *Indian Journal of Pharmacology*, 38:341.
- Murakami Y, Shoji M, Hanazawa S, Tanaka S, Fujisawa S. 2003. Preventive effect of bis-eugenol, a eugenol ortho dimer, on lipopolysaccharide-stimulated nuclear factor kappa B activation and

- inflammatory cytokine expression in macrophages. *Biochemical pharmacology*, 66:1061-1066.
- Nishijima H, Uchida R, Kameyama K, Kawakami N, Ohkubo T, Kitamura K. 1999. Mechanisms mediating the vasorelaxing action of eugenol, a pungent oil, on rabbit arterial tissue. *The Japanese Journal of Pharmacology*, 79:327-334.
- oztürk A, ozbek H. 2005. The anti-inflammatory activity of *Eugenia caryophyllata* essential oil: an animal model of anti-inflammatory activity. *European Journal of General Medicine*, 2:159-163.
- Park IS, Park SJ, Gil HW, Nam YK, Kim DS. 2011. Anesthetic effects of clove oil and lidocaine-HCl on marine medaka (*Oryzias dancena*). *Lab animal*, 40:45-51.
- Raghavenra H, Diwakr BT, Lokesh BR, Naidu KA. 2006. Eugenol--The active principle from cloves inhibits 5-lipoxygenase activity and leukotriene- C4 in human PMNL cells. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 74:23-27.
- Robbers JE, Tyler VE. 1999. *Tyler's Herbs of choice: the therapeutic use of phytomedicinals*: CRC. Saeed S,
- Tariq P. 2008. In vitro antibacterial activity of clove against Gram negative bacteria. *Pakistan J. Botany*, 40:2157- 2160.
- Sell AB, Carlini EA. 1976. Anesthetic action of methyleugenol and other eugenol derivatives. *Pharmacology*, 14:367-377.
- Sen P, Maiti PC, Puri S, Ray A, Audulov NA, Valdman AV. 1992. Mechanism of anti-stress activity of *Ocimum sanctum* Linn, eugenol and *Tinospora malabarica* in experimental animals. *Indian journal of experimental biology*, 30:592.
- Sepehri G, Shafeiee MN. 2006. Effect of Cuneiformis Nucleus Inactivation by Lidocaine Microinjection on the Analgesic Response of Morphine in Rats. *Iranian Biomedical Journal*, 10:21-26.
- Tyler VE, Brady LR, Robbers JE. 1988. *Pharmacognosy*. Lea & Febriger, Philadelphia, PA. Links.
- Waterstrat PR. 1999. Induction and recovery from anesthesia in channel catfish *Ictalurus punctatus* fingerlings exposed to clove oil. *Journal of the World Aquaculture Society*, 30:250- 255.
- Yang BH, Piao ZG, Kim YB, Lee CH, Lee JK, Park K, Kim JS, Oh SB. 2003. Activation of vanilloid receptor 1 (VR1) by eugenol. *J Dent Res*, 82:781- 785.
- Yu J, Hungju F. 1981. Studies on the essential oils of clove buds and clove leaves. *Zhong Caoyao*, 12:339-342.
- Zhang Y, Chen Y. 1997. Isobiflorin, achromone C-glucoside from cloves (*Eugenia caryophyllata*). *Phytochemistry*, 45:401-403.
- Zheng GQ, Kenney PM, Lam LKT. 1992. Sesquiterpenes from clove (*Eugenia caryophyllata*) as potential anticarcinogenic agents. *Journal of natural products*, 55:999-1003.