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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 inmice 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 cancerogenic effects of the plant and its components have also been reported (Zheng et al., 1992; Zhang and Chen, 1997; Kouidhi 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

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.

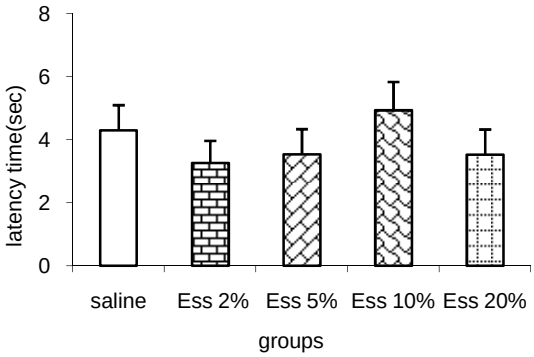

Figure 1. Comparison of basal reaction time between the animals of different groups. Data were shown as mean± SEM (n=10 in each group). There was no significant difference between groups.

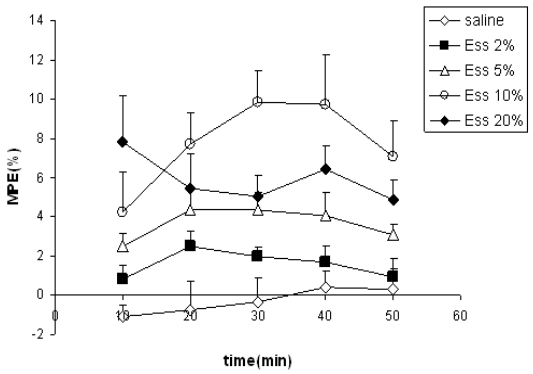

Figure 2. Comparison of MPE between the groups which received different concentrations of essential oil or Saline. Data were shown as mean± SEM (n=10 in each group). Repeated measures ANOVA test showed that MPE in animal groups treated by 5, 10 and 20% essential oil was significantly higher than saline 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-cariophyllene (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.

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