



Clinical effects of eugenol and lidocaine as anesthetic on histopathology and skin wound healing in rabbit

Moosa Javdani, Zahra Nikousefat

Department of Clinical Sciences, School of Veterinary Medicine, Razi University, Kermanshah, Iran

Abstract

Eugenol has been widely used in dental practice to relieve pain arising from a variety of sources. Several beneficial effects of eugenol such as its anti-inflammatory, antioxidant, anesthetic and vascular and muscle relaxant activity are recognized. It seems that there is no scientific study about eugenol effects on wound healing. The present study involved the clinical anesthetic effects of different doses of eugenol (2, 5 and 15%) with a routine anesthetic (lidocaine 20 mg/ml) in rabbits and histological study of their effects on the skin wound healing. The results of current study suggested the use of eugenol in 2 and 5 percentage concentrations for local anesthesia.

Introduction

Eugenol (the chief constituent of clove oil; also obtained from other sources) has been widely used in dental practice to relieve pain arising from a variety of sources, including pulpitis and dentinal hypersensitivity. Other effects of the eugenol such as its anti-inflammatory, antioxidant, anesthetic, vascular and muscle relaxant activity are recognized, but a concomitant irritant effect has been noted (Sell and Carlini, 1976; Ko et al., 1995; Dohi et al., 1991; Damiani et al., 2003). Also, there are some reports that investigated eugenol's anti-stress anticonvulsant and analgesic activities (Dallmeier Zelger et al., 1983; Prakash and Gupta, 2005).

The aim of present study was to compare the clinical anesthetic effects of different doses of eugenol (20, 50 and 100 mg/ml) with a routine anesthetic (lidocaine 20 mg/ml) in the rabbits and the histological study of their effects on the skin wound healing.

Materials and Methods

Eighteen New Zealand white rabbits, weighing 2 to 3 kg, were used in this study. They were randomly separated into three groups. In group one, eugenol 2% (1ml/1cm) subcutaneously were infiltrated on right thoracic paravertebral region of animal. In other groups, eugenol 5% and 15% were used. Lidocaine 2% was also used subcutaneously on left thoracic paravertebral

region of every animal in all groups as control group. Beginning of local anesthesia (absence of response to needle stimulation) and its duration (behavioral reaction of animal to present surgical incision) were recorded. The hair of anesthetic sites was closely shaved with a razor, and the surgical field was disinfected with povidone-iodine. All surgical procedures were performed under aseptic conditions by the same surgeon. A full skin incision of 3 cm in length was performed on the denervated region (eugenol and lidocaine site) and animal reactions were recorded. Then the incision was sutured primarily with non-absorbable suture (NUROLON* Nylon, 4/0 Ethicon, Inc.). In all surgical sites, eugenol and lidocaine (with mentioned dilution) were splashed with needle after suturing on wound site and it was repeated for six days (twice in a day). No postoperative antibiotics were given. Animals were housed in a temperature-controlled and ventilated room after experiments and were given unlimited access to food and water. All rabbits were sacrificed on the 14th day and evaluated for histologic examination of epithelization, edema, vascularity and presence of acute and chronic inflammatory cells. In all animals, the dorsal wounded area was cut into a 5 x 1 cm strip and subjected to histological laboratory. The biopsies were placed in 10% formaldehyde, embedded in paraffin, sectioned perpendicular to the wound and stained with hematoxylin-eosin for histological examination. Histopathologic samples were examined by using light microscopy (20x) and scored by using a

Corresponding author: Zahra Nikousefat, Department of Clinical Sciences, School of Veterinary Medicine, Razi University, P.O Box: 67156-85414, Kermanshah, Iran. Phone number: +988318329540

Table 1: Onset and duration times of local anesthetic effect of different dosage of eugenol

Group stage	Group 1 (mean±SE)	Group 2 (mean±SE)	Group 3 (mean±SE)	Control (mean±SE)
Onset time (second)	140.7±3.63 ^a	88.67±6.93 ^b	91.83±4.35 ^b	91.33±5.08 ^b
Duration time (minute)	53.67±2.72 ^b	80.17±4.18 ^a	84.17±4.65 ^a	86.44±5.40 ^a

^{a,b}The different letters show significant difference in each row (P<0.05)

modified Ehrlich-Hunt numerical scale (Ehrlich et al., 1973). Fibroblast content, collagen deposition vascularity and inflammatory cell infiltration were graded as 0 for absence, 1 for occasional presence and light scattering, 2 for abundance, and 3 for confluence of cells and fibers. Epithelial regeneration was scored as 0 for no epithelium, 1 for single-layer epithelium with partial closure, and 2 for multilayer epithelium with complete closure (Loewen et al., 2001). Parametric data were analyzed by one-way analysis of variance. Differences between groups were analyzed with independent-samples Student's *t*-tests. A level of P<0.05 was deemed to be statistically significant.

Results

Onset time of local anesthesia after SC injection of each drug and its duration of anesthetic effect is summarized in Table 1. There was significant difference between onset and duration of anesthetic time of group A with other dosage of eugenol and lidocaine. The onset time and duration of time were significantly high in the group treated with 2 and 5% eugenol respectively.

The epidermis showed no significant difference in wound healing among of all groups. Mono-nucleated lymphocytes were identical in the incision classified as control and group 1 (Fig. 1&2). The subcutaneous fat showed no significant difference in wound healing. In each sample mild, moderate and severe cellular infiltration were recorded. There was moderate to severe neutrophil accumulation in group 3 and 4 (Fig. 3&4). Secondary infection was recorded in 50 percent of samples of group 3.

Discussion

Natural products consist of compounds that are found in nature and produced by plants, animals or fungi. Molecules originating from nature have been counter parted as an important place in chemistry and in society at large. One this product is eugenol that is isolated from cloves. The Clove tree is a tropical evergreen that develops clusters of flowers. Eugenol is the principal compound responsible for giving cloves their distinctive aroma and taste.

Eugenol, the principle chemical constituent of clove oil, has recently been evaluated for its anesthetic and analgesic properties in fish and amphibians. Eugenol is a major component of root canal sealers and

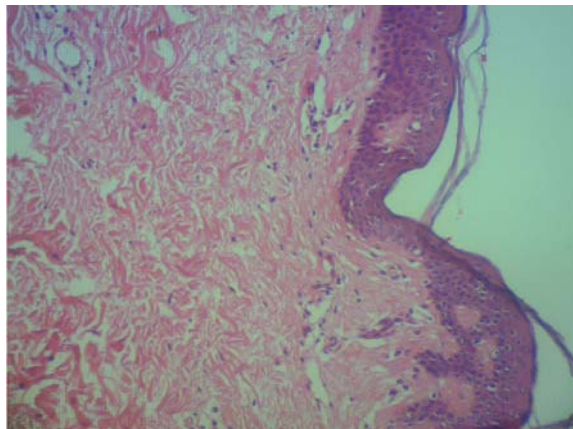


Fig 1: Epithelial regeneration with mild mononuclear cells and collagen deposition (Control)

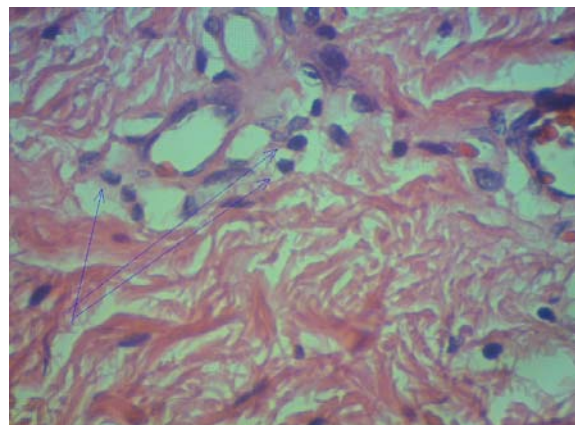


Fig 2: Mild mononuclear cells infiltration (Group 1)

retrograde filling materials in dentistry and has been widely used in dental clinics to relieve pain (Markowitz et al., 1992). Lee and others (2007) suggested that eugenol might be involved in periapical healing by impairing the functions of osteoblasts. Guénette and others (2007) investigated the effect of eugenol for anesthesia of African clawed frogs (*Xenopus laevis*). They showed when eugenol was administered as a single-bath immersion (dose 350 mg/L for 15 minutes, it may serve as an effective anesthetic in *X. laevis* frogs for short surgical procedures.

Eugenol produced a reversible, dose-dependent anesthesia in male Sprague-Dawley rats (Guenette et al., 2006). Leal-Cardoso and others (2002) suggested that eugenol induces relaxation of rat ileum by a direct action on smooth muscle via a mechanism largely independent of alterations of extracellular Ca^{2+} influx.

Also, Damiani et al. (2003) suggested that eugenol produces smooth muscle relaxation resulting from the blockade of both voltage-sensitive and receptor-operated channels that are modulated by endothelial-generated nitric oxide.

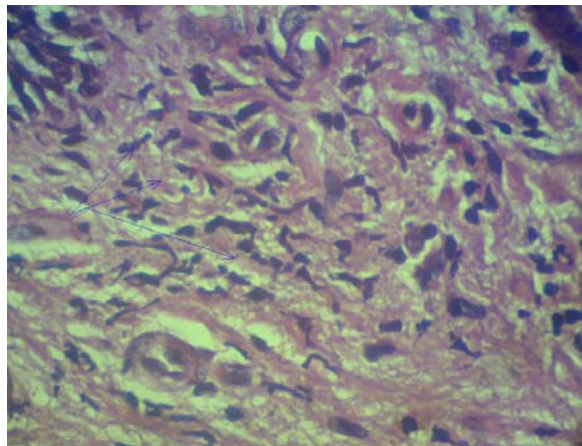


Fig 3: Epithelial regeneration with moderate to severe neutrophil accumulation and collagen deposition (Group 2)

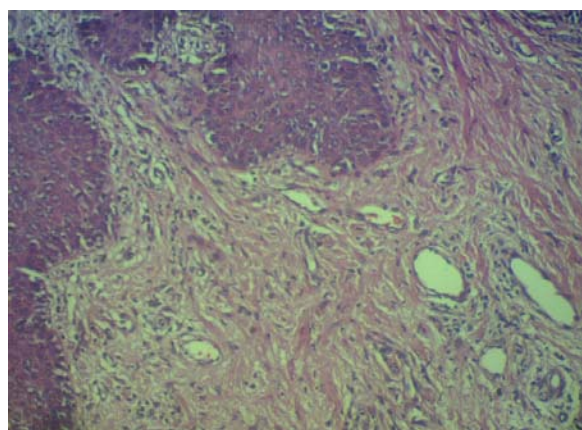


Fig. 4: Epithelial regeneration with moderate to severe neutrophil accumulation and collagen deposition (Group 3)

Eugenol can inhibit polymorphonuclear cell migration and can not induce chemotaxis of PMNs (Fotos et al., 1987). Also, eugenol has potent antiinflammatory and antirheumatic properties (Sharma et al., 1994). According to study of Kurian and others (2006) eugenol exerts antinociceptive activity in different experimental models of pain in mice. 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 indomethacin and other NSAIDs (Kurian et al., 2006). 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 (Brodin, 1985), 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 (Kurian et al., 2006). In cell lines stably expressing human N-type calcium channels, eugenol reportedly inhibited high-voltage-activated calcium currents (Lee et al., 2005).

Intraperitoneal administration of eugenol is also associated with hypothermia in rats and myorelaxation and anticonvulsant effects in mice (Dallmeier et al., 1983). Subcutaneous injection of 50 mg of eugenol daily for 7 days (total dose 1365 mg/kg bw) to partially hepatectomized male, Charles River rats, had no effect on rate of liver regeneration (Gershbein, 1977). In a limited study in mice, eugenol did not potentiate the tumorigenic effects of methylcholanthrene (Hitchcock, 1952).

It seems that there is no scientific study about eugenol effects on wound healing. The results of current study suggest that local anesthetic effects of different concentration of euogenol produce histopathologic changes such as lidocaine, but these changes in wound healing are not statistically significant. Finally, results of current study suggest use of eugenol in 2 and 5 percentage concentrations for local anesthesia.

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