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EFFECTS OF EUGENOL ON ACUTE CORNEAL PAIN OF MALE RATS

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ABSTRACT

Eugenol is extensively used in dentistry as analgesic and anti-inflammatory. Due to its small size and high density of sensory nerves, cornea is one of the most sensitive tissues of the body. Major sensory nerves of the cornea are poly-modal pain nerves, which respond to thermal, mechanical, or chemical stimuli. The aim of this study was to determine effects of the topical eye and intraperitoneal administration of eugenol on acute corneal pain in male rats. In this experimental study, Seventy-two male Wistar rats were randomly divided into 9 groups of 8 as follows: two topical and intraperitoneal control groups that received normal saline via corneal surface and intraperitoneal ways respectively. The positive control group received 3.5 mg/kg morphine. Three groups received eugenol via corneal surface and three groups intraperitoneally at doses of 3, 10, and 30 mg/kg. Forty µl of 5 M sodium chloride was used to induce corneal pain. One hour after drug administration, the number of eye rubbing in 30 seconds was used to measure acute corneal pain. Morphine significantly reduced eye rubbing caused by 5M sodium chloride. Different amounts of topical ocular and intraperitoneal eugenol significantly decreased acute corneal pain. Topical ocular concentration of 10 and intraperitoneal concentration of 30 showed the best analgesic effect. The analgesic effects of eugenol on acute pain of cornea can be considered in future research.

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Introduction

Due to its small size and high density of sensory nerves, cornea is one of the most sensitive tissues of the body. The majority of cornea sensory nerves are pain nerves, so that it is said that the density of its pain receptors is 300-600 times of that of the skin and 20-40 times of that of the dental pulp [1]. Pain nerves of the cornea are poly-modal that respond to thermal, mechanical, and or chemical stimuli [2]. Corneal pain is caused by most problems of cornea such as corneal ulcers, corneal abrasion, corneal dryness, after eye surgery, or excessive use of contact lenses [3]. Since cornea sensory nerves derive from the ophthalmic branch of trigeminal nerve, corneal pain is used to study trigeminal system [4]. It has been shown that neurons in the trigeminal nucleus highly respond to topical use of capsaicin, nicotine, and 5M saline in the corneal surface [5]. Neuropathic pain induced by these materials is shown by rubbing the eyes on the animal's part. Today, rubbing the eyes due to topical administration of 5M saline on the cornea is used as a sensitive animal model to study the mechanisms of acute corneal pain and trigeminal system [6, 7]. It was shown that intraperitoneal administration of Crocin can significantly reduce the acute corneal pain caused by 5M saline [8]. The analgesic effect of topical administration of Vanillosmopsis arborea baker on neuropathic pain induced by 5M saline has been examined in another study [9]. In most patients, eye pain is not controlled well and enough with the existing pain killers. Moreover, topical nonsteroidal anti-inflammatory drugs (NSAIDs) have gradual onset and little impact, and the side effects opioids are well known [9]. Eugenol (4-allyl-2-

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methoxyphenol) is transparent and almost colorless or yellow liquid that is of phenolic drugs and the main constituent of Eugenia caryophylata [10]. Several studies have shown antidepressant [11], anticonvulsants [12], and anti-inflammatory [13] effects of eugenol. Eugenol is widely used as analgesic and anti-inflammatory in dentistry [14], and its analgesic effects have been evaluated on a variety of animal pain models. The results of a study showed that topical injection of eugenol could inhibit both acute and chronic phases of formalin-induced pain [15]. It has been shown that intrathecal administration of eugenol has average onset of effect and prolonged analgesic effect [10]. Intraperitoneal administration of methyl eugenol derived from eugenol led to loss of corneal reflex in rabbits [3].

It has been a long time since when topical medications are used to reduce pain. Analgesic topical medications affect the damage tissues or nerves and control pain. As no studies were found that have examined the effects of eugenol on the corneal acute pain, the aim of this study was to investigate the effects of intraperitoneal injection of eugenol on acute corneal pain caused by 5M hypertonic saline. Moreover, given the priority of the topical administration over intraperitoneal injection, the other purpose of this study was to compare these two methods of administration on acute corneal pain caused by 5m saline in rats.

Materials and Methods

This experimental study was conducted after the approval of committee of ethics and research of Jahrom University of Medical Sciences on 72 male Wistar rats with an average weight of 150 to 170 g. These rats were randomly divided into the following groups: two ocular and intraperitoneal control groups that received normal saline as eye drops and intraperitoneally respectively. The positive control group received 3.5 mg morphine per kilogram of body weight. Three groups received eugenol (Grodab Chemie, Germany) topically via eye and three groups intraperitoneally at doses of 3, 10 and 30 mg per kg body weight. One hour after drug administration, the number of eye rubbing in 30 seconds was used to measure acute corneal pain. To induce acute corneal pain, 5M sodium chloride was used. To do so, one drop (40 micro liter) of 5M saline was poured in the rat cornea and then within 30 seconds, the number of rubbing the eye by the same hand (e.g. rubbing the right eye with the right hand) was counted. Reduction in the number of rubbing was considered as an indicator of analgesia and average rubbing of the eye was statistically analyzed in different groups [7].

The rats that did not receive 5M normal saline drops well were excluded from the study. The volume of administration to determine the topical effects of eugenol was 20 µl per eye that poured into the animals' eyes an hour before acute corneal-pain test animals [9]. To determine the effects of intraperitoneal injection of eugenol, half an hour before acute corneal-pain test, different specified values with constant volume of 0.5 ml were injected.

Statistical analysis:

Values were reported as mean ± SEM, and $p<0.05$ was considered as significant. One-way ANOVA test and back up Duncan test were used for comparison of the groups.

Results

According to the table 1and figure 1, the number of eye rubbing significantly decreased in morphine group compared to the control group ($p=0.000$). Comparison of 3 mg ocular eugenol group with the control group showed a significant reduction ($p= 0.001$), but compared to the morphine group increased significantly ($p=0.006$). Ocular administration of 10 mg of eugenol compared with the control group showed significant reduction ($p = 0.000$), on the other hand, showed no significant difference compared to the morphine group (Fig. 1). Prescription of eugenol eye drops as 30 mg showed significant reduction compared to control group ($p = 0.003$) and a significant increase compared to the morphine group ($p = 0.002$) (Fig. 1).

Comparison of 3 mg eugenol intraperitoneal group with the control group showed a significant reduction ($p = 0.009$), but showed significant increase compared to the morphine group ($p = 0.001$) (Figure 1). Intraperitoneal administration of 10 mg of eugenol compared with the control group showed significant reduction ($p=0.000$) and showed a significant increase compared to the morphine group ($p=0.013$) (Figure 1). Intraperitoneal injection of 30 mg of eugenol showed a significant decrease compared to the control group ($p=0.000$), while no significant change compared to the morphine group (Figure 1). Eugenol eye-drop administration with a concentration of 10 mg per kg of body weight showed the best analgesic effect. The best analgesic effect of intraperitoneal injection of eugenol was in 30 mg per kg body weight (Figure 1). There was no significant difference between identical concentrations of eugenol in both types of injection (Figure 1).

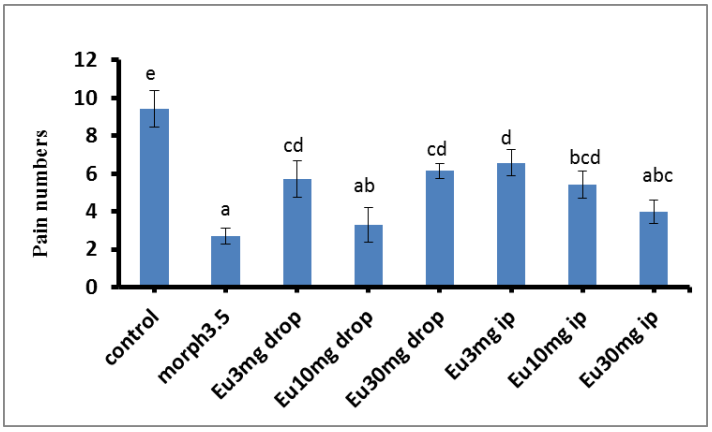

Figure 1: Pain numbers in different groups. Groups that do not have a common language have a significant difference at the level of $p < 0.05$.

Table 1: Pain numbers in different groups (Mean±SEM).

Groups	Mean±SEM
control	9.43±0.97
morph3.5	2.71±0.42
Eu3mg drop	5.71±0.97
Eu10mg drop	3.29±0.92
Eu30mg drop	6.14±0.4
Eu3mg ip	6.57±0.69
Eu10mg ip	5.43±0.72
Eu30mg ip	4±0.62

Discussion

According to the results of this study, the acute corneal pain due to 5M normal saline significantly reduced by eugenol administration in form of eye drops and intraperitoneal administration, and the best concentration for topical ocular administration was 10 mg / kg and 30 mg / kg in intraperitoneal administration.

Most corneal pain sensory fibers are poly-modal activated by mechanical, thermal, and chemical stimulators [2]. Corneal pain is transferred by primary afferent fibers to the posterior horn of the medulla oblongata, particularly trigeminal caudate nucleus. Conscious perception of pain needs transmission of neural activity to the higher centers of the brain. Ascending pain transmission primarily occurs through the thalamus path or para-brachial nucleus. Painful corneal surface stimulation stimulates the neuron cells in the rostral pole of caudate nucleus including para-brachial neurons that receive direct input of afferents of the cornea. The ascending output of this area is sent to the brain areas involved in emotional and haemostatics responses [16].

Many studies have examined the analgesic effect of eugenol on different types of pain, such as acute and chronic pain caused by formalin [15, 17], pain caused by heat [10], neuropathic pain [18], osteoarthritis [19]. Some studies have been done on anesthetic effects of eugenol and its derivatives showing that these effects are comparable with other local anesthetics such as lidocaine in some cases [20, 21].

In one study, it was found that oral and intraperitoneal administration of eugenol exert their analgesic effects by stimulating the opioid system and inhibition of glutamate receptors (such as the kainate and AMPA) and inhibition of TNF-α [14]. The analgesic effect of eugenol may be excreted through opioid receptors and adrenergic α_2, but it has been shown that serotonergic receptors (5-HT) do not have a role in it [22]. It is found that eugenol can affect primary sensory neurons in the ganglia, dorsal root of the spinal cord, so that through inhibition of voltage-dependent sodium channel, in fact, it inhibits the entry of pain to the central nervous system [23]. Moreover, the analgesic effect of methyl eugenol in the second phase of the pain caused by formaldehyde may be due to inhibition of N-Methyl-D-aspartic Acid (NMDA) receptors [17]. GABA A receptor can also modulate pain as a target molecule for eugenol [24]. It is suggested that eugenol and guaiacol apply their analgesic effects through capsaicin receptors located on sensory terminals in the spinal cord [25]. Intrathecal injection of eugenol in the dorsal horn of the spinal cord, where the vanilloid receptors are, could reduce neuropathic pain, allodynia, and

hyperalgesia [18]. Local anesthetic and analgesic effect of methyl eugenol that is of derivatives of eugenol has been shown. It seems that these effects are applied by inhibition of the peripheral sodium channels [26].

According to the studies that have been conducted in the past on the analgesic effect of eugenol, it can be concluded that, all or part of the mechanism mentioned can apply the analgesic effect of eugenol in controlling acute corneal pain by requiring more study.

Due to fewer side effects of topical vs. intraperitoneal administration on the one hand, and the need for less concentration of eugenol to achieve the best analgesic effect in topical administration on the other hand, it seems that this method of administration is preferred.

As intraperitoneal administration of normal saline had no effects on acute pain of the cornea caused by 5M saline (In fact, it had no difference with the control group), this group was not entered in charts and tables.

Moreover, based on the reference of previous studies, it was supposed to use 7 mg / kg of morphine for positive control [9]. Since after intraperitoneal injection of this amount, amount of analgesia was zero, 3.5 mg/kg dose was used.

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