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The Study of Antinociceptive Effect of Hydroalcoholic Extract of *Teucrium oliverianum* (A Plant Use in Southern Iranian Traditional Medicine) in Rat by Formalin Test

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Abstract: Objective: Antinociceptive and anti-inflammatory activities of hydroalcoholic extract of *Teucrium Oliverianum* were investigated by formalin test model. This study was conducted in on the male Wistar rats, weighting 150-180 g. The animals were divided into seven groups (n = 7) and recieved 200, 400, 600 and 800 mg kg^{-1} of hydroalcoholic extract of teucrium oliverianum intraperitoneally, respectively. Negative control group received normal saline (5 mL kg^{-1}) and the positive control groups received 2.5 mg kg^{-1} morphine and 300 mg kg^{-1} aspirin, intraperitoneally respectively. The results showed that all doses of extract have significant analgesic effect ($p<0.05$) in all studies times in comparison with negative control. The best result achieved with 600 mg kg^{-1} of extract. The result revealed that the analgesic effect of the extract (600 mg kg^{-1}) \was less than aspirin (300 mg kg^{-1}) on the second phase of pain and less than morphine (2.5 mg kg^{-1}) in both phases of the pain, more than aspirin in first phase of pain. One group of animals was treated with naloxone (1 mg kg^{-1}, i.p.) and suitable dose of extract (600 mg kg^{-1}, i.p.). Also, Naloxone inhibited analgesic effect of alcoholic extract of Teucrium Oliverianum. It can be concluded that the alcoholic extract of *Teucrium oliverianum* may exert its effect through opioid receptors, stimulating GABAergic system or promotes the release of endogenous opipeptides or decreasing free radicals.

Key words: *Teucrium oliverianum*, antinociceptive, Formalin test, rat

INTRODUCTION

Researching on plants which had been used as analgesics in traditional medicine still be seen as a fruitful and logical research area in search for new pain killers with low and possibly no side effects (Khalid *et al*., 2010). Pain is a bad and an unpleasant sense that almost everyone experiences during his/her life span and it is the reason that why scientists have been investigating the cure for this problem. A large amount of self-medications could be seen around the world (Sarahroodi and Arzi, 2009; Sarahroodi *et al*., 2010) and a lot of them are made by traditional or herbal medicine (Arzi *et al*., 2001; Sarahroodi *et al*., 2009) Overall, plants have been employed for many medical issues as well as pain relieving in Iranian and world history. Due to the emerging side effects of synthetic medicines, many studies have been conducted to find the plant derived potential medicinal compounds (Chevallier, 1996; Karim *et al*., 2011; Sohail *et al*., 2011a, b; Sohail and Sohail, 2011).

Teucrium oliverianum which belongs to the family Lamiaceae; usually grows in Mediterranean areas. This plant could be found in east south of Iran in Khoozestan province (Ahwaz, Susangerd, Hamidiah, Haft tappeh, Ramhormoz, Aqajari, Masjed Soleiman, Baghmalek and Behbahan). Various related species of this plant have been studied but there is no study on *Teucrium olivevianum.* Its aqueous extract possess potential antioxidant activity (Ljubuncic *et al*., 2006). Essential Oil and Methanol Extract of *Teucrium montanum* have antimicrobial Activities (Vukovic *et al*., 2007). Also the hydroalcoholic extract of *Teucrium polium* is a potent

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analgesic that could be an appropriate substitute for Hyosine and Indomethacin (Kabouche *et al*., 2007).

Different spices of *Teucrium* contains some components such as monoterpens, diterpens, stroles, saponines, Iridoids, poly phenols, flavnoids, alkaloids and volatile oils.

The aim of the present study was to investigate the antinociceptive properties of the hydroalcoholic extract of *Teucrium oliverianum* by using formalin test.

MATERIALS AND METHODS

Animals: Experiments were performed on 56 male Wistar rats weighting (150-180 g). Animals housed at 23±2°C and 40-50% humidity, under a 12: 12 h light-dark cycle, with free access to chow (prepaired from Khorakdam Co. Teharn) and tap water.

Formalin test: Rats were placed in an open Plexiglas observation chamber for 30 min to acclimate with their surroundings. Formaline (50 μL, 2.5%)was injected subcutaneously into the dorsal surface of the right hind paw 30 min after intraperitoneal(i.p.) injection of hydro alcoholic extract of *Teucrium oliverianum* (200, 400,600 and 800 mg kg^{-1}) , normal saline (5 mL kg^{-1}), morphine (2.5 mg kg^{-1}) and Aspirin (300 mg kg^{-1}). Finally one group received intraperitoneal injection of hydroalcoholic extract of *Teucrium oliverianum* (600 mg kg^{-1}) and Naloxon (1 mg kg^{-1}), after 30 min of formaline injection.

The animals were returned to the observation chamber.

Rats were observed from 0 to 5 min (first phase of pain) and from 15 to 60 min (second phase of pain) and a nociception score was determined for each period by giving score to injected limb during the observation time.

The first phase has one 5 min block that is divided to 20 of fifteen second parts, so each rat was studied for 20 times in this phase. But the second phase has 9 five min blocks and in each block rat was studied for 20 times.

Assessment: If rat can walk without any problem on its injected paw, its score will be zero. If it cannot put its injected paw on surface normally, its score will be 1, If the rat cannot put its injected paw on the surface and walks on its other paw, its score was 2 and at last if the animal has paw-jerk or it licks its injected paw, its score will be 3 (Dubuisson and Dennis, 1977).

Data analysis: Data are presented as Means±SEM of measurements made on seven animals in each group. Comparisons across three or more treatments were made using one-way ANOVA and Tukey's post hoc test or repeated measures two-way ANOVA with Bonferroni's post hoc test, when appropriate. Statistical differences were considered to be significant at $p<0.05$.

RESULTS

The antinociceptive effect of intraperitoneal injection of hydroalcoholic extract of *Teucrium oliverianum* (200, 600 and 800 mg kg^{-1}) was significantly ($p<0.05$) more than normal saline in both first and second phase.

This antinociceptive effect is dose dependent but there was no significant deference between 600 and 800 mg kg^{-1} of extract, so the 600 mg kg^{-1} of extract was selected as the most effective dose (Fig. 1).

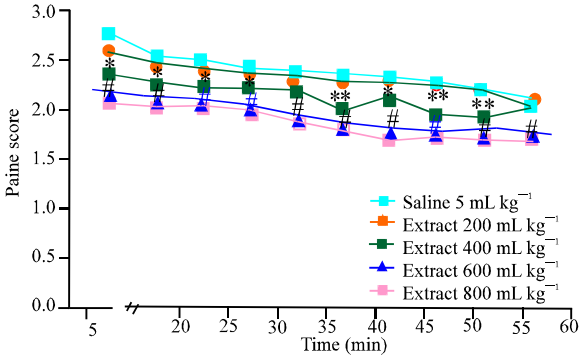

Fig. 1: Comparison of antinociceptive (phase 1 and 2) effect of *Teucrium oliverianum* extracts and normal saline in rats. Significant differences between extracts and normal saline are shown as *p<0.05, **p<0.01, #p<0.00. Data are Mean±SEM of seven mice

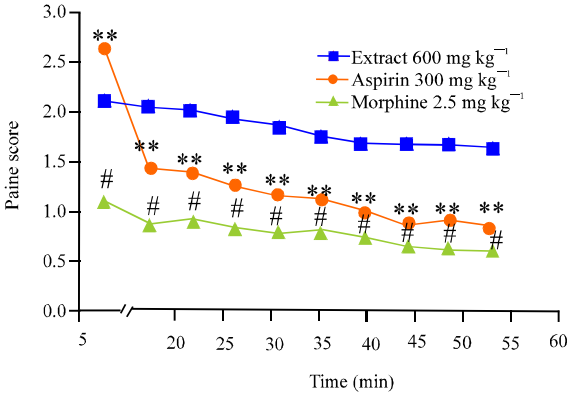

Fig. 2: Comparison of antinociceptive effect of Aspirin, morphin and *Teucrium oliverianum* extract (600 mg kg^{-1}) in first (0-5 min) and second (15-60 min) phase of pain. Significant differences between Aspirin and Morphine with Teucrium Oliverianum extract are shown as **p<0.01, #p<0.001. Data are Mean±SEM of seven mice

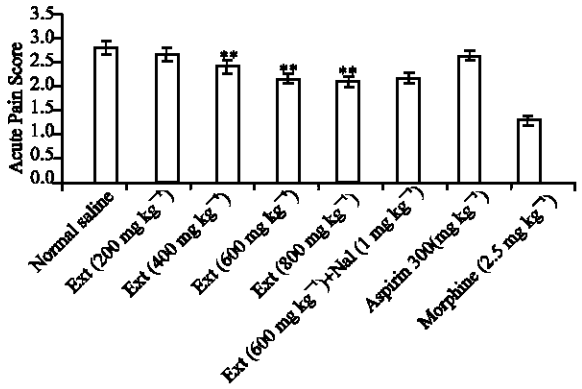

Fig. 3: Comparison of antinociceptive effect of *Teucrium oliverianum* extract, *Teucrium oliverianum* extract mg kg^{-1} plus Naloxan, morphine and aspisin with normal saline in first phase of pain (0-5 min) in rats, Significant differences between normal saline with *Teucrium oliverianum* extract are shown as **p<0.01. Data are Mean±SEM of seven mice

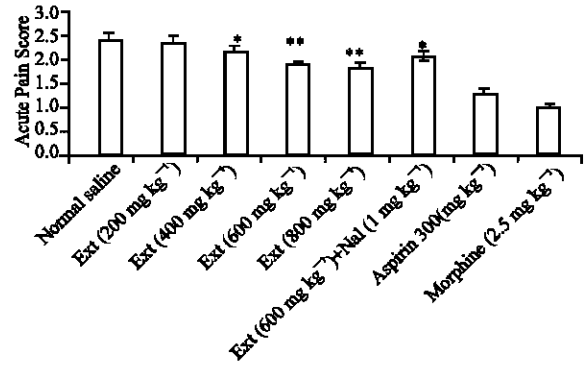

Fig. 4: Comparison of antinociceptive effect of *Teucrium oliverianum* extract, *Teucrium oliverianum* extract plus Naloxan, morphine and aspirin with normal saline in second phase of pain (15-60 min) in rats, Significant differences between normal saline with *Teucrium oliverianum* extract are shown as *p<0.05, **p<0.01. Data are Mean±SEM of seven mice

The antinociceptive effect of *Teucrium oliverionum* (600 mg kg^{-1}) was siginficantly ($p<0.001$) less than morphine (2.5 mg kg^{-1}) in both first and second phases of pain.

On the other hand Asprin showed its antinociceptive effect in second phase and for morphine it was for both first and second phase (Fig. 2) of pain. Also it's antinociceptive effect was significantly more than Asprine (300 mg kg^{-1}) ($p>0.01$) in first phase and in second phase it was significantly less($p<0.01$) than Asprine (Fig. 2).

The intraperitoneal injection of *Teucrium oliverianum* (600 mg kg^{-1}) and Naloxon (1 mg kg^{-1}) showed that Naloxon significantly ($p<0.05$) decrease antinociceptive effect of the plant extract in first (Fig. 3) and second phases of pain (Fig. 4).

DISCUSSION

One of the well known models of persistent pain is formalin test. Subcutaneous injection of formalin induces pain in both first and second phases. The first phase is cause of receptor response in rat's paw and the second phase induces by local irritation (Shibata *et al*., 1989). Central acting analgesic agents inhibit both phases of pain but non-central acting agents only inhibit the second phase (Shibata *et al*., 1989).

The first phase of pain which generates by activation of nociceptive neurons in periphery by direct action of formalin, is associated with neurogenic pain, while second phase that occurs with activation of ventral horn neurons associates with an inflammatory pain (Tjolsen *et al*., 1992; Leal *et al*., 2000). Pharmacological studies have suggested that intraperitoneal Injection of morphine (2.5-10 mg kg^{-1}) inhibits both first and second phase of pain in formalin test although that the same administration rout of Aspirin (300-400 mg kg^{-1}) only inhibits second phase of pain (Hunskaar *et al*., 1985). The other study by Hunskaar and Hole (1987) revealed that ip injection of Aspirin inhibits both phases of pain in mice in formalin test. This could because of prostaglandin inhibition in CNS or increasing serotonin although that it may because of down regulation in 5HT2 receptors, that can centrally reduces the pain. On the other hand reduction of irritation in irritated location can reduce peripheral pain.

Teucrium oliverianum decreases first and second phase of pain. This antinociceptive effect could be due to Borneol (monoterpen component) by inhibition of cholinesterase and GABA-A stimulating by increasing the release of GABA (Lynch, 2004; Almeida *et al*., 2001).

The antiinflamative and antinociceptive effect of *Teucrium oliverianum* in both phases could be cause of Squtel Larin component. The antinociceptive effect of Squtel larin is mediated by opioid, cholinergic and dopaminergic receptors. On the other hand this effect is cause of intracellular increase of calcium ion by inhibition of Ca/ATPase in reticulum sarcoplasmic and inhibition of voltage dependent Ca channels that inhibits Ca ion entrance to presynaptic spaces (Granger *et al*., 2005).

Also poly phenols and flavenoids that are present in this plant are antioxidants which could reduce the pain by decreasing free radicals concentration (Peana *et al*., 2006). Neochlordane and diterpenoids which exist in extract of Teucrium oliverianum are analgesic components too. Diterpenoids show their antinociceptive effect by stimulating of κ receptors, although that some of them are partial agonists of opioid receptors (Coll and Tandron, 2004). In nutshell it can be said that the alcoholic extract of *Teucrium oliverianum* may exert its effect through opioid receptors, stimulating GABAergic system or promotes the release of endogenous opipeptides or decreasing free radicals.

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