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Evaluation of analgesic effect of hydroalcoholic extract of cardamom fruit in male rat

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Abstract: Due to significance of pain management and adverse effects of synthetic analgesic drugs, the use of herbal medicine for pain relief has gained popularity worldwide. This study investigated the analgesic effects of hydroalcoholic extract of cardamom fruit in male rats. In this experimental-laboratory study, male Wistar rats were divided into 7 groups (6 rats in each group): control group (treated with normal saline) and the groups treated with 100, 200 and 400 mg/kg of hydroalcoholic extract of cardamom fruit, control group receiving morphine (1 mg/kg), control group receiving naloxone (2 mg/kg), and a group receiving "naloxone (2 mg/kg) + extract (200 mg/kg)". Tail flick and writhing tests were used to evaluate the analgesic effects of the extract. Data were analyzed using one-way ANOVA. 200 and 400 mg/kg of hydroalcoholic extract of cardamom fruit significantly reduced pain level compared to the control group in tail flick test and all three doses of the extract (100, 200 and 400 mg/kg) significantly reduced pain level in writhing test. From the results, it can be concluded that the hydroalcoholic extract of green cardamom fruit has analgesic effects and can reduce the sensation of pain caused by central and environmental factors. The use of green cardamom extract as an analgesic substance can be considered by researchers in the clinical fields.

Keywords: Green cardamom fruit, Pain, Male rat

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1 Introduction

Although the feeling of pain has always been considered as an unpleasant feeling by human, but it is one of the most important physical feelings that has an extraordinary role in preventing physiological and pathological injuries. Pain feeling results from a variety of stimuli, including mechanical, thermal, and chemical stimuli. Various environmental and genetic factors have a significant effect on the severity of pain (Lin et al., 2020; Tanveer et al., 2020).

Due to significance of pain management and adverse effects of synthetic analgesic drugs, the use of herbal medicine for pain relief has gained popularity worldwide. In this regard, green cardamom plant has been studied in terms of analgesic properties. The green cardamom plant with the scientific name, *Elettaria cardamomum*, is a perennial and evergreen herbaceous plant of the ginger family (Zingiberaceae). Cardamom is a small fruit about the size of a finger with dark skin and fragrant seeds. There are different types including black cardamom, white cardamom and green cardamom, and the green type has a sharper aroma. Green cardamom fruit extract is rich in starch, protein, waxes, estradiol and vitamin E. In traditional medicine, cardamom has warming and energizing properties and is involved in protecting the gastrointestinal tract, controlling cholesterol, relieving pain caused by cardiovascular problems, and relieving depression and improving blood flow (Asghar et al., 2021; Li et al., 2021), which indicates the soothing effects of cardamom. Studies show that green cardamom can also have analgesic effects. In this regard, seeds and oils of green cardamom can reduce gum pain (Alam et al., 2019). Also, studies have shown that dark cardamom plants can be effective in relieving pain by reducing stress (Ahmed et al., 2000; Shanmugam et al., 2011). Research findings indicate significant effects of cardamom plant extracts in the treatment of pain caused by osteoarthritis (Altman and Marcussen, 2001). Furthermore, dark cardamom plant extracts are impressive in reducing menstrual cramps and muscle aches (Black et al., 2010; Darwish et al., 2013; Nejat et al., 2017; Nicoll and Henein, 2009). Contrary to the research findings that show the soothing and beneficial effects of dark cardamom plants on pain, some research results have shown that dark cardamom plants do not have significant effects on pain treatment and can even have side effects. In this regard, other research findings have shown that oral consumption of cardamom species plants can have side effects such as increased bleeding and heartbeat, although this occurs when cardamom extract is used over time and in high concentrations in experimental and clinical samples (Fatemeh et al., 2017; Kafeshani, 2015). According to importance of recognition pain reasons and analgesic factors in order to find ways to relieve pain with less side effects and also considering that pathological pains have many complications (Lin et al., 2020; Tanveer et al., 2020) and regarding the results of previous research about analgesic effects of cardamom species plants (Alam et al., 2019; Kafeshani, 2015; Shanmugam et al., 2011) and considering that few studies have been carried out to study the analgesic effects of green cardamom fruit extract, the present study investigated the analgesic effects of hydroalcoholic extract of green cardamom fruit in male Wistar rat.

2 Materials and Methods

In this experimental-laboratory study, male Wistar rats weighing 200-250 g were used. The animals were prepared from Pasteur Institute of Iran and kept in standard conditions with suitable humidity and the light period (12 hours of light and 12 hours of darkness) and temperature conditions of 22±2 °C. The rats had free access to water and food and were kept in standard cages. This study was carried out fully according to the ethical guidelines of the International Association for the Study of Pain (IASP).

Cardamom fruit was prepared from Avicenna Medicinal Plants Center of Jihad Agricultural Organization of Hamadan Province after approval by the experts. The samples were kept in shade for 2 to 3 days and were dried completely. The samples were then pulverized with an electric mill. To preparing the extract with the desired concentration, 8 gr of dried extract was poured into 20 ml of distilled water twice and completely stirred by an electric mixer. This solution had a concentration of 400 mg per ml and other required doses were prepared from this solution. After filtering the solutions with a special syringe, they were injected to rats intraperitoneally. Based on other studies of researchers (Rhamanian et al., 2019) animals were divided into 7 groups (n = 6/ each group): the control group (normal saline recipient) and the groups treated with 100, 200 and 400 mg/kg of hydroalcoholic extract of cardamom fruit, morphine receiving group (1 mg/kg) (as positive control group and pain reliever) and naloxone receiving group (2 mg/kg) (as negative control and pain enhancer). In addition, in order to evaluate the anti-naloxone effect of the extract, a group was treated with "naloxone (2 mg/kg) + extract (200 mg/kg)".

In order to evaluate the analgesic effects of the extract, tail flick and writhing tests were used. Tail-flick test was performed to evaluate the analgesic activity of drugs and extracts using the TF-5500. In this test, the level of analgesia was measured based on the duration of the delay in the reaction of the tail to the heat-damaging tissue. During this test, an intense light beam is focused on the animal's tail and a timer starts. When the animal flicks its tail, the timer stops and the recorded time (delay or latency) is a measure of the pain threshold. The duration of delay in flicking the tail was measured three times and two minutes before the injection of normal saline, drug or extract. Then, twenty minutes after the injection, the delay time was measured three times during two minutes. Writhing test was performed by injecting acetic acid as a chemical stimulant of pain and to evaluate the environmental analgesic activity of drugs and extracts. During this test first the animals were placed in a standard glass box for 30 minutes to adjust to the environment. 15 minutes after injection, the number of abdominal contractions was recorded. The mean number of abdominal contractions was compared between groups.

For analyzing the data, $SPSS_{20}$ software was used. Kolmogorov-Smirnov test was used to determine the normality of the data. Statistical differences between groups were analyzed using one-way ANOVA and Tukey post hoc test. The results were presented as mean ± SEM and *P*-value less than 0.05 was considered as a significant level of statistical differences.

3 Results and Discussions

The results of tail-flick showed that at the time before drug or extract injection, the groups did not show significant difference in pain thresold. Normal saline injection did not have any effect on delay time. Morphine injection significantly increased the delay time ($p < 0.001$) and naloxone injection significantly reduced the delay time ($p < 0.01$). Injection of 100, 200 and 400 mg/kg of the extract caused a significant increase in delay time compared to the control group ($p < 0.01$, $p < 0.001$ and $p < 0.001$, respectively). On the other hand, the delay time increased with enhancing the concentration of the extract. Injection of 200 mg/ kg of the extract prevented the analgesic effect of naloxone and did not cause a significant change in delay time compared to the control group, but it was longer than the group receiving naloxone ($p < 0.01$) (Figure 1).

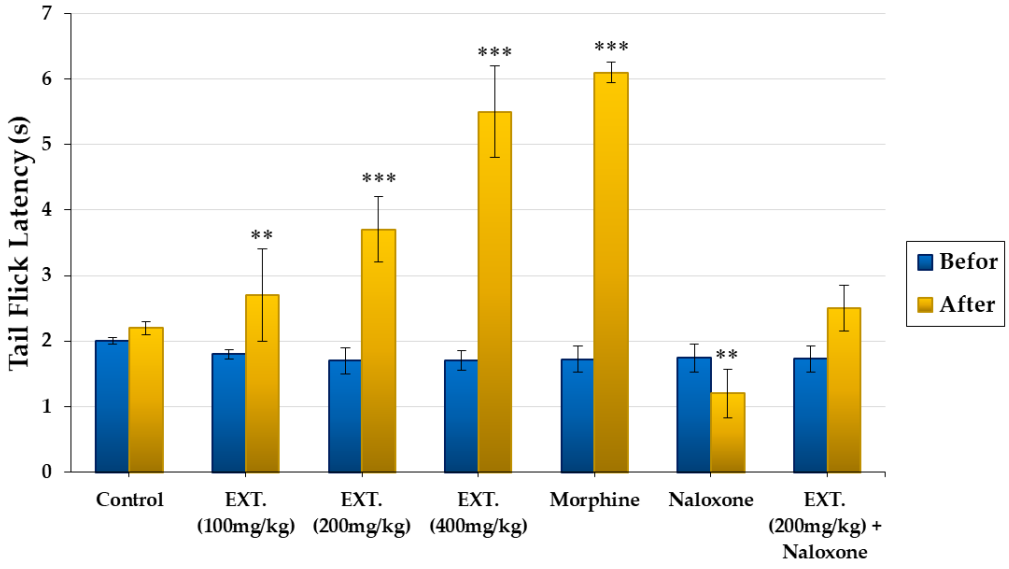

Figure 1: Results of tail-flick test in control and experimental groups. * indicates a significant difference compared to the control group (**: $p < 0.01$, ***: $p < 0.001$).

The results of the writhing test showed that normal saline injection had no significant effect on the number of writhing. Morphine injection caused a significant decrease ($p < 0.001$) and naloxone injection significantly increased the number of writhing ($p < 0.01$). Injection of 100, 200 and 400 mg/ ml of the extract caused a significant decrease in the number of writhing compared to the control group ($p < 0.001$). On the other hand, the number of writhing decreased with increasing the concentration of the extract. Co-injection of the extract (200 mg/kg) and naloxone prevented the analgesic effect of naloxone and reduced the pain threshold compared to control group (Figure 2).

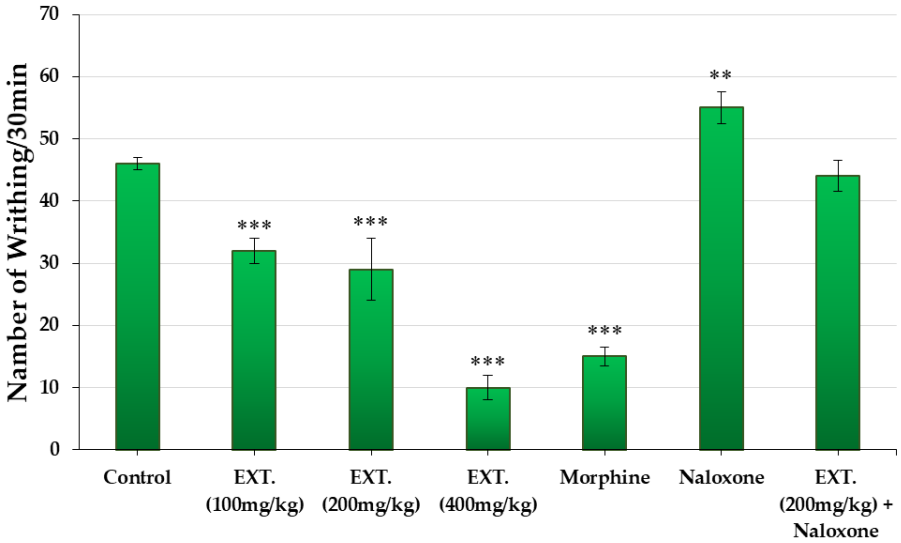

Figure 2: Results of writhing test in control and experimental groups. * indicates a significant difference compared to the control group (**: $P < 0.01$, ***: $P < 0.001$).

The study of pain and analgesic effects of herbal substances and compounds has been discussed in many aspects. Although some researchers emphasize the beneficial and soothing effects of some plant extracts (Ahmed et al., 2000; Altman and Marcussen, 2001; Shanmugam et al., 2011), there are researchers that concerned about the unknown or known side effects of plant extracts that can induce adverse effects (Fatemeh et al., 2017; Kafeshani, 2015). The analgesic effects of plant extracts were evaluated by means of tail-flick test to assess the central analgesic activity of the extract (Rhamanian et al., 2019) and pain evaluation by writhing test was evaluated in order to determine the environmental analgesic activity of the extract (Al-Zuhair et al., 1996). The results of both tail-flick and writhing test showed that the extract of green cardamom fruit has analgesic effects and this analgesic effect. In addition, cardamom fruit extract has both central analgesic and environmental analgesic effects. Other studies show the analgesic effects of cardamom species plants extracts, although experimental and laboratory studies on the analgesic effects of green cardamom extract are very limited. Some cardamom species plants can reduce the level of oxidative stress (Shanmugam et al., 2011) therefore have soothing effects. A study was performed in order to comparing novafen analgesic with cardamom herbal extract and the results showed that this extract can have analgesic effect on menstrual pains same as novafen (Rad et al., 2018). Research findings have also shown that consumption of some cardamom species plants can be effective in reducing muscle pain (Black et al., 2010). Investigation of the resin fraction of green cardamom shows that this fraction has antiseptic effects (Arpitha et al., 2019), therefore this drug can have possible analgesic effects. Furthermore, pharmacological study of green cardamom oil showed that green cardamom oil extract can significantly reduce the number of writhing test and in this regard has significant analgesic effects (Al-Zuhair et al., 1996). Research on the analgesic effects of hydroalcoholic extract of green cardamom seed through formalin test and tail-flick test showed that green cardamom seed has analgesic effects on acute and chronic pain (Rhamanian et al., 2019). The results of clinical trial studies also show that the seeds and oils of green cardamom can reduce gum pain (Alam et al., 2019). Although studies have focused on the analgesic effects of green cardamom extract, some studies have shown that cardamom species plants do not have

significant analgesic effects and may even they have side effects. In this regard, research findings have shown that oral consumption of cardamom species plants high concentrations can have significant side effects (Fatemeh et al., 2017; Kafeshani, 2015).

Green cardamom is rich in flavonoids to exerts its analgesic effects by reducing prostaglandin mediators. In fact, flavonoids reduce the activity of intracellular calcium by inhibiting the activity of NMDA receptors and thereby can reduce the activity of nitric oxide synthase enzyme and phospholipase A2 resulting in reduced NO and prostaglandin levels followed by a reduction in pain perseption (Rauf et al., 2016; Xue et al., 2019; Zakaria et al., 2018). The pain-relieving effects of green cardamom extract can be from the cineole found in the cardamom extract. Cineole has anti-inflammatory and analgesic properties (Santos and Rao, 2000). Green cardamom also contains chemical compounds including carbohydrates, proteins, minerals, lipids, essential oils, flavonoids, terpenoids and carotenoids which may contribute to analgesic effects of the extract (Alam et al., 2019); however, more studies are required to clarify the exact analgesic pathway involved in the extract effect on the pain threshold.

4 Conclusion

The results of this study showed that the extract of green cardamom fruit has analgesic effects. Considering that the results of tail-flick test and writhing test both showed the analgesic effects of green cardamom fruit extract, therefore it can be concluded that green cardamom fruit extract has its analgesic effects in both environmental and central nervous system pathways.

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Conflict of interests

The authors declare that there are no conflict of interests regarding publication of this paper.

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