

Research Article

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Effect of Hydroalcoholic Extract of Hyssop on Acute Pain in Male Rats Using Tail Flick Test

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com**Abstract****Background:** Iranian traditional medicine uses hyssop (*Hyssopus officinalis*) as an effective medicinal plant to reduce pain and inflammation in different diseases. Although the anti-inflammatory effect of this plant is proved, there is no study into its analgesic effects. Thus, this study aimed to investigate the analgesic effect of the hydroalcoholic extract from hyssop flowers and upper branches.**Methods:** This experimental study was conducted on 66 male rats that were divided into several groups including a saline control group, the groups of different doses of hyssop extract, morphine positive control group, the groups of hyssop extract plus morphine, and the most effective dose of the hyssop extract plus naloxone. All injections were administered intraperitoneally, and the pain was measured through the tail flick test.**Results:** Based on the results, 600 mg/kg was the most effective analgesic hyssop extract dose, and the most analgesic effect was observed at 45 minutes after administration. In addition, the administration of the most effective extract dose (600 mg/kg) plus morphine significantly improved the analgesic effects of morphine ($P < 0.001$). Finally, the administration of naloxone plus the most effective extract dose (600 mg/kg) significantly reduced the analgesic effect of the extract ($P < 0.05$).**Conclusion:** Overall, the hydroalcoholic extract of hyssop has analgesic effects that are probably applied through opioid receptors.**Keywords:** Hyssop, Pain, Tail flick test, Rat

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Introduction

Pain, as an unpleasant feeling and mental experience, is related to actual or potential tissue damage. In fact, pain is a warning sign by which patients and physicians notice a disorder in a part of the body (1,2). Two groups of analgesics are currently prescribed for pain relief. The first group of analgesics includes the non-steroidal anti-inflammatory medications, which are associated with major, specifically gastrointestinal, side effects. Opioid analgesics can result in nausea, constipation, and dependence (3). In addition to chemical drugs, medicinal plants are used for pain relief and occasionally yield a more favorable response. Hyssop (*Hyssopus officinalis*) is an herbaceous perennial plant belonging to the *Lamiaceae* family. It is a wild aromatic plant rich in essential oils and native to the eastern and southern regions of Europe, Turkey, Iran, and the Caucasus. Flowers and upper branches are the usable parts of the plant (4). The most important active substances of hyssop, which were analyzed in other studies, are chlorogenic acid, rosmarinic acid (5), caffeic

acid, methyl eugenol (6), and ferulic acid (7). Hyssop has been used in Iranian traditional medicine for reducing inflammation, specifically in the upper respiratory tract (8). Liver protective (10), anti-diabetic (11), and antioxidative effects (12) have been proven in addition to its antimicrobial effects (9). Regarding the importance of hyssop in traditional medicine and the lack of study into its analgesic effects, the present research intended to evaluate the analgesic effects of the hydroalcoholic extract of hyssop on male rats using the tail flick test.

Materials and Methods**Animals**

In this study, 66 male Wistar rats (180-250 g) were purchased from the Pasteur Institute of Iran and maintained under standard animal room conditions of 12/12 hours of light/darkness, the temperature of 22 ± 1 °C, and relative humidity of 50%-55%. The samples were supplied with specific food and water *ad libitum* and kept in metal cages. They further were accustomed to laboratory conditions



for at least 2 hours before the test, which was conducted according to the ethical guidelines of the International Association for the Study of Pain in Laboratory Animals (13). The present research project was approved by the Local Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1395.277). All experiments were performed according to the National Institutes of Health Publication (No. #85-23, revised in 1985) and the Declaration of Helsinki (last update: 2008) guidelines.

Experimental Groups

This experimental study was performed on 11 groups of male rats ($n=6$, each). The control and morphine (standard group) groups received normal saline (0.90% w/v of NaCl) as a vehicle and morphine intraperitoneally at a dose of 2.5 mg/kg, respectively. Hyssop groups received the extract intraperitoneally in different doses of 100, 200, 400, and 600 mg/kg. Moreover, hyssop plus morphine groups received the hyssop extract (100, 200, 400, and 600 mg/kg) following the administration of morphine (2.5 mg/kg), and finally, the group that received the most effective dose of the extract with naloxone (1 mg/kg).

Extract Preparation

A total of 3 kg hyssop was collected from the Avicenna Herb Garden in Hamadan in early summer 2017 and then approved by a botanical specialist in Tehran University. The herbal specimen was deposited in the herbarium (voucher No.: PMP-1328). After separating other plant parts, flowers and upper branches were dried under the shade at room temperature (25°C) and grounded using an electronical mill. Then, the dried powder of flowers and upper branches (100 g) was added to 70% ethanol (1 L) and maintained for 72 hours to extract its active compounds (14). Next, the solution was placed on a rotary evaporator to separate the solvent and then placed in a Petri dish under a safety cabinet to dry it out. After one week, the remaining extract was dissolved in an appropriate amount of physiological serum (0.9% sodium chloride).

Gas Chromatography and Mass Spectrometry Analysis

The composition of the applied *H. officinalis* aerial part hydroalcoholic extract in this study was determined by Gas chromatography and Mass spectrometry (GC-MS) (15,16).

Tail Flick Test

This test is performed using the tail flick device (LE7106 Harvard apparatus, USA). The test was conducted based on the study by D'Amour and Smith (17), and the tail flick latency time was measured in each group 15, 30, 45, 60, 90, 120, and 180 minutes after the last injection. The maximum cutoff time was 10 seconds for applying the Guidelines for Ethical Conduct in the Care and Use of non-Human Animals in Research. In other words, the emission

of the light beam was stopped to avoid tissue damage if the rat did not flick its tail after 10 seconds. Each animal was horizontally placed in a specific cage and one-third of its tail was subjected to the heating beam. After a while and the reduction of the anxiety of the animal, a light beam was emitted to the subject's tail and the tail flick latency time was recorded accordingly. Then, the sample was allowed to rest for five minutes, and the test was repeated for the second and third times. The mean tail flick latency time of all three replications was regarded as the latency time.

Statistical Analysis

Data were presented as the mean \pm SD and one-way ANOVA and the Tukey's comparison test were used for multiple comparisons using SPSS-19. In this study, $P < 0.05$ was considered statistically significant (18).

Results

In this experimental model, no fatalities were recorded 72 hours after the oral administration of various doses of the *H. officinalis* hydroalcoholic extract.

The screening of the chemical composition of the hydroalcoholic extract of *Hyssopus officinalis* aerial part revealed the presence of 20 constituents (Table 1).

According to the results of this study, the analgesic effects of the extract depend on the dose. The strongest and weakest analgesic effects were observed after 45 and 180 minutes, respectively. The latency time in the 600 mg/kg hyssop group was significantly longer compared to the 400 mg/kg group ($P < 0.05$). As a result, 600 mg/kg hyssop was the most effective dose (Figure 1). The lowest dose of the extract (100 mg/kg) had a significantly stronger analgesic effect in comparison with the control 45 minutes after the injection ($P < 0.001$). Based on data in Figure 2, using this extract dose plus morphine dramatically increased the

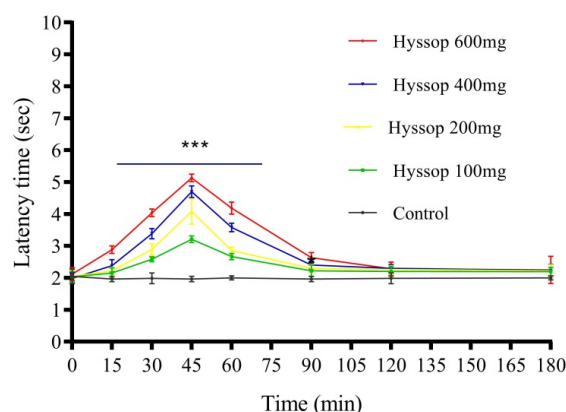


Figure 1. Comparison of the Analgesic Effect of Different Extract Doses Before and After Injection.

Note. Data are reported as the mean and standard deviation. The latency time of 15 minutes before the injection of each analgesic was regarded as the baseline response. $***P < 0.05$ in comparison between the extract doses of 400 and 600 mg/kg. Control: Negative control group (normal saline recipients).

Table 1. Chemical Composition of the *Hyssopus officinalis* Aerial Part Hydroalcoholic Extract

Phytochemical Components	Scan No.	Amount (%)	Retention Time (min)
1,8- Cineole	38	5.5	27.23
Germacrene	46	7.7	30.94
Cyclofenchene	55	4.3	30.87
Cnicin	75	1.8	5.64
p-Cymene	114	2.4	33.18
1,4-Cineole	123	2.9	29.07
Camphor	135	7.9	18.01
1,8-Cineole	144	7.4	18.33
Octahydro-7a-methyl- 1Hinden- 1-one	164	6.1	28.92
5-Methylspiro[3.5]- nonan-1-one	175	3.9	16.81
7a-Methyl-1,2,3,3a,4,7ahexahydro-5H-inden-5-one	184	3.9	33.97
Borneol	197	5.8	18.89
Perillyl aldehyde	211	4.6	29.46
Myrtenyl acetate	219	13.3	19.01
Dehydro/β-ionone	225	4.5	18.63
Cyclohexanol-2-methylene-3- (1-isopropyl) acetate [sic]	231	7.3	20.97
Bornyl acetate	243	1.7	11.63
Ascaridol	252	2.4	22.85
Nerolidol	265	4.1	30.97
Unidentified components	298	2.5	23.82 25.87

analgesic effect of morphine ($P < 0.001$).

No significant difference was observed in the latency time between the most effective extract dose group and morphine group (2.5 mg/kg) at different times before and after the injection (Figure 3).

There was a significant difference ($P < 0.05$) in the latency time between the 600 mg/kg hyssop group and the 600 mg/kg hyssop plus 1 mg/kg naloxone group at different times after the injection (Figure 4).

Discussion

In summary, the results indicated the effectiveness of the hydroalcoholic extract of hyssop in reducing pain. Based on the findings, all doses (100, 200, 400, and 600 mg/kg) of the hyssop extract were more effective compared to the control in controlling pain. In addition, the analgesic effects of hyssop increased by increasing the dose, and 600 mg/kg hyssop had the same analgesic effect as morphine. The administration of naloxone plus 600 mg/kg hyssop reduced the analgesic effects of the extract as compared to the group receiving the extract alone. In general, similar to morphine, as an analgesic compound, the hydroalcoholic extract of hyssop inhibited the response to pain in the tail flick test. As a result, this extract could probably exert its analgesic effects by affecting opioid receptors. Moreover, the reduced analgesic effect of this extract in combination with naloxone, as an opioid antagonist, showed the opioid mechanism of the compounds of the extract. The inhibitory effects of the extract can affect ligand-sensitive

channels through binding to pain receptors and blocking the voltage-gated calcium channels at the end of the presynaptic neuron. This mechanism reduces the release

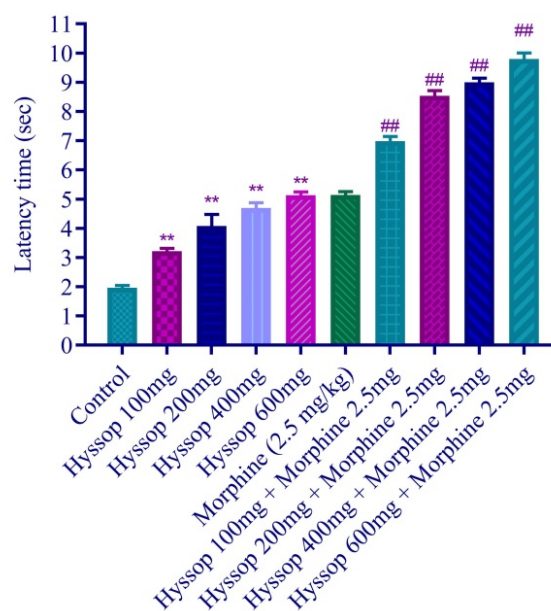


Figure 2. Comparison of the Analgesic Effect of Different Study Groups 45 Minutes After Injection.

Note. Data are reported as the mean and standard deviation. ** $P < 0.001$ compared to the control, ## $P < 0.001$ in comparison to morphine alone. Control: Negative control group (normal saline group).

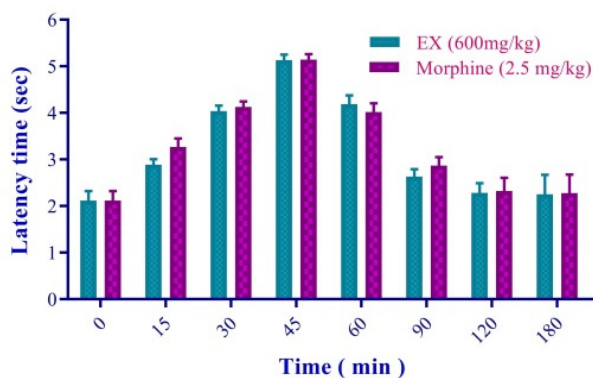


Figure 3. Comparison of the Analgesic Effect of the Most Effective Extract Dose (600 mg/kg) With 2.5 mg/kg Morphine at Different Times Before and After Injection.

Note. Data are reported as the mean and standard deviation. The latency time of 15 minutes before the injection of each analgesic was regarded as the baseline response.

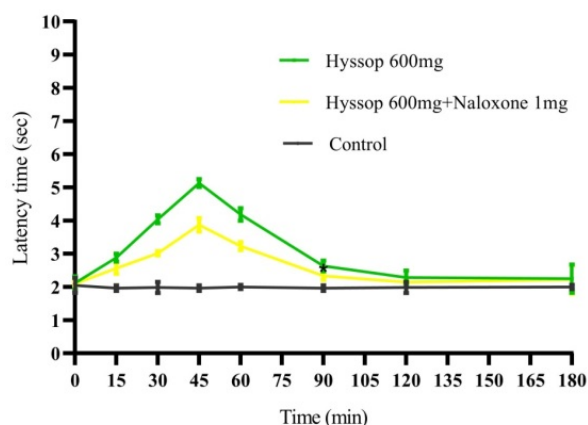


Figure 4. Comparison of the Analgesic Effect of the 600 mg/kg Hyssop Group With the 600 mg/kg Hyssop Plus the Naloxone Group.

Note. Data are reported as the mean and standard deviation. The latency time of 15 minutes before the injection of each analgesic was regarded as the baseline response. $***P < 0.05$ in comparison with the 600 mg/kg hyssop group and the 600 mg/kg hyssop plus the naloxone group.

of neurotransmitters and/or opens potassium channels, thereby causing hyperpolarization and presynaptic neuron inhibition (19).

The analgesic effects of the hyssop extract can be attributed to its compounds including flavonoids. There are many hypotheses about the mechanism of the analgesic effects of hyssop compounds. Studies on opioid and flavonoid receptors revealed their similarities. Thus, flavonoids can produce analgesic effects through binding to opioid receptors (20). The findings of another study demonstrated that ondansetron (serotonergic antagonists), haloperidol (dopaminergic antagonists), and yohimbine (adrenergic antagonist) could not inhibit the analgesic effects of flavonoids (21). It indicates the synergic role of these systems in producing analgesic effects. However,

the reasons for the analgesic effect of flavonoids are controversial.

Conclusion

Overall, the hydroalcoholic extract of hyssop has analgesic effects and can inhibit acute pain in male rats. Although the exact mechanism of hyssop effects continues to remain unknown, literature results attributed them to the flavonoid content of this plant. Finally, it is hoped that further studies pave the way for a better understanding of the effects and mechanism of the hydroalcoholic extract of hyssop, allowing for using this extract as an adjunctive therapy in addition to the routine analgesics, especially morphine, and/or alone for pain relief.

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Conflict of Interests

The authors declare that they have no conflict of interests.

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