

# Acute/Chronic Pain Relief: Is *Althaea officinalis* Essential Oil Effective?

Yosef Golshani,<sup>1</sup> Mohammad Zarei,<sup>2,\*</sup> and Saeed Mohammadi<sup>3,\*</sup>

<sup>1</sup>Department of Biology, Payam-Noor University, Hamadan Branch, Hamadan, IR Iran

<sup>2</sup>Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, IR Iran

<sup>3</sup>Department of Biology, Hamadan Branch, Islamic Azad University, Hamadan, IR Iran

\*Corresponding authors: Saeed Mohammadi, Department of Biology, Hamadan Branch, Islamic Azad University, Hamadan, IR Iran. Tel: +98-9187134858, Fax: +98-8138380464, E-mail: smiauhphd.sm@gmail.com; Mohammad Zarei, Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, IR Iran. Tel: +98-8138380462, Fax: +98-8138380464, E-mail: zarei@umsha.ac.ir

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## Abstract

**Background:** The *Althaea officinalis* (marshmallow) plant is traditionally used to treat skin burns and constipation and to reduce inflammation.

**Objectives:** The aim of this study was to evaluate the acute and chronic analgesic effects of the essential oil of *Althaea officinalis* leaves (EOAO) in adult male mice.

**Materials and Methods:** This experimental study used thirty-six adult male mice, which were randomly divided into six groups: a control, three groups treated with EOAO (18, 38, or 80 mg/kg, i.p.), a morphine group (1 mg/kg, i.p.), and a group treated with a combination of naloxone (1 mg/kg, i.p.) and 80 mg/kg EOAO. The analgesic effects of EOAO were evaluated by writhing, tail-flick, and formalin tests. The essential oil of the plant was prepared by the steady distillation method and its composition was analyzed by GC/MS.

**Results:** Significant antinociceptive effects were noted with doses of 38 and 80 mg/kg EOAO in the chronic phase response of the formalin test ( $P < 0.05$  versus control). Doses of 38 and 80 mg/kg EOAO had a significant analgesic effect in the writhing test ( $P < 0.05$  and  $P < 0.01$ , respectively, versus control). The 80 mg/kg dose of EOAO caused an enhancement in reaction time in the tail-flick test ( $P < 0.01$  versus control).

**Conclusions:** EOAO showed an analgesic effect that may involve both the central and peripheral nervous systems.

**Keywords:** Medicinal Herb, Pain, Essential Oil, Mouse, *Althaea officinalis*

## 1. Background

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or is described in terms of this type of damage (1, 2). Pain treatment is an important medical practice and the use of medicinal plants in pain relief has a long history. The use of plant-derived drugs, herbal remedies, and natural products has been increasing in recent years (3), so that 25% of the drugs in the world pharmaceutical market are now apparently derived from plants. This increased use is particularly evident in developing countries, which account for about 80% of the world population, according to the world health organization (WHO). Synthetic drugs are limited in availability in developing countries due to their high cost and minimal access, so the most common treatments are derived from medicinal plants. In recent years, these availability factors, coupled with the potential adverse side effects of synthetic drugs, have promoted much research into species of plants that have known positive effects as treatments for many human diseases (4, 5).

One highly utilized medicinal plant is *Althaea officinalis*

(marshmallow), an annual of the Malvaceae family (6) native to Asia, America, and Europe. The most important ingredients in this plant are pectin (7), starch (8), mucilage (9), and compounds such as coumarin (10), phytosterols, tannins, amino acids, and asparagine (11).

Marshmallow is used in traditional medicine to treat inflammation, skin burns, diarrhea, constipation, and abscesses (12). The polysaccharide in the plant induces human monocytes to produce a cytokine (interleukin-6) and tumor necrosis factor (TNF), which have anti-inflammatory activity and stimulate the immune system (13). The polysaccharide also displays antiviral (14), antimicrobial, and anti-fungal (15) properties. The antidiabetic (16) and antioxidant (17) activities of marshmallow have also been confirmed by modern medicine.

One unexplored facet of the medicinal use of marshmallow is the therapeutic value of its essential oil. Essential oils are biological and chemically active agents (18); therefore, the essential oil of *Althaea officinalis* leaves might also induce effects that could have potential therapeutic uses.

## 2. Objectives

Marshmallow has anti-inflammatory effects and pain is always associated with inflammation processes. However, the current scientific literature contains no information regarding the possible analgesic effects of essential oils of *Althaea officinalis* leaves. The aim of this study was therefore to use adult male mice to investigate the acute toxicity of the essential oil of *Althaea officinalis* leaves (EOAO) and its analgesic effects in the formalin, writhing, and tail-flick tests.

## 3. Materials and Methods

### 3.1. Plant Material Collection

In April 2015, two kg of fresh *Althaea officinalis* leaves were collected and botanically confirmed by the botanist at Bu-Ali Sina University of Hamadan. The EOAO was extracted from freshly chopped plant leaves by steam distillation and analyzed as previously described (19). Briefly, freshly plant leaves were placed in a glass flask that was connected at one end to a glass vessel filled with water and at the other end to a water-cooled condenser. The water was heated to boiling, and the steam percolated through the chopped leaves and collected in the condenser. After condensation, the aqueous portion of the essential oil with its solutes was separated from the oil portion. The composition of EOAO used in this study was determined by gas chromatography and mass spectrometry. It contained flavonoid compounds such as isoquercetin, kaempferol, coumaric acid, ferulic acid, vanillic acid, and a tannin compound identified as scopoletin (Table 1).

### 3.2. Design of Animal Experiments

Thirty-six male mice were purchased from the Pasteur Institute of Iran and kept under controlled room conditions of a 12 hour light/12 hour dark photoperiod and a temperature of  $22 \pm 1^\circ\text{C}$ . Animals were kept in steel cages with free access to food and water. At least 2 hours prior to experiments, animals were acclimated to the laboratory conditions. The experiments were conducted daily between the hours of 8 am and 12 pm. The study was approved by the research council of Payam-Noor university of Hamadan and was conducted in accordance with the ethical guidelines of the international association for the study of pain (19).

### 3.3. Drug Administration

The animals were randomly divided into six groups: a control group (receiving saline), three essential oil groups receiving low, medium, and high doses of EOAO (18, 38, and 80 mg/kg, respectively), a morphine group (morphine,

**Table 1.** Phytochemical Analysis of *Althaea officinalis* Essential oil

Major Components of Essential Oil	Values <sup>a</sup>
Pectins	10
Palmitic acid	7
Heptacosane	3.3
Nonacosane	7.2
Palmitic acid	6.8
Linoleic acid (omega-6)	18.0
Naphthalene decahydro 2,6-dimethyl	16.4
Hypolaetin-8-glucoside	1.5
Isoquercetin	5.4
Kaempferol	6.1
Caffeic acid	0.8
P-coumaric acid	1.6
5 $\beta$ , 13 $\beta$ -dihydroxynacosanylglodoleate	1.9
N-triacotanic acid	2.1
Tannins	5.5
Asparagine	7.1
Phytosterols	1.1
Coumarins, scopoletin	3

<sup>a</sup>Values are expressed as percentage.

1mg/kg), and an EOAO plus naloxone group (naloxone, 1mg/kg, and essential oil, 80 mg/kg). All injections were administered intraperitoneally.

### 3.4. Pain Tests

**Writhing Test:** On the experimental day, 30 minutes before running the experiments, the animals were placed in a standard experiment glass box for acclimatization. The essential oil was dissolved in a specific volume of sterile saline and injected. After 15 minutes, acetic acid (10 ml/kg, density 0.6%) was injected and the number of abdominal contractions (both legs stretched) was immediately counted for 30 minutes. Each animal was used only once. The writhing test in the control group was conducted after the injection of saline (i.p.) (20).

**Tail-flick test:** This experimental pain test used the TF-5500 model tail-flick device manufactured by Borj-e Sanat company, Iran. The test was conducted according to the previously presented pattern (21). The light intensity was equal to seven degrees (as displayed by the device) and the light exposure time was ten seconds (as the reference and the definite light times). In other words, if the animal did not pull its tail after ten seconds of exposure to the burning light radiation, the stimulus was stopped to prevent tissue

damage. The animal was placed horizontally in a special box and its tail was free. The time delay in pulling the tail was measured three times at two-minute intervals, before and 20 minutes after the injection of drugs/essential oils. The mean times were recorded and considered as time delays before and after treatment.

**Formalin test:** This test evaluates central and peripheral pain, based on the proposed model by Dubuisson and Dennis (22). The animals were placed in a special formalin test box one hour prior to the test for acclimatization to the conditions of the experiment. The movements of the animal were observed by placing a mirror at a 45° angle under the animal and opposite the observer. Thirty minutes after the i.p. injection of the drugs, 50  $\mu$ L of 2.5% formalin solution was subcutaneously injected into the right paw and the animal's behavior was scored for 60 minutes. Every 15 seconds, a motor response to pain was scored from zero to three as follows: Zero, when the animal could walk and maintain perfect balance with its weight distributed on both feet; one, when the animal could not bear its weight on the infected foot or favored it; two, when the animal lifted the painful paw and had no contact with the chamber floor; and three, when the animal licked, chewed, or violently shook the painful paw. The average scores of the first five minutes (acute phase) and 15 to 60 minutes (chronic phase) of each test were considered as the first and second phases of the formalin test, respectively (22).

### 3.5. Determination of Acute Lethal Dose

The acute toxicity was determined based on a previous experimental model (23). Various doses of the EOAO (18, 38, and 80 mg/kg) were injected into individual mice. The mortality rate of the mice was determined 72 hours after injection and the acute lethal dose (LD50) of the EOAO was calculated.

### 3.6. Drugs

Morphine sulfate, naloxone, acetic acid, and formaldehyde were obtained from Merck Germany.

### 3.7. Data Analysis

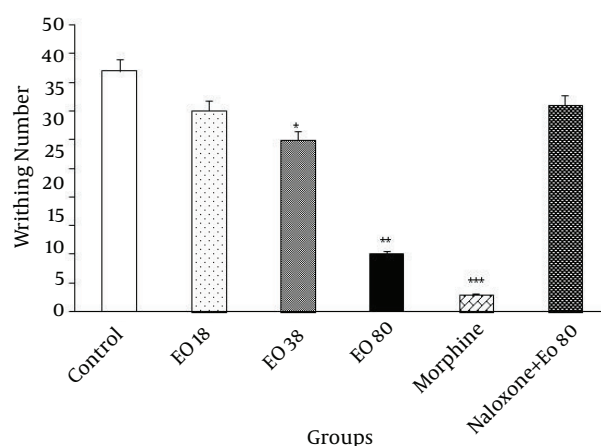
All data were expressed as mean  $\pm$  SEM. One way analysis of variance, followed by Tukey's post hoc test, was used for analysis of data, and  $P < 0.05$  was considered statistically significant.

## 4. Results

The EOAO showed no acute toxicity until doses of 600 mg/kg. Injection of doses of 38 and 80 mg/kg of the EOAO significantly reduced the number of writhing responses

when compared with the control group ( $P < 0.05$  and  $P < 0.01$ , respectively), whereas the 18 mg/kg dose of the EOAO had no effect. The number of writhing responses was also significantly reduced in the morphine group when compared with the control group ( $P < 0.001$ ). Injection of naloxone plus a dose of 80 mg/kg EOAO reduced the analgesic effects of the EOAO (Figure 1).

**Figure 1.** Effect of Different Doses of Essential Oil of *Althaea officinalis* Leaves (EOAO) on Acetic Acid Induced Writhing in Mice



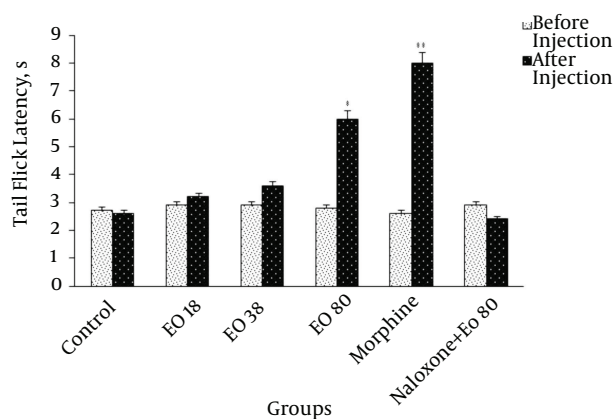
Injection doses of 38 and 80 mg/kg EOAO caused a significant reduction in the number of writhing responses when compared with control mice ( $P < 0.05$  and  $P < 0.01$ , respectively). A significant reduction was also observed in the number of writhing responses of morphine-treated mice when compared with control mice ( $P < 0.001$ ). As compared with control mice: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , ( $n = 6$ , mean  $\pm$  SEM).

In the tail flick test, the 80 mg/kg dose of the EOAO significantly enhanced the reaction time when compared with the control group ( $P < 0.05$ ), but the 18 and 38 mg/kg doses of the EOAO had no significant effect. Morphine showed a strong analgesic effect ( $P < 0.01$ ). Injection of naloxone plus an 80 mg/kg dose of the EOAO reduced the analgesic effects of the EOAO (Figure 2).

The formalin test had two pain phases: acute and chronic. The EOAO doses of 38 and 80 mg/kg significantly reduced pain in the chronic phase ( $P < 0.05$ ) but had no effect on the acute phase. Injection of the EOAO at 18 mg/kg did not result in any pain reduction in either the acute or chronic phases. Morphine injection significantly reduced pain in both the acute and chronic phases when compared with the control group ( $P < 0.01$ ). Injection of naloxone plus an 80 mg/kg dose of the EOAO reduced the analgesic effects of the EOAO (Figure 3).

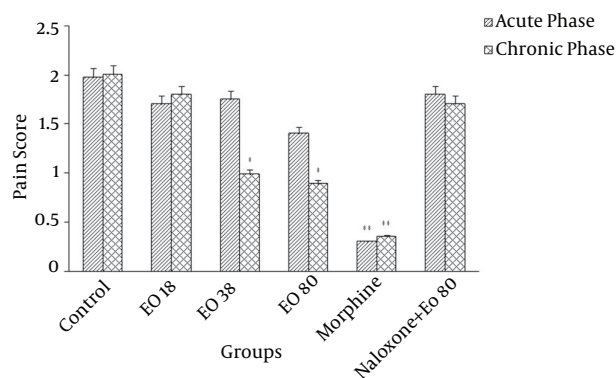
The control group was the metric for all comparisons. No between-group comparisons were performed.

**Figure 2.** Effect of Different Doses of Essential Oil of *Althaea officinalis* Leaves (EOAO) on the Reaction Time of Mice



An 80 mg/kg dose of EOAO caused a significant enhancement in the reaction time when compared with control mice ( $P < 0.05$ ). A significant enhancement was also seen in morphine-treated mice when compared with control mice ( $P < 0.01$ ). As compared with control mice: \* $P < 0.05$ , \*\* $P < 0.01$ , ( $n = 6$ , mean  $\pm$  SEM).

**Figure 3.** Effect of Different Doses of Essential Oil of *Althaea officinalis* Leaves (EOAO) on Formalin-Induced Nociception in Mice



Doses of 38 and 80 mg/kg EOAO significantly reduced pain in the chronic phase ( $P < 0.05$ ). Morphine injection significantly decreased pain in both acute and chronic phases when compared with control mice ( $P < 0.01$ ). As compared with control mice: \* $P < 0.05$ , \*\* $P < 0.01$ , ( $n = 6$ , mean  $\pm$  SEM).

## 5. Discussion

Medicinal plants are an important source of chemicals that have useful therapeutic effects (24, 25). The results of this study confirm the analgesic effects of the EOAO.

The writhing test is used to identify environmental pain triggers. The acetic acid used in this test can lead to activation of endogenous compounds, such as bradykinin, histamine, and substance P. Injection of the EOAO prevented the abdominal contractions induced by acetic acid and significantly reduced the number of writhing responses when compared with the control (Figure 1). One

possibility is that the analgesic essential oil inhibits the release of arachidonic acid metabolites, and guessed that the environmental effects of its management are supported by mechanisms (26).

The tail flick test is a specific test that evaluates analgesic effects on the spinal reflex, a central nervous system response. The 80 mg/kg dose of EOAO caused a significant enhancement in the latency time when compared with the control. Narcotic drugs, such as morphine and pethidine, can increase the latency time of withdrawal of the tail in the tail flick test (27, 28). In other words, the EOAO acted similar to morphine in inhibiting pain in the tail flick test. The EOAO is likely affecting the opioid receptors, particularly the  $\mu$  receptor, to exert its analgesic effect. The inhibitory effects of EOAO may involve its binding to pain receptors and sensitizing of ligand channels and voltage-dependent calcium ion channels at the end of the presynaptic neurons. These, in turn, would release neurotransmitters, thereby reducing the closure and opening of the channels, leading to hyperpolarization and inhibition of post-synaptic neuronal potassium flux (29).

The formalin test evaluates the analgesic effects on environmental (acute) and central (chronic) pain. Drugs such as morphine affect the central nervous system and inhibit both acute and chronic pain, whereas drugs such as aspirin only inhibit the peripheral pain of the chronic phase (30, 31). The EOAO doses of 38 and 80 mg/kg significantly reduced pain in the chronic phase.

Previous phytochemical studies have confirmed the presence of compounds such as flavonoids, hypolaetin 8-glucoside, isoquercetin, kaempferol, caffeic acid, coumaric acid, ferulic acid, vanillic acid (32), and tannins (10) in EOAO. Earlier papers also reported that flavonoids have an analgesic effect (33-35) due to inhibition of cyclooxygenase activity and the release of nitric oxide (36). Various flavonoids, such as kaempferol, are known to exert anti-inflammatory and anti-nociceptive effects (25, 37). Some results have indicated that inhibition of the activity of the N-methyl-D-aspartate receptor by flavonoids reduces intracellular calcium levels. Consequently, this decreases the activity of the calcium-related enzyme that synthesizes nitric oxide and of phospholipase A2. The resulting reduction in nitric oxide and prostaglandins, and especially prostaglandins  $E_2$  and  $F_2\alpha$ , result in the observed antinociceptive effects (38, 39). Other research indicates that a specific component of the tannins in *Althaea officinalis* could play a role in the analgesic and anti-inflammatory effects, as the analgesic effect of essential oils is also considered to be due to the tannins in them (40).

In summary, marshmallow leaf essential oil appears to have analgesic properties and therefore could be a good alternative to synthetic analgesic drugs. The flavonoids

and tannins in the EOAO were most probably responsible for the pain reduction by activating specific neural pathways. Identifying the precise mechanisms underlying the observed pain relief will require further research.

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## Footnotes

**Authors' Contribution:** Yosef Golshani designed and conducted this study. Saeed Mohammadi analyzed and interpreted the data and helped to write the manuscript. All authors read and approved the final manuscript.

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