

# In Vivo Antinociceptive Effects of Persian Shallot (*Allium hirtifolium*) in Male Rat

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**Background:** The tendency toward using herbal plants instead of synthetic drugs is increasing in recent years because of their lower adverse effects and high varieties of efficient components.

**Objectives:** In this investigation, analgesic effects of hydroalcoholic leaf extract of *Allium hirtifolium* were studied in male rats.

**Materials and Methods:** A total of 48 adult male rats were divided into eight groups: control, intraperitoneal 50, 100, and 200 mg/kg of extract, 200 mg/kg of *A. hirtifolium* extract plus aspirin, aspirin (1 mg/kg), morphine (1 mg/kg), and 200 mg/kg of *A. hirtifolium* extract plus naloxone (1 mg/kg). The analgesic effects of *A. hirtifolium* were assessed with writhing, tail-flick, and formalin tests. The data were compared with control by one-way ANOVA and Tukey post hoc test.

**Results:** *Allium hirtifolium* extract at (200 mg/kg dosage), alone and in combination with aspirin, had shown antinociceptive effect in writhing, tail-flick, and formalin tests ( $P < 0.01$ ). *Allium hirtifolium* extract (at 100 mg/kg dosage) had just shown analgesic effects on tail-flick and formalin (chronic phase) tests.

**Conclusions:** It looks that *A. hirtifolium* has antinociceptive effects that might be due to flavonoids and saponins in plant the analgesic effect of which was demonstrated previously.

**Keywords:** *Allium hirtifolium*; Formaldehyde; Saponins; Flavonoids

## 1. Background

Pain is a serious challenge in medicine and a somatic sensation such as touch, pressure, and proprioception, which has an important protective role in avoiding or treatment of actual or potential tissue damages. American Pain Society has reported that about 50 million people of different ages in the United States are affected by pain, which costs more than 25 million dollar to control. Today, nonsteroidal anti-inflammatory or opioid drugs are mostly used to control pain, but these drugs have a many adverse effects and cause gastrointestinal disorders, renal damages, and dependence. Therefore, people look for new drugs that have fewer adverse effects and are cheaper and easily available (1).

The herbal drugs have been used to treat pain and inflammation in Iranian traditional medicine; however, in most cases the origins and mechanisms of these herbal drugs are unknown. Evaluation of the pharmacologic effects of pure extracts of these plants can be used as a logical research strategy to find new drugs (2, 3).

Shallot, with the scientific name of *Allium hirtifolium*, is a perennial native herb in Iran, which belongs to the Liliaceae family and the *Allium* genus. This genus includes over 500 species. *Allium hirtifolium* is 80 to 120 cm high and has lanceolate leaves. The chive of *A. hirtifolium* has a storage tissue, and is used as a food flavoring worldwide (4). Plants belonging to the *Allium* genus are highly effective in the treatment of diabetes, arthritis, colds, stress, fever, headache, asthma, atherosclerosis, and inflammatory diseases (5-10). Recently, antioxidant, antifungal, antibacterial, anticancer, and anti-radiation characteristics of *A. hirtifolium* have been demonstrated (11-14).

The extract of *A. hirtifolium* is used to treat rheumatism and inflammation in traditional medicine (15). Considering the anti-inflammatory effects of *A. hirtifolium* in traditional medicine, the close relation of inflammatory processes with pain, and lack of studies on analgesic effects of *A. hirtifolium* in major databases, a study to assess its effects on pain was needed.

## 2. Objectives

This study aims to investigate the antinociceptive effects of hydroalcoholic extract of *A. hirtifolium* leaf using formalin, writhing, and tail-flick tests.

## 3. Materials and Methods

### 3.1. Preparation of Extract

In August 2013, 1 kg of fresh *A. hirtifolium* was obtained and then received approval by the botanist of Abu Ali Sina University, Hamadan. *Allium hirtifolium* was dried at room temperature (25°C) in the shade and then was powdered by mechanical grinder. A total of 100 g of powdered *A. hirtifolium* was placed in one liter of 80% methanol for 72 hours to extract the required active ingredients. The obtained mixture was placed in a rotary device to remove the solvent and then to dehydrate the substance, it was put in a dish and under a hood for one week. After a week, what was left in the bottom of the container, ie, extract, was dissolved in the appropriate amount of saline (0.9% saline) to treat rats with different doses of the extract.

### 3.2. Animals

A total of 48 male Wistar rats (weight, 250 - 220 g) were purchased from Pasteur Institute of Iran. The animals were kept at a controlled temperature of 22°C ± 1°C, with a light/dark cycle of 12 hours and relative humidity at 50% to 55%. The rats were kept in metal cages and had free access to food and water ad libitum. The animals were accustomed to lab conditions at least two hours before the test. The test was conducted between 8:00 A.M. to 12:00 P.M. and according to the ethical guidelines of the International Association for the Study of Pain in laboratory animals (16). The animals were randomly divided to eight groups of 6 rats: control group (received 0.9% saline), three extract groups treated with low, moderate, and high doses of shallot extract (respectively 50, 100, and 200 mg/kg), extract plus aspirin group (aspirin, 1 mg/kg; extract, 200 mg/kg), aspirin group (aspirin, 10 mg/kg), morphine group (morphine, 1 mg/kg), and extract plus naloxone group (naloxone, 1 mg/kg; and extract, 200 mg/kg). All injections were administered intraperitoneally.

### 3.3. Tests of Pain

**Writhing Test:** On the test day, 30 minutes before running the experiments, the animals were transferred into a standard experiment glass box to get used to the conditions. The obtained extract was solved in a certain amount of sterile saline and was injected. After 15 minutes, acetic acid on the scale of 10 mL/kg of the body weight with the density of 0.6% was injected and immediately after the injection of the acetic acid, the number of abdominal contractions was counted for 30 minutes (both legs stretched). It is also necessary to mention that each animal was used only once. In the control group, af-

ter the injection of saline (via i.p.) injection of saline, the writhing test was ran (17).

**Tail-flick test:** This test was conducted by the TF-5500 model of tail-flick device manufactured by Borj Sanat Company, Iran. The test was performed according to the previously presented pattern (18). Lightening intensity was equal to seven degrees (as displayed by the device) and lightening time was ten seconds (as the reference and the definite lightening time), meaning that if the animal did not pull its tail after ten seconds of exposure to the burning light radiation, the stimulus would be stopped to prevent tissue damage. The animal was placed horizontally in a special box and its tail was free. The delayed time in pulling the tail was measured three times at two-minute intervals before and 20 minutes after the injection of drugs/extracts and their means were recorded and considered as delayed time before and after treatment.

**Formalin test:** In this test, the proposed model by Dennis and Dubuisson was used. The animals were sent into a special formalin test box one hour prior to the test in order to get accustomed to the conditions of the experiment. In order to better observe the movements of the animal, a mirror with a 45° angle was placed under the animal and opposite the observer. Thirty minutes after the injection of the IP injection of the drugs, 50 µL 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 a perfect balance with its weight distributed on both feet; one, when the animal could not bear its weight on the injected foot or took care of 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 of first five minutes (acute phase) and 15 to 60 minutes (chronic phase) of each test were considered as the first and second phase of the formalin test, respectively (19).

### 3.4. Determination of Acute Lethal Dose

The acute toxicity was determined by the previous laboratory model (20). Various doses of the extract were injected to rats separately. Mortality rate of rats until 72 hours after injection was counted and the acute lethal dose (LD50) of the plant extract was determined.

### 3.5. Drugs

Morphine sulfate, naloxone, and aspirin were purchased from Daruo Pakhsh, Iran, and acetic acid and formalin were ordered from Merck, Germany.

### 3.6. Statistical Analysis

The data was presented as mean ± standard error of means (SEM). One-way analysis of variances (ANOVA) and

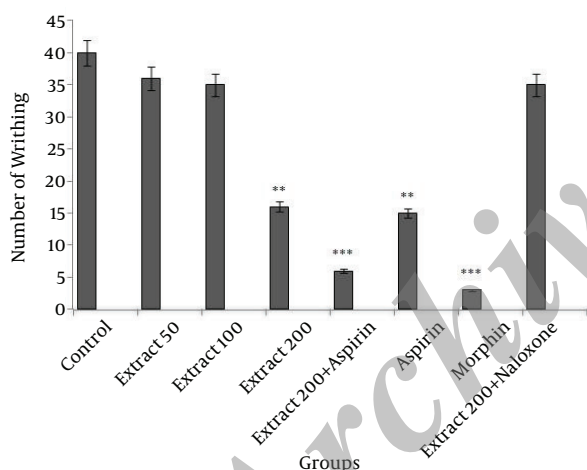
then Tukey post hoc tests was performed. After obtaining data from different groups, the results were analyzed and  $P < 0.05$  was considered as significant indicator. SPSS 18 (SPSS Inc, Chicago, Illinois, the United States) was used for data analysis.

## 4. Results

### 4.1. Writhing Test

Writhing test results showed significantly decreased writhing frequency in groups receiving 200 mg/kg extract alone or in combination with aspirin in comparison with the control group ( $P < 0.01$ ). In other words, shallot extract had prevented cramps caused by acetic acid. Moreover, the frequency of writhing within morphine and aspirin groups showed a significant decrease compared to the control ( $P < 0.001$  for both comparisons) (Figure 1).

**Figure 1.** Effect of Different Doses of the Hydroalcoholic Extract of *Allium hirtifolium* on Acetic Acid Induced writhing in Rats

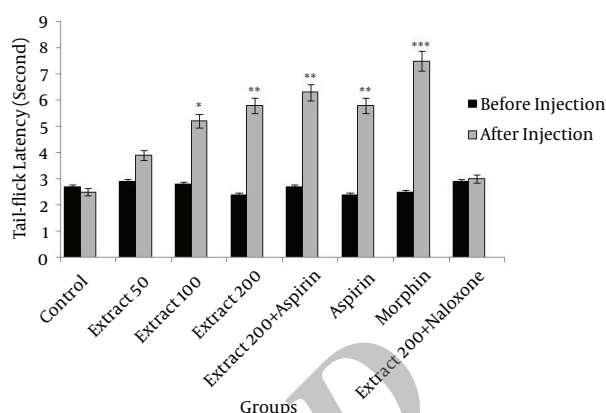


As compared with control: \*\*,  $P < 0.01$ ; and \*\*\*,  $P < 0.001$  ( $n = 6$ , Mean  $\pm$  SEM).

### 4.2. Tail-Flick Test

In this test, injection of 100 and 200 mg/kg of hydroalcoholic extract of shallot chives showed significant increase in tail-flick latency compared with the control group ( $P < 0.05$ ). Injection of 200 mg/kg of extract plus aspirin increased tail flick latency significantly in comparison with the control group ( $P < 0.01$ ). Meanwhile, injection of morphine and aspirin showed a significant increase in latency compared with the control group ( $P < 0.001$  and  $P < 0.01$ , respectively). As displayed in Figure 2, the result of this study showed that the medium and high doses of the shallot extract decreased pain caused by thermal stimulus in the tail -flick test.

**Figure 2.** Effect of Different Doses of the Hydroalcoholic Extract of *Allium hirtifolium* on the Reaction Time of Rats

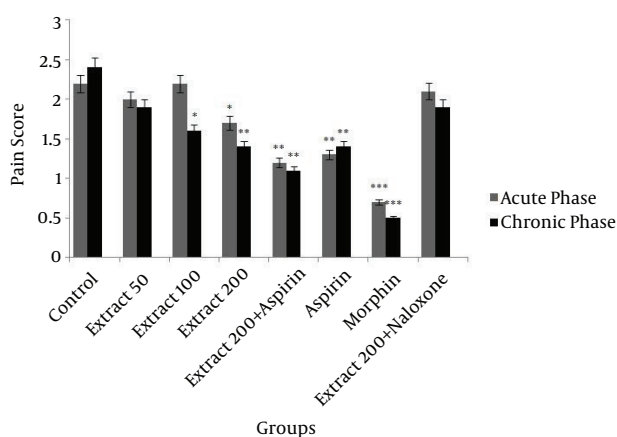


As compared with control: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; and \*\*\*,  $P < 0.001$  ( $n = 6$ , Mean  $\pm$  SEM).

### 4.3. Formalin Test

In formalin test, injection of 200 mg/kg of extract, 200 mg/kg of extract with aspirin, and injection of morphine and aspirin reduced pain scores in the acute phase of this test ( $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ , and  $P < 0.01$ , respectively). However, injections of 100 and 200 mg/kg of extract as well as 200 mg/kg of extract with aspirin resulted in significant decrease in pain scores in the chronic phase ( $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.01$ , respectively). Furthermore, injection of aspirin and morphine in the chronic phase of this test showed a significant decrease compared with the control group ( $P < 0.01$  and  $P < 0.001$ , respectively). Therefore, shallot extract controls the pain (Figure 3).

**Figure 3.** Effect of Different Doses of the Hydroalcoholic Extract of *Allium hirtifolium* on Formalin-Induced Nociception in Rats



As compared with control: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; and \*\*\*,  $P < 0.001$  ( $n = 6$ , Mean  $\pm$  SEM).

#### 4.4. Acute Toxicity

The individual dose required to kill 50% of a population of test animals, ie, LD50, was 2125 mg/kg.

### 5. Discussion

In the past, medicinal plants and herbal medicine were considered as rich sources for disease treatment worldwide (21). The results of this study confirm the analgesic effects of hydroalcoholic extract of shallot. In this study, writhing, tail-flick, and formalin tests were used to evaluate the analgesic effects of hydroalcoholic extract of shallot.

Writhing test is one of the most important tests used to screen potential analgesic compounds in which acetic acid is used. It is also a chemical stimulation, which is widely used to evaluate peripheral analgesic activity (22). According to Figure 1, shallot extract prevents cramps caused by acetic acid. Therefore, it can be assumed that its relief effects are supported by peripheral mechanisms. The IP injection of acetic acid can cause acute inflammation of the peritoneum (23). In this model, it appears that peripheral analgesic effects of shallot are indirectly produced by internal mediators such as bradykinin, serotonin, histamine, substance P, and prostaglandins because all these mediators are connected with stimulating peripheral nociceptive neurons (22).

Tail-flick test in which the thermal stimulation is used, is one of the most important parameters in evaluating the analgesic activity (15). As displayed in Figure 2, the result of this study shows that the medium and high doses of the extract decreased pain caused by thermal stimulus in the tail-flick test. Since tail-flick test is used to examine spinal reflexes and identify central analgesic direction (24, 25), it suggests that shallot extract creates its analgesic effects through the central nervous system.

The advantage of formalin test is its ability to distinguish compounds that operate through central pain pathways from peripheral pain pathways (26). Subcutaneous injection of formalin causes two pain-inflicting phases. The first one is neurogenic phase (acute), which is created around active nociceptive neurons under the direct influence of formalin. The second one is inflammatory phase (chronic), which is created through activation of ventral horn neurons on the surface of spinal cord (27). As displayed in Figure 3, shallot extract controlled pain, but its effect on the chronic phase was greater than on the acute phase. Inhibition of the chronic phase of the formalin test by the extract may be due to the inflammations, which cause chemicals such as prostaglandins F<sub>2α</sub> and E<sub>2</sub> to be released. In some cases certain amounts of these chemicals can sensitize central nociceptive neurons (28).

To evaluate the probable interaction between the shallot extract's analgesic effects and opioid system, naloxone (an antagonist of opioid system that prevents the activation of opioid receptors) was used (29, 30). The re-

sults of the present study demonstrated that naloxone reduces the analgesic effects of the extract. Therefore, it seems that the pain-relief effect of the extract is due to opioid receptors.

Drugs such as aspirin or morphine prevent the release of substance P from neurons. Unfortunately, the major disadvantage of these analgesic drugs is dependence and addiction. The repeated use of these drugs may reduce their efficacy due to phenomenon of tolerance. In behavioral tests of pain, nociceptive substances such as prostaglandins, bradykinin, and serotonin are released in location of the damaged tissues and pain on exertion. Mentioned drugs reduce effects of nociceptive substances that are produced due to tissue damage. It seems that the antinociceptive activity of shallot hydroalcoholic extract and fraction of the plant (containing amino acid derivatives and/or other cationic compounds) mechanism is similar to that of aspirin or morphine in modulating pain (31).

Existence of saponin and saponogenin compounds, sulfuric compounds such as thiosulfate, and flavonoids such as quercetin have been reported in different species of *Allium* (32). Moreover, phytochemical studies have proven existence of saponin triterpenoid compounds such as furostanol and spirostanol as well as flavonols such as kaempferol (33). Analgesic effects of saponins have been confirmed by some reports (34). There have been reports of inhibition of nitric oxide enzyme induction and synthesis of cyclooxygenase-2 by triterpenoids, which facilitates pain relief (35, 36). Flavonoids have many biologic effects on protein synthesis, cell differentiation, and angiogenesis in human (37). Besides, various types of flavonoids, both glycoside and non-glycoside types, have been reported to have anti-inflammatory and analgesic effects (37-39).

In conclusion, it seems that the analgesic effects of shallot extract are due to contained flavonoids and saponins. In this study, the relieved cramps, the increased time of tail lift by the rat, and the inhibition of both phases of the formalin pain proved the analgesic effects of shallot extract. These analgesic effects are probably due to inhibition of prostaglandins synthesis and inhibition of the central and peripheral nervous systems. Therefore, the shallot extract can be potentially used in the control of painful diseases.

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### Authors' Contributions

Saeed Mohammadi and Mohammad Zarei drafted the manuscript and conceived and designed the study. Mi-

noo Mahmoodi interpreted and performed the statistical analysis. Mohammad Mahdi Zarei and Mahtab Asgari Nematian collected the data. All authors read and approved the final manuscript.

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