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Assessment of the Antinociceptive, Anti-Inflammatory, and Acute Toxicity Effects of Solanum Dulcamara Essential Oil in Male Mice

A.R. Fallahzadeh (PhD)[1], S. Mohammadi (PhD)*[2]

1.Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, I.R.Iran

2.Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, I.R.Iran

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ABSTRACT

BACKGROUND AND OBJECTIVE: Pain is a complex set of unpleasant sensory, emotional, and cognitive experiences that are relieved with synthetic and herbal remedies. Of course, medicinal plants are more important than chemical drugs due to fewer side effects. Solanum dulcamara is one of the most important medicinal plants used in traditional Iranian medicine to treat rheumatic and inflammation pains. The aim of this study was to investigate the analgesic, anti-inflammatory and acute toxicity effects of Solanum dulcamara stem in male mice.

METHODS: In this experimental study, 84 male mice were used in 6 groups such as control and treated groups with essential oils of 30, 100 and 300 mg/kg. In pain assessment tests that included writhing (assessment of abdominal contractions), Tail Flick (assessment of tail jump duration), and formalin (assessment of pain associated with sole of foot), animals were given gavage or oral morphine (intraperitoneally) and naloxone (Intraperitoneally) with a dose of 300 mg/kg of essential oil. In the xylene test (to evaluate inflammation), the animals were divided into 5 groups: control, essential oil (oral) and dexamethasone (intraperitoneal).

FINDINGS: Use of 300 mg/kg essential oil in writhing tests (decreased from 41 in control to 13) and tail flick (increased from 2.8±0.2 seconds in control group to 6.1±0.5 seconds) showed significant analgesic effect ($p<0.01$). Also, in the xylene test, the use of 100 and 300 mg/kg of essential oil reduced the rate of ear inflammation in mice by 4.1±0.8 and 3.8±0.1, respectively, compared to the control group.

CONCLUSION: It seems that SDEO probably have both analgesic and anti-inflammatory effects in male mice.

KEY WORDS: *Anti-Inflammatory Agents, Pain, Solanum Dulcamara, Lethal Dose 50.*

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***Corresponding Author: S. Mohammadi (PhD)**

Address: Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, I.R.Iran

Tel: +98 81 32518064

E-mail: smiauhphd.sm@gmail.com

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Introduction

Pain is defined as a complex set of unpleasant sensory, emotional, and cognitive experiences resulting from tissue damage and manifestations of autonomic, psychological, and behavioral reactions (1). Inflammation is also one of the common complications of many diseases that weakens the immune system and delays the recovery of diseases (2). Inflammatory processes are strongly associated with pain. If chemicals released during the inflammatory process, such as bradykinin, prostaglandins can further stimulate pain receptors and lead to inflammatory pain (3). Because about 80% of the world's population lives in developing countries, and due to the high cost of synthetic drugs, there is a greater tendency than medicinal plants (4, 5).

Solanum dulcamara is a member of the Solanaceae family (6). Other common names of this plant in Iran are Jasmine berry, Gooseberry and Morelle douce. Solanum dulcamara is a shrub with ivy and ascending with wooden stems, its stems are alternate green and ovoid. This plant grows in Iran in Nowshahr, Lahijan and in the west of Iran on the wet slopes of Ashtaran Zorvand Mountain, Hamedan (7). In traditional Iranian medicine, the stems of this plant have been used as a narcotic compound and in cases of rheumatism, migraine and severe inflammation (8). Also, today, the antimicrobial effects of this medicinal plant have been well proven due to the presence of its alkaloid compounds (9). In the slender stems and fruits of Solanum dulcamara, the solanine glycoside alkaloid and the solanidine alkaloid are present (10).

Today, in various studies, the analgesic and anti-inflammatory effects of plants of the same family as the Solanum dulcamara plant, such as: Solanum incanum, Solanum sisymbriifolium, Solanum lycopersicum, Solanum violaceum, Solanum anomalum, have been proven in mice. On the other hand, in all these studies, the use of plants belonging to the Solanum dulcamara family did not show any acute toxicity (11-15). Due to the chemical composition of Solanum dulcamara such as alkaloids and also due to the proof of analgesic and anti-inflammatory effects of this plant family, acute toxicity, analgesic and anti-inflammatory effects of Solanum dulcamara have not been evaluated yet. The present study investigated the acute toxicity, analgesic effects (using tail-flick, formalin and writhing tests) and anti-inflammatory effects (xylene test) of the Solanum dulcamara on male mice.

Methods

In this experimental laboratory study, the Solanum dulcamara was prepared in July 2018 from the slopes of Alvand mountain in Hamedan and then approved by the botanist of Bu Ali Sina University of Hamedan (Herbarium number: 12845). The stems were then separated from the stems and fruits of the plant and dried at room temperature (25 °) in the shade. Plant essential oil was extracted using water distillation method and using Clevenger machine (16).

Animals and their grouping: 84 male mice (20-30 g) were purchased from Pasteur Institute of Iran and were kept in standard animal room conditions under light period of 12 hours light, 12 hours dark (light period starts from 7:00 AM), temperature conditions 22±1 ° C. Animals were kept in metal cages with free access to water and special food. The animals were accustomed to laboratory conditions at least 2 hours before the experiment. The experiment was performed between 8:00 AM and 12:00 PM. Experiments were performed on laboratory animals in accordance with the ethical guidelines of the International Association for the Study of Pain and the Ethics Committee of Tehran University of Science and Research (IR: IAU. SRB. REC. 1397. 317) (17). In pain assessment test such as: formalin, writhing and tail flick tests, animals were divided into 6 groups of 6: control group (normal saline), morphine group (1 mg/kg, intraperitoneal injection), and treated groups with low, medium and high doses of essential oil of Solanum dulcamara (30, 100 and 300 mg/kg, respectively, by oral injection or gavage) and naloxone-treated group (1 mg/kg, intraperitoneal injection) with high dose of extract (300 mg/kg). In the xylene inflammatory test, the animals were divided into 5 groups of 6, including: control group, essential oil (30, 100 and 300 mg/kg by oral injection or gavage) and dexamethasone (10 mg/kg, intraperitoneal injection).The doses used in this experiment were determined according to previous studies (18, 19) and also based on the acute toxicity test performed in the present study, so the use of these doses was completely safe.

Determination of acute toxicity (Lethal Dose 50: LD50): Determination of acute toxicity was performed based on the previous laboratory model (5, 20). Different doses of essential oil were injected orally (gavage) and separately into mice. Animal mortality was counted up to 72 hours after injection and LD50 of essential oil was determined.

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Inflammation test: Inflammation test was performed using xylene in the ears of mice. Two hours after xylene administration, the animals were killed and both ears were removed. Using a cork driller, 7 mm incisions were taken from both left and right ears and weighed, and the difference in weight between the incisions of the left and right ears was determined. The benchmark in this test was the difference in ear weight of mice (21).

Pain tests

Writhing test: In this test, the essential oil of the plant stem dissolved in sterile physiological serum at doses of 30, 100 and 300 mg/kg was injected intraperitoneally at a rate of 2 ml/kg. After 15 minutes, acetic acid in a volume of 0.1 ml/kg with a concentration of 1% was injected intraperitoneally and 5 minutes after acetic acid injection, the number of abdominal contractions was counted. The measurement criterion in this test was the number of abdominal contractions in male mice (22, 23).

Tail flick test: This test was performed using the tail jump machine, model TF-5380 made by Iran Sanat Tower Company. The test was performed based on the previously presented model (24, 25). The light intensity used was 7 and the reference time of 10 seconds was used as the cut-off time. Animals that had a delay time of more than 6 seconds in at least two of the above three tests were excluded. The benchmark in this test is the duration of the tail jump of male mice.

Formalin test: In this test, a Plexiglas box was used. In order to better observe the animal's movements, a mirror with a 45 degree angle was placed below it. 30 minutes after oral injection of the drugs, 50 µl of 2.5% formaldehyde was injected subcutaneously into the sole of the right foot of the animal and the animal's behavior was scored for 60 minutes. The average of 5 minutes at the beginning of each test was considered as the first phase of formalin test (acute phase) and the average of 15-60 minutes of the test was considered as the second phase of formalin test (chronic phase). The measurement criteria in this test were based on the score obtained from licking the sole of the foot, lifting the leg or caring the injected leg for male mice (26).

Drugs: Morphine sulfate and naloxone were prepared from Daropakhsh company (Iran) and acetic acid, formalin and xylene from Merck company (German).

Statistical analysis: The data were presented as the mean standard error of Mean±S.E.M and one-way analysis of variance was used followed by Tukey test and $p<0.05$ was considered significant.

Results

Acute toxicity: During this test, no mortality was observed in mice 72 hours after injection of different doses of essential oil.

Xylene test: According to the results of xylene test, the use of doses of 50 and 100 mg/kg of essential oil of this plant significantly reduced inflammation with $p<0.05$, $p<0.01$ with 34.2% and 47.9% inhibition percentage, respectively compared to the control group (Table 1).

Table 1. Effect of Solanum dulcamara essential oil and dexamethasone injection on xylene-induced inflammation in male mice

Groups	Dosage (mg/kg)	Inflammation of the mouse ear	Inhibition Percentage
Control	10	7.6±0.4[a]	-
Low dose of extract	20	7.1±0.1[a]	6%
Medium dose of extract	100	4.9±0.8[b]	34.2%
High dose of extract	200	3.4±0.1[c]	49.7%
Dexamethasone	15	2.2±0.2[d]	56.2%

Writhing test: Also, the results of the study in the writhing test showed that the use of high dose of essential oil (300 mg/kg), reduced the number of writhing by $p<0.01$ compared to the control group. In this laboratory model, it was found that the use of naloxone with a high dose of essential oil reversed the analgesic effects of the essential oil alone. On the other hand, the use of a dose of 300 essential oil in comparison with the morphine group showed a significant difference at the level of $p<0.05$ (Figure 1).

Tail Flick test: In Tail Flick test, the use of 300 mg/kg essential oil showed a significant analgesic effect ($p<0.01$) compared to the control group and the duration of tail jump reaction in mice (tail- flick latency) increased from 2.8±0.67 seconds to 5.8±0.79 seconds. In this experiment, the combined use of naloxone with a high dose of essential oil reversed the analgesic effects of the essential oil. Morphine useage increased the duration of tail jump reaction in mice from 2.8±0.59 seconds in the control group to 8.1±2.1 seconds ($p<0.001$) (Figure 2).

Formalin test: The results of formalin test showed that injection of 300 mg/kg of essential oil in both chronic and acute phases of pain causes a significant analgesic effect ($p<0.01$) compared to the control group and the pain score was approximately decreased by 1.5 points in comparison to control group (Figure 3).

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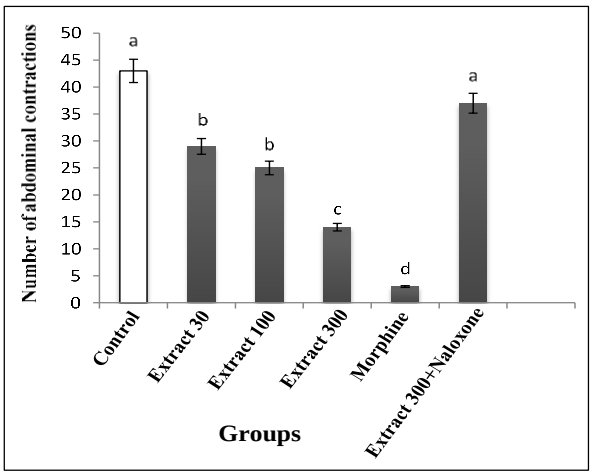

Figure 1. Comparison of the mean number of Writhing (abdominal contractions) of male mice with different concentrations of Solanum dulcamara essential oil in acetic acid test

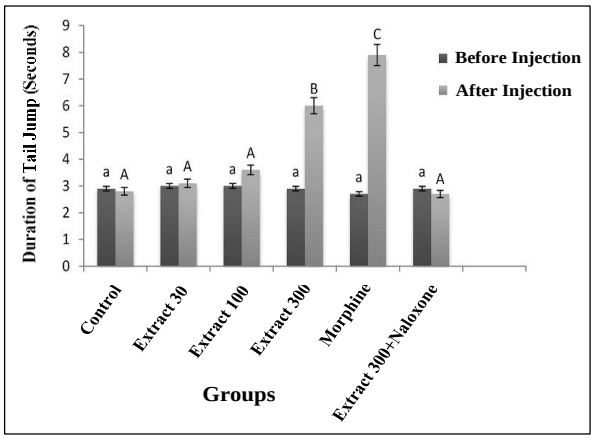

Figure 2. Mean comparison of different concentrations of essential oil in the Tail Flick test

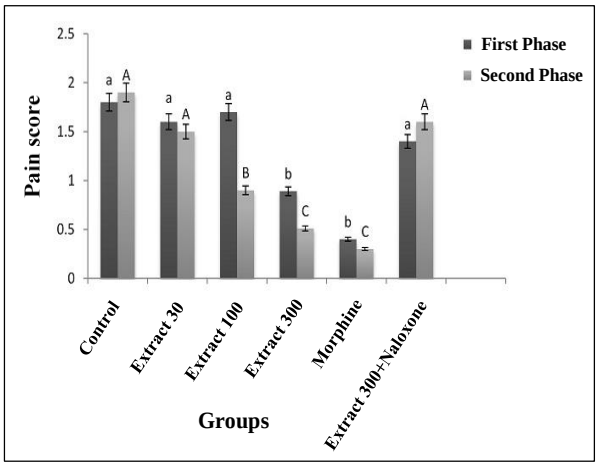

Figure 3. Comparison of the mean pain scores of male mice with different concentrations of essential oil of the Solanum dulcamara stem in the formalin test

Discussion

In this experimental study, the use of Solanum dulcamara essential oil showed significant analgesic and anti-inflammatory effects in male mice. In the study of Mwonjoria et al. on Solanum incanum, a member of the Solanaceae family, the stem essential oil of this plant (at doses of 100 and 300 mg/kg) showed significant analgesic effects when evaluated by acetic acid test (27). In the present study, the essential oil of the Solanum dulcamara, as in the previous study, prevent acetic acid-induced cramps at doses of 100 and 300 mg/kg. However, the difference between the present study and the previous study was that the use of 30 mg/kg of essential oil could show a significant analgesic effect in the writhing test, but in the previous study, this dose of essential oil did not show a significant analgesic effect. In another study by Ndebia et al. (28), the use of Solanum torvum leaf extract in a writhing test did not reduce the number of abdominal contractions in rats, which contradicts the results of the present study.

The results of the present study show that the injection of medium and high doses of essential oil reduces the pain caused by the thermal stimulus in the tail flick test. In a study conducted by Pandurangan et al., the use of Solanoum trilobatum stem essential oil increased the duration of tail jumping in rats, which was similar to the results of the present study (29). In another study by Kaushik et al., the use of Solanum nigrum stem essential oil in the Tail Flick test did not increase tail jump duration in male rats, which contradicted the results of the present study (30). Since the Tail flick test is used to evaluate spinal reflexes and identify the central analgesic pathway (31), it can be suggested that Solanum dulcamaraessential oil has central analgesic effects.

The results of the present study show that the essential oil of the Solanum dulcamara reduces the second phase of formalin test more than the first phase. In a study, the analgesic effect of Solanum torvum was confirmed using a formalin test, during which it was found that the extract of this plant reduces pain in the second phase of the formalin test, and these effects are mainly through flavonoids and alkaloids in essential oils. The results of this study were similar to the present study (32). In another study conducted by Nasrin et al., was found that Solanum sisymbriifolium essential oil in the first phase of formalin test reduced the pain score more than the second phase, which was contrary to the results of the present study (12). Xylene induction of edema test is one of the useful models to evaluate anti-

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inflammatory agents. In this model, after the induction of xylene, vasodilation and consequently acute edema of the skin occurred (33). According to the results of the present experiment, ear inflammation in mice was reduced by injecting essential oil, especially at a dose of 300 mg/kg, which indicates the anti-inflammatory effect of essential oil. One study demonstrated the anti-inflammatory effect of Solanum paranense using xylene test, which was almost in agreement with the present study (34). In another study conducted by Wang et al., was found that the leaf essential oil of Solanum nigrum at a dose of 300 mg/kg did not reduce inflammation in the ears of mice in the xylene test, which contradicted the results of the present study (35).

As mentioned, the important chemical compounds of this plant were mainly alkaloid compounds such as solanine (10). Studies conducted by Zakaria et al. found that solanine in Solanum nigrum, which is also an important alkaloid of the Solanum dulcamara, has a significant analgesic effect (36). Perhaps part of the analgesic effect shown by the Solanum dulcamara essential oil is related to the presence of this important alkaloid.In a general conclusion from the present experiment, it can be seen that the orally use of Solanum dulcamara stem essential oil can possibly inhibit inflammation by inhibiting inflammatory mediators and also reduce pain in male mice by inhibiting central and peripheral systems.

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