

## Aloe vera Aqueous Extract Effect on Morphine Withdrawal Syndrome in Morphine-Dependent Female Rats

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**Background:** *Aloe vera* is a medicinal herb used as an anti-inflammatory and sedative agent.

**Objectives:** The current study aimed to evaluate the effect of *Aloe vera* aqueous extract on morphine withdrawal symptoms in morphine-dependent female rats.

**Patients and Methods:** The current research was performed on 40 female Wistar-Albino rats which were made dependent on morphine using Houshyar protocol and were randomly divided into five groups (A, B, C, D, and E). Group A did not receive any agent in the period of handling but other groups (B, C, D and E) received 5, 10, 20 and 40 mg/kg of *Aloe vera* aqueous extract by gavage, three times daily for a week, respectively. Withdrawal symptoms, stool form, agitation, disparity, floppy eyelids, and body mass variations were checked for 10 days. The obtained data were analyzed using SPSS v.11 software, and Friedman, Kruskal-Wallis, and Mann-Whitney statistical tests. Statistical difference was considered significant ( $P < 0.05$ ).

**Results:** The results of the present study showed that agitation, disparity, and floppy eyelids in group E were significantly higher than those of others groups; however, these symptoms in group C were significantly lower than those of the other groups.

**Conclusions:** The results of the present study revealed that the *Aloe vera* aqueous extract had various effects on morphine withdrawal syndrome in morphine-dependent female rats.

Keywords: Morphine; Substance Withdrawal Syndrome; *Aloe vera*; Rats

### 1. Background

*Aloe vera* is a cactus-like enduring plant which belongs to the Liliaceae family. It grows in hot and dry climates of Asia, Africa, and other tropical regions of the world (1). The extract of *Aloe vera* contains many components such as acemannan (polysaccharide) which has an anti-inflammatory effect on oral aphthous ulceration (2-5). *Aloe vera* gel powder is also used to treat peptic ulcers and has a cytoprotective property (6, 7). Furthermore, there is evidence which supports the uses of *Aloe vera* topical agents or *Aloe vera* dressings to treat severe and prolonged wounds (8). Shin et al. revealed that the *Aloe* extract complex could improve syndromes related to diabetes and insulin resistance in mice fed with a high-fat diet (9). There was evidence that topical *Aloe* gel can provide safe and effective treatment for the management of dermatitis in infants (10). Experimental studies have reported that *Aloe vera* leaf gel has a protective anti-hyperglycemic effect on patients with hyperlipidemic diabetes type 2 (11). Researches have clearly shown that many medicinal herbal extracts suppress morphine withdrawal in laboratory animals (12). Hajhashemi et al. indicated that *Aloe littoralis* have apparent wound-healing and anti-inflammatory effects on rats (2). Topical administration of *Aloe vera* gel

is also suggested as an agent to treat surgical scars (13). Oral administration of *Aloe vera* is recommended to relieve prolonged non-cancer pains, mainly those caused by osteoarthritis (14, 15). In addition, *A. vera* (200 and 400 mg/kg, p.o.) significantly decreases the second phase of the formalin-induced pains (15). Moreover, application of *Aloe vera* ointment on the surgical sites effectively reduces postoperative pains after hemorrhoidectomy (16). However, photochemical screening shows that the *Aloe vera* has anti-PLA<sub>2</sub> antibodies and anti-inflammatory effects on animals (17, 18). Since *Aloe vera* has anti-inflammatory and nociceptive effects, the current study aimed to evaluate the effect of *Aloe vera* aqueous extract on morphine withdrawal symptoms in morphine-dependent female rats.

### 2. Objectives

The present study aimed to evaluate the effect of *Aloe vera* aqueous extract on morphine withdrawal symptoms in morphine-dependent female rats.

### 3. Patients and Methods

The current study was performed on 40 adult Wistar-

Albino female rats. The rats were required from animal house of Zahedan University of Medical Sciences. The animals weighed 200 to 250 g and aged five to seven months. The rats were separately housed in cages (one rat in each cage, and had free access to water and food. They were maintained in a room at  $23 \pm 2^\circ\text{C}$  with 12 hours, 6 AM to 6 PM, constant light (timer model: SUL180a, AC220V, China), and humidity of 45% to 70%; the air was adequately recycled. All animals were fed with standard rodents' diet during the experiment for one week. After a five-day habituation, the rats were made morphine-dependent through Houshyar protocol (19) and then were randomly divided into five groups (A, B, C, D and E, n = 8). Group A did not receive any agents during the trial period, but the other groups (B, C, D and E) received 5, 10, 20 and 40 mg/kg of *Aloe vera* aqueous extract three times daily for a week, respectively. Morphine withdrawal such as: floppy eyelids, stability, stool form and agitation were checked three times daily (19-21). For each situation, the animals received scores.

### 3.1. Morphine Withdrawal

**Floppy Eyelid:** Animals with open binocular score one; animal with half-open binocular received score two, and those which had two blindfolds scored three.

### 3.2. Balance

well-balanced animals scored "one"; sleepy animals scored "two"; those which could not stand on their legs scored "three", and animals experienced high off situation scored "four".

**Stool Form:** Moderately hard stool, loose stool, and very loose stool scored from one to three, respectively.

### 3.3. Agitation (Jumping)

When an animal was quiet, the score was one, but restless animals were given score two and the score three were given to the animals which were agitated, stripped and did not allow gavage. Data were recorded.

*Aloe vera* aqueous extract with the following specifications was purchased from Baridge Essence Co. (Kashan, Iran): pH = 4.41, specific gravity 1.003, effect substance = 0.57% w/v, sterile and the extract followed the USP standards.

Data were analyzed employing SPSS v.11. The Statistical tests of Kruskal-Wallis, Friedman and Mann-Whitney

were also used. P < 0.05 was considered as the level of significance.

## 4. Results

The results of the current study showed that weight in the groups which received 5 mg/kg and 10 mg/kg of *Aloe vera* aqueous extract significantly increased compared with those of the other groups (P = 0.001) (Table 1). On the other hands, equilibrium in the group which received 10 mg/kg of *Aloe vera* aqueous extract daily significantly decreased (P = 0.01) (Table 2) compared with the other groups; however, this parameter in the group which received 40 mg/kg *Aloe vera* aqueous extract significantly increased compared with the control group (P = 0.01) (Table 2). In addition, the results of the present study showed that the floppy eyelids in the group which received 10 mg/kg of *Aloe vera* aqueous extract was significantly lower (Table 3) than that of the control group, but this parameter in the group which received 40 mg/kg *Aloe vera* aqueous extract was significantly higher than that of the control group (P = 0.03) (Table 3). Agitation in the group which received 10 mg/kg *Aloe vera* aqueous extract significantly decreased (P = 0.01) (Table 4) compared with the control group, but this parameter in the group which received 40 mg/kg *Aloe vera* aqueous extract significantly increased compared with the control group (Table 3). Type of stool did not show any significant differences in the groups.

**Table 1.** The Comparison of Weight Gain on the First, Fourth and Tenth Days of Experiment in the Control and Intervention Groups (n = 8)<sup>a,b</sup>

| Weight/<br>Groups   | Primary       | Fourth Day                | Tenth Day                  | P Value |
|---------------------|---------------|---------------------------|----------------------------|---------|
| <b>A (Control)</b>  | 200.1 + 4.22  | 213 + 9.00                | 196.1 + 10.23              | > 0.05  |
| <b>B (5 mg/kg)</b>  | 207.5 + 11.89 | 226.2 + 16.3 <sup>c</sup> | 225.5 + 15.39 <sup>c</sup> | 0.001   |
| <b>C (10 mg/kg)</b> | 206.1 + 16.88 | 216.3 + 13.7 <sup>c</sup> | 215 + 14.76 <sup>c</sup>   | 0.01    |
| <b>D (20 mg/kg)</b> | 202.8 + 24.09 | 220.7 + 22.3              | 213.3 + 20.14              | > 0.05  |
| <b>E (40 mg/kg)</b> | 208 + 17.52   | 219.5 + 18.83             | 205.2 + 17.39              | > 0.05  |

<sup>a</sup> Data are presented as Mean ± SD

<sup>b</sup> Based on Kruskal-Wallis, Friedman and Mann-Whitney tests the diversity of weight in B and C groups which respectively received 5 mg/kg and 10 mg/kg *Aloe vera* aqueous extract significantly increased compared with their primary weight

<sup>c</sup> P < 0.05

**Table 2.** The Effect of *Aloe vera* Aqueous Extract on Equilibrium in the Control and Intervention Groups During the Experiment (n = 8)<sup>a,b</sup>

|              | <b>A (Control)</b> | <b>B (5 mg/kg)</b> | <b>C (10 mg/kg)</b> | <b>D (20 mg/kg)</b> | <b>E (40 mg/kg)</b> | <b>Total</b> |
|--------------|--------------------|--------------------|---------------------|---------------------|---------------------|--------------|
| <b>1</b>     | 6 (75)             | 6 (75)             | 7 (87, 5)           | 5 (62, 5)           | 0                   | 24 (60)      |
| <b>2</b>     | 2 (25)             | 2 (25)             | 1 (12, 5)           | 2 (25)              | 3 (37, 5)           | 10 (25)      |
| <b>3</b>     | 0                  | 0                  | 0                   | 0                   | 0                   | 0            |
| <b>4</b>     | 0                  | 0                  | 0                   | 1 (12, 5)           | 5 (62, 5)           | 6 (15)       |
| <b>Total</b> | 8 (100)            | 8 (100)            | 8 (100)             | 8 (100)             | 8 (100)             | 40 (100)     |

<sup>a</sup> Data are presented as No. (%)

<sup>b</sup> Based on Kruskal-Wallis, Friedman and Mann-Whitney tests, Equilibrium rate was lower in group B but higher in group E compared to that of the control group.

**Table 3.** The Effect of *Aloe vera* Aqueous Extract on Floppy Eyelids in the Control and Intervention Groups During the Experiment (n = 8)<sup>a,b</sup>

|              | <b>A (Control)</b> | <b>B (5 mg/kg)</b> | <b>C (10 mg/kg)</b> | <b>D (20 mg/kg)</b> | <b>E (40 mg/kg)</b> | <b>Total</b> |
|--------------|--------------------|--------------------|---------------------|---------------------|---------------------|--------------|
| <b>1</b>     | 7 (87.5)           | 6 (75)             | 8 (100)             | 5 (62.5)            | 3 (37.5)            | 29 (72.5)    |
| <b>2</b>     | 0                  | 0                  | 0                   | 1 (12.5)            | 1 (12.5)            | 2 (5)        |
| <b>3</b>     | 1 (12.5)           | 2 (25)             | 0                   | 2 (25)              | 4 (50)              | 9 (22.5)     |
| <b>Total</b> | 8 (100)            | 8 (100)            | 8 (100)             | 8 (100)             | 8 (100)             | 40 (100)     |

<sup>a</sup> Data are presented as No. (%).<sup>b</sup> Based on Kruskal-Wallis, Friedman, and Mann-Whitney tests, floppy eyelid rate was lower in group B but higher in group E compare to that of the control group.**Table 4.** The Effect of *Aloe vera* Aqueous Extract on Agitation in the Control and Intervention Groups During the Experiment (n = 8)<sup>a,b</sup>

|              | <b>A (Control)</b> | <b>B (5 mg/kg)</b> | <b>C (10 mg/kg)</b> | <b>D (20 mg/kg)</b> | <b>E (40 mg/kg)</b> | <b>Total</b> |
|--------------|--------------------|--------------------|---------------------|---------------------|---------------------|--------------|
| <b>1</b>     | 7 (87.5)           | 7 (87.5)           | 8 (100)             | 6 (75)              | 0                   | 29 (72.5)    |
| <b>2</b>     | 1 (12.5)           | 1 (12.5)           | 0                   | 2 (25)              | 3 (37.5)            | 2 (5)        |
| <b>3</b>     | 0                  | 0                  | 0                   | 0                   | 5 (62.5)            | 9 (22.5)     |
| <b>Total</b> | 8 (100)            | 8 (100)            | 8 (100)             | 8 (100)             | 8 (100)             | 40 (100)     |

<sup>a</sup> Data are presented as No. (%).<sup>b</sup> Based on Kruskal-Wallis, Friedman and Mann-Whitney tests, agitation rate was lower in group B but higher in group E compare with that of group A.

## 5. Discussion

*Aloe vera* belongs to Liliaceae family and has anti-nociceptive activity. The results of the current study showed that the morphine withdrawal symptoms such as agitation, imbalance, and floppy eyelids in morphine-dependent group B was significantly lower than those of the control group; however, these values in morphine-dependent group E increased compared with those of the control group A in the treatment period. In addition, the average weight in groups B and C was significantly higher than that of the control group. The stool form did not show any significant differences in the groups.

*Aloe vera* is an adaptive plant with anti-inflammatory and useful effects on sunburns. Its extract contains several elements such as vitamins, enzymes, minerals, amino acids, salicylic acids, and aloin (22). There exists enough evidence confirming that *Aloe vera* gel could be used as a safe and effective treatment to manage dermatitis in infants and this may be due to the idea that *Aloe vera* extract components can inhibit cyclooxygenase enzyme and affect the prostaglandin biosynthesis (10). The results of the current study revealed that administration of high doses of *Aloe vera* extract in group E increased the symptoms of morphine withdrawal syndrome in morphine-dependent female rats. The results of the experiments revealed that concentration-dependent *Aloe vera* extract caused depolarization of the muscle fiber membrane resting potential, and increased excitatory functional potentials following the electrical stimulus of the isolated excitatory axon in the crayfish (23). To the best of our knowledge, no similar studies have been previously conducted to compare the results with; however, a part of the current study findings can be compared to those of the study by Rathor

et al. which revealed that oral administration of *Aloe vera* gel (200 and 400 mg/kg) in rats significantly decreased the second phase of the formalin-induced pain, while it did not show any significant effects on the first phase (15). In addition, the current study finding is in agreement with that of Cowan et al. who reported that oral administration of *Aloe vera* aqueous extract could be used as a sedative agent to treat chronic non-cancer pains, chiefly in osteoarthritis (14). A part of the current study results was similar to those of Khedmat et al. who reported that *Aloe vera* gel extract can decrease the abdominal pain/distress in patients suffering from constipation (24). Furthermore, the current results indicated that in group E withdrawal symptoms were significantly higher than the control group, which was in disagreement with those of Cowan et al. (14). This may be due to different administered concentrations used in the treatment period and the period of the experiment. The findings of the present study showed that the effect of oral administration of *Aloe vera* aqueous extract on morphine-dependent female rats in groups B and C caused a significant increase in the weight gain. This finding is in disagreement with those of Misawa et al. who discovered that the administration of *Aloe vera* gel powder in the diet of male Sprague-Dawley rats can reduce body fat accumulation and weight gain (25). This difference may be due to the diversity of the animals used, different animal sexes, or the diverse physiologic conditions of the animals (18). The current study results are in line with those of Rishi et al. and Doherty et al. which revealed that *Aloe vera* contained many components such as phenolic which has anti-inflammatory and nociceptive effects which inhibit phospholipase A2

and decrease prostaglandins and prostacyclin production, and relieve pain in rats (18, 26). The results of the tests revealed that *Aloe vera* may decrease prostaglandin E2 production from arachidonic acid through inhibition of cyclooxygenase pathway (27). The current study results revealed that the value of withdrawal symptoms in morphine-dependent group E, which received high dose of *Aloe vera* extract, were significantly higher than that of the control group A. In addition, administering high doses (100, 200, and 400 mg/kg, p.o.) of *Aloe vera* significantly decreased the depression symptoms in mice, by forced swim test, compared with the control group (28).

To evaluate depression, the forced swim test and tail suspension test were performed, and to assess locomotor activity, the Rota Rod test and photoactometer were used. It was concluded that *Aloe vera* aqueous extract had different effects in morphine withdrawal syndrome in morphine-dependent female rats. Further studies are needed to find out the mechanism of these biological effects and also the active constituents responsible for the effects.

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## Authors' Contributions

Main Author: Mohammad Reza Shahraki, co-authors: Hamideh Mirshekari, Azame Sabri.

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