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Antinociceptive Effect of Oral and Intraperitoneal Administration of Alcoholic *Viscum Album* Fruit Extract in Male Rats

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ABSTRACT

Objective: The present study was designed to investigate the analgesic effect of oral and i.p. administration of alcoholic *Viscum album* (V.A) fruit extract as a rich source of alkaloid substances.

Materials and Methods: Experimented animals were divided into control and treatment groups. The treatment rats received different doses of the V.A fruit extract, which was prepared from alcoholic smashed fruits. Then, the animals from each group were subjected to pain scoring experiments such as hot plate and formalin tests.

Results: The results of the experiments i.e., antinociceptive effect of i.p. V.A extract (50, 100 and 200 mg/kg) and oral application method V.A (6.25% food pellet) were compared with others and morphine sulfate and naloxone as positive and negative control groups, respectively. The extract at doses over than 100 mg/kg (i.p.) could potentially alleviate the pain in hot plate and both phases of the formalin test. Besides, there was a marked antinociceptive effect of the extract (over than 200 mg/kg) in oral method in hot plate and both phases of the formalin tests. Meanwhile, the effective doses of morphine sulfate as positive control were observed at doses over than 15 mg/kg (i.p.).

Conclusion: In summary, by comparing the analgesic effect of different doses of morphine sulfate with V.A fruit extract in i.p and oral conditions and regarding to the extract LD_{50}, it is concluded that the V.A fruit extract has a potent, semi-absorbable, and nearly safe ingredient which can exert a potential analgesic effect in acute and chronic pain

1. Introduction

The medicinal plant *Viscum album* (V.A) has been introduced as an analgesic plant in Iranian folk medicine (1). Recently, it has been used as a narcotic and local anesthetic drug in many societies (2-4). Also, in some nations young people use it leaves by smoking for hallucination purpose (2). However, the analytic experiments have shown the presence of rich alkaloids in V.A, especially antimuscarinic component (5, 6). It is obvious that these alkaloids in V.A are more abundant in V.A fruits than other parts of the plant (7). Other studies have also shown that there is an interaction between opioid and cholinergic systems (8, 9). However, it is evident that the role of cholinergic system on the pain was mediated by its effect on inhibitory opioid pathways (10, 11), centrally acting through spinal cord and brain stem opioid receptors (12).

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Since the role of opioid receptors in antinociception, especially in acute pain has been established, regarding interaction of these receptors with cholinergic system and introduces of the V.A fruit extract as a rich source of anticholinergic gradient, this herb may be a good candidate for therapeutically analgesic purposes.

In addition, regarding many side effects and insufficiency of chemical analgesic drugs and recommendation of herbal medicine (1), the present study was proposed to investigate the analgesic effect of V.A fruit extract in hot plate and formalin tests administered i.p. or via oral method.

2. Materials and Methods

2.1. Preparation of crude extract

The fruits of medicinal plant *Viscum album* were obtained from the local market and scientifically identified by the Department of Botany of Shaheed Beheshti University (SBU). One hundred grams of cleaned V.A fruits was crushed and mixed at a ratio of 1 to 4 with methanol. The mixed complex was set aside for 24 h at room temperature. Then, it was filtered three times through a mesh. The alcohol of filtered solution was evaporated at 50^{o}C in tissue organ bath. Finally, 8-12 g concentrated residue remained in the container, which was used for preparation of extract doses (mg/kg).

2.2. Animals

Male NMRI rats at weight ranges of 300-350 g (Razi Institue, Iran) were used in our experiments. Four animals were housed in each cage with free access to food and water. The laboratory temperature and light-dark cycling was 24±2 ^{o}C and 12/12 h, respectively.

2.3. Antinociceptive tests

The V.A fruit extract at 50, 100 and 200 mg/kg, i.p. were given to the rats 15-20 min prior to measuring the pain. Also, in oral method, doses of 6.25% were prescribed to the animals via food pelletes for 3 weeks. In positive control tests, morphine sulfate (15 mg/kg; i.p.) were used.

Then, in order to assess the pain, all of the animals were subjected to the hot plate and formalin tests. The saline injected rats were used as control group.

2.4. Hot plate test

Antinociception was assessed with a hot plate apparatus (Harvard, UK). The rats were acclimated in the turn-off hot plate apparatus before scoring the pain, 4-5 times with 5 min interval. The time between standing of the animals on the turned-on hot plate (54^{o}C) till licking of burned paw was measured and considered as the pain score. Each animal was tested 5 times with 5 min interval. The animal's paw was prevented from tissue injury, because in our experiments the duration of the test was not over than 30 s. In treatment group, we calculated the percentage of Maximum Possible Effect (MPE) of the extract with the following formula

$$MPE = \frac{Test\ Latency(s) - Baseline}{Cutoff\ \ Time(s) - Baseline} \times 100$$

(13).

In this formula, baseline and test latency are the pain threshold time before and after the extract application, respectively, and the cut-off time is the maximum time that the animals are permitted to stay on the hot plate apparatus (30 s).

2.5. Formalin test

Formalin test introduced by Dubuisson and Dennis (1977) was used in our experiments. In this method formaldehyde (50 µl, 2.5%) was injected subcutaneously into the plantar surface of hind paw, and then the animal was placed in a Plexiglas chamber (30×30×30 cm) which had a mirror with 45^{o} angle underneath in order to accurate observation. In the treatment groups, the V.A fruit extract was administered intraperitoneally and orally, prior to the formaldehyde injection, 15-20 and 40-50 min, respectively. Before the experiments, all animals were brought to the test chamber 5 times with 5 min interval in order to adapt to the environment.

The behavioral pain reactions due to formalin injection were detected and recorded for 1 h. The first 10 min, post-formalin injection is known as the early phase (acute phase), and the period between 15-60 min is as the second or chronic phase.

2.6. Statistical analysis

The result of each dose of the extract was expressed as mean±S.E.M. The differences were estimated by ANOVA and followed by Tukey's test. We considered the probability of $p<0.05$ as a significant difference.

Table 1. Effect of Va fruit extract, morphine-sulfate and naloxone in Hot plate test

Treatment	Dose	Hot plate latency (s)			
	(mg/kg)	Pre-drug	Post-drug	P	*n*
Saline		17 ± 0.82	18 ± 0.91		10
Morphine	5	18 ± 0.25	16 ± 0.91		7
	10	19 ± 0.33	14 ± 0.11	*	9
	15	24 ± 0.68	13 ± 0.83	**	7
Naloxone	5	19 ± 0.60	21 ± 0.11		6
	10	21 ± 0.14	19 ± 0.19		7
	20	17 ± 0.98	18 ± 0.63		9
Va, i.p	50				
	100				
	200				

3. Results

3.1. Hot plate test

3.1.1. Intraperitoneal method

Figure 1 shows the antinociceptive effect of different doses of V.A fruit extract in i.p. method which was compared with control groups. Each dose was administered to 8-12 rats. However, in contrast to the weak analgesic effect of the extract at doses of 50 and 100 mg/kg, it could have exerted a marked antinociceptive effect at a dose of 200 mg/kg.

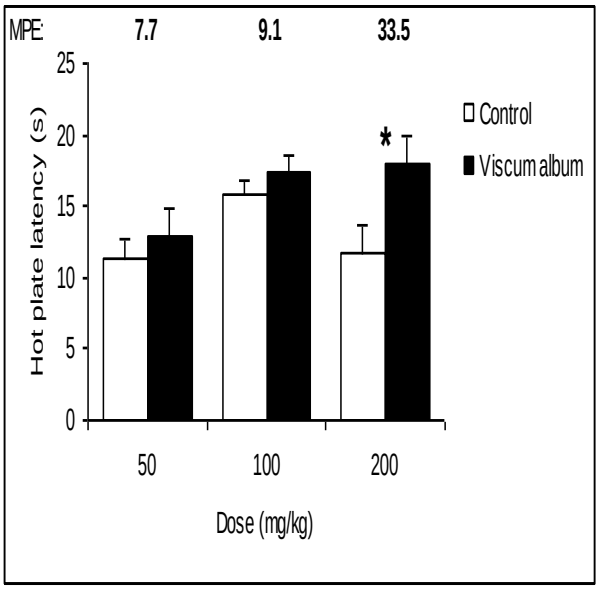

Fig. 1: Comparison of acute pain for different doses of V.A extract with control rats. Bars show mean of hot plate latency ± SEM. n=8, * p< .0.5.

3.2. Formalin test

3.2.1. Intraperitoneal method

Administration of the extract at doses of 50 and 100 mg/kg could not produce marked analgesic effect in acute (Fig. 2A) and chronic (Fig. 2B) formalin phases. However, dose of 100 mg/kg could lead to moderate ($p<0.05$) and higher doses over than 200 mg/kg could yield significant analgesic effects in both first (A) and second (B) phases of formalin test ($p<0.01$ and $p<0.001$).

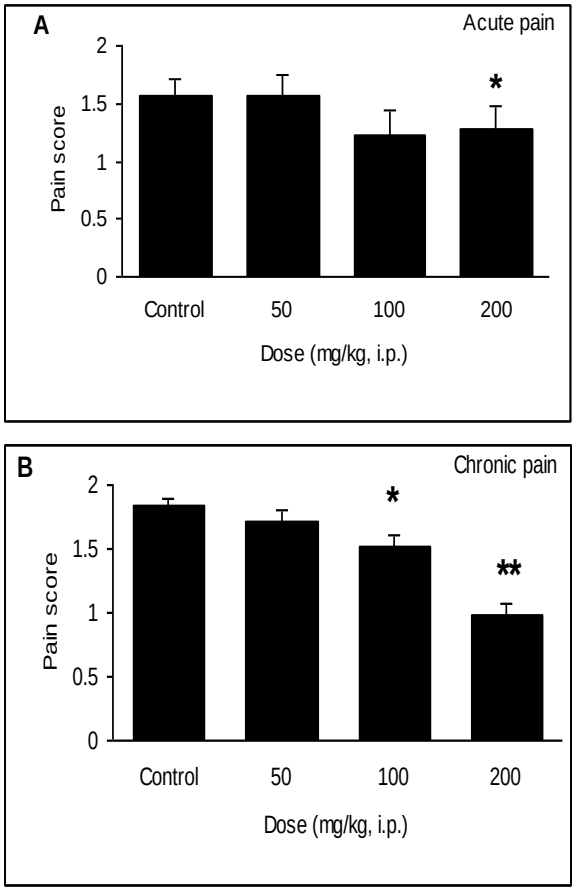

Fig. 2: Comparison of acute (A) and chronic (B) pain for different doses of V.A extract with control rats. Bars show mean of pain score ± SEM. n=8, *, ** show p< .0.5 and 0.01, respectively.

3.2.2. Oral method

In figure 3, the effect of oral feeding of the plant (6.25% food pellete) were compared with control group. As shown, there was no significant effect between control and treatment groups in acute and chronic models. The formalin pain score (as mean±S.E.M) in control, extract treatment and positive control groups (morphine sulfate) were compared with others (Fig. 4). As shown, application of the extract (i.p.) and morphine sulfate could markedly diminishe the chronic formalin pain. However, the late period of chronic phase (40-60 min) could significantly be attenuated by the oral application method.

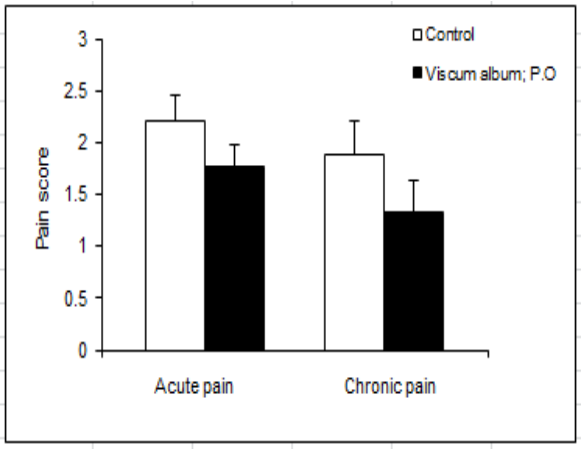

Fig. 3: Comparison of acute (A) and chronic (B) pain in oral application method. Bars show mean of pain score ± SEM. n=8

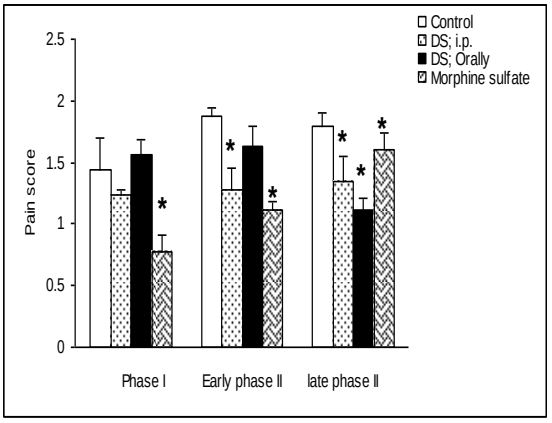

Fig. 4: Comparison of acute (A) and chronic (B) pain in control, injected, and oral groups with respect to positive control animals (morphine sulfate). Bars show mean of pain score ± SEM. n=8, * shows $p<0.05$.

4. Discussion

Pain as a real complaint in clinical setting has different causative factors. Although there are many analgesic drugs for prescription, but because of many complexities including broad side effects, different origins of pain and weak potency of many conventional drugs (14, 15), medicinal plant substitution has been recommended for this purpose (16).

In the present study, we used alcoholic V.A fruit extract, because it contains a rich source of alkaloids in comparison with other parts of the plant (3). Because these alkaloids which are mainly antimuscarinic components (4) can interact with opioid system (8, 9), they could be responsible for V.A analgesic effect. The usage of V.A extract for local anesthesia in some nations (1, 17) and analgesic effect of some species of *Viscum* like *Fastuosa* and *Ceratocaula* (3) are consistent with our report. However, the extract could exert the analgesic effect in both hot plate and formalin tests. Because the acute and chronic pain, respectively were mediated through central nervous system and peripheral mechanisms (18, 19), it is concluded that the extract alleviated the pain through both central and peripheral mechanisms. However, since the analgesic effect of the extract in i.p. form was very potent than oral routes, so it may be concluded that the V.A fruit effective component could not pass through the gastrointestinal wall. Moreover, the comparison of the analgesic effect of V.A fruit extract with morphine sulfate as positive control revealed that in spite of antinociceptive effect of morphine sulfate in acute phase (hot plate and phase I formalin test) which is consistent with other reports (20-22), this plant could not exert a potent analgesic effect in acute pain. In contrast, the chronic phase of formalin pain and especially its late period could significantly be attenuated by V.A fruit extract. Regarding the inflammatory events for phase II of formalin pain, through release of the local mediators like prostaglandins, kinin, interleukins, substance p and potassium (19), it can be concluded that this herb may have the modulatory effect on mentioned inflammatory mediators.

In addition, it is concluded that the alcoholic V.A fruit extract could markedly diminish the acute hot plate and chronic formalin pain (especially its late phase). However, the effective components could not pass through the gastrointestinal system successfully, and regarding the high distance between LD_{50} and its effective dose, this herb can be introduced as an analgesic medicinal plant. However, other analytic experiments for isolation and purification of antinociceptive components are needed to be carried out.

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