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Anti-nociceptive 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 for investigation of the analgesic effect of oral and intraperitoneal (i.p.) administration of alcoholic *Viscum album* L (V. album) fruit extract as a rich source of alkaloid substances.

Materials and Methods: Experimented animals were divided to control and treatment groups. The treatment group received different doses of the 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., anti-nociceptive effect of V. album extract in i.p. (50, 100 and 200 mg/kg) and oral application method V. album (6.25% food pellet) were compared with others and morphine sulfate and naloxone as positive and negative control groups, respectively. However, the extract over than 100 mg/kg i.p. could potentially alleviate the pain in hot plate and both phases of formalin test. Besides, there was a marked anti-nociceptive effect of the extract (over than 200 mg/kg) in oral method in hot plate and both phases of formalin tests. In our next experiments, the effective doses of morphine sulfate as the positive control drug were obtained over than 15 mg/kg; i.p.

Conclusion: In summary, by comparing the analgesic effect of different doses of morphine sulfate with V. album fruit extract in i.p. and oral conditions and regarding the extract LD50, it is concluded that the V. album fruit extract has a potent, semi-absorbable, and nearly safe ingredient which can exert a potential analgesic effect in acute and chronic pain.

Keywords: *Viscum album*, Formalin test, Hot plate

1. Introduction

The medicinal plant *Viscum album* 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 alkaloid especially anti-muscarinic component in the extract (5, 6). It is obvious that these alkaloids are more abundant in the fruits than other part 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). Since the role of opioid receptors in anti-nociception, especially in acute pain has been established, regarding interaction of these receptors

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with cholinergic system and introduces of the V.album fruit extract as a rich sources 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. album fruit extract in hot plate and formalin tests using i.p and oral methods.

2. Materials and Methods

2.1. Preparation of the crude extract

The fruits of plant were obtained from the local market and scientifically identified by the department of botany of Shaheed Beheshti University (SBU). One hundred grams of cleaned fruits was crushed and mixed at a ratio of 1 to 4 with methyl alcohol. The mixed complex was set aside for 24 h in laboratory temperature. Then, it was filtered three times through a mesh. The alcohol of filtered solution was evaporated in a 50°C tissue organ bath. Finally, 8-12 g concentrated residue remained in the container, which was used for preparation of extract doses.

2.2. Animals

Male NMRI rats (200-230 g) (Razi Institue, Iran) were used in our experiments. Four animals were housed in each Plexiglas cage with free access to food and water. The laboratory temperature and light-dark cycling was 24±2°C and 12 h, respectively.

2.3. Anti-nociceptive tests

The extract in 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 animals' food for 3 weeks. For positive control, morphine sulfate (15 mg/kg; i.p.) was used. 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 the control group.

2.4. Hot plate test

Anti-nociception 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°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 following formula (13).

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

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 plexiglass chamber (30×30×30 cm) which has a mirror with 45° angle underneath in order to accurate observation. In the treatment groups, the extract was administered intraperitoneally and orally, prior to the formalin injection. 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±SEM. The differences were estimated by ANOVA and followed by Tukey's test. We considered the probability of $P<0.05$ as a significant difference.

3. Results

3.1. Hot plate test

As shown in Table 1, V. album at doses of 100 and 200 mg/kg increase hot plate latency like the morphine with doses of 10 and 15 mg/kg.

Table 1. Effect of *V. album* 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±0.11	29±0.33	*	9
	15	24±0.68	33±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
V. album, i.p.	50	20±0.60	18±0.60		9
	100	22±0.83	32±0.50	**	8
	200	21±0.90	29±0.60	**	8

* and ** show the difference in comparing to pre-drug latency groups with probability of P< 0.05 and 0.01, respectively.

3.1.1. Intraperitoneal method

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

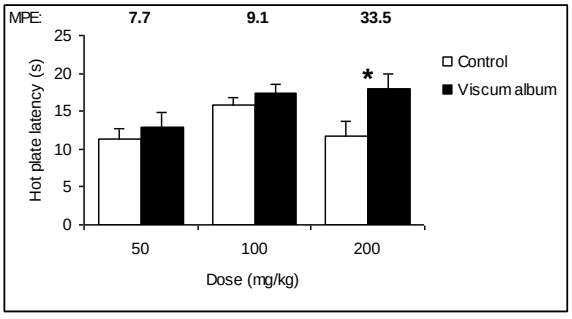

Fig.1. Comparison of acute pain in different doses of V. album with control rats. Bars show mean of hot plate latency ± SEM. n=8, * = P< 0.05.

3.2. Formalin test

3.2.1. Intraperitoneal method

Administration of the extract in doses of 50 and 100 mg/kg could not produce the 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 0.001).

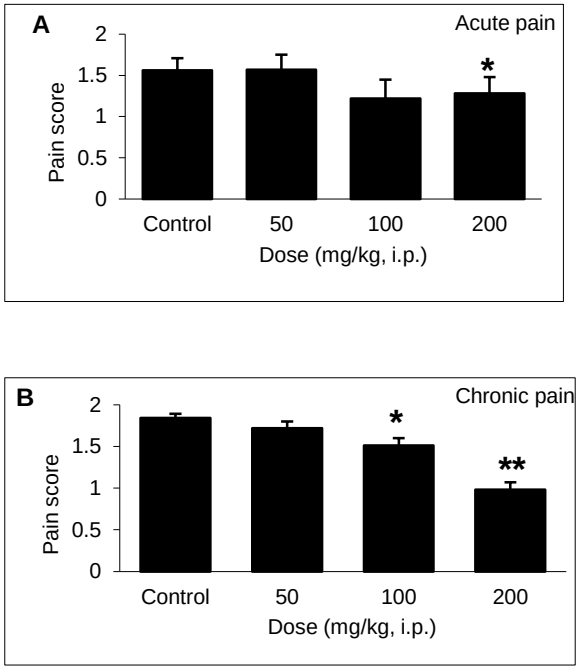

Fig.2. Comparison of acute (A) and chronic (B) pain in different doses of the extract with control rats. Bars show mean of pain score ± SEM. n=8, *, ** respectively show P< .0.5 and 0.01.

3.2.2. Oral method

In figure 3, the effect of oral feeding of the plant (6.25% food pellet) 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±SEM) in the control, extract treatment and positive control groups (morphine sulfate) were compared with others (Fig. 4) As shown, application of the extract and morphine sulfate could markedly diminish the chronic formalin pain. However, the late period of chronic phase (40-60 min) could significantly attenuated by the oral application method.

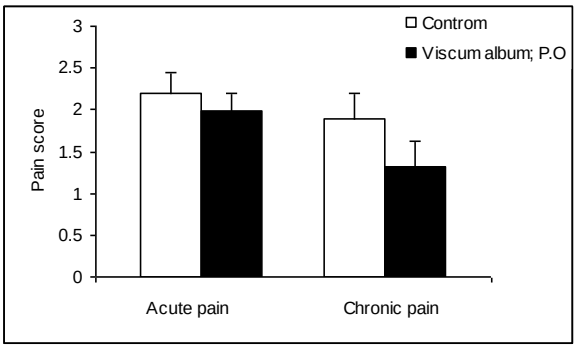

Fig 3. Comparison of acute (A) and chronic (B) pain in oral application method. Bars show mean of pain score ± SEM. n=8

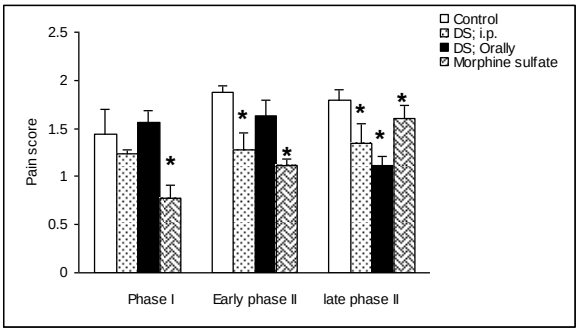

Fig.4. Comparison of acute (A) and chronic (B) pain in control, injected, oral groups regarding positive control animals (morphine sulfate). Bars show mean of pain score ± SEM. n=8, * show P< 0.05.

4. Discussion

Pain as a real complaint in clinical training, has different causing 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. album 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 anti-muscarinic components (4) can interact with opioid system (8, 9), it is presumed that the suggestive mechanism for V. album analgesic effect was carried out via its alkaloids. The usage of V. album 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 concluded that the extract alleviated the pain through both central and peripheral mechanisms. However, since the analgesic effect of the extract in i.p method was very potent than oral, So, it may be concluded that the V. album fruit effective component could not pass through the gastrointestinal, successfully. Moreover, the comparison of the analgesic effect of V. album fruit extract with morphine sulfate as positive control test, revealed that in spite of anti-nociceptive effect of morphine sulfate on acute pain (hot plate and phase I formalin test) which is consistent with other reports (20-22), it 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 attenuated by the extract. Regarding the inflammatory origins for phase II of formalin pain, through release of the local mediators like prostaglandins, kinnin, interlukins, and potassium (19), it can be concluded that this herb may have the modulatory effect on mentioned inflammatory mediators.

It is concluded that the alcoholic V. album fruit extract could markedly diminish the acute hot plate and chronic formalin pain (specially its late phase). However, the effective components could not pass through the gastrointestinal system successfully, and regarding the high distance between LD50 and its effective dose, this herb can be introduced as an analgesic medicinal plant. However, other analytic experiments for isolation and purification of anti-nociceptive components need to be carried out.

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