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ANTINOCICEPTIVE ACTIVITIES OF *VICIA CANESCENCE* AERIAL PARTS

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Abstract

Some pharmacological activities have been reported in Papilionaceae family. The aim of present study was to investigate antinociceptive activities of *Vicia canescence* aerial parts. Antinociceptive activity of the extract was determined by hot plate and writhing tests in mice. Impairment in mouse coordination was evaluated by rota-rode test. Extract produced statistically significant inhibition of nociception when compared to the control group. At all tested doses, extract reduced the writhing count at a dose-dependent mode, and showed an extremely significant effect when compared to the control. Extract at the dose of 200 mg kg-1 showed the same activity of diclofenac 50 mg kg-1 ($p>0.05$) and at 400 and 800 mg kg-1 showed higher activity than diclofenac 50 mg ($p<0.001$). Extract, in all tested doses significantly increased the pain threshold in hot plate thermal test. Extract at 800 mg kg-1 showed the same activity of diclofenac 50 mg kg-1 at 30th minutes ($p>0.05$). Extract did not induce any locomotor impairment in mice at any tested doses. Extract was safe and did not exhibit any toxicity up to 2.5 g kg-1. This study indicates the potential therapeutic use of *V. canescence* as a potent antinociceptive agent.

Key words: *Vicia canescence*; Writhing; Hot plate, locomotor impairment, Nociception.

Introduction

Pain is still one of the main health problems for the world's population. Current drugs such as NSAIDs and opioids may not be used in all cases, for their undesirable adverse effects and in some cases unsafe potency. As a result, the search for alternative pain remedies seems necessary and beneficial. A need for new pain relief remedies is stronger than ever and warrants the serious search for new and more useful compounds in this area. Among the various medicinal plants, some endemic and edible species are of particular interest because they may be used for producing raw materials or preparations containing phytochemicals with significant health benefits [4-6]. *Vicia genus* (Papilionaceae) has 45 species in Iran [1]. V. Sativum has protective role in cancer invasion and metastasis, insecticidal activity and also shows hepatoprotective activity [2]. Antioxidant activities of *V. sativum* [2], *V. faba* [3], *V. cracca* and *V. sativa* [4] and *V. canescence* [5] have been reported previously. Also anti-inflammatory and antinociceptive activity of *V. sativa* [6] and antimicrobial and cytotoxic activity of *V. faba* [7] have been reported. We have recently reported good antioxidant, antidepressant and antihemolytic activities of *V. sojakii* [8,9]. To the best of our knowledge, antinociceptive of *V. canescence* have not been reported to date and nothing was found about these activities. The aim of the present work was to determine the antinociceptive activities of V. canescence aerial parts in mice in the thermal and chemical models of analgesia in order to understand the usefulness of this plant in medicine.

Methods

Plants materials and preparation of extract

V. canescence aerial parts were collected, in July 2014 from Sari, Iran. The sample was authenticated by Dr. B. Eslami and the voucher specimen was deposited (No. 545) have been deposited in the Sari School of Pharmacy herbarium. Plant material was dried under dark conditions at r. t. for 2 weeks. The dry material was milled, obtaining 2-3 mm particles and then extracted by methanol for 24 h at room temperature. The extracts were then separated from the sample residues by filtration through Whatman No.1 filter paper and repeated three times. The resulting extracts were concentrated over a rotary vacuum at 35-40 °C until a crude solid extracts were obtained which then were freeze dried (MPS-55 Freeze-drier, Cperon, Korea) for complete solvents removal.

Animals

All experiments were performed on male Swiss mice (21 ± 2 g) obtained from Institute Pasteur. Animals were randomly housed in polypropylene cages at an ambient temperature, 25 ± 1°C and 45-55% relative humidity, with a 12 h light: 12 h dark cycle (lights on at 7 a.m.). The animals had free access to standard pellet and water and libitum. Experiments were conducted between 9:00 and 14:00 h. The experiments were conducted according to the norms of Committee for the Purpose of Control and Supervision of Experiments in Animal. Each animal was used once only. Seven mice were used in each experiment.

Writhing test

The abdominal constriction was induced by i.p. injection of 0.3% acetic acid [10]. Animals were pretreated with vehicle (3 ml kg-1, i.p.), extract (100-800 mg kg-1, i.p.) 30 min before the acetic acid injection. Diclofenac (50 mg kg-1 i.p.) was used as the reference drug. After challenges, pairs of mice were placed in separate plexiglas cages and the number of abdominal constrictions and stretches were cumulatively counted 8 min after acetic acid injection in each mouse over a period of 20 min.

Hot plate test

The extract was given at 100-800 mg kg-1, i.p. to the animals as a single dose. Mice were placed on a thermostatically controlled hot plate apparatus (Harvard, UK) maintained at 52 ± 0.5°C and the reaction time for licking or kicking of the fore or hind paws through was recorded with a stop watch. Mice which did not show any reaction after 15 sec, were discarded. Reaction time before and at 15, 30, 45 and 60 min after administrations of the extract was recorded. A cut-off time of 45 s was imposed to avoid tissue damage [11-13].

Motor coordination by Rota rod test

Effect on motor coordination was assessed using Rota rod apparatus (Harvard, UK) at a rotating speed of 16 rpm [11]. Only those animals that demonstrated the ability to remain twice on the revolving rod for at least 45 s were selected. Test was carried out in groups of 6 animals after i.p. administration of extracts at dose of 800 mg kg-1. The number of falls from the rod was counted for 45 s. The animals were observed before and 15, 30, 45 and 60 min after administration of each extract.

Non-fatal dose

Three mg kg-1 doses of extracts were injected to

separated groups of seven. After 48h, any mortality was considered as the maximum non-fatal dose.

Statistical Analysis

Results are expressed as means ± SD. One-way analysis of variance (ANOVA) for writhing test or repeated-measures ANOVA (for hot plate and rota-rod tests) followed by Newman-Keuls multiple comparisons tests were used. Differences with $p<0.05$ were considered significant.

Results and Discussion

The acetic acid induced writhing method has been widely used for the evaluation of peripheral antinociceptive activity and is able to determine the antinociceptive effect of compounds at dose level that might appear inactive in other methods like the tail flick test. However it is known that acetic acid induced constriction may be considered a non selective antinociceptive model, since acetic acid indirectly induces the release of endogenous mediators stimulating nociceptive neurons sensitive to NSAIDs [14]. Our results indicated that extracts, at the all tested doses reduced the writhing count at a dose-dependent mode, and showed an extremely significant effect when compared to the control. Extracts, in all tested doses (100-800 mg kg-1), reduced significantly the writhing count when compared to the control groups ($p<0.001$, Figure 1). The effect was dose dependent. Extracts at the dose of 200 mg kg-1 showed the same activity of diclofenac 50 mg kg-1 ($p> 0.05$, Figure 1). At the dose of 400 and 800 mg kg-1 showed higher activity than diclofenac 50 mg ($p< 0.001$, Figure 1). Number of writhing was decreased by diclofenac in magnitude of 62.0% which was lower that of leaf extract at the dose of 400 or 800 mg kg-1 (84.8 and 93.0 %, respectively, $p>0.001$). In the present experiments, the extract demonstrated significant analgesic activities, against thermal nociception. Extract, in all tested doses (100-800 mg kg-1) significantly increased the pain threshold in hot plate thermal test (Table 1). Extract at 800 mg kg-1 showed the same activity of diclofenac 50 mg kg-1 at 30th minutes ($p>0.05$). This activity was somewhat lower than morphine at 30th minutes ($p<0.05$, Table 1). Pretreatment of animals with naloxone, decreased the antinociceptive effect produced by morphine, but did not affect the action caused by extract. This finding indicates that the mechanisms involved in the analgesic properties of the extract in both writhing and hot plate tests seem to be related to the non-opioid models [14].

The animals treated with extract remained on the rotating rod for 1 minute in doses of 1200 mg kg-1. Extracts did not induce any locomotor impairment in mice at any tested doses. Remaining of treated animals on the rotating rod in all tested doses, indicate that extracts do not induce any deleterious effect on motor coordination and confirms that analgesic activity is not due to muscle relaxation or sedation. Vicia canescence contain phenols and flavonoids [5], it is possible that these compounds are responsible compounds for antinociceptive activities of this plant. In conclusion, these results introduced Vicia canescence as easily accessible source of natural products. This study indicates the potential therapeutic use of this plant as a potent antinociceptive agent. Elucidation of exact mechanism of action and active components responsible for good activities requires further investigations.

Acknowledgments

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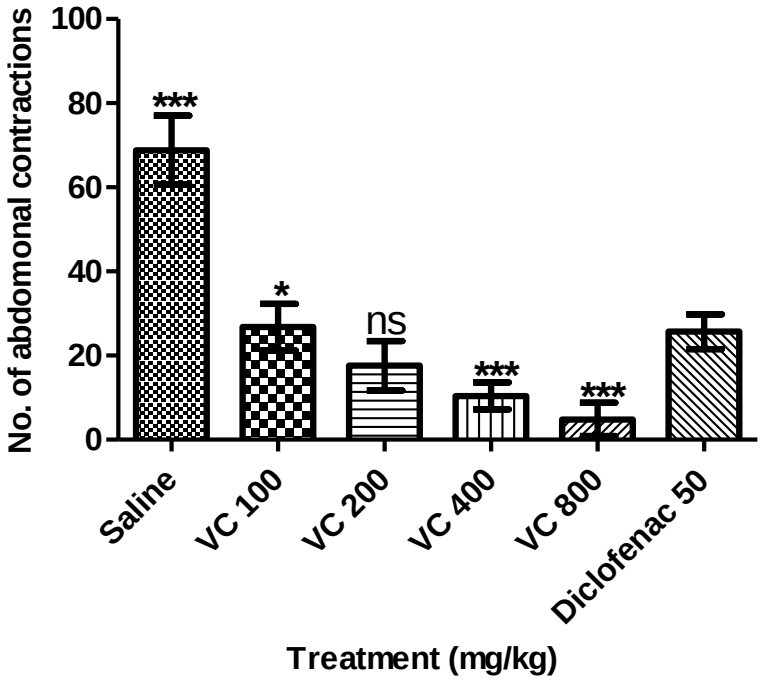

Figure 1. Antinociceptive activity of Vicia canescence extract in mice (Writhing test). Values are mean ± SD. (n = 7). All groups were different from control with *p < 0.05, ***p < 0.001.

Table 1. Antinociceptive activity of *Vicia canescence* extract in mice (Hot plate method).

Treatment	Dose (mg kg^{-1})	0	15	30	45	60
Control		6.2 ± 0.7	7.1 ± 1.5	6.8 ± 1.1	6.8 ± 0.7	7.2 ± 1.4
VC	100	5.5 ± 1.1	8.2 ± 0.6^{ns}	10.1 ± 1.3***	7.9± 0.6^{ns}	7.7 ± 0.6^{ns}
	200	6.3 ± 0.7	9.6 ± 2.1*	11.8 ± 1.3***	10.9 ± 1.5***	9.8 ± 1.7*
	400	5.9 ± 0.8	10.2 ± 1.7***	13.2 ± 0.3***	10.5 ± 1.1***	8.9 ± 1.1*
	800	6.9 ± 0.7	9.7 ± 1.5^{ns}	14.3 ± 1.1***	11.7 ± 1.7***	8.5 ± 1.6^{ns}
Morphine	5	6.2 ± 0.5	15 ± 2.1***	16.5 ± 1.0***	12.3 ± 1.1***	9 ± 1.6^{ns}
Diclophenac	5	5.8 ± 0.6	13 ± 0.6***	14 ± 0.9***	12.4 ± 0.4***	11.7 ± 1.5***

Data are expressed as mean ± *SD* (*n* = 7). ***Groups were different from control group with *p* < 0.001, ** *p* < 0.01, * *p* < 0.05, ns, not significant.