

ANTIINFLAMMATORY AND ANTINOCICEPTIVE ACTIVITIES OF *VICIA FABAE* HULLS

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Abstract

Some pharmacological activities have been reported in Fabaceae family. The aim of present study was to investigate antiinflammatory and antinociceptive activities of *Vicia faba* hulls. Inhibition of carrageenan induced edema was used to evaluate anti-inflammatory activity. Antinociceptive activity of the extract was determined by hot plate and writhing tests in mice. Impairment in mouse coordination was evaluated by rota-rod 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 400 mg kg⁻¹ showed the same activity of diclofenac 50 mg kg⁻¹ ($p > 0.05$). 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 produced statistically significant inhibition of edema induced by carrageenan at all doses (>200 mg kg⁻¹) when compared to the control groups ($p < 0.001$). The effect was dose-dependent. No statistically significance was observed between extract at 400 mg kg⁻¹ and diclofenac (50 mg kg⁻¹). at this dose, extract was equipotent with diclofenac ($p > 0.05$). Extract did not induce any locomotor impairment in mice at any tested doses. Extract was safe and did not exhibit any toxicity up to 3 g kg⁻¹. This study indicates the potential therapeutic use of *V. Faba hulls* as a potent anti-inflammatory and antinociceptive agent.

Key words: *vicia faba*, writhing, hot plate, locomotor impairment, nociception, carrageenan.

Introduction

Pain and inflammation are two of the main health problems. Because of undesirable adverse effects, current drugs such as NSAIDs are unsafe in some cases. 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 species are of particular interest because they may be used for producing raw materials or preparations containing phytochemicals with significant health benefits. [1-3]. *Vicia faba* L., (Fabaceae) is pulse crop commonly grown in many parts of the world. It has its origin in the East, and their consumption is popular in South America, Argentina and China. [4] It is a source of energy, protein, folic acid, niacin, vitamin C, magnesium, potassium, iron and dietary fibre. Due to the high levels of lysine in their protein they are an adequate complement to the protein of cereals. [5] They have great potential in the snack food industry. [6] Iranian climate and favoured geographical locations have contributed to the diversity of medicinal plants. There are vast expanses of *V. faba* bean fields in northern of Iran. Good HIV-1 reverse transcriptase activity has been reported. [7] Recently, good antioxidant activity has been reported for *V. faba* bean and hulls (Boudjou et al., 2013). Antioxidant activities [8,9], antimicrobial and cytotoxic activity of *V. faba* [10] have been reported.

Recently we have reported very good nitric oxide scavenging activity of *V. faba* hulls. [9] Because NO transmits signals from vascular endothelial cells to vascular smooth muscle cells and plays an important role in vital physiologic functions such as inflammation, this extract was selected for assay of antiinflammatory activity. [11] To the best of our knowledge, antiinflammatory and antinociceptive of *V. faba* hulls have not been reported to date and nothing was found about these activities. The aim of the present work was to determine the antiinflammatory and antinociceptive activities of *V. faba* hulls 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

Vicia faba L. beans and hulls were collected, in May 2015 from Sari, Iran. The sample was authenticated by Dr. B. Eslami and the voucher specimen was deposited (No. 1137) have been deposited in the

was dried under dark conditions at r.t. for 2 weeks. The dry material was milled, obtaining 2-3 mm particles. Samples were extracted with methanol in an ultrasonic cleaning bath by indirect sonication at a frequency of 60 kHz and a temperature of $25 \pm 3^\circ\text{C}$ for 1 h to yield ultrasonic extracts. The extracts were then separated from the samples residue by filtration. The resultant extracts were concentrated in a rotary evaporator until a crude solid extracts were obtained which were freeze-dried for complete solvent removal and used as ultra-sonic extracts. [9].

Animals

Experiments were performed on male Swiss mice (21 ± 2 g) or Wistar rats (180-200 g) (Institute Pasteur). Animals were housed at an ambient temperature and 45-55% relative humidity, with a 12 h light: 12 h dark cycle. The animals had free access to standard pellet and water and libitum. Experiments were conducted between 9:00 and 14:00. All the experimental procedures were conducted in accordance with the NIH guidelines of the Care and Use of Laboratory Animals. The Institutional Animal Ethical Committee (IAEC) of Mazandaran University of Medical Sciences also approved the experimental protocol. Each animal was used once only. Seven mice were used in each experiment. Mice (or rats) were divided into different groups ($n = 7$) and 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 $52 \pm 0.5^\circ\text{C}$ 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.

Antiinflammatory activity

Carageenan (50 µL of 1% suspension, Sigma Chemicals Co. USA) was injected into the sub planar tissue of the right hind paw of each rat. Extract (100-800) or diclofenac (50 mg kg⁻¹) was administered i.p. to rats 1 hour before carageenan injection. Volume of edema was measured prior and 3 h after carageenan injection. Degree of swelling was the ratio of the volume of hind paw before to after carageenan treatment. [15,16]

Non-fatal dose

Three mg kg⁻¹ doses of extract 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

Extract in all doses (>200 mg kg⁻¹), reduced significantly the writhing count when compared to the control group ($p < 0.001$, Fig. 1). Extract at the dose of 400 mg kg⁻¹ was equipotent with diclofenac 50 mg kg⁻¹ ($p > 0.05$). At 800 mg kg⁻¹ was the most potent one but showed the same activity as diclofenac 50 mg kg⁻¹ ($p > 0.05$). Extract, in all tested doses (100-800 mg kg⁻¹) significantly increased the pain threshold in hot plate thermal test in 30th minutes (Table 1). Extract at the dose of 800 mg kg⁻¹ was the most potent one and showed the same activity as diclofenac ($p > 0.05$). Naloxone did not reverse the effect of extract. The animals treated with extract remained on the rotating rod for 1 minute in dose of 800 mg kg⁻¹. Extracts did not induce any locomotor impairment in mice at all. Extract produced statistically significant inhibition of

edema induced by carrageenan at all doses (>200 mg kg⁻¹) when compared to the control groups ($p < 0.001$, Fig.2). The effect was dose-dependent. No statistically significance was observed between extract at 400 and 800 mg kg⁻¹ and diclofenac (50 mg kg⁻¹). Extract at these doses were equipotent with diclofenac ($p > 0.05$).

Discussion

There are some reports concerning the anti-inflammatory and antinociceptive effects of *Vicia* spp. [18-20] There are some reports concerning the anti-inflammatory effect of flavonoids. [21] *V. faba* contain phenols and flavonoids [9], it is possible that these compounds are responsible compounds for anti-inflammatory activity. The 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. [19] Our results indicated that extract at all tested doses reduced the writhing count at a dose-dependent mode, and showed an extremely significant effect when compared to the control. Number of writhing was decreased by diclofenac in magnitude of 62.0% which was lower than that of leaf extract at the dose of 800 mg kg⁻¹ (74.3 %, $p > 0.05$). In the present experiments, the extract demonstrated significant analgesic activities, against thermal nociception. Extract, in all tested doses 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. [21] The animals treated with extract remained on the rotating rod for 1 minute in dose of 800 mg kg⁻¹. Extract did not induce any locomotor impairment in mice at tested dose. Remaining of treated animals on the rotating rod in the highest tested dose, 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.

Extract produced potent and statistically significant inhibition of edema induced by carrageenan at all doses when compared to the control groups (Fig. 2). Extract at 400 mg kg⁻¹ showed the same activity of diclofenac ($p > 0.05$). This finding indicates the potential therapeutic use of this plant as a potent anti-inflammatory agent. Since this effect was not reversed by naloxone, it is concluded that mechanism underlying the anti-inflammatory action of the extract might be unrelated to the opioid system and other mechanisms such as interaction with PG biosynthesis or other mediators should be considered.

In conclusion, these results introduced *Vicia faba* hull as easily accessible source of natural products. This study indicates the potential therapeutic use of this plant as a potent anti-inflammatory and antinociceptive agent. Elucidation of exact mechanism of action requires further investigations.

Acknowledgments

This research was supported by a grant from the research council of Mazandaran University of Medical Sciences, Iran.

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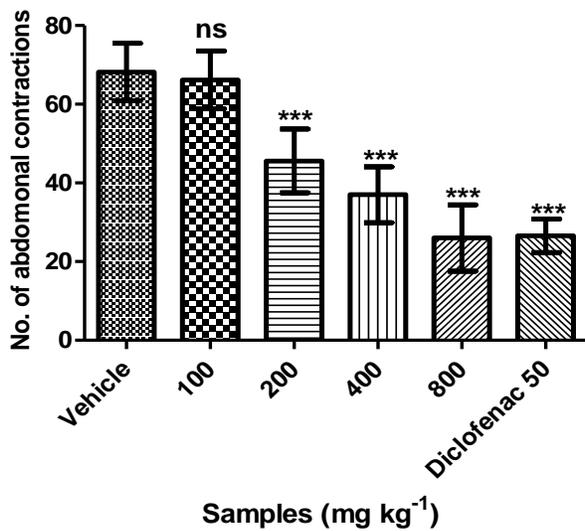


Figure 1. Antinociceptive activity of *V.faba* in mice (Writhing test). Values are mean \pm SD. (n=7), ***p<0.001, ns, not significant with respect to control.

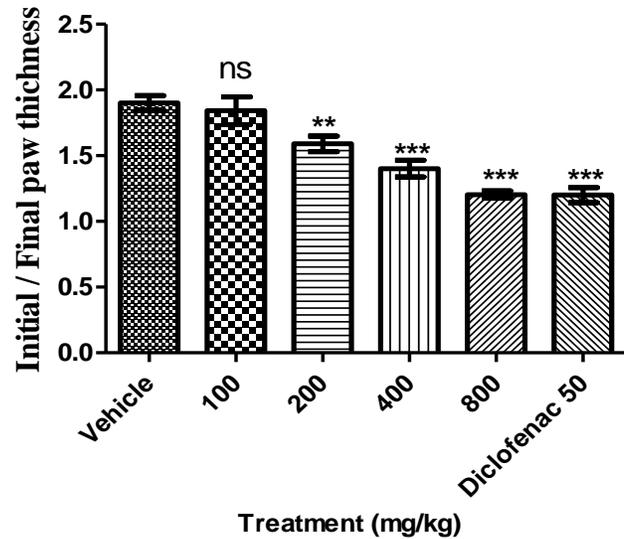


Figure 2. Antiinflammatory activity of *V. faba* hull on carrageenan induced paw edema in rats. Values are mean \pm SD (n = 7).

Table 1. Antinociceptive activity of *V. faba* hull in mice (Hot plate method).

Treatment	Dose (mg kg ⁻¹)	0	15	30	45	60
Control		6.2 \pm 0.2	7.1 \pm 1.6	6.8 \pm 0.2	6.8 \pm 0.2	7.2 \pm 1.3
extract	100	5.4 \pm 0.9	7.8 \pm 0.9 ^{ns}	8.9 \pm 0.5*	8.2 \pm 0.7 ^{ns}	7.5 \pm 0.8 ^{ns}
	200	5.4 \pm 0.5	8.2 \pm 1.6 ^{ns}	10.1 \pm 1.2***	9.2 \pm 1.4*	7.7 \pm 1.1 ^{ns}
	400	5.6 \pm 0.5	9.2 \pm 1.2**	11.6 \pm 0.6***	10.3 \pm 0.7***	8.5 \pm 0.8 ^{ns}
	800	5.3 \pm 0.5	11.5 \pm 0.7***	13.4 \pm 0.9***	11.1 \pm 1.7***	9.1 \pm 1.6 ^{ns}
Morphine	5	6.2 \pm 0.5	15 \pm 2.0***	16.5 \pm 1.0***	12.3 \pm 1.0***	9.0 \pm 1.6*
Diclophenac	5	5.8 \pm 0.6	13 \pm 0.6***	14 \pm 0.9***	12.4 \pm 0.4***	11.7 \pm 1.5***

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