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ANTIDEPRESSANT ACTIVITY OF VICIA FABA HULLS

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Abstract

Many biological activities have been reported in Fabaceae family. The aim of present study was to investigate antidepressant activities of Vicia faba hulhs extract. Antidepressant activities of methanolic extract was evaluated by modified forced swimming test (FST) and tail suspension tests (TST) in male Swiss albino mice. Extract showed significant antidepressant activities in both models. Extract shortened remarkably the immobility period in both FST and TST and exhibited a dose dependent activity ($p<0.001$). Extract in all tested doses decreased significant the duration of immobility during FST as compared to control group. But at 1200 mg kg-1, this effect was not comparable with that of imipramine ($p<0.05$). Extract in all tested doses showed significant activity in increasing in climbing time as compared to control group ($p<0.01$ and $p<0.001$). Imipramine also increased climbing time respect to the control group significantly ($p<0.01$) but extract at 1200 mg kg-1 showed better activity than imipramine in increasing climbing time ($p<0.001$). Extract showed significant activity in decreasing in swimming time as compared to the control group. There was no difference between this dose and control group ($p>0.05$). The effect of extract was not comparable with that of imipramine ($p<0.001$). Extract in all tested doses showed significantly and dose dependently decreased in the immobility time in TST as compared to control mice. Extract even at 800 mg kg-1 did not show the same activity as imipramine ($p < 0.05$). The non-fatal doses of extracts were over 3.0 g kg-1. Our study indicates that extract showed significant antidepressant activity. It seems this effect is mainly mediated by inhibition of reuptake of catecholamines. This result introduces this plant as easily accessible source of natural antidepressant.

Key words: Vicia faba, Forced swimming test, Tail suspension tests, Hull, Antidepressant, Immobility period, Climbing time.

Introduction

Depression is a common disorder with high lifetime rates with high morbidity and mortality. Because the mechanism of depression is quite complex, different synthetic chemical antidepressants have been introduced. Current antidepressant treatments are efficacious, however, just for 50% of the patients and it often takes more than 5-8 weeks until the patients respond to the treatment. [1] Accordingly, it is necessary to research and develop more effective antidepressants. There are a large number of herbal medicines whose therapeutic potential have been assessed in a variety of animal models. Most assessments of herbal antidepressant activity were conducted using forced swimming test (FST) and the tail suspension test (TST). These studies have provided useful information for the development of new pharmacotherapies from medicinal plants for use in clinical for treatment of depression. Plants, such as Hypericum perforatum [2], Vicia sojakii [3], Hibiscus esculentus [4], corn silk [5] and Feijoa sellowiana [6] may be important sources of new antidepressant drugs.

Vicia faba L., (Fabaceae) is commonly grown in many countries. Its origin is in the East but its consumption is popular in South America, Argentina and China. [7] It is a source of energy, protein, folic acid, niacin, vitamin C, magnesium, potassium, iron and dietary fibre. [8] V. faba has a great potential in the snack food industry. [9] 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. [10] Recently, good antioxidant activity has been reported for V. faba bean and hulls. [11,12] Also, antiinflammatory and antinociceptive [13], antimicrobial and cytotoxic activity of V. faba [14] have been reported. Recently we have reported very good nitric oxide scavenging activity of V. faba hulls. [12] Because NO is so important in CNS, and plays an important role in physiologic functions such as depression [15], this extract was selected for assay of antidepressant activity. To the best of our knowledge, antidepressant activities of V. faba hulls have not been reported to date and nothing was found about these activities. The aim of present study was to investigate antidepressant activities of *Vicia faba* hulhs extract. Antidepressant activities of methanolic extract was evaluated by modified forced swimming test (FST) and tail suspension tests (TST) in male Swiss albino mice.

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 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. Samples were extracted with methanol in an ultrasonic cleaning bath by indirect sonication at a frequency of 60 kHz and a temperature of 25 ± 3°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. [12]

Animals

Experiments were performed on male Swiss mice (21 ± 2 g) (MAZUMS Institute of Experimental Animal Research). 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 were divided into different groups (n = 7) and used in each experiment.

Forced swimming test (FST)

The mouse was dropped into a glass cylinder (20 cm in height and 14 cm in diameter) containing 15-cm-deep water at 24-25□C and left there for 6 min. After the initial 2-3 min of vigorous activity, the animals show a period of immobility by floating with minimum movements. Each mouse was judged to be immobile when it stopped struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. The duration of immobility, climbing and swimming behaviors during the final 4-min interval of the swimming test were measured. [3,4] Control group was treated with Tween 80 plus 0.9% (w/v) saline solution. The other groups received an i.p. injection of extract (100-1200 mg kg-1) in Tween 80 plus 0.9% (w/v) saline solution and imipramine (10 mg kg-1)

(from Darupakhsh Co., Tehran, Iran), one hour before the experiment. The animals were used only once. A decrease in the duration of immobility is indicative of an antidepressant effect.

Tail suspension test (TST)

The tail suspension test was carried out as per the established method. Groups of 7 animals are treated with the extract (100-800 mg kg-1) by i.p. injection 30 min prior to testing. For the test the mice are suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. After 2-3 min of vigorous activity characterized by struggling movements, attempts to catch the adhesive tape, or body torsions or jerks, the mice hung passively and completely motionless. Immobility was defined as the absence of any limb or body movements, except for those caused by respiration or when they hung passively and completely motionless. The duration of immobility is recorded for a period of 5 min. Imipramine (10 mg kg-1) was used as positive control. [3,4] A decrease in the duration of immobility is indicative of an antidepressant effect.

Non-fatal dose

Three mg kg-1 doses of extract were injected to separated groups of seven. After 72 hours, any mortality was considered as the maximum non-fatal dose. [3]

Statistical Analysis

Results are expressed as Means ± SD. One-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparisons tests was used. Differences with $p<0.05$ were considered significant.

Results

Table 1 showed the effect of extract on the duration of immobility during FST. Extract in all tested doses showed significant activity as compared to control group. But even at the highest dose, 1200 mg kg-1, the effect was not comparable with that of imipramine ($p<0.05$). Extract in all tested doses showed significant activity in increasing in climbing time as compared to control group ($p < 0.01$ and $p < 0.001$). Imipramine also increased climbing time respect to the control group significantly ($p < 0.01$) but extract at 1200 mg kg-1 showed better activity than imipramine in increasing climbing time ($p < 0.001$). Extract in all tested doses showed significant activity in decreasing in swimming time as compared to the control group. But this effect was not dose dependent. Extract at highest dose, 1200 mg kg-1, increased swimming time again. There was no difference between this dose and control group ($p > 0.05$). Imipramine increased swimming time respect to the control group significantly ($p<0.001$). The effect of extract was not comparable with that of imipramine ($p<0.001$). Extract in all tested doses showed significantly and dose dependently decreased in the immobility time in TST as compared to control mice. Extract even at 800 mg kg-1 did not show the same activity as imipramine ($p < 0.05$) (Figure 1). The non-fatal doses of extracts were over 3.0 g kg-1. No mortality was observed after 72 hours.

Discussion

There is an increasing interest in the study of the antidepressant effect of medicinal plants, because conventional antidepressants in market provide a complete remission just for the half of individuals. [1] There are many published papers that showed polyphenolic compounds such as flavonoids have antidepressant activity. [16-18]

NO has been associated with a variety of physiological processes in the human body since it was identified as a signal molecule. It transmits signals from vascular endothelial cells to vascular smooth muscle cells. It also plays an important role in vital physiological functions. [19,20] In the nervous system, NO works as an atypical neural modulator that is involved in neurotransmitter release, neuronal excitability and learning and memory. [21] CNS activity of some plants may be partially mediated by the NO pathway. [22] There is some evidence that strongly suggests involvement of the NO signalling pathway in CNS disorders. [23,24] Recently we have reported high phenolic contents and very good nitric oxide scavenging activity of V. faba hulls. [12] Based on this observation, this extract was selected for assay of antidepressant activity.

FST and TST are the two well established animal models of depression which are used to screen the potential drugs for antidepressant activity. Behavioral despair was proposed as a model to test for antidepressant activity. It was suggested that mice forced to swim in a restricted space from which they cannot escape are induced to a characteristic behavior of immobility. This behavior can be reduced by agents that are therapeutically effective in human depression. There is a significant correlation between clinical potency and effectiveness of antidepressants in both models. [25,26] FST is the most widely used tool for assessing antidepressant activity preclinically.

However, the major drawback of the traditional FST is that it is unreliable in the detection of the effects of selective 5-HT reuptake inhibitors, [27,28] which are the most widely prescribed antidepressant drugs today. Several modifications have been made to enhance the sensitivity of the traditional FST. [27,28] These alterations enabled investigators to distinguish specific behavioural components of active behaviors: (1) climbing behavior, upward-directed movements of the forepaws along the side of the swim chamber; (2) swimming behavior, the movement throughout the swim chamber that also includes crossing into another quadrant; and (3) immobility as in the traditional test. The major advance of the this modified FST is that it reveals that catecholaminergic agents decrease immobility with a corresponding increase in climbing behavior, whereas 5-HT-related compounds decrease immobility but increase swimming behavior. [27,28] Extract in all tested doses showed significant activity as compared to control group.

Extract in all tested doses showed significant activity in increasing in climbing time as compared to control group ($p< 0.01$ and $p< 0.001$). Imipramine also increased climbing time respect to the control group significantly ($p< 0.01$) but extract at 1200 mg kg-1 showed far better activity than imipramine in increasing climbing time ($p< 0.001$). It seems V. faba extract has antidepressant activity by inhibition of reuptake of catecholamines.

Extract in all tested doses showed significant activity in decreasing in swimming time as compared to the control group. But this effect was not dose dependent. Extract at highest dose increased swimming time again. Swimming time was the same as control group ($p > 0.05$). It seems, V. faba extract have not any activity on the reuptake of 5-HT. Imipramine increased swimming time significantly ($p<0.001$).

TST has been described as a facile means of evaluating potential antidepressants. [26] The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by the tail. Extract in all tested doses showed significantly and dose dependently decreased in the immobility time in TST as compared to control mice. Imipramine was very effective and extract even at the highest tested dose, 800 mg kg-1 did not show the same activity as imipramine ($p < 0.05$) (Figure 1). In conclusion, our studies indicate that *V. faba* hull extract showed significant antidepressant activity. It produced dose dependent effect on both FST and TST. It seems, this effect is mainly mediated by inhibition of reuptake of catecholamines. However, further studies are necessary for complete understanding its antidepressant mechanism. This study introduced *V. faba* hull as easily accessible source of natural antidepressant.

Acknowledgments

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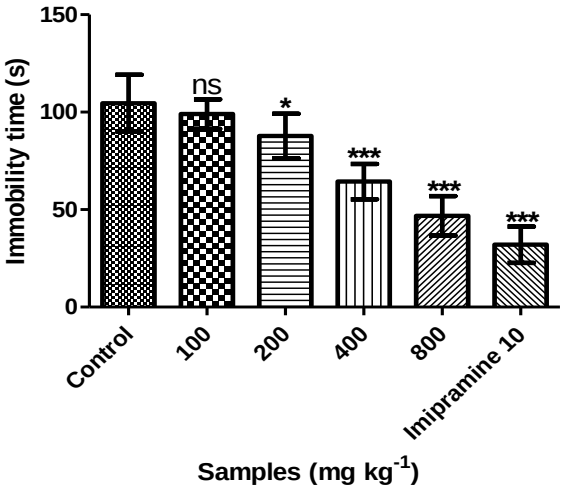

Figure 1. Antidepressant activities of *V. faba* in TST. Groups are different from imipramine (***P<0.001) except for 800 mg kg-1 (*P<0.05).

Table 1. Antidepressant activities of *V. faba* in modified FST.

Group	Dose (mg kg^{-1})	FST		
		Immobility (s) [a,b]	Climbing (s) [a,c]	Swimming (s) [a,d]
Control	-	203.8 ± 19.7	6.5 ± 1.5	30.0 ± 3.1
Extract	100	168.8 ± 25.9*	52.2 ± 25.9**	19.1 ± 2.7**
	200	164.4 ± 34.1*	59.6 ± 32.7**	16.0 ± 4.7***
	400	158.6 ± 29.6*	59.6 ± 21.2**	22.4 ± 7.7**
	800	155.4 ± 16.8*	61.6 ± 12.2**	23.4 ± 5.4**
	1200	86.0 ± 28.3***	127.2 ± 24.1***	26.8 ± 6.7^{ns}
Imipramine	10	44.0 ± 6.5***	43.6 ± 3.4**	152.0 ± 3.2***

[a]Data are expressed as mean ± SD (*n* = 7), respect to control group. [b] Groups were different from imipramine with ***P<0.001 except for 1200 mg kg^{-1} (*P<0.05). [c] Groups were not different from imipramine [ns] P>0.05 except for 1200 mg kg^{-1} (***P<0.001). [d] Groups were different from imipramine with ***P<0.001.