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Antidepressant and antihemolytic activities of *Vicia sojakii*

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Abstract. – OBJECTIVES: Many pharmacological activities have been reported in *Vicia* (V.) genus. The aim of present study was to investigate antidepressant and antihemolytic activities of aerial parts of *V. sojakii*.

MATERIALS AND METHODS: Antidepressant activity of methanolic extract was evaluated by forced swimming test (FST) and tail suspension tests (TST) in male Swiss albino mice. Antihemolytic effect of extract was also determined.

RESULTS: Extract showed good antidepressant activity in both FST and TST. It shortened remarkably the immobility period in FST and TST and exhibited a dose dependent activity. Extract in 125 mg kg^{-1} showed significant activity as compared to control ($p < 0.05$) in both test. Extract at 1500 mg kg^{-1} showed the same activity of imipramine 15 mg kg^{-1} ($p > 0.05$) in FST. Extract show good antihemolytic activity against H_2O_2 induced hemolysis.

CONCLUSIONS: Our report indicated the *V. sojakii* aerial parts extract was safe and showed remarkable antidepressant activity in FST and TST in mice. It also exhibited good antihemolytic activity. These results introduced *V. sojakii* as an easily accessible source of natural antidepressant.

Key Words:
Antihemolysis, Forced swimming test, Tail suspension test.

Introduction

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. Depression is a common chronic condition in clinical practice and affects about 121 million people worldwide and is a leading cause of disability, according to WHO[1]. Although there are many effective antidepressants available today, the current armentarium of therapy is often inadequate with unsatisfactory results in about one third of all subjects treated[2]. This necessitates the development of newer and more effective antidepressants from traditional medicinal plants. *Vicia* (V.) genus (*Papilionaceae*) has 45 species in Iran. *V. sativum* has protective role in cancer invasion and metastasis and also shows hepatoprotective activity[3]. Antioxidant activities of *V. sativum*[3], *V. faba*[4] and *V. canescence*[5] have been reported previously. Also antiinflammatory and antinociceptive activity of *V. sativa*[6] and antimicrobial and cytotoxic activity of *V. faba*[4] have been reported. *V. sojakii* is native to Iran. We have recently reported its good antioxidant activity[7]. To best of author knowledge there is no report on its antidepressant activity. The aim of this study was to determine the antidepressant activity by FST and TST and antihemolytic activity of *V. sojakii* aerial parts in male Swiss albino mice in order to understand the usefulness of this plant in medicine.

Material and Methods

Plant Material and Preparation of Freeze-Dried Extract

V. sojakii aerial parts were collected from north of Iran. The sample was authenticated by Dr. B. Eslami and the voucher specimen was deposited (No. 1149) in Sari School of Pharmacy Herbarium. Aerial parts were dried and coarsely ground. Powder was extracted by maceration using methanol. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained, which was then freeze-dried for complete solvent removal (yield: 16.5%).

Animals

Male Swiss albino mice (20 ± 2 g) were randomly housed in groups of 10 in polypropylene cages at an ambient temperature, 25 ± 1°C and 45-55% relative humidity, with a 12 h light: 12 h

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dark cycle (lights on at 7 a.m.). The animals had free access to standard pellet and water and *libitum*. Experiments were conducted between 8:00 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. NMRI (Naval Medical Research Institute) mice were divided into five different groups (n = 10 per group) and tested in FST and TST.

Forced Swimming Test

Mouse was dropped into a glass cylinder (20 cm in height and 12 cm in diameter) containing 8-cm-deep water at 24-25°C and left there for 6 min. The duration of immobility during the final 4-min interval of the swimming test was measured[2]. Control group was treated with Tween 80 plus 0.9% (w/v) saline solution. The other groups of mice received an *i.p.* injection of extract (125, 250, 500, 1000 and 1500 mg kg^{-1}) in Tween 80 plus 0.9% (w/v) saline solution and imipramine (15 mg kg^{-1}), 1h before the experiment. Imipramine was utilized as positive control.

Tail Suspension

Male mice weighing 20-25 g are housed in plastic cages for at least 10 days prior to testing in a 12 h light cycle with food and water freely available. Animals are transported from the housing room to the testing area in their own cages and allowed to adapt to the new environment for 1 h before testing. Groups of 10 animals are treated with the extract (125, 250, 500, 1000 and 1500 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. The duration of immobility is recorded for a period of 5 min. Mice are considered immobile when they hang passively and completely motionless for at least 1 min. Imipramine (15 mg kg^{-1}) was used as positive control[2].

Maximum Non-Fatal Dose

Different doses of extract were injected to separated groups of seven. After 48 h, the highest dose that did not induce any mortality was considered as the maximum non-fatal dose.

Scavenging of Hydrogen Peroxide

Extract (0.1-1 mg ml^{-1}) in distilled water were added to a H_2O_2 solution [0.6 ml, 40 mM in phosphate buffer (pH 7.4)]. The absorbance of H_2O_2 at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging was calculated as follows: $[(A_o - A_1)/A_o] \times 100$ where A_o was the absorbance of the control and A_1 was the absorbance in the presence of the sample of extract and standard[8,9].

Antihemolytic Activity Against H_2O_2 Induced Hemolysis

Erythrocytes were isolated from male Wistar rats and diluted with PBS (phosphate buffered saline) to give 4% suspension. Antihemolytic activity of the extract was assessed as described recently[8,9]. The erythrocytes were 1 g of extract/ml of saline buffer was added to 2 ml of the erythrocyte suspension and the volume was made up to 5 ml with saline buffer. The concentration of H_2O_2 in the reaction mixture was adjusted to bring about 90% of hemolysis after 240 min. Extend of hemolysis was determined by measuring the absorbance at 540 nm corresponding to hemoglobin liberation.

Statistical Analysis

Experimental results are expressed as means ± SD. All measurements were replicated thrice. The data were analyzed by analysis of variance ($p < 0.05$) and the means separated by Duncan's multiple range test.

Results

Table I shows the result of effect of extract on the duration of immobility during FST. The extract in all of doses show significant activity as compared to control group. The extract at the dose of 1500 mg kg^{-1} showed the same activity of imipramine at 15 mg kg^{-1}, in FST ($p < 0.05$). In addition, extract significantly and dose dependently decreased the immobility time as compared to control mice TST. But at the dose of 1500 mg kg^{-1} it showed weaker activity than imipramine at 15 mg kg^{-1}, in decreasing immobility period ($p < 0.05$). As the maximum non-fatal dose, extract did not induce any mortality until 4000 mg kg^{-1} for 48 hours. Extract was capable of scavenging H_2O_2 in a concentration dependent manner. IC_{50} was 984.1 ± 34.4 mg ml^{-1}. The IC_{50} values for vitamin C and BHA were 21.4 and 52.0 mg ml^{-1}, respectively. Lipid oxidation of

Table I. Antidepressant activities of *V. sojakii* in FST and TST.

Group	Dose (mg/kg)	Duration of immobility (s), FST	Duration of immobility (s), TST
Control	–	164.2 ± 1.3	178.6 ± 9
Extract	125	157.7 ± 4.8*	171.6 ± 9.4*
Extract	250	150.1 ± 6.1**	164.3 ± 7.3*
Extract	500	122.9 ± 5.0***	142.6 ± 13.0**
Extract	1000	100.5 ± 4.7***	121.3 ± 7.2***
Extract	1500	93.3 ± 8.6***	94.0 ± 10.7***
Imipramine	15	88.2 ± 3.0***	82.0 ± 9.6***

[a]Data are expressed as mean ± SD (n = 10). Groups are different from control group (**$p < 0.01$, ***$p < 0.001$).

rat blood erythrocyte membrane mediated by H_2O_2 induces membrane damage and subsequently hemolysis. The extract showed good antihemolysis activity (IC_{50} = 679.4 ± 25 μg ml^{-1}). Vitamin C exhibited better activity (IC_{50} = 235 ± 9 μg ml^{-1}).

Discussion

Behavioral despair was proposed as a model to test for antidepressant activity by Porsolt et al[10]. 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 reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. The extract in all of doses show significant activity as compared to control group.

Tail suspension test has been described by Steru et al[11] as a facile means of evaluating potential antidepressants. 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. The extract significantly and dose dependently decreased the immobility time as compared to control mice. The extract showed good antidepressant activity.

Scavenging of H_2O_2 by extract may be attributed to its phenolics, and other active components[7] which can donate electrons to H_2O_2 and neutralizied it to water. Extract was capable of scavenging H_2O_2 in a concentration dependent manner. Although H_2O_2 itself is not very reactive, it can cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is important in food systems.

Hemolysis has a long history of use in measuring free radical damage and its inhibition by antioxidants but only few studies have been performed with erythrocytes. In this study, we used a biological test based on free radical-induced erythrocyte lyses in rat blood. This assay is useful either for screening studies on various molecules and their metabolites, especially on the one hand molecules have a redox activity and on the other hand molecule having a long-term action[9].

Conclusions

Our study indicated *V. sojakii* was safe and showed remarkable antidepressant activity in FST and TST models. It also exhibited good antihemolysis activity. Further investigation of individual compounds and the mechanisms of activity are needed.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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