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Antidepressant activity of *Hibiscus esculentus L.*

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Abstract. – OBJECTIVES: *Hibiscus (H.) esculentus L.* (Okra) is distributed from Africa to Asia, Southern European and America and widely used as food. The aim of present study was to investigate antidepressant activity of Okra seeds and leaves.

MATERIALS AND METHODS: Antidepressant activity of methanolic extracts were evaluated by forced swimming test (FST) and tail suspension tests (TST). Also, total phenol and flavonoid contents were measured by Folin Ciocalteau and AlCl3 assays, respectively.

RESULTS: Phenol and flavonoid contents of extracts were determined as gallic acid and quercetin equivalents from a calibration curve, respectively. Extracts showed good antidepressant activity in both FST and TST. The extracts shortened remarkably the immobility period in FST and TST and exhibited a dose dependent activity. Seeds extract in 250, 500 and 750 mg kg^{-1} showed significant activity as compared to control ($p < 0.001$). Both extracts at 750 mg kg^{-1} showed similar activity as imipramine 15 mg kg^{-1} ($p > 0.05$) in TST. Extracts contained high amount of phenol and flavonoids. No mortality has been observed up to 2 g kg^{-1} for seeds and 2.5 g kg^{-1} for leaves.

CONCLUSIONS: These results introduced *H. esculentus* seeds and leaves as an easily accessible and edible source of natural antidepressant.

Key Words:
Antidepressant, Forced swimming test, *Hibiscus esculentus L.*, Tail Suspension test.

Introduction

Depression constitutes the second most common chronic condition in clinical practice[1]. It is one of several devastating illness affecting mood, along with mania, hypomania, and bipolar disorders[2]. Approximately two-thirds of the anxious or depressed patients respond to the currently available treatments but the magnitude of improvement is still disappointing[3]. Although there are many effective antidepressants available today[4], current armentarium of therapy is often inadequate with unsatisfactory results in about one third of all subjects treated[4]. This necessitates the development of newer and more effective antidepressants from traditional medicinal plants whose psychotherapeutic potential has been assessed in a variety of animal models[5]. *Hibiscus (H.) esculentus L.* (Okra), a tropical to subtropical plant that is widely distributed from Africa to Asia, Southern European and America[6] belongs to the family of Malvaceae. It is common vegetable in most regions of Greece, Turkey and Iran, especially in the northern[7]. Okra is primarily a southern vegetable garden plant, grown for its immature pods, which are consumed when cooked either alone or in combination with other foods. The seeds of mature okra are roasted and ground and used as a coffee substitute in Turkey[8]. The seeds of mature pods are sometimes used for chicken feed and have been used on a small scale for the production of oil[6]. Studies have confirmed okra seeds as a good source of oil and protein[9]. In addition Okra seeds have good antioxidant and antihypoxic activities[8]. Antihemolytic and antioxidant activity of okra leaves have been reported recently[10]. There are many published papers that showed polyphenolic compounds such as flavonoids have antidepressant activity[11-14]. Because of good antioxidant activities of Okra seeds and leaves, it was nominated for assay of antidepressant activity. To the best of the author's knowledge, antidepressant activity of *H. esculentus* has not been reported to date and nothing was found about mechanism /or antidepressant activity of this plant. The aim of the present work was to determine the antidepressant activity by forced swimming test (FST) and tail suspension test (TST) in order to understand the usefulness of this plant as a foodstuff as well as in medicine.

Materials and Methods

Plant Materials and Preparation of Freeze-dried Extract

Seeds and leaves of *H. esculentus* were collected from Dashte-Naz area, north of Sari, Iran, in summer 2008. After identification of the plant,

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a voucher (No. 358 and 359) has been deposited in the Faculty of Pharmacy Herbarium. These parts were dried at r. t. and coarsely ground before extraction. 100 g of each part was extracted by percolation method using methanol. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained which were then freeze-dried for complete solvent removal. Yields were 10% for seeds and 18% for leaf, respectively.

Chemicals

Gallic acid, quercetin, Folin-Ciocalteau, $AlCl_3$, sodium carbonate and potassium acetate were purchased from Merck (Darmstadt, Germany). Imipramine was purchased from Darupakhsh Co (Tehran, Iran). All other chemicals were of analytical grade or purer.

Determination of Total Phenolic and Flavonoid Content

Total phenolic compound content was determined by the Folin-Ciocalteau method[15]. Samples were mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent for 5 min and 2.0 ml of 75 g/l sodium carbonate were then added. The absorbance of reaction was measured at 760 nm after 2 h of incubation at room temperature. Results were expressed as gallic acid equivalents. Total flavonoid was estimated according to method of our recent papers[15]. Briefly, 0.5 ml solution of extract in methanol were mixed with 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water and left at r. t. for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (UV-Visible EZ201, Perkin Elmer, Norwalk, CA, USA). Total flavonoid contents were calculated as quercetin from a calibration curve.

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 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 mice were divided into five different groups (n = 10 per group) and tested in FST and TST.

Forced Swimming Test

The 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[13]. Control group was treated with Tween 80 plus 0.9% (w/v) saline solution. The other groups of mice received an intraperitoneal (*i.p.*) injection of extracts (125, 250, 500 and 750 mg kg^{-1}) in Tween 80 plus 0.9% (w/v) saline solution or imipramine (15 mg kg^{-1}), 1h before the experiment. Imipramine was utilized as positive control of the test.

Tail Suspension Test

Male mice weighing 20-25 g are used preferentially. They are housed in plastic cages for at least 10 days prior to testing in a 12h 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 and 750 mg kg^{-1}) by intraperitoneal (*i.p.*) injection 30 minutes 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 minutes. Mice are considered immobile when they hang passively and completely motionless for at least 1 minute. Imipramine (15 mg kg^{-1}) was used as positive control of the test[13].

Non-fatal Dose

Different doses of extracts 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[7].

Statistical Analysis

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

Results

The total phenolic contents of extracts was in order to: leaves (68.81 ± 3.9) > seeds extract (58.4 ± 2.6) mg gallic acid equivalent g^{-1} of extract by reference to standard curve. Also, total flavonoid contents was in order to: leaves (49.3 ±

Table I. Antidepressant activities of Hibiscus esculentus extracts 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
Seed extract	125	151.62 ± 1.9*	155.48 ± 1.5**
	250	130.4 ± 7.1***	146.6 ± 4.58***
	500	104.7 ± 6.5***	109.4 ± 7.5***
	750	85.6 ± 5.1***	83.4 ± 2. 8***
Leaf extract	125	154.7 ± 5.9 ns	162.9 ± 2.1 ns
	250	140.3 ± 9.1**	150.6 ± 8.1**
	500	118.6 ± 6.11***	12.3 ± 4.5 ***
	750	103.8 ± 4.5***	87.7 ± 5.3***
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 ($^{ns}p > 0.05$,$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

2.2) > seeds extract (23.9 ± 1.2) mg quercetin equivalent g^{-1} of extract, by reference to standard curve. Table I showed the result of effect of extracts on the duration of immobility during FST. The seeds extract in 250, 500 and 750 mg kg^{-1} show significant activity as compared to control group ($p < 0.001$). Leaves extract in 500 and 750 mg kg^{-1} show significant activity as compared to control group ($p < 0.001$). The seeds extract at the dose of 750 mg kg^{-1} showed the same activity as imipramine at 15 mg kg^{-1} ($p > 0.05$). In TST, leaves extract in 500 and 750 mg kg^{-1} show significantly and dose dependently decreased the immobility time as compared to control mice ($p < 0.001$) also seed extract showed show significant activity as compared to control group at 250, 500 and 750 mg kg^{-1} (Table I). The seeds and leaves extracts at the dose of 750 mg kg^{-1} showed the same activity as imipramine at 15 mg kg^{-1}, in decreasing immobility period ($p > 0.05$). The non-fatal doses of seeds and leaves extracts were 2000 and 2500 mg kg^{-1}, respectively.

Discussion

This plant showed high total phenol and flavonoid contents. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities[16]. Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases[17]. Behavioral despair was proposed as a model to test for antidepressant activity[5,17]. It was suggested that mice or rats 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. TSThas been described as a facile means of evaluating potential antidepressants[18]. 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 mice display after active and unsuccessful attempts to escape when suspended by the tail. The extracts showed good antidepressant activity. They produced dose dependent effect on both FST and TST.

Conclusions

Our studies indicate that the seeds and leaf extracts of *H. esculenta* showed a remarkable antidepressant activity in forced swimming and tail suspension tests. Further investigation of individual compounds and the mechanisms of activities are needed.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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