ANTIDEPRESSANT ACTIVITY OF CORN SILK

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Summary

Corn silk (CS) is widely used in Iranian traditional medicine. The aim of present study was to investigate antidepressant activity of CS by forced swimming (FST) and tail suspension tests (TST). Phenol and flavonoid contents were measured by Folin Ciocalteu and $AlCl_3$ assays, respectively. Hydro alcoholic extract of Corn silk (125, 250, 500 and 1000 mg kg^{-1}) was studied for its antidepressant activity by FST and TST. Immobility period during the experiments were noted. Phenol and flavonoid contents of the extract were determined as gallic acid and quercetin equivalents from a calibration curve, respectively. Extract showed good antidepressant activity in FST and TST. The extract shortened remarkably the immobility period during the FST and TST and exhibited a dose dependent activity. All test groups were significantly different form control group ($P<0.001$) in both tests. Extract at 1500 mg kg^{-1} showed similar activity as imipramine 10 mg kg^{-1} ($p> 0.05$) in TST. CS extract contained a significant amount of phenol and flavonoids. No mortality has been observed up to 4000 mg/kg. These results introduced *CS* aerial parts as an easily accessible and edible source of natural antidepressant and antioxidants.

Keywords: Antidepressant, Corn silk, Forced Swimming Test, Tail Suspension Test.

Introduction

Depression constitutes the second most common chronic condition in clinical practice and will become the second leading cause of disease burden worldwide by the year 2020 (1). Approximately two-thirds of the anxious or depressed patients respond to the currently available treatments but the magnitude of improvement is still disappointing (2). Although there are many effective antidepressants available today (3,4), the current armentarium of therapy is often inadequate with unsatisfactory results in about one third of all subjects treated. 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). In recent years, considerable attention has been directed towards the identification of plants with antioxidant ability that may be used for Human consumption (6-8). Diuretic, as well as antilithiasic, uricosuric, and antiseptic, properties are traditionally attributed to Corn silk (CS), stigma/style of Zea mays Linne (Poaceae/ Gramineae), which has been used in many parts of the world for the treatment of edema as well as for cystitis, gout, kidney stones, nephritis, and prostatitis (9,10). CS contains proteins, vitamins, carbohydrates, Ca^{2+}, K^{+}, Mg^{2+} and Na^{+} salts, volatile oils, and steroids such as sitosterol and stigmasterol, alkaloids, saponins, tannins, and flavonoids (9,10). Phenolic compounds present in CS are anthocyanins, p-coumaric acid, vanillic acid, protocatechuic acid, derivatives of hesperidin and quercetin, and bound hydroxycinnamic acid forms composed of p-coumaric and ferulic acid (11). There are also reports about antioxidant activity of CS (10,12). The constituents in the volatile extract and petroleum ether, ethanol, and water extract of CS exhibited clear antioxidant activities (13). To the best of the author's knowledge, antidepressant activity of CS has not been reported to date and nothing was found about mechanism /or antidepressant activity of CS. Therefore, the aim of the present work is 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

Animals: Swiss albino mice (20 ± 2 g) of either sex were randomly housed in groups of six in polypropylene cages at an ambient temperature of 25± 1^{o}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 *ad libitum*. Each animal was tested once. All of the experiment conducted between 8:00 and 14:00 h. Mice were divided into five different groups (n = 8 per group) and tested in FST and TST. The experiments were conducted in accordance to the ethical guidelines regarding investigation with laboratory animals (NIH guidelines of the Care and Use of Laboratory animals) and were also approved by the Ethical Committee for Animal Experimentation of Mazandaran University of Medical Sciences.

Plant material: CS (dried cut stigmata of Zea mays L, Poaceae flowers) used for this investigation was collected in January 2008 and authenticated by Dr. Bahman Eslami (Department of Biology, Islamic Azad University of Qhaemshahr, Iran) and the voucher specimen was deposited in the Sari School of Pharmacy herbarium (No. HS280). CS was dried at room temperature and an ethanol-water (1:1) extraction was performed using maceration method by soaking in the solvent mixture. The extract was collected after removing the solvent and lyophilization.

Determination of total phenolic and flavonoid content: Total phenolic compound content was determined by the Folin-Ciocalteau method (14-16). The extract sample (0.5 ml of different dilutions) was 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 paper (17-19). 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 room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (Perkin Elmer). Total flavonoid contents were calculated as quercetin from a calibration curve.

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 ºC and left there for 6 min. The duration of immobility during the final 4-min interval of the swimming test was measured (2-4). Control group was treated with solvent. The other groups of mice received an interperitoneal (i.p.) injection of extract (125, 250, 500 and 1000 mg kg^{-1}) in Tween 80 plus 0.9% (w/v) saline solution and imipramine (5 and 10 mg kg^{-1}), 1 h before the experiment. Imipramine was utilized as positive control of the test.

Median lethal dose: LD_{50} was assumed using 50% deaths within 72h after i.p. administration of the extract at different doses. Male Swiss mice weighing 20-25 g (10 per group) were used in this experiment (20).

Tail suspension test: This test is a variant of the behavioural despair test in which immobility is induced by suspending a mouse by its tail. On day 3, 1 h after the extract treatment, mice were hung individually on a wire in an upside down posture so that its nostril just touches the water surface in a container. After initial vigorous movements, the mouse assumes an immobile posture and the period of immobility during a 5 min observation period were noted (21). This test is reliable and rapid screening method for antidepressants including those involving the serotonergic system (22).

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

Results

Total phenol and flavonoid contents: Total phenol compounds, as determined by Folin Ciocalteu method, are reported as gallic acid equivalents by reference to standard curve ($y = 0.0063x$, $r^2 = 0.987$). The total phenolic content of CS was 118.94 ± 2.78 mg gallic acid equivalent/g of extract. The total flavonoid contents was 58.22 ± 1.34 mg quercetin equivalent/g of extract, by reference to standard curve ($y = 0.0067x + 0.0132$, $r^2 = 0.999$).

Effect of CS on immobility in FST: The result of effect of hydroalcoholic extract of CS on the duration of immobility during forced swimming test is shown in Table 1. The extract shortened remarkably the immobility period during the forced swimming test in comparison with negative control and exhibited a dose dependent antidepressant activity. ANOVA analysis followed by Newman–Keuls multiple comparisons test shows that all test groups (except for lowest dose) were significantly different form control group ($P<0.001$).

Imipramine 5 mg/kg, had shortest immobility period that was not comparable with CS extract (P<0.001). No mortality has been observed up to 4000 mg/kg.

Effect of CS on immobility time in TST: CS (500 and 1500 mg kg^{-1}) significantly (*P*< 0.001) and dose dependently decreased the immobility time as compared to control mice (Table 1). The extract at the dose of 1500 mg kg^{-1} showed the same activity as imipramine at 10 mg kg^{-1} (*P*> 0.05), in decreasing immobility period.

Table1. Effect of hydroalcoholic extract of Corn silk on the duration of immobility during forced swimming test and Tail suspension test.

Group	Dose (mg/kg)	Duration of immobility (s), FST	Duration of immobility (s), TST
Control	-	150.2 ± 8.9	157.8 ± 12
Corn silk	125	146.8 ± 6.2*	---------
Corn silk	250	120.2 ± 7.6**	---------
Corn silk	500	72.0 ± 4.3**	134.4 ± 7.2*
Corn silk	1000	57.6 ± 3.1**	97.2 ± 6.7**
Corn silk	1500	---------	79.2 ± 3.4**
Imipramine	5	21.6 ± 0.7**	---------
Imipramine	10	14.4 ± 0.5**	73.8 ± 4.2*

ANOVA followed by Newman–Keuls multiple comparisons test shows that all test groups are significantly different from control group (*P<0.01, **P<0.001). Values are Mean ± SD (N = 8).

Discussion

Total phenol compounds were determined as gallic acid and the total flavonoid contents as quercetin equivalent/g of extract. 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 (23). Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases (24, 25). CS extract showed high level of Total phenol and flavonoids contents. The swimming test has been widely employed to evaluate the effect of various agents on the central nervous system (CNS), such as CNS depressants, antidepressants, sedative-hypnotics, psychostimulants, euphorics, nootropics, adaptogens, etc. The immobility seen in rodent during swimming reflects behavioral despair as seen in human depression (3). The swimming test has also been used extensively to assess the anti stress activity of plants in mice (26). The forced swimming test is a classic animal model for antidepressant drug screening (3,4). CS extract shortened remarkably the immobility period during the FST in comparison with negative control. The effect was dose dependent. Tail suspension test represents the behavioral despair model, claimed to reproduce a condition similar to human depression. The test is based on the observation that animals, following initial escape oriented movements, develop an immobile posture when placed in an inescapable chamber. The immobility is thought to reflect either a failure of persistence in escape-directed behavior (i.e. behavioral despair) or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli. It has been argued that the TST is less stressful than FST and has greater pharmacological sensitivity (27).

Remarkably, TST detects the anti-immobility effects of a wide array of antidepressants, including tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRI), monoamine oxidase inhibitors (MAOI), electro-convulsive shock (ECS), and even atypical antidepressants. Thus, the activity of *CS* could involve one of the mechanisms of the established agents as described above. CS extract significantly ($P< 0.001$) and dose dependently decreased the immobility time as compared to control mice. The extract at the dose of 1500 mg kg^{-1} showed the same activity as imipramine at 10 mg kg^{-1} ($P> 0.05$), in decreasing immobility period. Also, extract was safe and no mortality has been observed up to 4000 mg/kg.

Conclusions

The finding of the present investigation suggests the antidepressant activity of CS in FST and TST models of depression. CS significantly reduced the immobility period in both FST and TST. The extract also had high level of phenol and flavonoids and was so safe at least up to 4000 mg/kg. However, further studies are necessary for complete understanding the antidepressant activity of CS. Such identified potential and natural constituents could be exploited as cost effective food additives for human and animal health.

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References

1. An overview of a strategy to improve the mental health of underserved populations. Social Change and Mental health, WHO, (May 1977, revised May 1999). Available from: http://www.who.int/entity/mental_health/media/en/46.pdf. Accessed August 17, 2009.

2. Ebrahimzadeh MA, Hadizadeh F, Hosseinzadeh H, Hajipoor M, Nabavi SM. Antioxidant and antidepressant effects of four novel Bupropion analogues. *Pharmacologyonline* 2009; 2: 317-322.

3. Hadizadeh F, Ebrahimzadeh MA, Hosseinzadeh H, Motamed-Shariaty V, Salami S, Bekhradnia AR. Antidepressant and antioxidant activities of some 2-benzoxazolinone derivatives as Bupropion analogues. *Pharmacologyonline* 2009; 1: 331-335.

4. Mahmoudi M, Ebrahimzadeh MA, Ansaroudi F, Nabavi SF, Nabavi SM,Antidepressant and antioxidant activities of *Artemisia absinthium* L. at flowering stage. Afr J Biotechnol in press.

5. Zhang ZJ. Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. *Life Sci* 2004; 75: 1659-1699.

6. Ebrahimzadeh MA, Nabavi SM, Nabavi SF. Correlation between the *in vitro* iron chelating activity and poly phenol and flavonoid contents of some medicinal plants. Pak J Biol Sci 2009; 12(12): 934-938.

7. Ghasemi K, Ghasemi Y, Ebrahimzadeh MA. Antioxidant activity, phenol and flavonoid contents of 13 Citrus specis peels and tissues. Pak. J. Pharm. Sci., 2009; 22 (3): 277-281.

8. Ebrahimzadeh MA, Nabavi SF, Nabavi SM, Eslami B. Antihypoxic and antioxidant activity of *Hibiscus esculentus* seeds. Grasas Y Aceites 2010; 61(1): 30-36.

9. Grases F, March JG, Ramis, M, Costa-Bauza A. The influence of Zea mays on urinary risk factors for kidney stones in rats. *Phytother Res* 1993; 7:146-149.

10. Ebrahimzadeh MA, Pourmorad F, Hafezi S. Antioxidant Activities of Iranian Corn Silk. *Turk J Biol* 2008; 32: 43-49.

11. Kaur G, Alam MS, Jabbar Z, Javed K, Athar M. Evaluation of antioxidant activity of Cassia siamea flowers. *J Ethnopharmacol* 2006; 108: 340-348.

12. Maksimovic ZA, Kovacevic N. Preliminary assay on the antioxidative activity of Maydis stigma extracts. *Fitoterapia* 2003; 74: 144-147.

13. El-Ghorab E, El-Massry KF, Shibamoto T. Chemical composition of the volatile extract and antioxidant activities of the volatile and nonvolatile extracts of egyptian Corn Silk (Zea mays L.) *J Agr Food Chem* 2007; 55(22): 9124- 9127.

14. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Bahramian F. In vitro antioxidant activity of *Phytolacca americana* berries. *Pharmacologyonline* 2009; 1: 81-88.

15. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B. In vitro antioxidant and free radical scavenging activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. *Phcog Mag* 2009; 4(18):122-126.

16. Dehpour AA, Ebrahimzadeh MA, Nabavi SF, Nabavi SM. Antioxidant activity of methanol extract of *Ferula assafoetida* and its essential oil composition, *Grasas y Aceites* 2009; 60(4): 405-412.

17. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Eslami B. Antioxidant activity of aqueous extract of *Pyrus boissieriana* fruit. *Pharmacologyonline* 2009; 1: 1318-1323.

18. Ebrahimzadeh MA, Bahramian F. Antioxidant activity of *Crataegus pentaegyna subsp. elburensis* fruits extracts used in traditional medicine in Iran. *Pak J Biol Sci* 2009; 12(5): 413-419.

19. Ebrahimzadeh MA, Nabavi SF, Nabavi SM. Antioxidant activities of methanol extract of *Sambucus ebulus* L. Flower. *Pak J Biol Sci* 2009; 12 (5): 447-450.

20. Morteza-Semnani K, Mahmoudi M, Riahi G. Effects of essential oils and extracts from certain *Thymus* species on Swimming Performance in Mice. *Pharm Boil* 2007; 45(6): 464-467.

21. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacolog*y 1985; 85: 367-370.

22. Bhattacharya SK, Satyan KS, Ramanathan M. Experimental methods for evaluation of psychotropic agents in rodents: II- antidepressants. *Indian J Exp Biol* 1999; 37: 117–123.

23. Ebrahimzadeh MA, Nabavi SF, Nabavi SM. Antihemolytic and antioxidant activity of *Hibiscus esculenus* leaves Pharmacologyonline 2009; 2: 1097-1105.

24. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A. Bekhradnia AR. Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica Mey. Pharmacologyonline* 2008; 2: 560-567.

25. Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR. Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. Afr. J. Biotechnol. 2008; 7 (18): 3188-3192.

26. Maity TK, Mandal SC, Saha BP, Pal M. Effect of *Ocimum sanctum* roots extract on swimming performance in mice. *Phytother Res* 2000; 14: 120-121.

27. Thierry B, Steru L, Simon P, Porsolt RD. The tail suspension test: ethical considerations. *Psychopharmacology* 1986; 90: 284–285.