EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 9
204-208
Year: 2022

PHYTOCHEMICAL AND ANTIDEPRESSANT ACTIVITY OF AQUEOUS EXTRACT OF *CUCURBITA PEPO* LEAVES IN MICE

***Sajja Ravindra Babu, Mariam John Mammen, Burra Sai Praveen, K. Bhavani, Nidhi Jha and Pendyala Ruchitha**

Department of Pharmacology, Malla Reddy Institute of Pharmaceutical Sciences, Secunderabad, India.

***Corresponding Author: Sajja Ravindra Babu**
Department of Pharmacology, Malla Reddy Institute of Pharmaceutical Sciences, Secunderabad, India.

Article Received on 25/06/2022 **Article Revised on 15/07/2022** **Article Accepted on 05/08/2022**

ABSTRACT
The aim of the present study was to determine the antidepressant activity of leaves of *Cucurbita pepo* (Pumpkin) compared with the standard drug: Fluoxetine (20 mg/kg) in normal mice. The phytochemical test provides an overview of the chemical composition of *Cucurbita pepo* and shows the presence of alkaloids, flavonoids, tannins, saponins, carbohydrates and terpenoids. Animal testing results showed the administration of *Cucurbita pepo* produced a significant ($p < 0.01$) decrease of the duration of immobility time of mice exposed to Forced Swimming Test and Tail Suspension Test. Dose of 300 mg/kg, p.o of Aqueous extract of *Cucurbita pepo* administered to mice produced significant antidepressant effect in both FST and TST than Aqueous extract of *Cucurbita pepo* 100 mg/kg, p.o and their efficiencies were found to be comparable to fluoxetine in dose of 20 mg/kg, p.o. The flavonoids components of CPAE might be interacting with 5-HT in mediating the antidepressant effect of *Cucurbita pepo*.

KEYWORDS: Antidepressant, *Cucurbita pepo*, Forced Swim Test, Tail Suspension Test.

1. INTRODUCTION
Depression is classified as a mood disorder. It may be described as feelings of sadness, loss, or anger that interfere with a person's everyday activities. It's also fairly common. The Centers for Disease Control and Prevention (CDC) Trusted Sources estimates that 8.1 percent of American adults ages 20 and over had depression in any given 2-week period from 2013 to 2016.[1]

There is substantial evidence that nerve growth factors such as brain-derived neurotrophic factor (BDNF) are critical in the regulation of neural plasticity, resilience, and neurogenesis. The evidence suggests that depression is associated with the loss of neurotrophic support and that effective antidepressant therapies increase neurogenesis and synaptic connectivity in cortical areas such as the hippocampus. BDNF is thought to exert its influence on neuronal survival and growth effects by activating the tyrosine kinase receptor B in both neurons and glia.

The monoamine hypothesis of depression suggests that depression is related to a deficiency in the amount or function of cortical and limbic serotonin (5-HT), norepinephrine (NE), and dopamine (DA). In addition to the monoamines, the excitatory neurotransmitter glutamate appears to be important in the pathophysiology of depression. A number of studies of depressed patients have found elevated glutamate content in the cerebrospinal fluid of depressed patients and decreased glutamine/glutamate ratios in their plasma.[2]

2. MATERIALS AND METHODS:
2.1. Collection and Authentication of Plant Materials
The leaves of *Cucurbita pepo* were collected from the premises of Farm & Agricultural land in Moinabad. The leaves of *Cucurbita pepo* were cleaned and air dried at room temperature for one week and ground into a fine powder by grinder. Powdered samples were collected and stored in air-and water-proof containers protected from direct sunlight and heat until required for extraction.

2.2. Experimental Animals
Swiss mice (20-30g) either sex werw selected and the animals were maintained in a well ventilated room with 12:12 hours light dark cycle in polypropylene cages. Standard pellet fed and tap water provided ad libitum throughout the experimentation period. Animals were acclimatised to laboratory conditions a week prior to the initiation period. Animals were acclimatised to laboratory conditions a week prior to initiation of experiment.

2.3. Preparation of Plant Extract

The fresh leaves were washed with distilled water, shade dried and the leaves were completely powered by the use of the grinder. The air dried and powdered plant materials (50gm) were extracted with water by using Soxhlet apparatus for 8 hours at a temperature not exceeding the boiling point. Then the extract was collected from Soxhlet apparatus and kept at room temperature for air-drying. The residues were collected, weighed and stored at 4^0 C for future use.

2.4. Preliminary Phytochemical Screening

Phytochemical tests were done to find out the presence of bioactive chemical constituents such as alkaloids, terpenoids, flavonoids, carbohydrates, tannins, saponins and steroids amino acid compounds by the following procedure.

2.5. Experimental Procedure

2.5.1. Forced Swim Test (FST): Forced swim test was proposed as a model to test antidepressant activity by postal et.al.[3] Mice were individually forced to swim in open glass chamber (25✕15✕25 cm) containing fresh water to height of 15 cm and maintained at 26 ± 1^0C. At this height of water, animals were not able to support themselves by touching the bottom or the side walls of the chamber with their hand paws or tail.

Each animal showed vigorous movements during the initial 2 minutes period of the test. The duration of immobility during the next 4 minutes of the total 6 minutes testing period. Mice were considered to be immobile when they ceased struggling and remained floating motionless in water, making only those movements necessary to keep their head above water.[4-5]

GROUPING

Group 1: Administered saline orally for 7 days and severed as control

Group 2: Fluoxetine (20mg/kg) was administered orally for 7 days and served as standard

Group 3: Administered Aqueous extract of *Cucurbita pepo*(100 mg/kg) orally for 7 days

Group 4: Administered Aqueous extract of *Cucurbita pepo*(300 mg/kg) orally for 7 days

The response time was observed after 30 minutes of administration of the drug given orally.

2.5.2. Tail Suspension Test (TST)

The total duration of immobility include by tail suspension was measured according to a method described as a means of evaluating potential antidepressant.[6-7] Each mouse was individually suspended to the edge of the table, 50 cm above the floor, by adhesive tape placed approximately 1cm from the tip of the tail. Each animal which was under test was both acoustically and visually isolated from other animals during the test. The total period of immobility was recorded manually for 6 minutes. Animal was considered to be immobile when it didn't show any body movements, hung passively and completely motionless. The test was conducted in a dim lighted room and each mouse was used only once in the test.[8-9]

GROUPING

Group 1: Administered saline orally for 7 days and served as control

Group 2: Fluoxetine (20 mg/kg) was administered orally for 7 days and served as standard

Group 3: Administered Aqueous extract of *Cucurbita pepo* (100 mg/kg) orally for 7 days

Group 4: administered Aqueous extract of *Cucurbita pepo* (300 mg/kg) orally for 7 days

The response time was observed after 30 minutes of administration of the drug given orally.

2.6. Statistical Analysis

Results were reported as mean ±SEM. Statistical analysis was performed using a one-way analysis of variance (ANOVA). Data was considered statistically significant at $p<0.05$. When data was found to be very (i.e.,$p<0.001$) or highly (i.e.,$p<0,001$) significant, this was indicated in the results. All statistical analyses were performed using graph pad software.

3. RESULTS

3.1. Preliminary phytochemical screening

Preliminary qualitative phytochemical analysis of *Cucurbita pepo* aqueous extract revealed the presence of alkaloids, carbohydrates, terpenoids, saponins, flavonoids, phenols and glycosides.

3.2. Effect of CPAE on immobility time in mice Tail suspension test (TST) and Forced swim test

The aqueous extract of *Cucurbita pepo* (CPAE) showed antidepressant-like effects in prophetic animal models, namely forced swimming and tail suspension tests. CPAE (100 and 300mg/kg body weight) or the synthetic antidepressant drug, Fluoxetine (20 mg/kg), was orally administered to the mice once daily for 7 days. The extract (100 and 300mg/kg body weight) significantly reduced the duration of immobility time in the forced swimming test after 7 days treatment (Table 2 and Fig 2). Dunnett's post hoc analysis demonstrated that the test treatments significantly decreased the immobility time in comparison to the control group ($p<0.05$, $p<0.01$). Likewise, the extract reduced the duration of immobility time in the tail suspension test (Table 1 and Fig 1). Post hoc analysis confirmed that the extract significantly decreased the immobility time in comparison to the control group ($p<0.05$, $p<0.01$).

Table I: The effect of CPAE on immobility time in mice Tail suspension test (TST)

GROUPS	TREATMENT	DOSE (mg/kg)	IMMOBILITY TIME (Sec)
I	Control	10ml/kg	172±1.949
II	Fluoxetine	20	75±1.36**
III	CPAE	100	135.5±1.40*
IV	CPAE	300	93.16±1.40**

Values are expressed as mean ± SEM. Comparison between control vs all other treated groups. Statistical test done by one-way ANOVA followed by dunnett test. *p<0.05 and **p<0.01 when compared with the control group. CPAE: *Cucurbita pepo* extract. ANOVA: Analysis of Variance. SEM: Standard error of the mean.

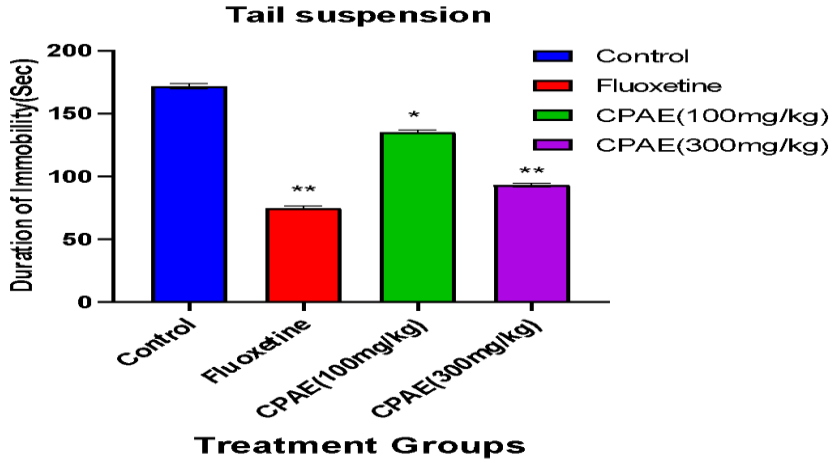

Fig 1: Effect of CPAE on immobility time Tail suspension test (TST) in mice.

Results are expressed as mean ± SEM. The differences were analyzed using one-way ANOVA followed by the Dunnett's test. For statistical significance, *p<0.05 and **p<0.01 when compared to the control group. CPAE: *Cucurbita pepo* aqueous extract. ANOVA: Analysis of variance; SEM: Standard error of the mean.

Table II: Effect of CPAE on immobility time in mice Forced swim test (FST).

GROUPS	TREATMENT	DOSE (mg/kg)	IMMOBILITY TIME (Sec)
I	Control	10ml/kg	158.16±1.16
II	Fluoxetine	20	97±0.96**
III	CPAE	100	123±1.46*
IV	CPAE	300	105±1.63**

Values are expressed as mean ± SEM. Comparison between control vs all other treated groups. Statistical test done by one-way ANOVA followed by dunnett test. *p<0.05 and **p<0.01 when compared with the control group. CPAE: *Cucurbita pepo* extract. ANOVA: Analysis of Variance. SEM: Standard error of the mean.

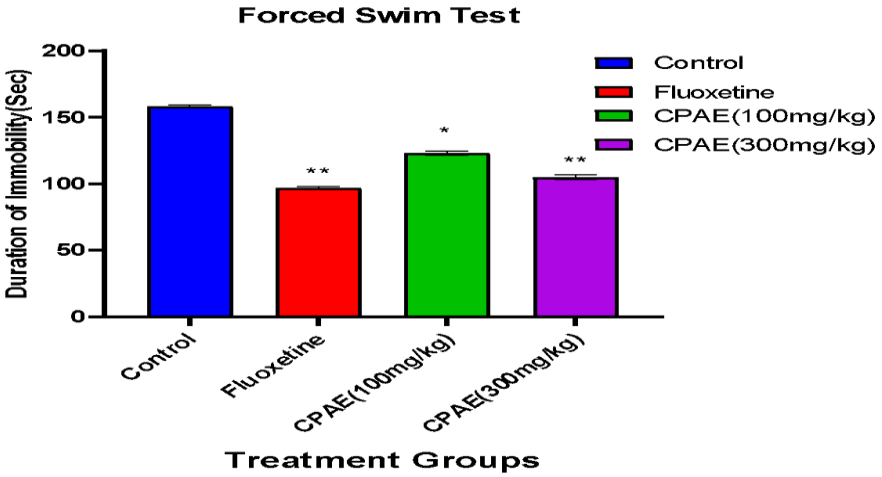

Fig 2: Effect of CPAE on immobility time in mice Forced swim test (FST) in mice.

Results are expressed as mean ± SEM. The differences were analyzed using one-way ANOVA followed by the Dunnett's test. For statistical significance, *p<0.05 and **p<0.01 when compared to the control group. CPAE: *Cucurbita pepo* aqueous extract. ANOVA: Analysis of variance; SEM: Standard error of the mean.

4. DISCUSSION

On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess the stress-precipitated behaviors. The two most widely used animal models for antidepressant screening are forced swimming and tail suspension test. These tests are quite sensitive and relatively specific to all major classes of antidepressants. In TST, immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. Similarly, in FST mice are forced to swim in restricted space from which they can not escape. This induces a state of behavioral despair in animals, which is claimed to reproduce to conditions similar to human depression. It has been seen that TST is less stressful and has higher pharmacological sensitivity than FST.[10-11]

Results showed that the administration of the CPAE produced a diminution of the duration of immobility time of mice exposed to both FST and TST. In the present study, the CPAE (300mg/kg, p.o) administered to mice produced significant antidepressant effect in both FST and TST than CPAE (100mg/kg, p.o) and their efficiencies were found to be comparable to fluoxetine (20 mg/kg, p.o).

The results of our preliminary phytochemical shows that aqueous extract of the whole plant of *Cucurbita pepo* contained alkaloids, flavonoids, tannins, saponins, carbohydrates and terpenoids. Phytochemical components, especially alkaloids, saponins, flavonoids, and carbohydrates have been reported to have antidepressant activity.[12-13]

Plants with antidepressant activity that contain flavonoids, polysaccharides, alkaloids, saponins and polyphenols include *Momordica cymbalaria*[14], *Passiflora foetida*[15] and *Eclipa alba*[16]. Therefore, the observed antidepressant effect observed with CPAE could be due to the presence of one or more of these secondary metabolites. In this work, the exact mechanism of antidepressant activity of aqueous extract of the whole plant of *Cucurbita pepo* was not very clear, therefore we suggest further work to ascertain their possible mechanism of action.

From all the above, the antidepressant activity of aqueous extract of the whole part of *Cucurbita pepo* was found to be significant at 300 mg/kg, p.o. The flavonoids components of CPAE might be interacting with 5-HT in mediating the antidepressant effect of *Cucurbita pepo*.

5. CONCLUSION

The CPAE contain alkaloids, flavonoids, tannins, saponins, carbohydrates and terpenoids. From the above findings, the antidepressant activity of CPAE was significant at 300 mg/kg, p.o. In FST and TST. Shortening of immobility time in the forced swimming and tail suspension tests was indicating CPAE acting either by enhancement of central 5-HT and catecholamine neurotransmission. However, further studies are needed to elicit its exact mechanism of action and to identify the active ingredient as a potent and efficacious antidepressant agent.

6. ACKNOWLEDGEMENT

The authors are thankful to the management and principal of Malla Reddy Institute of Pharmaceutical sciences, Secunderabad, for providing necessary facilities to carry out the present research work.

7. CONFLICT OF INTEREST

We declare that we have no conflict of interest.

8. REFERENCES

1. James M. Ritter, Rod Flower, Graeme Henderson, Yoon Kong Loke, David MacEwan, and Humphrey P. Rang; Rang and Dale's Pharmacology 8th Edition.
2. Bertram G.Katzung. Basic & Clinical Pharmacology. 14th ed. McGraw Hill Education (India) Private Limited; 2018
3. Porosolt RD, Bertin A, Jerltre M. Behavioural despair in mice: A primary screening for Anti-depressants. Archives Internationales de Pharmacodynamics de Therapie 1977; 229: 327 - 336
4. John F Cryan, Rita J Valentino, Irwin Lucki. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test Neurosci Biobehav Rev, National Library of Medicine, 2005.
5. Benoit Petit-Demouliere, Franck Chenu, Michel Bourin. Forced swimming test in mice: A review of antidepressant activity; Psychopharmacology 2005
6. Lucien Steru, Raymond Chermat, Bernard Thierry, and Pierre Simon. The tail suspension test: A new method for screening antidepressants in mice; Psychopharmacology Springer-Verlag 1985.
7. Dhingra D, Sharma A. Antidepressants like activity of Glycyrrhiza glabra L. in mouse model of immobility tests. Progress Psychopharmacol Bio Psychiatry 2006; 449-454.
8. Dhingra D, Valecha R. Screening for Antidepressants like activity of Convolvulus pluricaulis choisy in mice. Pharmacology Online 2007; 262-278.
9. Rodrigues AL, da Silva GL, Mateussi AS, Fernandes ES, Miguel OG, Yunes RA, Calixto JB, Snatos AR. Involvement of monoaminergic system in the Antidepressants like effect of hydroalcoholic extract of Siphocampylus Verticillatus. Life Sci

2002; 1347-1358.
10. Pemminati S, Gopalakrishna HN, Shenoy AK, Sahu SS, Mishra S, Meti V, Nair V. Antidepressant activity of aqueous extract of fruits of Emblica offinalis in mice. Int J Appl Bio and Pharm Technol 2010; 449-54.
11. Trease GE, Evans MC. Textbook of Pharmacognosy. 12th ed. London: Bailliare Tindall; 1983. P.336.
12. Fred-Jaiyesimi AA, Oredipe AB. Antidepressant activities of methanol extract, petroleum ether and ethyl acetate fractions of Morus mesozygia stem bark. Pharmacol pharm 4: 100-103; 2013 .
13. Hamid HA, Ramli ANM, Yusoff MM. Indole Alkaloids from plants as potential leads for Antidepressant drugs: A mini review. Front Pharmacol; 2017.
14. Parvathi S, Kumar VJ. Studies on chemical composition and utilization of the wild edible vegetable athalakkai (Momordica tuberosa). Plant Foods Hum Nutr 57: 215-222. 2002.
15. Abdel-Fattah AFM, Matsumoto K, Gammaz HAK, Watanabe H. Hypothermic effect of harmala alkaloid in rats: Involvement of serotonin mechanism. Pharmacol Biochem Behav 52:421-426; 1995.
16. Mishra G, Singh P, Verma R, Kumar S, Srivastav C, et al. Traditional uses, phytochemistry and pharmacological properties of Moringa oleifera plant: An overview. Der Pharmacia Lettre 3: 141 - 164; 2011.