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Research Article

EVALUATION OF ANTIDEPRESSANT LIKE ACTIVITY OF *CUCURBITA PEPO* SEED EXTRACTS IN RATS

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ABSTRACT

Objectives

To date the search for novel pharmacotherapy from medicinal plants for psychiatric illness was significantly progressed. And the present study was performed to evaluate the effect of aqueous and alcoholic extracts of *Cucurbita pepo* on methyl isobutyl ketone induced rats.

Methods

Alcoholic and aqueous extracts were orally administered in rats for 30 days after the induction of depression. The antidepressant activity was examined using Forced Swim Test (FST) in rats. Enzymic and non- enzymic antioxidant status were also analysed in control and experimental groups.

Key findings

The results showed that the extracts decreased immobility time with the increase swimming time (or improved behavioral activity) and significantly increased the enzymic and non-enzymic antioxidant status in brain and serum and also HDL and LDL levels in serum. It also showed that after standard (i.e.,) Imipramine, the aqueous extract is more potent compared to the alcoholic extract.

Conclusions

Thus, it may be concluded that *C. pepo* seed extract possess significant antioxidant and antidepression activity. Therefore, *C.pepo* may be served as a potential resource for natural psychotherapeutic agent against depression. However, further studies, were still required.

Keywords: Depression; *Cucurbita pepo,* Antioxidant, Forced Swim Test

INTRODUCTION

According to world health report, about 450 million people suffer from a mental or behavioural disorder [1]. This amounts to 12.3% of the global burden of disease, and predicted to rise up to 15% by 2020 [2]. Depression can be described as a mood, a state of being or energy level that includes lack of motivation, a sense of hopelessness and a lack of physical energy. It is an emotional status that can result from many aspects of our life. It is often a disabling disease that affects a person's work, family, school life, sleeping and eating habits, general health, and ability to enjoy life. A state of constant depression may suggest persistent stress or a biochemical imbalance.

Increased oxidative stress occurs in major depression, as evidenced by defective plasma antioxidant defenses in conjunction with enhanced lipid peroxidation [3-5]. The tripeptide glutathione (GSH), as a redox regulator participates in the maintenance of oxidant homeostasis and the cellular detoxification of reactive oxygen species (ROS) in brain cells [6]. GSH depletion has been shown to affect mitochondrial function probably via selective inhibition of mitochondrial complex I activity [7]. Therefore, compromised GSH system in the brain has been considered as a relevant index of neuronal oxidative stress [8]. Vitamin C is essential for mental and emotional well-being. Depletion or deficiency of this vitamin may trigger depression, irritability, neurological, cardiac disorders, anxiety and fatigue. Anxiety and excitement have shown the increased rate of breakdown of ascorbic acid (Vitamin C) [9].

The forced swimming test (FST) is a well validated model for experimental depression[10], widely employed to predict antidepressant efficacy and to determine depression-like behavior in animals after exposure to other stressors [11-13]. Total serum cholesterol and LDL levels were found to lower in depressed patients. Lipid peroxidation is increased in depressed condition [14]. Enzyme levels also get altered in serum and brain of depressed patients when compared to normal people [15].

Currently, different therapeutic regimens are employed to treat anxiety and depressive disorders, but their clinical uses are limited by their side effects such as psychomotor impairment, potentiation of other central depressant drugs and dependence liability. Therefore, herbal therapies should be considered as alternative / complementary medicines. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plants research has progressed constantly worldwide. This has been reflected in a large number of herbal medicines whose psychotherapeutic potential has been assessed in a variety of animal model [16].

The species, *Cucurbito pepo* is a cultivated plant of the genus *Cucurbita*. It includes varieties of squash and guord. The seeds and pulp of *C. pepo* is used for medicinal values as it irritates the intestinal tract of parasites and worms. It is also used to treat urinary tract problem and gastritis and to remove tapeworms and roundworms from the intestine. Pumpkin is a gourd like squash of the genus *Cucurbita* and the family Cucurbitaceae, has high antioxidative, antidepressive, anti-helminthic activity and antimicrobial activity [17].

But till now no scientific works have been reported on its antidepressant activity. In light of above information, the present study has been undertaken to study the antidepressant and antioxidant effects of the alcoholic and aqueous extracts of C. pepo on depression induced rats.

METHODS AND MATERIALS

Plant material

The seeds of *Cucurbita pepo* were collected from the local areas of Coimbatore district and authentically, certificate was obtained from the Botanist, Kongunadu College of Arts and Science, Coimbatore and the voucher specimen of *C. pepo* were preserved in the

Department of Biochemistry, Dr. NGP Arts and Science College, Coimbatore.

Preparation of plant extract

The collected seeds were washed well with water. 50 grams of the dried seeds were grounded well to fine powder. The powdered seeds were successfully extracted with alcohol and distilled water. The extraction was continued for 24 hours. Then the extract was filtered using Whatmann No: 1 filter paper.

Chemicals

Methyl isobutyl ketone (100mg/kg) was used as the depression inducer and Imipramine (30mg/ kg) was used as the standard drug in this study which was purchased from the Purani Hospitals, Coimbatore, India. The dosage range used here is found to be optimum from various pilot experiments. All other reagents used were of analytical grade.

Preparation of the Standard Drug

Imipramine was used as the reference drug for evaluating the antidepressant activity. Imipramine was powdered and made into suspension in distilled water using Tween 80 as the suspending agent.

Animals

Male albino Wistar rats (weighing 150-200 g) were obtained from the KMCH College of Pharmacy, Coimbatore, India. The animals were housed in large cages and fed with standard pellet diet supplied by National Institute of Nutrition Laboratory Information Centre for Animal Science, Hyderabad and water ad libitum.

The animals were acclimated to standard environmental conditions of temperature (22± 5° C) and humidity (55 ± 5%) and 12 hrs light/dark cycles throughout the experimental period. Animals used in the present study were approved by the institutional ethical Committee for the Purpose of Control and Suspension of Experiments on Animals (CPCSEA) under Ministry of Animal Welfare Division, Government of India, New Delhi

Experimental design

All rats were randomly divided into 5 groups. Each group contained 4 rats. Group I was assigned as control and group II as depressive control. Rats were starved for 24 hours and divided into control and experimental groups. For the induction of depression, each rat in the experimental group (II to V) was injected with methyl isobutyl ketone intra peritoneally (100 mg/ kg weight, i.p.). The induction was made twice daily for 30 days.

From 31st day onwards, after the confirmation of depression, group III and IV was treated with oral administration of alcoholic and aqueous extracts (100mg/ kg) of seeds of *C.pepo* respectively for 30 days. Group V was treated with standard drug Imipramine (30mg/ kg) for 15 days.

Forced swim Test

In order to assess the antidepressant activity of plant extracts, the modified Forced Swim Test [10] was conducted. In the first trial, the rats which has not yet treated were forced to swim in a glass aquarium (25cm in diameter, 40 cm in height containing 12cm high freshwater at 23 ± 1º C). In the next exposure, antidepressant activity of alcoholic and aqueous extracts was assessed after 30 days of treatment. Two sessions were conducted, an initial 15 minute training session (pre -test session) followed 24 hr later by a 5 minute test session. During the test session, the immobility time, swimming and climbing times were observed by a trained observer. The total duration of immobility was measured during the 5 min test. Upon removal from the water, rats were towel dried and finally returned to their cage.

Separation of Serum and Plasma

At the end of the experimental period, the rats were sacrificed by cervical decapitation. The serum and plasma were separated for further analysis.

Preparation of Brain Homogenate

The brain is carefully dissected out from the rat and it is homogenized with substrate buffer and the homogenate is filtered.

Biochemical Analysis

The key enzymes and total lipid peroxidation that indicates the antioxidant status of animals were analysed using established biochemical procedures

Enzymic antioxidants

Estimation of super oxide dismutase (SOD)

Superoxide was assessed by the method of **[18]**. This method is based on the development of a red coloured compound when diazonium compound reacts with Naphthylamino group whose absorbance is measured at 543 nm.

Estimation of catalase (CAT)

The activity of catalase was assessed by the method of **[19]** . The values of CAT activity are expressed as micromole of H_2O_2 utilised per minute per milligram protein. Dichromate was reduced to chromic acetate when heated in the presence of H_2O_2. The chromic acetate thus produced was measured colorimetrically at 610 nm. The catalase preparation was allowed to split H_2O_2 for different period of time. The reaction was stopped at specific time intervals by the addition of dichromate and the remaining H_2O_2 was determined by measuring chromic acetate colorimetrically.

Glutathione peroxidase (GPx)

Glutathione peroxidase (GPx) was assayed according to the method of [20] with some modifications. A known amount of serum sample was allowed to react with H_2O_2 in the presence of GSH for a specific time period, then the remaining GSH was allowed to react with DTNB and the yellow colour developed was measured at 412 nm.

Non-enzymic antioxidants

Reduced Glutathione (GSH)

Total reduced glutathione (GSH) was determined by the method of [21]. The serum sample was precipitated with TCA and then it is allowed to react with DTNB and phosphate buffer. The absorbance was read at 410 nm.

Ascorbic Acid

Ascorbic acid (Vitamin C) was assessed by the method of [22]. The amount of Vitamin C is expressed as µg/ mg tissue.

Lipid peroxidation

The levels of tissue thiobarbituaric acid reactive substances (TBARS) were measured using the method of [23]. The pink chromogen was measured at 534 nm.

Triglycerides

The level of triglycerides was measured by [24]. The intensity of the chromogen formed is proportional to the triglycerides concentration in the sample when measured at 510nm.

The total protein content was determined by the method of [25] using bovine serum albumin as the standard at 660 nm.

Statistical analysis

The level of significance in the variation of parameters between two groups was determined by performing student's "t" test.

RESULTS

The phytochemicals present in the seeds of C. pepo are represented in Table 1.

Table 1: Phytochemical study in the seeds of *C. pepo*

S.No	Tests	Presence or absence in seeds of *C. pepo*
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Carbohydrates	+
5	Phenols	-
6	Steroids	+
7	Glycosides	+
8	Resins	+
9	Thiols	-
10	Tannins	+

Both aqueous and alcoholic extracts show significant changes in the immobility time in forced swim test (FST), which is shown in figure. The immobility time of depressed rats (group II) is found to be increased when compared to the normal rats (group I). On treatment with extracts of *C. pepo*, the immobility time significantly decreases when compared to the depressed rats.

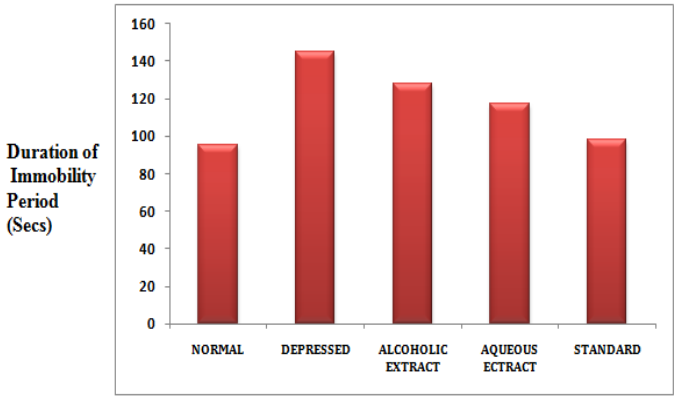

Table 2 gives the activity of SOD in serum and brain of control and experimental rats. There was a significant decrease in SOD activity of depressed rats (group II) when compared to the normal rats (group I). During treatment, the values were found to be reversed. Group III and IV was treated with the alcoholic and aqueous extracts of seeds of *C. pepo* respectively, where it shows a significant increase in the level of SOD in serum and brain of these rats when compared to that of depressed (group II) rats. In rats treated with standard drug (group V), the activity of SOD in serum and brain was increased close to that of group I. Treatment with aqueous extracts shows a better activity in serum and brain when compared to that of alcoholic extracts of seeds of *C. pepo.*

Table 2: Activity of superoxide dismutase (SOD) in Brain and Serum of rats under different conditions

Groups	Conditions	SOD in Brain (mg/dl)	SOD in Serum (mg/dl)
Group I	Normal	13.49± 0.1890*	28.47 ± 0.1038*
Group II	Depressed	5.66 ± 0.0051	14.52 ± 0.0764
Group III	Alcoholic Extract	12.17 ± 0.0223*	26.96 ± 0.15*
Group IV	Aqueous Extract	9.87 ± 0.0020*	29.57 ± 0.257*
Group V	Standard	13.57 ± 0.2650*	28.42 ± 0.0751*

* Significance at 1% level

Table 3 shows the catalase activity in serum and brain of rats. Decreased enzyme activity was found in group II (depressed) rats, compared to that of group I (normal) rats. The activity was reverted after treatment. There was significant increase in the activity of catalase in serum and brain of group III and IV, which was treated with the alcoholic and aqueous extracts of seeds of *C. pepo* respectively, when compared to that of group II.

Table 3: Activity of Catalase in Brain and Serum of rats under different conditions

Groups	Conditions	Catalase in Brain (mg/dl)	Catalase in Serum (mg/dl)
Group I	Normal	1018.07 ± 2.264*	707.97 ± 1.19*
Group II	Depressed	335.36 ± 2.410	326.7 ± 1.15
Group III	Alcoholic Extract	543.71 ± 1.43*	612.81 ± 1.435*
Group IV	Aqueous Extract	797.21 ± 1.73*	887.33 ± 1.665*
Group V	Standard	1070.16 ± 8.21*	711.9 ± 1.96*

* Significance at 1 % level

Table 4 represents the activity of glutathione peroxidase (GPx) in serum and brain of rats under different conditions. In group II (depressed) rats the activity of GPx was significantly decreased, than that of group I (normal) rats. In the treatment groups, (group III and IV), the activity was significantly increased when compared to that of group II rats.

Table 4: Activity of glutathione peroxidase (GPx) in brain and serum of rats under different conditions

Groups	Conditions	GPx in Brain (mg/dl)	GPx in Serum (mg/dl)
Group I	Normal	19..9 ± 0.52*	8.93 ± 0.02*
Group II	Depressed	5.60 ± 0.05	3.2 ± 0.03
Group III	Alcoholic Extract	20.79 ± 0.04*	7.47 ± 0.03*
Group IV	Aqueous Extract	21.49 ± 0.07*	6.03 ± 0.02*
Group V	Standard	21.97 ±0.14*	8.67 ± 0.04*

* Significance at 1 % level

Table 5indicates the reduced glutathione activity in serum and brain of various groups of rats. The levels of reduced glutathione were significantly decreased in group II (depressed) rats, when compared to that of group I (normal) rats.The reverse was noticed during the treatment. In group III and IV, the activity of glutathione was significantly increased when compared to that of group II rats. The level of reduced glutathione in serum and brain of groupV (Standard drug) was significantly increased to that of group I (normal) rats.

Table 5: Activity of reduced glutathione (GSH) in Brain and Serum of Rats under different conditions

Groups	Conditions	GSH in Brain (mg/dl)	GSH in Serum (mg/dl)
Group I	Normal	1.024 ± 2.57*	0.760 ± 0.97*
Group II	Depressed	0.6703 ± 3.58	0.4785 ± 2.36*
Group III	Alcoholic Extract	0.89374 ± 0.49*	0.8165 ± 2.03*
Group IV	Aqueous Extract	0.9934 ± 1.16*	0.6785 ± 1.94*
Group V	Standard	1.0163 ± 3.5*	0.7620 ± 1.9*

* Significance at 1 % level

The amount of Vitamin C in serum and brain of rats under different conditions was shown in table 6. The concentration of Vitamin C was significantly decreased in serum and brain of group II (depressed) rats, when compared to that of group I rats. The condition was reversed during treatment. In groups III and IV, the concentration of Vitamin C was significantly increased when compared to that of group II (depressed) rats and similar to that of group V (standard drug) rats.

Table 6: Amount of Vitamin C in Brain and Serum of Rats under different conditions

Groups	Conditions	Vitamin C in Brain (mg/dl)	Vitamin C in Serum (mg/dl)
Group I	Normal	2.07 ± 0.020*	1.704 ± 0.003*
Group II	Depressed	0.515 ± 0.025	0.248 ± 0.003
Group III	Alcoholic Extract	2.165 ± 0.025*	2.105 ± 0. 15*
Group IV	Aqueous Extract	2.105 ±0.025*	2.16 ± 0.02*
Group V	Standard	2.43 ± 0.020*	2.42 ± 0. 13*

* Significance at 1 % level

Table 7 represents lipid peroxidation in serum and brain of rats. There was significant increase in the level of LPO in group I (depressed) rats, when compared to that of group I (normal) rats. The lipid peroxidation was significantly decreased in serum and brain of group III and IV rats when compared to depressed rats (group II).

Table 7: Lipid Peroxidation in Brain and Serum of Rats under different conditions

Groups	Conditions	Lipid Peroxidation in Brain (mg/dl)	Lipid Peroxidation in Serum (mg/dl)
Group I	Normal	9.743 ± 0.31*	82.79 ± 0.34*
Group II	Depressed	11.878 ± 0.125	104.34 ± 0.16
Group III	Alcoholic Extract	7.7± 0.06*	81.835 ± 0.075*
Group IV	Aqueous Extract	7.4 ± 0.07*	78.73 ± 0.09*
Group V	Standard	9.7 ± 0.01*	83.225±0.095*

*Significance at 1 % level

Table 8 shows the estimation of serum lipid profile of rats under different conditions. In group II (depressed) rats; the level of total cholesterol HDL and LDL was significantly decreased when compared to that of group I. The reverse in levels was observed in the treatment groups. The level of triglycerides and total cholesterol was significantly increased in group III and IV as that of group V rats. The treatment with alcoholic and aqueous extracts of seeds of *C. pepo* shows similar results as that of standard drugs.

Table 8: Estimation of Serum Lipid profile under different conditions of rats

Groups	Conditions	Triglycerides (mg/dl)	Total Cholestrol (mg/dl)	High Density lipoprotein (mg/dl)	Low Density lipoprotein (mg/dl)

I	Normal	76.00 ± 1.0*	152.50 ± 25*	85.30 ± 0.3*	51.20 ± 0.2*
II	Depressed	71.77 ± 0.15	62.50 ± 0.5	36.14 ± 0.15	12.80± 0.2
III	Alcoholic Extract	74.51 ± 0.15*	151.50 ± 0.36*	84.79 ± 0.13*	52.02 ± 0.01*
IV	Aqueous Extract	74.30± 0.08*	151.07 ± 0.25*	84.03 ± 0.025*	52.14 ± 0.025*
V	Standard	74.51 ± 0.41*	151.60 ± 0.38*	84.33 ± 0.015*	52.60 ± 0.085

DISCUSSION

In the present study, aqueous and alcoholic extract of *Cucurbita pepo* produced significant antidepressant- like effect in mice in Forced Swim Test (FST) and its efficacy was found to be similar to Imipramine. This test is quite sensitive and relatively specific to all major classes of antidepressant drugs [26]. In FST, rats are forced to swim in restricted space from which they cannot escape. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression [27]**.** Our results show that *C. pepo* seed extracts can decrease immobility time in forced swim test. It is found that *C. pepo* can produce antidepressant like activity at a dose of 100mg/kg body weight after 30 days of treatment. The decrease in the immobility time is accompanied with the increase in swimming time. The precise mechanisms by which *C. pepo* extracts may produce antidepressant like effect are not completely understood.

The decreased SOD activity in depressed group may be because of highly reactive oxygen metabolites (ROMs) production. Erythrocyte SOD levels were suggested to be a hall mark for depression[3]. Therefore, antidepressant therapeutic intervention may be associated with a critical oxidative process along with alleviating the depressive symptoms. However, the present work provides evidence for the cumulative antioxidant promoting effects with more universal mechanism of action that not only involves SOD, a potential target of antidepressant regulation, but also extends to other enzymic and non-enzymic antioxidants [28, 29] . There is evidence of derangement of oxidant and antioxidant defense systems in depression[4].

The reactive oxygen species (ROS) like hydroxyl radicals, superoxide anion, hydrogen peroxide and nitric oxide, produced during normal cellular metabolic functions, produce oxidative damage in brain [30,31]. The brain is more vulnerable to oxidative stress because of its elevated consumption of oxygen and the consequent generation of large amounts of ROS.

Interestingly, our results evidenced a parallel increase in both (superoxide dismutase) SOD and catalase (CAT), the most important antioxidant enzymes in response to treatment with seed extracts to depressed animals. The increase in activity may provide an effective defense from the damaging effects of not only superoxide anion and hydrogen peroxide (neutralized by their enzymes) but also from damaging and highly reactive hydroxyl radical generated by Fentons reaction of the former radicals [32]. The restraint stress reduced the levels of SOD, CAT and enhanced lipid peroxidation (LPO) [33]**.** The decrease in the activity of glutathione peroxidase (GPx) could be due to its exhausted adoptive response to counter the effect of increased oxidative stress [34]**.** The increased level of LPO observed in depressed rats, indicates an excessive formation of free radicals and activation of LPO system. TBARS, produced as a by products of LPO that occurs in hydrophobic care of blood membrane [35] Thus, antidepressant like activity of *C. pepo* extracts might be due to inhibition of lipid peroxidation.Vitamin C is an anti stress vitamin and may counter too much adrenalin. Vitamin C blocks the behavioural response to dopamine and enhances the effect of neuraloptic drugs. States of depression and anxiety is associated with psychiatric disorders are probably accentuated by inadequate intake vitamin C [36] **.**

According to results of phytochemical screening and the literature, the antidepressants like potential might be due to the presence of alkaloids, glycosides and flavanoids. Flavanoids glycosides are mostly hydrolysed into their aglycons by mucosal and bacterial enzymes in the intestines, and then converted to conjugated metabolites during the absorption process [37,38] Transportation of these metabolites into the brain tissues via the blood brain barrier and their effect on the central nervous system (CNS) has been recently argued [39,40] **.** Therefore, one of the antidepressant mechanism of *C. pepo* is thought to involve flavanoids and glycosides which reach the brain tissues through the metabolizing process, protecting brain function from CNS disturbance and consequently, exerting an antidepressant effect. Thus, extracts of *C. pepo* may have potential therapeutic value for the management of depressive disorders. Further study is required to identify the particular components present in this extract responsible for its antidepressant like activity.

CONCLUSION

Antidepressant drugs used for the depression treatment may cause side effects such as vomiting, nausea, irritation etc. To overcome this, natural medicines are used for treatment which will have very less side effects. *Cucurbita pepo* is one of the plants used in traditional a medicine which was proved to possess antidepressant like activity of *C. pepo* in rats in our present study. The result is similar to that of standard drugs. However, further studies are needed to characterize the mechanism of the antidepressant effect of *C. pepo* and extend these results before the safe application in humans.

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