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Research Paper

ANTIDEPRESSIVE ACTIVITY OF PROCESSED PUMPKIN (*CUCURBITA MAXIMA*) SEEDS ON RATS

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The aim of the present study was to determine the anti depressive activity of raw, autoclaved, boiled, germinated and roasted pumpkin seeds powder extract compared with the standard drug: Imipriamine (30 mg/kg) in normal and depressed control rats. Methyl Isobutyl Ketone (100 mg/kg) was used as the depression inducer in rats. The result shows that there was a significant ($P < 0.01$) decrease in duration of immobility time (sec) of all experimental rats compared with depressed control group in Force Swimming Test and a significant ($P < 0.01$) decrease in duration of immobility time (sec) of all experimental rats compared with depressed control group for Tail Suspension Test.

Keywords: Depression, Pumpkin seeds, Methyl isobutyl ketone, Force swimming test, Tail Suspension test

INTRODUCTION

Major depression, one of the affective disorders, is a mood disorder characterized by a sense of inadequacy, despondency, decreased activity, pessimism, anhedonia and sadness where these symptoms severely disrupt and adversely affect the person's life, sometimes to such an extent that suicide is attempted or results. Irritability, insomnia, lethargy, agitation and anxiety often accompany depression. Depression and anxiety disorders are common public health problems with 17% lifetime prevalence (Levinson, 2006).

Major depressive disorder is defined by episodes of depressed mood lasting for more than 2 weeks accompanied by additional symptoms including disturbed sleep and appetite, reduced concentration, excessive guilt, and suicidal thoughts (Mill and Patrons, 2007). According to world health report, about 450 million people suffer from a mental or behavioral disorder (WHO, 2001). This amounts to 12.3% of the global burden of disease, and predicted to rise up to 15% by 2020 (Reynolds, 2003).

Pumpkin belongs to the family of Cucurbitaceae. The pumpkin plant, along with its seeds, has been used in the traditional medicine in many countries, including India. Pumpkins supply calcium, iron, vitamin A, oil (25-55%, rich in unsaturated oleic

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and linoleic acids), protein (25-35%) with high amounts of arginine, aspartate and glutamic acid, although it is deficient in lysine and sulphur-containing amino acids (Tsai *et al.,* 2006). The chemical constituents from seeds contain 30% unsaturated fatty oil (linoleic and oleic fatty acids). Triterpenoids, flavonoids, coumarins, saponines, cucurbitacins, vitamins, minerals, notably zinc amino acid. The highly unsaturated fatty acid composition of pumpkin seed makes it well-suited for improving nutritional benefits from foods. Pumpkin seed oil has been implicated in providing many health benefits (Fu *et al,* 2006).

Due to the purported L-tryptophan content of pumpkin seeds, they have been suggested to help remedy depression (Eagles, 1990). Tryptophan is an essential amino acid present in pumpkin seed, and 5-hydroxytryptophan (5-HTP) is the intermediate metabolite of tryptophan in the formation of the neurotransmitter serotonin. Both tryptophan and 5-HTP are promoted as treatment for depression. The most common nutritional deficiencies seen in mental disorder patients are of omega-3 fatty acids, B vitamins, minerals, and amino acids that are precursors to neurotrans-mitters (Hibbeln , 2008; Eby, 2006).

Rats show similar depression symptoms to humans, and react to anti-depression medicines similarly as well, according to Stanford University professor Karl Deisseroth. Rats thus are often used in studies investigating causes of and treatments for depression. Depression symptoms can be induced in rats by creating stressful situations the animals cannot escape or control, or by electric prodding in the brain. The objective of the present study was to explore the anti depressive activity of processed cucurbita maxima seed extracts on rats.

MATERIALS AND METHODS

The anti depressive activity was determined by producing depression in rats. Depression was induced by injecting Methyl Isobutyl Ketone (100mg/kg). Anti- depressive activity of the raw and processed (germinated) cucurbita maxima seeds extracts was assessed by Forced -Swimming Test and Tail Suspension Test and compare with Imipriamine (30 mg/kg)-standard drug, normal and depressed control rats.

Preparation of Extract

About 100 g of the powdered samples of raw, autoclave, boiled, germinated and roasted pumpkin seeds was mixed with 500 ml of distilled water, in a conical flask. The mixture was stirred severally and covered overnight at room temperature and then filtered using Whatman filter paper, No 1.The filtrate was then evaporated to dryness using digital heating drying oven (DHG-9030A) preset at 50°C and was later stored at 4°C. The yield of the extract was 8.5% w/w.

Induction of Depression

Methyl Isobutyl Ketone (100mg/kg) was used as the depression inducer and Imipriamine (30 mg/kg) was used as the standard drug in this study. The dosage range used here is found to be optimum from various pilot experiments (Umadevi *et al.,* 2010).

Preparation of the Standard Drug

Imipramine was used as the reference drug for evaluating the antidepressant activity. Imipramine are dissolved in double distilled water and injected in a volume of 0.1 ml/100 g rat body weight. The chemical is purchased from commercial sources (Carla Gambarana, 2000).

Experimental Animals

Male Wister rats (weighing 120-140 mg) were

used in this study. The experimental protocol was approved by Central Animal Research Facility of The Littlie Flower Medical Research Center (LFMRC), Angamaly, Kerala, which was registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, Registration No. 496/01/9/CPCSEA.

Rats were housed in polypropylene cages (30 × 22 × 14 cm). Paddy husk was used as bedding material, which was changed on alternative days. It kept in a well aerated room with exhaust and ceiling fans. The rats were acclimated to standard environmental conditions of temperature (25 ± 5°C) and humidity (55 ± 5° C) and 12hrs dark/light cycle throughout the experimental period. The each rat fed about 25gm of standard pellet diet per day which supplies by National Institute of Nutrition Laboratory Information Centre for Animal Science, Hyderabad.

Experimental Design

All rats are randomly divided into 8 groups. Each group contains 6 animals. Group 1 was assigned as Control (normal), Group II as depressive control, and Group III was given standard drug Imipramine and Group IV to Group VIII was supplemented with raw, autoclave, boiled, germinated and roasted pumpkin seed powder extract respectively. Rats were starved for 12 hr. For induction of depression each rat in the experimental group (II to V) was injected with Methyl Isobutyl Ketone Intraperitoneally (100mg / kg weight, ip). The induction was made twice daily for 30 days.

From 31st day onwards, after the confirmation of depression by FST and TST, group IV to group VIII was treated with oral administration of aqueous extract (100 mg/kg) of raw and processed pumpkin (cucurbita maxima) seeds respectively for 30 days. Group III was treated with standard drug imipramine (30mg/kg) for 30 days.

Evaluation of Antidepressant Activity

Two different tests like Forced -Swimming Test and Tail suspension test were employed to study antidepressant activity.

Forced -Swimming Test

In order to assess the antidepressant activity of processed pumpkin seeds, the modified Forced -Swimming Test was conducted (Porsolt *et al.,* 1977). Measurement of immobility time was carried out by observing the motoric activity of rat, which were placed in a pool of water. A glass cylinder, 25 cm in diameter, height 23 cm, was filled with water to a height of 12 cm.The temperature of the water was 23±1°c. In the first trial, the rats which have not yet treated were forced to swim in the glass aquarium. In the next exposure, antidepressant activity of processed seeds was assessed after 30 days of treatment. Two sessions were conducted; an initial 15 min training session (pre-test session) followed 24 hr. later by a 5 min test session. During the test session, the immobility time, swimming and climbing times were observed by a trained observer. The total duration of the immobility was measured during the 5 min test. Upon removal from the water, rats were towel dried and finally returned to the cage. Shorter immobility time is an indicator of the stronger antidepressant effect of the tested substances.

Tail Suspension Test

The tail suspension test was the second method for assessing the antidepressant effect of the processed pumpkin seed extracts. This model is based on the principle that suspending rat suspended upside down leads to a characteristic

behavior of immobility after initial momentary struggle. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective on human depression. On the 30th day of treatment tail suspension test was conducted after 40 min of drug administration (Steru *et al.,* 1985). Rats were suspendered on the edge of a table 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period (Rodrigues *et al.,* 2002). Animals were considered to be immobile when it did not show any movement of the body and hanged passively. The total duration of immobility was recorded during the next 4 min of total 6 min test (Dhingra Dand Sharma, 2005). Duration of immobility was compared with control and within the groups.

Statistical Analysis

The collected data was compiled and analyzed by using statistical methods. Descriptive statistics, ANOVA and Correlation is computed using s statistical software SPSS version 15.0. Duncan's multiple range tests were applied to determine the significant differences between the effects of processed pumpkin seed extracts on depressed rats.

RESULTS

Effect of Processed Pumpkin (*Cucurbita maxima*) Seeds

Extracts on Force Swimming Test (FST)

The immobility parameter of force swimming test was recorded for confirmation of depression and analysis the treatment effect on the animals. The immobility parameter was recorded after 24 hr. of preliminary forced swimming test, before and after the supplementation for 30 days of the supplementation (Table 1).

Mean duration of immobility was significantly reduced by Imipramine (group III) as compared to the depressed control ($P < 0.01$). Similarly, the duration of immobility observed in rats pretreated with the processed pumpkin seeds extracts was also reduced. Decrease in the immobility due to pumpkin seeds extracts was found to be significant

Table 1: Antidepressant Effect of Pumpkin (*cucurbita maxima*) Seeds Extract on Force Swimming Test (FST)

S. No.	Treatment Group		Forced-Swimming Test Immobility Time			
		Initial (s)	Final (s)	Mean Differences	*t*-Value	Significant
1.	Group-I (Normal)	72.25±1.436	75.75 ± 1.43^{bc}	–3.50±.95743	–3.658	0.035*
2.	Group-II (depressed control)	162.00±2.483	155.0 ± 2.48^{c}	6.50±7.708	0.843	0.461^{NS}
3.	Group-III(Imipramine)	165.50±2.020	68.25 ± 1.80^{b}	97.25±1.7969	54.113	0.000**
4.	Group-IV(Raw)	164.00±1.471	70.25 ± 2.09^{b}	77.25±2.9261	28.70	0.000**
5.	Group- V(autoclaved)	160.25±1.750	83.00 ± 4.453^{c}	95.500±3.570	26.400	0.000**
6.	Group-VI (Boiled)	165.25±.629	69.75 ± 3.42^{b}	106.50±1.84	26.745	0.000**
7.	Group-VII(Germinated)	164.00±1.471	57.50 ± 1.258^{a}	93.00± 3.240	57.617	0.000**
8.	Group-V(Roasted)	163.25±1.652	101.25 ± 1.25^{d}	63.50± 2.629	24.145	0.000**

Note: Values represents the mean ± SEM,*$P < 0.05$, ** $P<0.01$, when compared to initial readings of the treatment animals. $^{a\text{-}d}$ values in the same column with different superscripts are significantly different at ($p < 0.05$).

when compared to ($P < 0.01$) depressed control group. Group-I (normal) shows significant ($P < 0.05$) increased the immobility time after the supplementation. Group III (Imipramine), Groups-IV (raw), Group-V (autoclaved), Group VI (boiled), Group-VII (germinated) and Group-VIII (roasted) animals had highly significant ($P < 0.01$) in decreasing the duration of immobility compared with the initial analysis, i.e., before supplementation. Among the processed samples germinated pumpkin seeds supplementation shows highly effect in depression reduction. Statistically significant reduction in duration of immobility was observed in all treated animals.

EFFECT OF PROCESSED PUMPKIN SEEDS EXTRACTS ON TAIL SUSPENSION TEST ON DEPRESSED RATS

The immobility parameter of tail suspension test was recorded for confirmation of depression and analysis the treatment effect on the animals. Results of Tail suspension test showed no significant differences in Group-I and Group-II (Normal and Depressed control) animals compared to initial immobility time on Tail Suspension Test (Table 2). Group-III (Imipramine) and Group-IV (Raw), Group-VI (Boiled), Group-VII (Germinated) and Group-VIII (Roasted) showed a significant at $p < 0.01$ level and group V showed at $p < 0.05$ level reduction on immobility time in Tail Suspension Test. All the processed pumpkin seeds supplemented groups showed significant reduction of the immobility time compared to initial time. The mean difference between initial and final immobility time in rats revealed that germinated pumpkin seeds supplemented rats had higher difference followed by Imipramine, boiled, raw, roasted and autoclaved pumpkin seeds supplemented groups.

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

Table 2: Effect of Processed Pumpkin Seeds on Tail Suspension Test on Depressed Rat

S. No.	Treatment Group		Forced-Swimming Test Immobility Time			
		Initial (s)	Final (s)	Mean Differences	*t*-Value	Significant
1.	Group-I (Normal)	104.00±.707	104.25 ± 0.85^{b}	–0.2500±1.314	–0.19	0.861NS
2.	Group-II (Depressed)	140.750±2.25	140.50 ± 2.21^{c}	0.250±2.495	0.100	0.927NS
3.	Group- III(Imipramine)	143.00±2.67	103.50 ± 1.19^{b}	39.500±1.936	20.39	0.000**
4.	Group-IV(Raw)	141.250±3.145	109.50 ± 0.95^{c}	31.750±3.705	8.56	0.003**
5.	Group-V (Autoclaved)	160.25±4.45b	119.00 ± 1.75^{c}	24.750±4.697	5.269	0.013*
6.	Group-VI(Boiled)	140.25±.3.42	103.25 ± 0.62^{b}	37.0±3.162	11.70	0.001**
7.	Group- II(Germinated)	139.250±2.322	97.25 ± 1.79^{a}	42.000±2.549	16.47	0.000**
8.	Group-VIII(Roasted)	142.00±1.25	110.50 ± 1.65^{d}	31.500±1.500	21.00	0.000**

Note: Values represents the mean ± SEM,*P< 0.05, ** P<0.01, when compared to initial readings of the treatment animals. $^{a\text{-}d}$ values in the same column with different superscripts are significantly different at ($p < 0.05$).

will have very less side effects. Pumpkin (*Cucurbita maxima)* seeds raw as well as processed could reduce the depression. The result was similar to that of standard drugs.

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