

**A RESEARCH ON EVALUATION OF ANTIANXIETY ACTIVITY OF PUMPKIN
(*CUCURBITA MAXIMA*) SEEDS IN MICE**

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Article Received on 03/04/2019

Article Revised on 24/04/2019

Article Accepted on 14/05/2019

ABSTRACT

This study was performed to investigate the anxiolytic effects of aqueous extract of *Cucurbita maxima* seeds (AECM) in mice using the elevated plus-maze model (EPM) and light dark model. The extract administered orally in two different doses of 100mg/kg and 200mg/kg, were able to increase the time spent and the number of arm entries in the open arms of the elevated plus-maze, also increases the time spent by mice in the illuminated side of the light-dark test, in comparison with control animals. This effect was comparable to that of the diazepam (1.0mg/kg i.p.). These results indicate that AECM is an effective anxiolytic agent.

KEYWORDS: *Cucurbita maxima*, EPM, AECM.

INTRODUCTION

Anxiety is a psychological and physiological state characterized by somatic, emotional, cognitive, and behavioural components, associated with significant disability (including educational and occupational) which has a negative impact on the quality of life. Anxiety is a complex trait with a genetic contribution likely to be equally complex.^[1] Anxiety disorders are present in up to 13.3% of individuals in the U.S. and constitute the most prevalent subgroup of mental disorders.^[2] Currently, the most widely prescribed medications for anxiety disorders are the Benzodiazepines. However, the clinical uses of Benzodiazepines are limited by their side effects such as psychomotor impairment, potentiation of other central depressant drugs and dependence liability. Therefore, the development of new herbal medications possessing anxiolytic effect without the complication of BZD would be of great importance in the treatment of anxiety-related disorders.^[3]

Ayurveda is one of the traditional medicinal systems of Indian. The philosophy behind *Ayurveda* is preventing unnecessary suffering and living a long healthy life. In traditional system of medicine, *Cucurbita maxima* is used in the treatment of heart disease, osteoporosis, bladder dysfunction, urinary diseases, diabetes and arthritis. The plant is credited with anthelmintic, carcinogenic activity.

The seeds that come from pumpkins are packed with L-Tryptophan which is an amino acid that is a precursor to melatonin, which is essential for sleep. The seeds also contain fats that convert to 5-HTP, which is metabolized into serotonin. Many people who experience the symptoms of anxiety have low Serotonin levels in their brains, which is often the root cause of the symptoms.^[4] However, the underlying mechanism anxiolytic needs to be investigated. The present investigation was undertaken to explore the anxiolytic effect of action of the AECM in animal models.

MATERIALS AND METHODS

Collection and authentication of Plant Material

The seeds of *Cucurbita maxima* belonging to the family cucurbitaceae were collected from Mangalore, Karnataka in November 2018. it was authenticated by Mrs. Mary K M, HSST(Botany) sr. grade CHSS. Chattanchal, Kasaragod, Kerala It is preserved in the departmental library for future reference.

Extraction and Preliminary Phytochemical Screening

For the present study the *Cucurbita maxima* seeds are collected from authentic source. About 100 g of dried and powdered pumpkin seeds was mixed with 200 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 and was later stored at 4°C.^[5] The dried extracts were subjected to

various chemical tests to detect the presence of different phytoconstituents present in them.

EXPERIMENTAL MODELS

Swiss albino mice weighing 18-30 gm, were used for the study. The mice were inbred in the central animal house of the Department of Pharmacology, Srinivas College of Pharmacy, Mangalore, under suitable conditions of housing, temperature, ventilation and nutrition were used for anxiolytic activity. They were kept in clean dry cages week before the beginning of the experiment to acclimatize with the experimental conditions. The animals were fed with standard pelleted diet (Lipton India Ltd., Mumbai) and distilled water *ad libitum* was maintained at 21°C-23°C under a constant 12hrs light and dark cycle. The animal care and experimental protocols were in accordance with CPCSEA /IAEC.

EXPEERIMENTAL DESIGN

The animals were divided into six groups of Swiss albino mice, each comprising six animals. Group I served as control and received 0.05ml/10g of Normal saline orally Group II served as standard and received 1 mg/kg Diazepam(ip). Group III and IV served as low and high dose received 100mg/kg and 200mg/kg *Cucurbita maxima* seeds extract orally for 21days respectively, after 21 days dosing period the animal's anxiety level was observed by screening methods such as elevated plus maze, light dark model.

Elevated plus-maze test^[6]

The elevated plus-maze comprised two open (30 cm×5 cm×0.25 cm) and two enclosed (30 cm×5 cm×15 cm) arms that radiated from a central platform (5 cm×5 cm) to form a plus sign. The maze was constructed of black painted wood. A slight raised edge on the open arms

(0.25 cm) provided additional grip for the animals. The plus-maze was elevated to a height of 40 cm above floor level by a single central support. The experiment was conducted during the dark phase of the light cycle (9:00–14:00 h). The trial was started by placing an animal on the central.

Light dark test^[7]

The apparatus consisted of two 20 cm×10 cm×14 cm plastic boxes: one was dark and the other was transparent. The mice were allowed to move from one box to the other through an open door between the two boxes. A 100W bulb placed 30 cm above the floor of the transparent box was the only light source in the room. A mouse was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse stepped into the dark box. The apparatus was cleaned thoroughly between trials.

STATISTICAL ANALYSIS

Results are prepared as Mean ± SEM. One way ANOVA was used for multiple comparison followed by multiple comparison tests. For all tests a "P" value of 0.05 or less was considered for statistical significance.

Effect of AEPA on Elevated plus maze

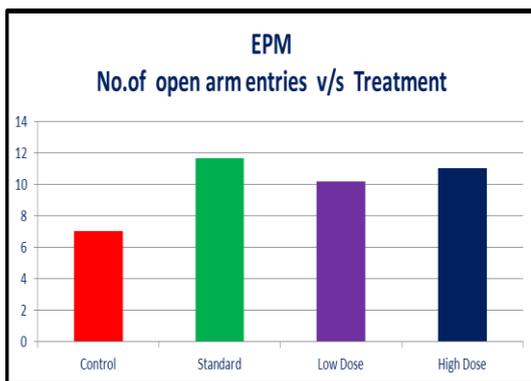
In EPM(Table no.1) animals treated with two doses of AECM (100mg/kg and 200 mg/kg). The time spent in the open and closed arms, and entries in the open and closed arms were compared with control & also Diazepam (1mg/kg) showed significant increase in the time spent in the open arms and increase in number of entries in open arm (Graph 1& 4). Furthermore, AECM 100 and 200 mg/kg had decrease in time spent and number of entries in closed arm (graph 2 &3).

Table No. 1: Effect of AECM on EPM in mice.

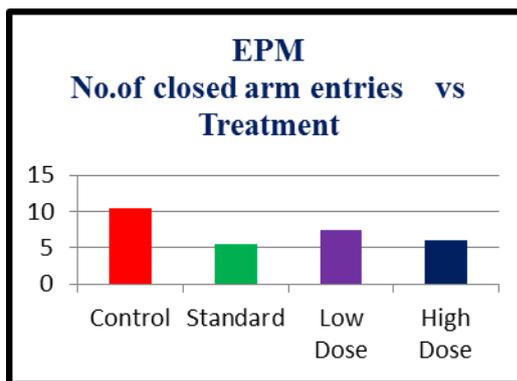
Group No.	Drug Treatment	Dose (mg/kg)	Number of entries (mean±SEM)		Time spent in sec (mean±SEM)	
			Open arm	Closed arm	Open arm	Closed arm
I	Control	0.05ml/10g	7.00 ± 0.5774	10.5 ± 0.4282	35.08± 0.3741	198.7 ± 2.141
II	Diazepam	1	12.67±0.333***	5.5±0.4282***	98.17±0.6009***	139.3± 0.3651***
III	AECM	100	10.17± 0.7032	6.5 ± 0.7638**	72.5± 3.819***	165.8 ± 3.005**
IV	AECM	200	11.42± 0.548**	7.84 ± 1.457**	83.14±0.356***	150.5±0.3635***

Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Tukey's test.

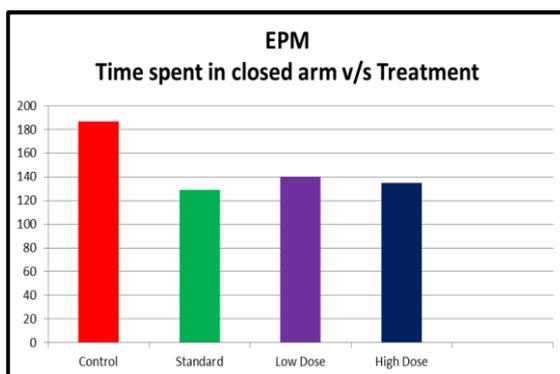
*P < 0.05, **P < 0.01, ***P < 0.001 vs. control



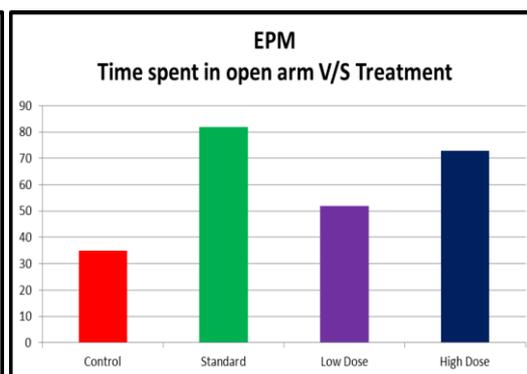
GRAPH 1



GRAPH 2



GRAPH 3



GRAPH 4

Effect of AECM on Light dark model

In LDT, (Table No. 2) animals treated with two doses of AECM (100 and 200 mg/kg) & diazepam showed reduced time spent but increase in number of entries in dark chamber and with concomitant increase in time &

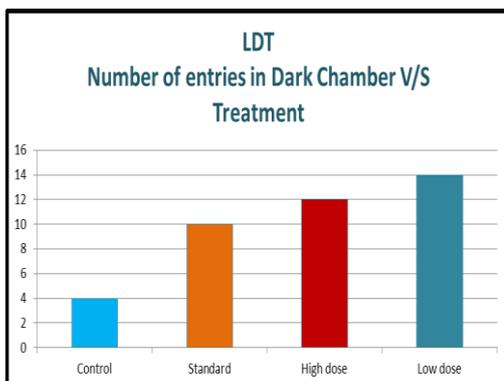
number of entries in light chamber when compared with control. Animals treated with high dose (200 mg/kg) shows more significant results when compared with low dose (100 mg/kg).

Table No. 2: Effect of AECM on Light Dark transition model.

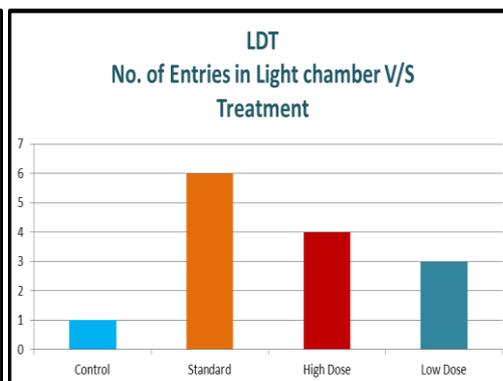
Group No.	Drug Treatment	Dose (mg/kg)	No. of entries (Mean±SEM)		Time spent in min (Mean±SEM)	
			Dark	Light	Dark	Light
I	Control	0.05ml/10g	4.167 ± 0.2171	1.167± 0.2171	7.083 ± 0.201	1.233 ± 0.1838
II	Diazepam	1	10.72 ± 0.3609***	6.867±0.3180***	3.55± 0.2930***	6.383± 0.3114***
III	AECM	100	12.5± 0.7638	3.551 ± 0.652*	6.3± 0.3651***	3.5 ± 0.2191
IV	AECM	200	14.88 ± 0.2568***	4.25 ± 0.3819**	4.324 ± 0.524***	5.58± 0.225**

Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Tukey’s test.

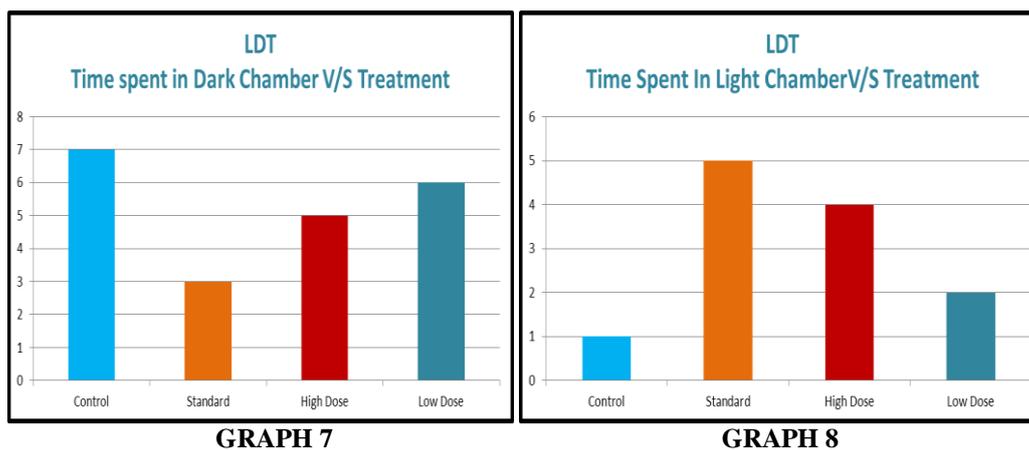
*P < 0.05, **P < 0.01, ***P < 0.001 vs. control



GRAPH 5



GRAPH 6



DISCUSSION

The purpose of this study was to evaluate the anxiolytic effect of *Cucurbita maxima* seeds by using anxiolytic models. The main finding of present investigation suggests the anxiolytic activity of cucurbita in different animal models of anxiety. Despite the widely popular use of *Cucurbita maxima* seeds for treating nervous disorders, there is an absence of scientific reports about the evaluation of its pharmacological effects. In this work, it was demonstrated that the administration of *Cucurbita maxima* seeds in mice was able to induce anxiolytic effects.

Anxiety may be seen in many types of situations, ranging from the anxiety that may accompany one's employment to the acute anxiety that may be seen during withdrawal from alcohol. Although a certain amount of anxiety is normal, excess anxiety interferes with day-today functioning and can cause undue stress.^[8] The literature review on pathophysiology of anxiety revealed that the anxiety occurs due to many reasons and many hypothesis has been proposed on it.

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