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Anxiolytic Effects of *Pisum Sativum* Seed Extracts in Animal Models.

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ABSTRACT

Pisum sativum has been used traditionally for treatment of anxiety neurosis, depression, and irritability. Yet, the plant has never been subjected to systematic biological investigation. The present study is aimed to evaluate the anxiolytic activity of ethanolic seed extract of *Pisum sativum* in animal models. *Pisum sativum* had increased number of entries and time spent in open arms while they decreased the number of entries and duration of time spent in closed arm of the EPM. In a similar fashion, the diazepam increased the percentage of time spent and percentage of arm entries in the open arms. The results suggest that ethanolic extract of *Pisum sativum* seems to possess anxiolytic effect comparable to that of Diazepam.

Keywords: Anxiety, *Pisum sativum*, Elevated plus maze.

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INTRODUCTION

Anxiety is considered as a common and significant psychiatric disorder which affects about 10–30% of the general population [1-3]. Anxiety is a state of excessive fear characterized by motor tension, apprehension, sympathetic hyperactivity and vigilance syndromes, leading to impairment of memory, intelligence and psychological function [4]. Till date, BZDs are the most preferred treatment for the management of anxiety disorders, but they have side-effects such as sedation, muscle relaxation, amnesia, and dependence [5,6]. About 43% of patients with anxiety use some form of complimentary therapy like herbal medicines, massages, folk remedies and homeopathy etc. Among complementary therapy, the most popular treatments include herbal medicines [7].

Pisum sativum belongs to the *Leguminosae* family. The plant also contains Tannins, Flavones, glycoflavones, flavonols, palmitic acid, calcium and phenolic acids [8].

Aim

To study the ability of *Pisum sativum* Seed Extracts to ameliorate anxiety in swiss mice by elevated plus maze test.

MATERIALS AND METHODS

Preparation of Ethanolic extract

The seeds of *Pisum sativum* belonging to the family Fabaceae purchased from the local market in Chennai. The seed is dried and finely powdered in a mechanical mixer. Ethanolic extract of *Pisum sativum* (EEPS) seed is prepared from the powder.

Selection of animals

Adult albino mice of either sex weighing 25 to 30 gm will be divided into 5 groups of 6 animals each. All animals were maintained at a controlled temperature ($25 \pm 1^\circ\text{C}$) and a 12-h dark/light cycle. Animals had free access to water and food.

Selection of Dose

A dose of 150mg/kg, 300mg/kg and 450mg/kg of Ethanolic extract of *Pisum sativum* seeds are taken for Anxiolytic activity.

Table 1: Drugs/Dose of the drugs, groups and number of rats in each group

Drugs/Dose of the drugs	Groups	Number of rats
Normal saline	I	6
Diazepam(1mg/kg)	II	6
<i>Pisum sativum</i> Seed Extracts (150mg/kg, i.p)	III	6
<i>Pisum sativum</i> Seed Extracts (300mg/kg, i.p)	IV	6
<i>Pisum sativum</i> Seed Extracts (450mg/kg, i.p)	V	6

Elevated plus maze (EPM)[9]

Mice were randomly allocated to the following groups: control (normal saline.), diazepam (1 mg/kg, i.p.) and ethanolic extract from *P.sativum* (150, 300 and 450 mg/kg, i.p.), as in table 1 that received treatment acute with single dose before of open field test. This test has been widely validated to measure anxiety in rodents [8]. The elevated plus maze (EPM; $30 \times 6 \times 6$ cm, each arm) made of wood and consisting of two open and two closed arms across each other, respectively is placed 60 cm above the ground level. After treatment, the animal was placed at the center of the plus maze with its nose in the direction of one of the closed arms, and observed for 5 min, following the parameters: number of entries in the open and closed arms, and time spent in each of them. The time spent in both open and closed arms, and the number of entries into both

open and closed arms were counted for a period of 5 mins. An entry was defined as having all four paws within the arm.

Statistical Analysis

The data were analyzed by one-way ANOVA and Posthoc comparisons were performed by applying Dunnet’s multiple comparison test. P <0.05 was considered statistically significant.

OBSERVATION

Table 2: Effect of administration of EEPS on rat behaviour in elevated plus maze

Group (n=6)	time spent in sec		
	open arm	closed arm	neutral zone
Control	28.1±2.3	223.6±10.8	48.1±11.2
Diazepam	136.5±4.1**	103.6±4.3	45.5±2.8
EEPS 150 mg/kg	65.8±2.1	162.5±3.8	71.6±2.5
EEPS 300 mg/kg	81.5±2.5	118.5±3.2	99.8±4.1
EEPS 450mg/kg	110.4±6.3**	130.1±9.0	59.6±3.22

n=6. The observation are mean ± SEM * <0.05, ** p< 0.01, as compared to control

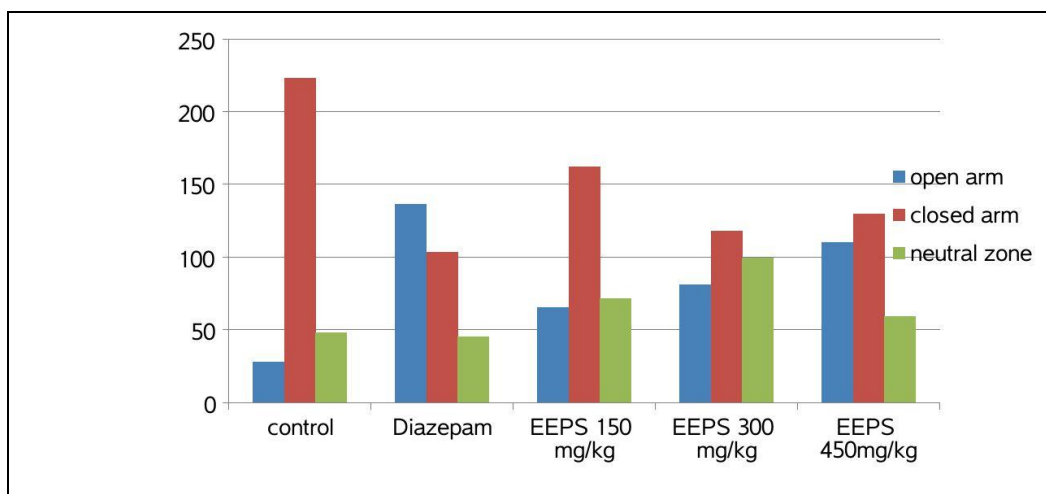


Figure 1: Effect of Normal saline (control), Diazepam1mg/kg , EEPS (150mg/kg), EEOC (300mg/kg), EEPS (150mg/kg) on the time spent in open and closed arm and Neutral zone in elevated plus maze (EPM).

Table 3: Effect of administration of EEPS on rat behaviour in elevated plus maze

Group	No of entries	
	Open arm	Closed arm
Control	2.17±0.3	27.1±8.3
Diazepam	13.7±0.7**	28.1±5.4
EEPS 150 mg/kg	7.17±0.4	38.33±10.1
EEPS 300 mg/kg	10.5±1.4*	35.3±14.2*
EEPS 450mg/kg	11.3±1.2**	30.1±9.2

n=6. The observation are mean ± SEM * <0.05, ** p< 0.01, as compared to control

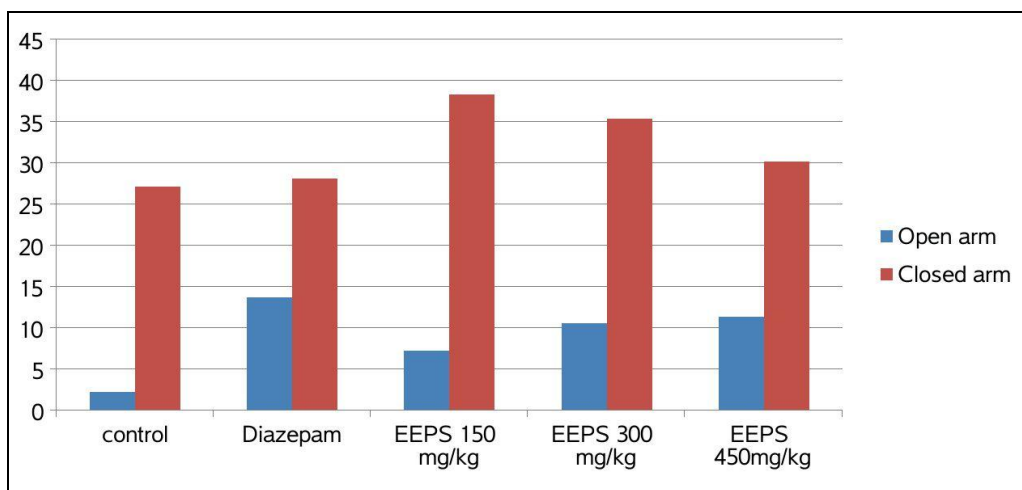


Figure 2: Effect of Normal saline (control), Diazepam 1mg/kg (DZP), EEPS 150mg/kg , EEPS 300mg/kg, EEPS 450mg/kg on the number of entries in open and closed arm in elevated plus maze (EPM).

RESULTS

Results obtained were studied statistically by calculating standard error of mean (SEM) by using ANOVA . Results indicate that EEPS in the dose of 450mg/kg significantly increased the number of open arm entries, time spent in the open arms when compared to vehicle treated group. In contrast with control group , Diazepam (1mg/kg) and EEPS (450mg/kg) had shown significant increase in time spent in open arm.

Diazepam (1mg/kg), EEPS (300mg/kg, 450mg/kg) groups significantly increased the number of open arm entries compared to control group.

DISCUSSION

Animals whenever subjected to unknown environment exhibits a particular form of behavioral inhibition, termed as anxiety. Traditional medicine has well established documentation regarding the usage of phytochemicals especially secondary metabolites in the treatment of psychotic disorders, most of which directly or indirectly affect the CNS, serotonin, GABA and BZA Neurotransmitter activities[10]

Anxiety has become a very important area of research in psychopharmacology in this decade. This increase of interest is the result of a rapid growth of scientific studies and the discovery of new drugs that alter anxiety in animal models.^[11] The elevated plus-maze is a test for the identification of anxiolytic or anxiogenic effect of a drug in rodents. In the elevated plus maze, the open arms are more fear provoking than the closed arms. The total ratio of entries, time spent and rearing behaviour in open arms to closed arms reflects the safety of closed arms with relative fearfulness of open arms.

In the present study, group V that received EEPS at the dose of 450 mg/kg showed a significant increase in the time spent in open arms. They showed a decrease in time spent in closed arms of elevated plus maze. All these suggest that decreased fear, an increased exploratory behaviour and the behavioral disinhibitory effect of EEPS at the dose of 450 mg/kg comparable to diazepam, the standard anxiolytic.

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