

## EVALUATION AND COMPARISON OF ANXIOLYTIC EFFECT OF FLAXSEED OIL AND PERILLA OIL IN MICE

Kumar Sandeep, Nain Parminder\*, Nain Jaspreet, Kumar Sunil  
M.M. College of Pharmacy, M.M. University, Mullana, Ambala, India

Article Received on:17/02/2011 Revised on:18/03/2011 Approved for publication:06/04/2011

\*Parminder Nain, Asst Prof., M.M.College of pharmacy, M.M. University, Mullana, Ambala, India  
Email: Parminder.nain26@gmail.com

### ABSTRACT

The Purpose of this study was to evaluate and compare the putative anxiolytic-like activity of Flaxseed Oil and Perilla Oil using elevated plus maze (EPM) and light-dark exploration test in mice. Flaxseed Oil is also known as linseed oil. Flaxseed Oil obtained from the dried ripe seeds of the *Linum usitatissimum*, (Linaceae). Perilla Oil is also known as Yegoma oil. It is obtained from seed of *Perilla frutescens* (Lamiaceae). Male mice were treated orally with the different doses of Flaxseed Oil (0.5-2.0 ml/kg), Perilla Oil (0.3-1.2ml/kg) and diazepam respectively, 1 hour before behavioral evaluation. Oral administration of 1.5 and 2.0ml/kg of Flaxseed Oil and 0.9, 1.2 ml/kg of Perilla Oil significantly ( $p<0.05$ ) increased the number of entries and time spend in light arena of light-dark exploration test as well as these significantly ( $p<0.05$ ) increased number of entries and time spent in open arm of elevated plus maze test. The anxiolytic effects of herbal oils compared with that of control group without any treatment and further, the results are quite comparable to diazepam (2mg/kg, p.o.). These results indicate that both oils have anxiolytic profile. The comparison between maximum significant effect of Flaxseed Oil and Perilla Oil at doses 1.5ml/kg and 0.9ml/kg respectively showed that Perilla Oil as more anxiolytic effect with low dose as compared with Flaxseed Oil.

**KEYWORDS:** Anxiety, Flaxseed Oil, Perilla Oil, Elevated plus maze.

### INTRODUCTION

Anxiety is an exaggerated feeling of apprehension, uncertainty, and fear. It is an unpleasant state of tension with an anticipation of imminent danger<sup>1</sup>. Anxiety disorders are considered the most common psychiatric diagnoses, and represent a significant disease burden affecting between 10–30% of the general population<sup>2,3,4</sup>. So anxiety becomes a very important area of research interest in Psychopharmacology during this decade<sup>5</sup>. Benzodiazepines are the most preferred treatment till date for the effective management of anxiety disorders but these compounds have well known side-effects such as sedation, muscle relaxation, amnesia, and dependence<sup>6,7</sup>. It has been estimated that 43% of anxiety sufferers use some form of complementary therapy mainly herbal medicines, massages, folk remedies, self help groups and homeopathy<sup>8</sup>.

### MATERIALS AND METHODS

#### Material

#### Herbal Oil

Perilla Oil gifted by Dubio Co., Ltd Korea while Flaxseed Oil was purchase from New Delhi (India).

#### Drugs

Diazepam dose of 2 mg/kg, i.p. was used as a reference standard<sup>9,10</sup>. Other chemical of analytical grade were purchased market of Ambala.

#### Animals

Swiss albino mice (22-25 g) were used to study the anxiolytic effect. The animals were housed in groups of six mice per cage and maintained at  $24^{\circ}\text{C} \pm 1^{\circ}\text{C}$ , with relative humidity of 45-55% and 12:12 hour's dark/light cycle. The experiments were carried out between 08:30 and 13:00h. The animals had free access to food (Standard Chew pellets) and water ad libitum. Food, not water, was withdrawn before night and during night the experiment. The Institutional Animal Ethics Committee (IAEC) approved all the experimental protocols.

#### Assessment of Anxiolytic Activity

#### Schedule Treatment

The anxiolytic activity of Perilla Oil and Flaxseed Oil were examined using Elevated Plus Maze (EPM), and Light and Dark. The animals will divided into twenty groups, consisting of six mice per group in mice. Group 1 was control without treatment; Group 2 received Diazepam 2 mg/kg of mice, Group 3 to 6 received Flaxseed Oil (0.5, 1.0,1.5 and 2.0 ml/kg of mice respectively) and Group 7 to 10 received Perilla Oil (0.3,

0.6, 0.9 and 1.2 ml/kg respectively) for light and dark exploration test.

Group 11 was control without treatment; Group 12 received Diazepam 2 mg/kg of mice, Group 13 to 16 received Flaxseed Oil (0.5, 1.0, 1.5 and 2.0 ml/kg of mice respectively) and Group 17 to 20 received Perilla Oil (0.3, 0.6, 0.9 and 1.2 ml/kg respectively) for elevated plus maze test.

### Model Used

#### Light/Dark Exploration Test

The apparatus consisted of two acrylic boxes. Two distinct chambers, a black chamber (20 x 30 x 30 cm) painted black and other open chamber made up transparent acrylic (30 x 30 x 30cm). The two chambers are connected through a small open doorway (8 x 8 cm) situated on the floor level at the centre of the partition<sup>11</sup>. Each mouse was placed individually in the of the light compartment and observed for the next 5 minutes for the numbers of the crossing between two compartment and time spend in the light and dark compartment.

#### Elevated Plus Maze

This model of anxiety has been used extensively for evaluation of novel anxiolytic agents and to investigate psychological and neurochemical basis of anxiety<sup>12</sup>. The method is rapid, simple, selective, and can evaluate both anxiogenic and anxiolytic agents under identical experimental conditions. An anxiogenic agents will increase the time spent and entries in the closed arm, whereas an anxiolytic agents are tested against anxiogenic drug induced perturbations. This method is said to be ethologically valid since it utilizes fear of balancing on the open and elevated maze arm as the stimulus. The plus-maze apparatus, consisting of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof, with the plus maze elevated (25 cm) from the floor used to observed anxiolytic behavior in mice<sup>13,14</sup>. Each mouse was placed at the center of the elevated plus maze with its head facing the open arm. During the 5-min experiment, the behavior of the mouse was recorded as: (1) No. of entries in to open arm, (2) and time spent by mice in open arm. During the entire experiment, mice were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of the plus maze could invoke maze anxiety.

#### STATISTICAL ANALYSIS (ANOVA)

Results was expressed as mean  $\pm$  S.E.M. The statistical analysis of data was done using the one way analysis of variance (ANOVA) followed by Tukey's test. A probability level (p value) less than 0.05 is considered statically significant.

## RESULT

### Light and Dark Model

Diazepam-treated mice significantly increased the time spent in light compartment and also increased number of entries in light compartment. Different dose of herbals oils significantly change time and numbers of entries in light compartment and produced anxiolytic activity in light and dark experiments on mice [Table 1].

### Elevated Plus Maze

Oral administration of different doses of Perilla Oil and Flaxseed Oil produced a significant increase in permanence in open arms of the plus maze [Table 2], suggesting an anxiolytic effect of these herbal oils. Animals treated with diazepam (2mg/kg, i.p.) spent more time in the open arms of the maze.

## DISCUSSION

In elevated plus-maze, mice show natural aversion to open and high spaces and, therefore, spend more time in the enclosed arms, which forms the basis for its use in the measurement of anxiety. The behavioral results of the present study showed that the native mice spent a significantly lesser time in the open arms, and also entered in it less frequently than the enclosed arms. The preference is likely to reflect an aversion towards the open arms caused by fear or anxiety.

In the present study, we found that herbal oils like Perilla Oil and Flaxseed Oil increased the percentage of open entries and time spent in open arms and thus showed anxiolytic-effect in these models. The herbal oils produced prominent anxiolytic activity in this experiment. However these oils show less anxiolytic activity than diazepam on mice. On the other hand Perilla Oil was more anxiolytic than Flaxseed Oil. Thus they have better profile for an anxiolytic agent. There is considerable interest in the development of new anxiolytics that do not induce sedative effects and do not inhibit locomotion.

In conclusion, present study confirms the anxiolytic activity of herbal oil on elevated plus maze and light and dark model in mice. In the same way, identification of compounds responsible for biological activity could be used as prototype(s) to design substances with anxiolytic activity. It may be alpha-linolenic acid which produces prominent anxiolytic activity. Although further major active components and precise anxiolytic mechanism need to be identified.

## REFERENCES

1. Barar FSK. Essential of Pharmacotherapeutic. New Delhi: S. Chand and Company Ltd; 2005. p. 89.
2. Rice DP, Miller LS. Health economics and cost implications of anxiety and other mental disorders in the United States. Br J Psychiatry 1998; 173:4-9

3. Greenberg PE, Sisitsky T, Kessler RC, et al. The economic burden of the anxiety disorders in the 1990s. *J. Clin. Psychiatry* 1999; 60:427-435.
4. Wittchen HU and Hoyer J. Generalized anxiety disorder: nature and course. *J. Clin. Psychiatry* 2001; 62:15–21.
5. Eisenberg RB, Davis SL, Ettner S, Appel S, Wilkey M, Rompay V, et al. Trends in alternative medicine used in United State: Results of a follow up national survey. *J. Am. Med. Assoc.* 1998; 280:1569-75.
6. Jordan AD, Kordik CP, Reitz AB, Sanfillipo PJ. Novel anxiolytic agents- 1994 to present. *Expert Opin Ther Pat.* 1996; 6:1047–60.
7. Rickels K and Schweizer E. The treatment of generalized anxiety disorder in *Psychopharmacol. Psychopharmacol Bull.* 1996; 22:93-99.
8. Eisensberg DM, Davis RB, Ettner SL, Appel S, Wilney S, Rompay MV and Kessler RC. Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. *JAMA.* 1998; 280 (18):1569-759.
9. Crawley JN, Goodwin FK. Neuropharmacologic specificity of a simple animal model for the behavioral actions of benzodiazepines. *Pharmacol Biochem Behav* 1981; 15:595-09
10. Amborgi LC, Giachetti A. Some Factors influencing conditioned and spontaneous behavior of rats in light dark box test. *Physiul Behav* 1986; 36: 97-101
11. Costall B, Domeney AM, Gerrard PA, Kelly ME, Naylor RJ. Anxiolytic profile in rodent and primate model of anxiety. *J Pharmacy Pharmacol* 1988; 40:302-5.
12. Pellow S, Chopin P, File SE and Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci Methods* 1985; 14:149–67.
13. Kulkarni SK, Reddy DS. Animal behavioral models for testing anti-anxiety activity. *Met Finds Clin Pharmacol* 1996; 18:219-40.
14. Vogel HG, Vogel. WHO. *Drug Discovery and Evaluation.* Germany Heidelberg, Springer 1999; p. 378-90.

**Table 1: Effect of Flaxseed oil and Perilla oil on behavior of mice using light and dark exploration test**

Group No.	Treatment	Dose	No. of Entry in light arena Mean ± S.E.M	Time spent (sec) in light arena Mean ± S.E.M
1.	Control	-----	7.3±0.79	79.8±8.4
2.	Diazepam	2mg/kg	15.8±0.80***	157.39±8.2***
3.	Flaxseed Oil	0.5ml/kg	8.2±0.81	86.3±7.3
4.	Flaxseed Oil	1.0ml/kg	9.5±0.75	94.7±9.1
5.	Flaxseed Oil	1.5ml/kg	11.7±0.92**	119.4±7.3**
6.	Flaxseed Oil	2.0ml/kg	11.4±0.59*	113.7±3.52*
7.	Perilla Oil	0.3 ml/kg	8.5±1.3	89.7±7.7
8.	Perilla Oil	0.6 ml/kg	10.3±1.47	106.0±10.3
9.	Perilla Oil	0.9 ml/kg	13.2±1.25**	130.85±7.3**
10.	Perilla Oil	1.2 ml/kg	12.1±0.69*	120.62±10.1*

Each value represents as the mean ± S.E.M (n=6), \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs control.

Table 2: Effect of Flaxseed oil and Perilla oil on behavior of mice using elevated plus (EPM) test

Group No.	Treatment	Dose	No. of Entry in open arm Mean $\pm$ S.E.M	Time spent (sec) in open arm Mean $\pm$ S.E.M
11.	Control	-----	4.5 $\pm$ 0.79	31.1 $\pm$ 1.2
12.	Diazepam	2mg/kg	10.4 $\pm$ 0.69***	68.7 $\pm$ 2.3***
13.	Flaxseed Oil	0.5ml/kg	5.1 $\pm$ 0.49	34.4 $\pm$ 1.4
14.	Flaxseed Oil	1.0ml/kg	5.9 $\pm$ 0.44	38.7 $\pm$ 1.8
15.	Flaxseed Oil	1.5ml/kg	7.5 $\pm$ 0.53*	46.9 $\pm$ 4.1**
16.	Flaxseed Oil	2.0ml/kg	7.1 $\pm$ 0.78	45.6 $\pm$ 2.7**
17.	Perilla Oil	0.3 ml/kg	4.75 $\pm$ 0.43	36.1 $\pm$ 5.4
18.	Perilla Oil	0.6 ml/kg	6.1 $\pm$ 0.34	41.8 $\pm$ 2.7
19.	Perilla Oil	0.9 ml/kg	7.94 $\pm$ 0.62**	51.7 $\pm$ 2.9**
20.	Perilla Oil	1.2 ml/kg	7.59 $\pm$ 0.67*	48.7 $\pm$ 3.8**

Each value represents as the mean  $\pm$  S.E.M (n=6), \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs control.

Source of support: Nil, Conflict of interest: None Declared