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Original Research Article

Evaluation of anxiolytic activity of encapsulated flax seed oil alone and as an adjuvant in Swiss Albino mice

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ABSTRACT

Background: Anxiety is a psychological and physiological state characterized by somatic, emotional, cognitive, and behavioural components with displeasing feeling of fear and concern. Among all the antianxiety drugs benzodiazepines are commonly employed drugs for the treatment but they do have limitations. Considering the high prevalence of anxiety disorders and lack of an ideal anxiolytic drug, search for better anxiolytic drugs continue. Medicinal plants are an inexhaustible source and continue to get explored in the search for new drugs.
Methods: Antianxiety activity of Flax seed oil was evaluated in mice using Light-Dark Arena model and Elevated Plus Maze model. Encapsulated Flax seed oil (10ml/kg and 20ml/kg), Diazepam (1mg/kg), Normal saline (10ml/kg) and combination of Encapsulated Flax seed oil and Diazepam (10ml/kg + 1mg/kg) were given orally to the randomly divided 5 groups of 6 animals each. Number of entries and time spent in light arena of Light-Dark Arena model and in open arm of Elevated Plus Maze model were noted and compared among the 5 groups. Observations were analysed using ANOVA and Post hoc Tukey's test.
Results: Encapsulated Flax seed oil alone as well as an adjuvant to Diazepam showed significantly increased number of entries and time spent in light arena of Light-Dark Arena model (<0.05). It also showed significantly (<0.05) increased time spent but not number of entries in open arm of Elevated Plus Maze model.
Conclusions: Encapsulated Flax seed oil showed anxiolytic property in Light-Dark arena model and Elevated plus maze model.

Keywords: Antianxiety activity, Diazepam, Encapsulated flax seed oil

INTRODUCTION

Anxiety is a psychological and physiological state characterized by somatic, emotional, cognitive, and behavioural components with displeasing feeling of fear and concern.[1] It is one of most the common psychological disorders and represent a significant disease burden affecting between 10-30% of general population.[2] Hence treatment of anxiety is an important area of research interest in psychopharmacology.

Among the drugs available for the treatment of anxiety, benzodiazepines are commonly preferred drugs, but they have their limitations.[3] Due to lack of an ideal anxiolytic drug the search for better anxiolytic drugs continue.

Flax seed also known as common flax or linseed, *Linum usitatissimum*, is a member of the genus *Linum* in the family Linaceae. It is a food and fibre crop cultivated in cooler regions of the world. Flax seed oil is a colourless to yellowish oil obtained from the dried, ripened seeds of the flax plant by Cold Pressed extraction method. It is a source of healthy fat, antioxidants and fibre, and is claimed to lower the risk of diabetes, cancer and heart diseases as well as aid in neurotransmission.[4]

Hence in this study we aimed at determining the antianxiety property of encapsulated Flax seed oil using 2 models namely the Light-Dark Arena model and the Elevated Plus Maze model.

Objectives

- To determine the antianxiety efficacy of Flax seed oil in Swiss albino mice.
- To determine the antianxiety efficacy of Flax seed oil as an adjuvant to Diazepam in Swiss albino mice.

METHODS

Flax seed oil

Encapsulated Flax seed oil is a colourless to yellowish oil obtained from dried, ripened seeds of the flax plant. It was purchased from Sattvic Foods (Cold Pressed Flax seed oil) and identified and authenticated by the pharmacist of our institute.

Selection of animals

Healthy Swiss albino mice which were previously unused and weighing 20-30g were obtained from the Animal house attached to the Department of Pharmacology, J J M Medical College, Davangere. The animals were fed on a standard pellet diet and water. They were acclimatized for 7 days before commencement of the study in standard laboratory conditions of 12 h day and night rhythm, maintained at 25±30ºC and 50-70% humidity.

Screened animals were divided into 5 groups of 6 animals each for each model.

The drugs administered orally were:

- *Group I:* Control mice (Normal saline 10mL/Kg)
- *Group II:* Standard (Diazepam 1mg/Kg)
- *Group III:* Encapsulated Flax seed oil (10mL/Kg)
- *Group IV:* Encapsulated Flax seed oil (20mL/Kg)
- *Group V:* Encapsulated Flax seed oil with Diazepam (10mL/Kg + 1mg/Kg)

The antianxiety property was assessed by the Light-Dark Arena model and the Elevated Plus-Maze model.

a) Light-Dark Arena model

The apparatus consists of an open top wooden box with two chambers of specific dimensions separated by a partition wall and connected by a small open door measuring 7.5 x 5cm at the floor level in the centre of wall. The light chamber measuring 20 x 30 x 35cm is painted white and illuminated with 100 Watt bright light source located 17cm above the box.

Each mouse is placed in the centre of the light arena of the apparatus and is allowed to explore for 5 minutes. The number of entries and total time spent in each chamber are recorded. Animals spend more time in the light chamber and show more loco-motor activity after treatment with anxiolytics.

b) Elevated Plus Maze model

The apparatus consists of two open arms, 25 × 5 x 0.5cm (L X W) painted in black, and two closed arms 25 × 5 × 16cm (L X W X H) painted in white with an open roof. The apparatus is elevated 50cm above the floor. The two open arms are opposite to each other and are illuminated by a 40 W bulb positioned 20cm below each open arm. The mice were placed in the centre of maze facing one of the open arms. During 5 minutes the number of entries and total time spent in the open arms was recorded. The maze was cleaned with a paper towel after each trial.

RESULTS

All the values were expressed as mean ± standard error mean (SEM). Intergroup difference was statistically determined by ANOVA followed by Tukey's post-hoc test analysis. A 'P' value less than 0.05 was taken as the level of significance.

Light-Dark Arena model

In this model efficacy was measured by an increase in the mean values of the 2 parameters i.e. the number of entries into the Light Arena and the total time spent in the light arena of the Light-Dark Arena apparatus.

Table 1: Effect of the test drugs in the Light and Dark arena model. Results are expressed as Mean value±SD (standard deviation).

		No. of entries light arena			Total time spent light arena		
Group	**Drugs**	**Mean**	**SD**	**ANOVA**	**Mean**	**SD**	**ANOVA**
1	Control (NS 10mL/Kg)	3.50	1.05		32	5.55	
2	Standard (Diazepam 1mg/kg)	5.50	1.38		106.33	11.96	
3	Test drug (Flax seed oil 10mL/Kg)	5.67	1.37	F= 4.295 P< 0.009	65.33	9.18	F= 61.869 P< 0.001
4	Test drug (Flax seed oil 20mL/Kg	6.17	1.17		105.67	9.07	
5	Test drug (Flax seed oil 10mL/Kg + Diazepam 1mg/Kg)	5.50	1.05		84.67	11.43	

Table 2: Tukey's Post Hoc Multiple Comparison test (between groups with p <0.05) for Number of Entries in the light arena of the Light Dark Arena Model.

Groups compared	Level of Significance
Group-1 vs Group-2	P<0.05
Group-1 vs Group-3	P<0.03
Group-1 vs Group-4	P<0.007
Group-1 vs Group-5	P<0.05

When compared to the Control group encapsulated Flax seed oil showed significant anxiolytic activity at both the doses and also as an adjuvant to Diazepam by showing an increase in the number of entries in the Light arena and also by increasing the total time spent in the Light arena. However, when comparing the Adjuvant Group (Encapsulated Flax seed oil 10mL/Kg + Diazepam 1 mg/Kg) with the Standard Group (Diazepam 1mg/Kg) there was a reduction in the number of entries and the total time spent in the Adjuvant group. (Table 1, Table 2, Table 3 and Figure 1 and 2).

Table 3: Tukey's Post Hoc Multiple Comparison test (between groups with p<0.05) for Time spent in the light arena of the Light Dark Arena Model.

Groups Compared	Level of Significance
Group-1 vs Group-2	P<0.000
Group-1 vs Group-3	P<0.000
Group-1 vs Group-4	P<0.000
Group-1 vs Group-5	P<0.000
Group-2 vs Group-3	P<0.000
Group-2 vs Group-5	P<0.006
Group-3 vs Group-5	P<0.01
Group-3 vs Group-4	P<0.000
Group-4 vs Group-5	P<0.008

Table 4: Effect of the test drugs in the Elevated Plus Maze model. Results are expressed as Mean value±SD (standard deviation).

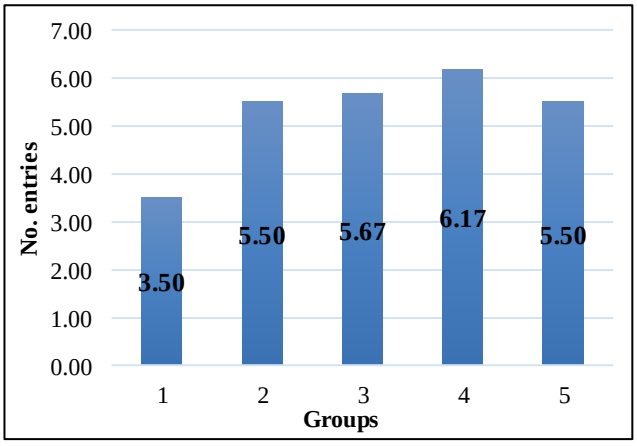
		No. of entries open arm			Total time spent open arm		
Groups	**Drugs**	**Mean**	**SD**	**ANOVA**	**Mean**	**SD**	**ANOVA**
1	Control (NS 10mL/Kg)	4.33	1.03		36.33	4.32	
2	Standard (Diazepam 1mg/Kg)	6.33	1.03		93.67	8.71	
3	Test drug (Flax seed oil 10mL/Kg)	4.83	1.47	F= 2.535 P< 0.06	53.67	7.55	F= 28.75 P< 0.001
4	Test drug (Flax seed oil 20mL/Kg)	6.17	1.72		82.33	19.08	
5	Test drug (Flax seed oil 10mL/Kg +Diazepam 1mg/Kg)	5.50	1.04		5.50	5.84	

Table 5: Tukey's Post Hoc multiple comparison test (between groups with p <0.05) of time spent in the open arm of the Elevated Pluz maze.

Groups compared	Level of significance
Group-1 vs Group-2	P<0.000
Group-1 vs Group-5	P<0.000
Group-2 vs Group-3	P<0.000
Group-2 vs Group-5	P<0.000
Group-3 vs Group-4	P<0.001
Group-4 vs Group-5	P<0.001

Elevated Plus Maze model

In this model efficacy was measured by an increase in the mean values of the 2 parameters i.e. the number of entries into the Open arm and the total time spent in the Open arm of the Elevated Plus Maze apparatus. When compared to the Control group encapsulated Flax seed oil alone (10 and 20 mL/Kg) and as an adjuvant to Diazepam showed a statistically significant increase in the time spent in the open arms.

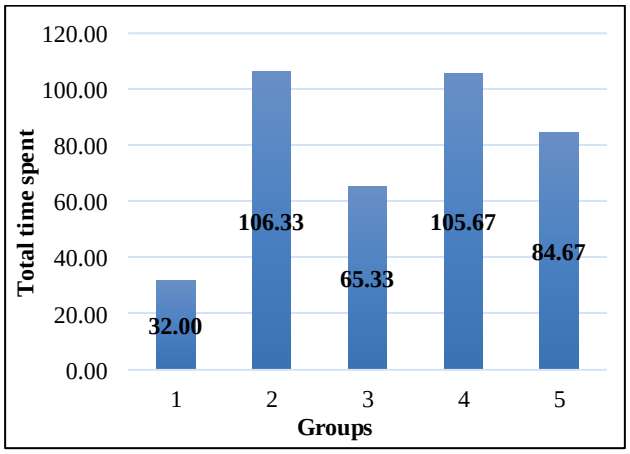


Group 1- NS 10mL/Kg, Group 2-Diazepam-1mg/Kg, Group 3-Flax seed oil-10 mL/Kg), Group 4- Flax seed oil 20mL/Kg), Group 5- Flax seed oil 10mL/Kg+ Diazepam 1mg/Kg.

Figure 1: Mean number of entries in light arena of the Light-Dark Arena model.

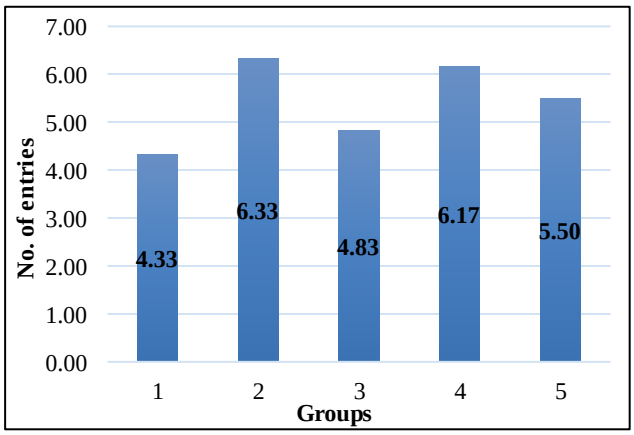
But when it came to the number of entries into the open arm the increase in the number of entries was not

statistically significant. However when comparing the Adjuvant Group (Encapsulated Flax seed oil 10mg/Kg + Diazepam 1 mg) with Standard (Diazepam 1mg/Kg) there was a reduction in the number of entries and the total time spent in the Adjuvant group. (Table 4, Table 5 and Figure 3 and 4).



Group 1- NS 10mL/Kg, Group 2-Diazepam-1mg/Kg, Group 3-Flax seed oil-10 mL/Kg), Group 4- Flax seed oil 20mL/Kg), Group 5- Flax seed oil 10mL/Kg+ Diazepam 1mg/Kg

Figure 2: Mean time spent in the light arena of the Light-Dark Arena model by the Groups.

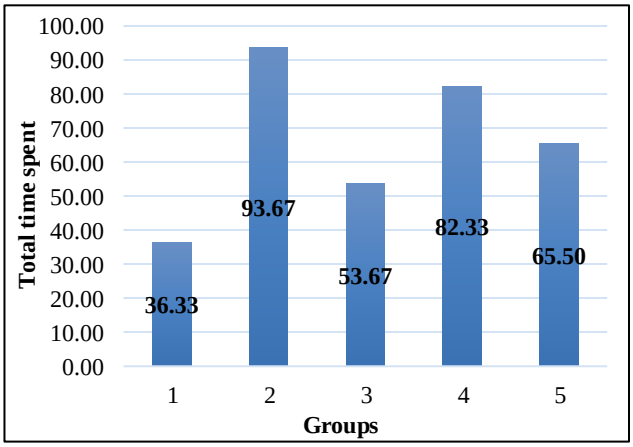


Group 1- NS 10mL/Kg, Group 2-Diazepam-1mg/Kg, Group 3-Flax seed oil-10 mL/Kg), Group 4- Flax seed oil 20mL/Kg), Group 5- Flax seed oil 10mL/Kg+ Diazepam 1mg/Kg.

Figure 3: Mean number of entries in the open arm of the elevated plus maze model by the Groups.

DISCUSSION

Anxiety disorders are characterised by changes in mood, behaviour and somatic function. Cognition symptoms of anxiety are commonly associated with depression, panic disorder, agoraphobia and other specific phobias like obsessive-compulsive disorder, eating disorders and some personality disorder.[5] The brain amygdala appears key in modulating fear and anxiety. In the central nervous system, the major mediators of the symptoms of anxiety disorders appear to be norepinephrine, serotonin, dopamine and gamma-amino butyric acid (GABA). Peripherally, the autonomic nervous system, especially the sympathetic nervous system, mediates many of the symptoms.[6] The anxiolytic activity of Diazepam is due to its GABA facilitatory action through GABA-A receptor.[3]



Group 1- NS 10mL/Kg, Group 2-Diazepam-1mg/Kg, Group 3-Flax seed oil-10 mL/Kg), Group 4- Flax seed oil 20mL/Kg), Group 5- Flax seed oil 10mL/Kg+ Diazepam 1mg/Kg

Figure 4: Mean time spent in the open arm of the elevated plus maze model by the Groups.

Flax seed has recently gained attention primarily because it is a rich source of alpha-linolenic acid (ALA), the phytoestrogen, lignans. ALA gets converted endogenously to longer chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Omega-3 fatty acids also called n-3 polyunsaturated fatty acids (PUFAs) are essential in normal brain development and acts as anti-inflammatory in our body, its deficiency can cause neurologic dysfunction.[7] DHA is a component of the cell membranes of neurons. It makes the cell membranes more fluid and enables the signals to travel faster and more fluidly between the cells in the brain.[8]

In this study Light-Dark Arena model and Elevated Plus Maze model were used to assess the anxiolytic activity of Flax seed oil. Swiss albino mice naturally show aversion to light, high or open spaces, and hence spend more time in enclosed or dark spaces. Behavioural change showing an increase in the number of entries and total time spent in the light arena and open arm is deemed as a reduction in anxiety and this forms the basis for its use in the antianxiety screening models.

Encapsulated Flax seed oil showed a dose dependant anxiolytic activity in both in the Elevated Plus Maze model and the Light-Dark Arena model. At a dose of 20mg/Kg it was comparable with the standard Dr.ug Diazepam 1mg. These results correlate with studies done by Nadhi et al where Encapsulated Flax seed oil showed anxiolytic activity in the Open-Field model and Vogel's conflict test in rats.[8] A similar study done by Kumar et al. between flax seed oil and perilla oil also showed anxiolytic property of

Flax seed oil in the Light-Dark arena model and the Elevated Plus model in rats.[9]

Our study also evaluated the role of Flax seed oil as an adjuvant to Diazepam. When Flax seed oil was used as an adjuvant to Diazepam the efficacy was lower than with either Encapsulated Flax seed oil or Diazepam used alone. In fact there was a reduction in the efficacy of Diazepam when combined with Encapsulated Flax seed oil. Thus there is an apparent antagonistic effect when Encapsulated Flax seed oil is used together with Diazepam.

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