EVALUATION OF ANXIOLYTIC POTENTIAL OF *LINUM USITATISSIMUM* OIL IN WISTAR RATS

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The objective of the present study was to evaluate the anxiolytic potential of the *Linum usitatissimum* (Flax seed) oil (5ml and 2.5ml/kg) in wistar rats by using Elevated plus maze (EPM) model and 5ml and 10 ml/kg in Lithium induced head twitches model. Experiments were carried out on white inbred Wistar rats (180-200 g). The efficacy of the oil at both the animal models was compared with the standard anxiolytic drugs Diazepam (0.5 mg/kg). The result showed that the oil significantly increased the number of entries and time spent in the open arm in the elevated plus maze. Similarly in Lithium induced head twitches model administration of oil decreases the no. of head twitches. Present study confirms that the extract showed significant anxiolytic activity at both dose levels which is comparable with standard anxiolytic Diazepam.

Keywords: Anxiolytic, Flax seed, Elevated plus maze, Diazepam

INTRODUCTION

Today we are witnessing a great deal of public interest in the use of herbal remedies. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years, and have served humans as well as valuable components of seasonings, beverages, cosmetics, dyes, and medicines. Consumption of fruit and vegetables, as well as grains, has been strongly associated with reduced risk of cardiovascular disease, cancer, diabetes, Alzheimer disease, cataracts, and age related functional decline [1–2]. Human anxiety is defined as a feeling of apprehension, uncertainty or tension stemming from the anticipation of imagined or unreal threat. [3] Anxiety effects one-eighth population worldwide and has become an important research area in the field of psychopharmacology. [4] Pharmacological therapies play an important role in the therapeutic concept. Benzodiazepines (BZDs), barbiturates, tricyclic antidepressants (TCA's) have been used for long time to treat anxiety disorders. Among them Benzodiazepines have been the most widely used anxiolytics in various practices for many years [5].

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MATERIAL AND METHODS

Plant Material

Dried ripe seeds of *Linum usitatissium* were procured from new market Bhopal. The identity of the seeds was confirmed by Botanist, Pinnacle Biomedical Research Institute (PBRI), Bhopal, India.

Drugs and Chemicals

All the chemicals used for the present study were of LR grade, employed for extraction of the plant material (seeds). Diazepam was used as a standard drug for evaluation of anti-anxiety activity.

Extraction of oil

For the extraction of oil of linseed seeds the maceration has been performed, briefly, 500 gm of dried ripe seeds of linseed were crossed to form coarse powder, which was mixed uniformly with 400 ml of petroleum ether. Mixture was kept in a closed plastic container for seven days with occasional shaking & mixing the liquid. After seven days it was filtered by using double layered muslin cloth. Filtrate & marc were obtained. Filtrate was filtered again with what man's filter paper. Yellow colored clear filtrate was obtained containing linseed oil & traces of petroleum ether. The filtrate was taken in a beaker & kept over rectangular water bath at 50^0C for 3 days (6-7 hrs per day).Then the beaker containing liquid was kept in hot air oven for 6 hrs at 45-50^0C for

complete removal of traces of petroleum ether. Yellow colored oil was obtained which was subjected for phytochemical screening[6-7].

S. No.	Chemical constituent	Inference
1.	Carbohydrates	Absent
2.	Alkaloids	Absent
3.	Glycosides	
	a. Cardiac Glycoside	Present
	b. Saponin Glycoside	Absent
	c. Coumarin Glycoside	Absent
	d. Flavonoids Glycoside	Absent
	e. Cyanogenetic Glycoside	Absent
4.	Phytosterols	Present
5.	Fixed oil	Present
6.	Proteins	Absent
7.	Tannins & phenolic compounds	Absent

Table no.1:Phytochemical Screening

Animals

Wistar albino rats (either sex), weighing around 120 gm were used for the present study. Animals were procured from institutional animal house facility, PBRI, Bhopal, India. The animals were housed in polypropylene cages in groups of six in each cage maintained at 25±2^{o}C (temperature) with 12 h light and 12 h dark cycle, fed with standard laboratory pellet diet and water *ad libitum*. The animals were acclimatized to laboratory condition one week prior to the initiation of the experiments. All conditions were maintained as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for the use and care of experimental animals. The experiments were conducted as per permission of Institutional Animal Ethical Committee (IAEC) of PBRI (Reg. No. 1283/c/09/CPCSEA) Bhopal, India. Animals were randomly grouped into four groups six animals in each group. Group-I (vehicle control), Group-II (linseed il 5ml/kg/p.o.), Group-III (linseed oil 2.5ml/kg/p.o.) and Group-IV (Diazepam 0.5mg/kg).

Elevated Plus Maze [10-11]

Placing an animal in an unaccustomed position or a new environment induces anxiety in animal which alters its exploratory behavior. A rat when placed on the elevated plus maze prefers to remain in the enclosed arm. The animal spends more time in the enclosed arm and the no. of entries in the open arm is also decreased. If the drug has anxiolytic effect, then the rat will spend more time in the open arm and the number of entries in the open arm shall also increase.

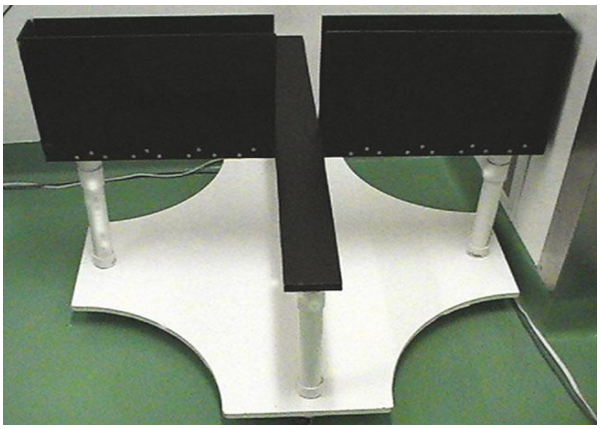

Fig no-1: Elevated plus maze

Rat became anxious when placed on an elevated surface. The platform was elevated 50 cm from the ground. The EPM consisted of two open arms (25x5 cm) crossed with two closed arms (25x5x 20 cm) as shown in the figure no 1. The arms were connected with a central square of 5x5 cm in a dimly illuminated room. The standard and the test drugs were administered to each animal via oral route one hour prior to the initiation of the experiment. The animals were placed individually in the center of the EPM facing the closed arm and the time spent in the open and closed arms was recorded for a period of six minutes. Statistical analysis was done with One way ANOVA followed by Dunnett's test.

Lithium Induced Head Twitches[12-13]

Animals were randomly grouped into four groups six animals in each group were fasted overnight prior to the initiation of experiment but drinking water was provided *ad libitum*. Group-I (vehicle control), Group-II (linseed oil 5ml/kg/p.o.), Group-III (linseed oil

10ml/kg/p.o.) and Group-IV (Diazepam 0.5mg/kg). The animals were treated with vehicle/test substances 60 mins prior to i.p injection of 200mg/kg of lithium carbonate. The prevention of head twitches due to standard drug/test substance was recorded up to sixty mins after lithium treatment. The lithium induced head twitching is used to assess the effect of drugs influencing second messenger system like phosphatidyl inosotol (IP) pathway which is responsible to release serotonin from the serotonergic neurons that stimulates the serotonin $5HT_2$ receptors and produces head twitches. Statistical analysis was done with One way ANOVA followed by Dunnett's test.

Treatment group	Mean ± SEM
Vehicle	27 ± 9.372
Extract (5 ml/kg)	$230.25 \pm 6.884^{a,b,c}$
Extract (2.5 ml/kg)	$200.5 \pm 5.809^{a,c}$
Diazepam (0.5 mg/kg)	322 ± 7^{a}

Table no. 2: Effect of different concentrations of drugs on antianxiety activity using Elevated plus maze (EPM open arm).

a - Significant variation as compared to vehicle treated group (P < 0.05), b – Non significant variation as compared to lower dose (P > 0.05), c - Significant variation as compared to diazepam (P < 0.05)

Treatment group	Mean ± SEM
Vehicle	120.5 ± 19.27
Extract (5ml/kg)	$71.25 \pm 11.8^{b,c}$
Extract (10ml/kg)	$58 \pm 6.42^{a,d,e}$
Diazepam (0.5mg/kg)	9.5 ± 2.102^{a}

Table no. 3: Effect of different concentrations of drugs on antianxiety activity by lithium induced head twitches in rats.

a - Significant variation as compared to vehicle treated group (P < 0.05),b - Non significant variation as compared to vehicle treated group (P > 0.05),c – Significant variation as compared to diazepam (P < 0.05),d – Non significant variation as compared to lower dose (P > 0.05) ,e – Non significant variation as compared to diazepam (P > 0.05)

DISCUSSION:

By using Elevated plus maze apparatus for assessment of anxiolytic activity of extract (table no. 2 and graph 1) it was discovered that the extract was having significant antianxiety activity at both doses i.e. 2.5 ml/kg and 5 ml/kg dose as compared to vehicle treated group (P<0.05). Diazepam was also having significant anxiolytic activity at 0.5 mg/kg (P<0.05). When the effect of extract at the two selected doses was compared, a non-significant variation was found between them. Anxiolytic activity of extract at 5 ml/kg was significantly less as compared to that of diazepam (0.5 mg/kg).When the effect of the extract was investigated for its activity on lithium induced head twitches (table no.3 and graph 2) it was revealed that the extract was not having significant effect at 5 ml/kg dose as compared to vehicle treated group (P>0.05), but the extract was showing significant effect at 10 ml/kg as compared to vehicle treated group (P<0.05). Significant activity was observed for diazepam as at 0.5 mg/kg dose as compared to vehicle treated group (P<0.05). The activity of extract at 10 ml/kg was not significantly different from 5 ml/kg, thus the effect was not found to be dose dependent at the selected doses. The effect of extract at 10 ml/kg dose was non-significantly different from diazepam (0.5 mg/kg) although the effect was less.

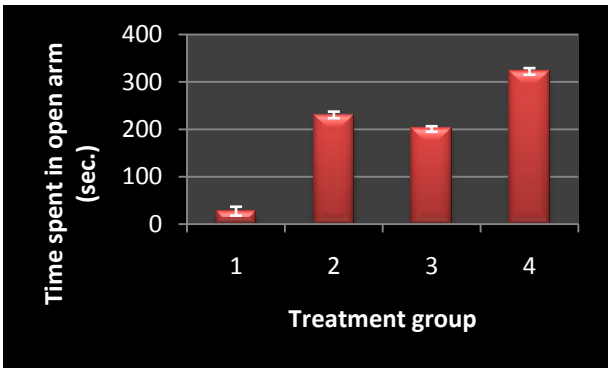

Fig: 2 Result showing effect of different concentrations of drugs on antianxiety activity using Elevated plus maze (EPM open arm)

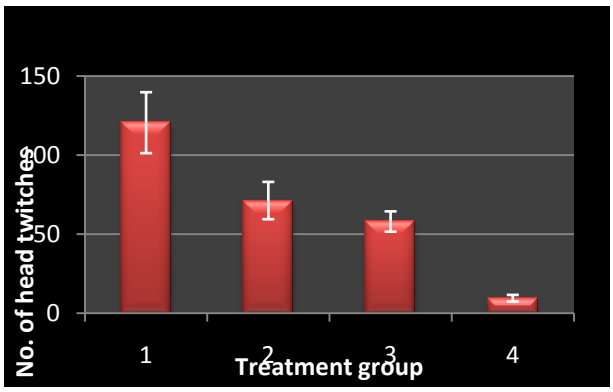

Fig: 2 Result showing effect of different concentrations of drugs on antianxiety activity by lithium induced head twitches in rats.

CONCLUSION

Anxiety disorders, though ubiquitous, are responsive to treatment. By using knowledge of the pathophysiology, pharmacologic regimens with various mechanisms of action can be implemented, leading to a positive outcome. From this research work it can be concluded that the administration of the oil has an effect on behaviour and on neurochemical parameters in various brain regions of the animal. In Elevated plus maze test, administration of oil, increased the time spent in open arm as compared to the vehicle treated group. Increase in the time spent in open arm suggests that the oil has anxiolytic activity. Similarly in Lithium induced head twitches model administration of oil, decreased the number of head twitches. Decrease in no. of head twitches reveals that the oil has antagonistic effect on 5-HT_2. Thus the potential of *Linium usitatissimum* seed oil in anxiety need further investigations for confirmation of mechanism of action. Being easily available and daily consumable part of normal diet *Linium usitatissimum* seed may a good alternative in anxiety.

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