

The Effect of Phaseolus Vulgaris L. Fixed Oil on the Behavioural Activity of Swiss Albino Mice

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Author's Contribution

All the authors contributed significantly to the research that resulted in the submitted manuscripts

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ABSTRACT

Background: Phaseolus vulgaris commonly known as kidney bean has been studied previously for pharmacological activities like antidiabetic, antioxidant, analgesic, anti-inflammatory, antifungal and antibacterial properties. Various extracts of the seeds have been researched except fixed oils.

Objective: Therefore objective of the present study is to investigate the behavioural activity of fixed oil extracted from Phaseolus vulgaris seeds.

Methodology: Hole board, light and dark, stationary rod and open field tests were used for behavioral evaluation. Four groups were made with seven mice in each: (1) Control = Normal saline 2ml/kg (2) Phaseolus vulgaris fixed oil = PVFO 2ml/kg (3) PVFO 4ml/kg (4) Diazepam (1mg/kg) as standard. All treatments were given orally, 30 minutes prior to the performance of tests. In hole board test PVFO 4ml/kg decreased the number of head poking ($p \leq 0.01$).

Results: In light and dark test PVFO in both doses decreased the time spent in light compartment ($p \leq 0.01$). In open field test number of peripheral lines crossed were decreased ($p \leq 0.05$) whereas in stationary rod test no effect was observed by any dose of PVFO.

Conclusion: Results indicate that Phaseolus vulgaris fixed oil does not affect memory or learning in mice and may possess a dose dependent sedative hypnotic effect.

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INTRODUCTION

Anxiety is considered most commonly occurring stress allied disorder among the individuals and categorized as stress induced psychiatric disorder. Anxiety is described as inner instability associated with nervous behavior, thoughts and somatic complaints, if remain untreated can lead to anxiety disorders which can affect the lives inducing psychosomatic diseases [1]. Unhealthy and complicated life style is one of the basic reason of anxiety and other stress related

disorders. Clinical and preclinical researches confirmed that anxiety can be generated due to interruption in central nervous system neurotransmitters activity. Serotonin (5HT) is the major neurotransmitter whose availability and regulation in central nervous system is responsible for triggering the anxiety and depression. Beside 5HT other neurotransmitters like norepinephrine (NE) or noradrenaline (NA), dopamine (DA), glutamate, and brain-derived

neurotrophic factor (BDNF) may also be involved [2]. BDNF has a role in growth and survival of neurons.

Fixed oils are thick viscous non volatile liquids which are esters of fatty acids. Fatty acids are essential component of body and fundamental part of cell membrane. Long chain polyunsaturated fatty acids (PUFA), which cannot be synthesized inside the body are complemented through dietary sources in forms of linolenic acid and alpha linolenic acids. Linoleic acid (LA) metabolize into omega-6 family of fatty acids, while alpha-linolenic acid (ALA) metabolizes to form omega-3 family. The PUFAs (n-3) are mostly found in green leafy vegetables, walnuts, fish and some seeds (chia, flax), while PUFAs (n-6) are mostly present in plants seeds and oils which include sunflower, corn ,cottonseed and some others [3] . LA and ALA are precursor fatty acids and undergo process of desaturation and elongation and convert into extremely unsaturated, long-chained arachidonic acid (AA) and docosahexaenoic acid (DHA), that process is carried out by microsomal enzyme system. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can be obtained directly from fish and fish oil supplements [4].

Omega 3 PUFAs such as EPA and DHA have a role in treatment of depression. The deficiency of n-3 polyunsaturated fatty acid specifically docosahexaenoic acid can lead to depressive and anxious behavior [5, 6]. Linolenic acid and alpha linolenic acid are beneficial for maintaining the healthy mood state [7]. It is also confirmed by an epidemiological report that vegetarian diet only contained triglycerides oil which are mainly composed of Linolenic acid and alpha linolenic acid have more efficient effect on mood as compared to diet which contains fish oils comprised of DHA and EPA [8]. PUFAs are also involved in regulating certain processes in central nervous system such as adjusting blood glucose and food consumption as well as these fatty acids take part in process of apoptosis,

regulating emotional behavior, neurotransmission and neuroinflammation [9]. Furthermore, dietary PUFAs (n-3) control various neurotransmitter operations, as well as signal transmission, responsiveness, and phospholipid alteration [10]. PUFAs (n-3) are neuroprotective , required for mental development, avoidance of neuronal death, and the inhibition of neuroinflammation [11].

Phaseolus vulgaris or kidney beans are commonly consumed in dry seed form. They have high nutritious value and contain protein, fiber, and B vitamins. Beans are thought to be an important source of unsaturated fatty acids, including palmitic, oleic, and linoleic acids. Linolenic acid made up 43.1% of fatty acids in beans [12].The seeds and pods of *Phaseolus vulgaris* possess therapeutic effects and are used as folk remedy for number of diseases. This study is conducted to evaluate the potential sedative, anxiolytic and learning behavior of fixed oil of *Phaseolus vulgaris* (PVFO).

MATERIAL AND METHODS

Collection of plant material and extraction

2 kg Seeds of *Phaseolus vulgaris* were purchased from local market and cleaned from foreign material and dirt. The seeds were identified and authenticated by a taxonomist Department of Botany, University of Karachi, then crushed and extracted with hexane (4L). Solvent was evaporated at room temperature, a clear yellow color oil was left as residue. The oil was stored at room temperature in dark area.

Animals

Swiss albino mice were used for behavioral studies in this experiment. Healthy mice of either sex weighing around 20-25gm were provided by animal house of Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi. Animals were housed in propylene plastic cages in a controlled

environment of 12 hour alternate light and dark cycle, 50-60% humidity and room temperature of 26-29°C. Standard diet was provided to the animals and were given free access to food and water. Mice were acclimatized with the laboratory conditions for a week. Experiments were carried out in accordance with the international guidelines of animal ethics and study was submitted to and approved by institutional ethical committee, Department of Pharmacology in 2017 and Board of advanced studies (BASR), University of Karachi in 2015.

Dosing

Mice were divided into four groups (n= 7) and received oral dosing as follows.

- (1) Control = Normal saline 2ml/kg
- (2) Phaseolus vulgaris fixed oil 2ml/kg = PVFO I
- (3) Phaseolus vulgaris fixed oil 4ml/kg= PVFO II
- (4) Diazepam (1mg/kg) as standard

BEHAVIORAL STUDIES

Hole board test

Hole board method was used to examine the exploratory behavior of animals. Hole board is basically a wooden box (35×45×45cm) rectangular in shape having 3 holes (3cm diameter) in each side of box and roof of box is made up of transparent glass to observe the movement of mice. After 30 minutes of oral dosing each mice was kept individually in hole board and number of head pokes were counted for a period of 10 minutes [13].

Light and dark test

The apparatus consisted of a rectangular box with 2 compartments, one is dark while the other is illuminated with a light bulb. A small opening for transition is provided between compartments. After 30 minutes of dosing the time spent in lit compartment was noted [14].

Open field test

For assessment of exploration, anxiety and locomotion open field apparatus was used [15]. It was constructed of white plywood and measured 72 ×72 cm with 36 cm walls. Lines were drawn on the clear plexiglas floor of apparatus with a marker. After 30 minutes of dose administration mice were placed in centre of apparatus and number of lines crossed in periphery, visits to central squares and frequency of rearing were observed for 5 minutes. Only one mouse was placed in apparatus at a time, and the apparatus was cleansed with 95% ethanol after each reading.

Stationary rod

The stationary rod test is basically used to evaluate the locomotor activity, memory and learning skills [16]. Stationary rod as name indicated consists of a horizontal rod which is made up of stainless steel with 2 platform at each end of rod. At start mice were trained on stationary rod to move from 1 platform to other for a period of three days. After 30 minutes of dosing the time taken by mice to reach from one platform to another platform was recorded.

STATISTICAL ANALYSIS

Data is analyzed by using SPSS version 20. One way ANOVA is applied for comparing means followed by post hoc test. All values in tables are mentioned as mean ±SEM.

RESULTS

Hole board test

PVFO 2ml/kg had no effect in hole board test whereas PVFO 4ml/kg and Diazepam(1mg/kg) decreased head pokes ($p \leq 0.01$) (Table and figure I).

Table 1: Hole board test

Groups	Dose	No of head pokes in 10 minutes
Control	2ml/kg	31.66±0.33
PVFO- I	2ml/kg	29.00±3.51
PVFO –II	4ml/kg	7.66±1.20**
Diazepam	1mg/kg	9.66±4.25**

Values are expressed as mean± SEM ; N=7 ; PVFO= Phaseolus vulgaris fixed oil; *= p≤0.05 as compared to control; **= p≤0.01 as compared to control

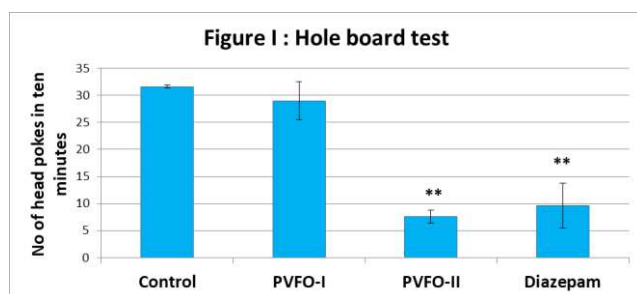


Figure 1: Hole board test

*= p≤0.05 as compared to control; **= p≤0.01 as compared to control

Light and dark test

PVFO 2ml and PVFO 4ml/kg decrease time spent in light compartment markedly than control (p≤0.01). A highly significant increase is observed in time spent in light compartment and number of transitions by standard drug diazepam (p≤0.01) (Table and figure II).

Table 3: Open field test

Groups	Dose	No of central square entry	No of peripheral lines crossed	Frequency of Rearing
Control	2ml/kg	5.66±1.76	228.66±6.76	30.00±2.64
PVFO- I	2ml/kg	2.66±0.66	90±7.00*	10.33±4.05
PVFO- II	4ml/kg	3.00±1.52	109.33±18.04*	16.66±7.26
Diazepam	1mg/kg	1.33±1.33	108.66±39.49*	1.00±1.00**

Values are expressed as mean± SEM ; N=7 ; PVFO= Phaseolus vulgaris fixed oil; *= p≤0.05

Table 2: Light and Dark test

Groups	Dose	Time spent in light compartment (min)	Frequency of Transition between compartment
Control	2ml/kg	3.83 ±0.23	06±0.57
PVFO- I	2ml/kg	1.91±0.29**	11.33±0.66
PVFO- II	4ml/kg	1.54±0.22**	10.66±1.85
Diazepam	1mg/kg	6.34±0.08**	18.33±1.45**

Values are expressed as mean± SEM ; N=7 ; PVFO= Phaseolus vulgaris fixed oil; *= p≤0.05 as compared to control; **= p≤0.01 as compared to control

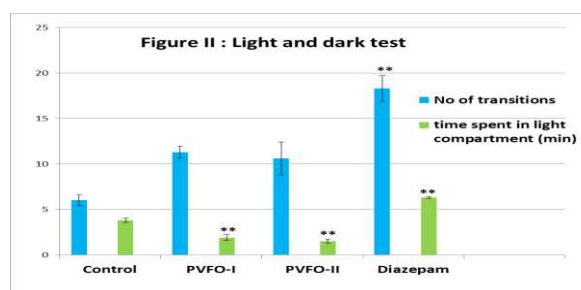


Figure 2: Light and dark test

*= p≤0.05 as compared to control; **= p≤0.01 as compared to control

Open field test

The number of peripheral lines crossed were decreased with PUFO in both doses (2ml/kg and 4ml/kg) as compared to control (p≤0.05). Similar result was observed with diazepam which also decreased number of rearings (p≤0.01) (Table and figure III a,b,c).

as compared to control; **= p≤0.01 as compared to control

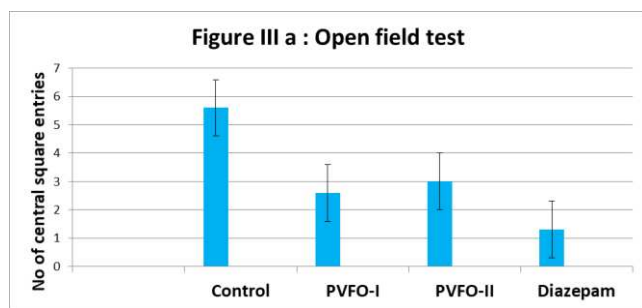


Figure 3(a): Open field test

*= $p \leq 0.05$ as compared to control; **= $p \leq 0.01$ as compared to control

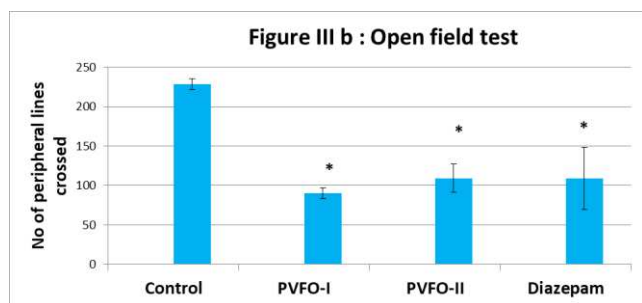


Figure 3(b): Open field test

*= $p \leq 0.05$ as compared to control; **= $p \leq 0.01$ as compared to control

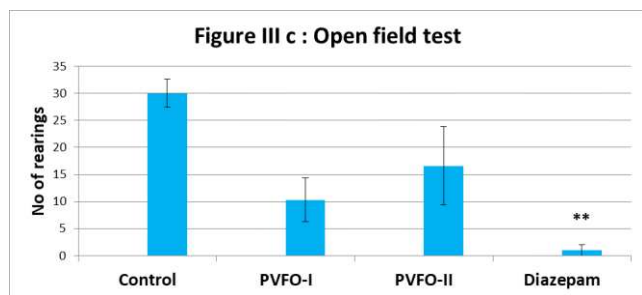


Figure 3(c): Open field test

*= $p \leq 0.05$ as compared to control; **= $p \leq 0.01$ as compared to control

Stationary rod test

No effect was produced in stationary rod test by PVFO, while diazepam showed significant increase in travel time as compared to control ($p \leq 0.05$) (Table and figure IV).

Table 4: Stationary rod test

Groups	Dose	Travel time (sec)
Control	2ml/kg	10.66±1.20
PVFO- I	2ml/kg	11.33±1.66
PVFO-II	4ml/kg	17.33±2.18
Diazepam	1mg/kg	27.66±1.45*

Values are expressed as mean± SEM ; N=7 ; PVFO= Phaseolus vulgaris fixed oil; *= $p \leq 0.05$ as compared to control; **= $p \leq 0.01$ as compared to control

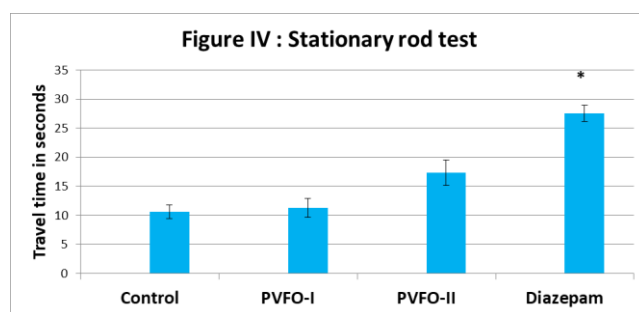


Figure 4: Stationary rod test

*= $p \leq 0.05$ as compared to control; **= $p \leq 0.01$ as compared to control

DISCUSSION

In present study *Phaseolus vulgaris* fixed oil (PVFO) was assessed for behavioral activity in mice. Pathophysiology of anxiety is concerned with dysregulation of dopaminergic, serotonergic adrenergic and GABAergic neurosystems and availability of central nervous system neurotransmitters [17]. The most important class of anxiolytics comprise of Benzodiazepines which produce action through binding with receptors, present on the GABA pentameric complex. Beside the fact that benzodiazepines are most commonly recommended therapy from last few decades for different anxiety disorders they also have some obvious side effects including sedation, ataxia,

amnesia and can also lead to pharmacological dependence [18].

In hole board PVFO 4ml/kg decreased head pokes and there is inverse relationship between number of head pokes and anxiety [13]. As Diazepam 1 mg /kg also produced similar effects in hole board test therefore it may be considered a dose dependent sedative effect of PVFO.

PVFO 2ml and PVFO 4ml/kg decreased time spent in light compartment. Rodents' innate behavior is aversive to light [14] and anxiolytic drugs such as Diazepam increase time spent in light compartment. PVFO is not anxiolytic according to this finding as Diazepam 1mg/kg increased time spent in light compartment and number of transitions. This effect of PVFO in light and dark test can also be correlated with the dose dependent sedative effect. The mechanism behind sedative effect of PVFO may be related with potential histaminergic activity which needs further research.

In the open field test if there is reduction in mobility and rearing it indicates anxiety[19]. The number of peripheral lines crossed were decreased with PUFO in both doses (2ml/kg and 4ml/kg) but as similar effects were observed with diazepam 1mg/kg therefore it may be considered a dose dependent effect.

No effect was produced in stationary rod test by PVFO, while diazepam 1mg/kg showed significant increase in travel time as compared to control.

In current study PVFO (2ml and 4ml/kg) did not produce anxiolytic actions in hole board, light and dark and open field tests but decreased number of head pokes , decreased time spent in light area and decreased number of lines crossed . Therefore it is concluded that like benzodiazepines PVFO may also possess a dose dependent sedative and anxiolytic effect. It has no effect on memory and learning in 2 and 4ml/kg doses. The mechanism for the behavioral effects of PVFO can be traced by

confirming presence of fatty acids like LA and ALA which are involved in mood regulation , neuroinflammation and neurotransmission in CNS [20,21]. A chronic or sub chronic study is further required to ascertain behavioral effects of PVFO.

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