REPEATED ORAL ADMINISTRATION OF FLAXSEEDS INDUCED ANTIDEPRESSANT AND ANXIOLYTIC EFFECTS IN RATS

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Abstract

Omega-3- fatty acids found in flaxseed is good for maintaining brain health as about 60 percent of the brain consists of lipids (fats) which make up the lining, or cell membrane, of every brain cell. The types of fats present in the brain influence its structure and functions, Flaxseeds are the richest plant source of omega-3 fatty acid (α-linolenic acid) and the phytohormone lignans. It is also an essential source of high-quality protein and dietary fiber. Additionally, flaxseed has potential to be a source of phenolic compounds. The present study was designed to investigate the effects of flaxseeds from neurobehavioral aspects. The effect of oral intake of flaxseeds was also monitored on glucose and cholesterol level. Flaxseeds were given orally to rats for a period of 4 weeks. Open field apparatus used to monitor locomotor activity, forced swim test to assess depression like symptoms and elevated plus maze to determine anxiogenic behaviors. Significant changes were observed in open field activity. A significant decrease in depression like symptoms and anxiety were also exhibited by test rats as compared to the control rats while significant improvement in memory was observed in flax treated rats. Flax seeds treated rats also exhibited a significant decrease in glucose and cholesterol level.

Introduction

Natural products for therapeutic purposes are used by about 1 billion people mostly in developing countries in the treatment of deadly diseases such as cancer, Alzheimer disease and Malaria. The use of natural foods as medicines has increased dramatically in recent years. Flax seeds contain PUFA, so they are expected to be useful for health. But unluckily they generate toxic derivatives since they frequently go through auto oxidation (Zuk *et al.*, 2012). Flax, with the binomial name Linum usitatissimum, is a member of the genus Linum in the family Linaceae, is an annual herb. Flax seeds also linseeds come from flax plant has a long history used as a food and a drug. It is also cultivated for oil and fiber (Madhusudhan, 2009). Flaxseeds are a rich source of micronutrients, dietary fiber, manganese, vitamin B_1, and the essential fatty acid alpha-linolenic acid, also known as ALA or omega-3.

The beneficial properties of consuming flaxseeds are protecting against various cancers like prostate cancer, breast cancer, colon cancer and lowering blood sugar, blood pressure and blood cholesterol to reduce risk of heart disease and diabetes, also protect against radiation because flaxseeds have antioxidant and anti-inflammatory properties (Steven and Ehlrich, 2013). It has been reported that inflammatory response, insulin resistance, and diabetes development are attenuated by antioxidants (Chun *et al.*, 2008; Vinayaga-Moorthi *et al*., 2006). Blocking DNA scissions, peroxidation of lipid and reduction of ROS are the activities exhibited by Flaxseed component such as lignan, secoisolariciresinol diglucoside (SDG) (Prasad 2000; Lee *et al.*, 2008). Serum TNF-α, IL-1 β, IL-6, CRP, glucose, or concentration of glycosylated haemoglobin or an elevated sensitivity of insulin hormone in humans are significantly reduced by supplementation of flaxseed (Caughey *et al.*, 1996; Bleodon *et al.*, 2008). Moreover inflammation, oxidative lung damages, peroxidation of lipid, and hyperinsulinemia in animals are also reduced by flaxseed (Oghborn *et al*., 2006; Lee *et al.*, 2009; Fukumitsu *et al.*, 2008). Flaxseeds not only have preventive role against cardiovascular diseases, cancer, and diabetes but also play role in enhancement of spatial memory (Akhtar *et al*., 2013). Polyphenols from flax seed with omega-3 fatty acids were able to reduce all the chronic mild stress effects tested compared to polyphenols from pomegranate peel (Naveen *et al*., 2013). Patients suffer from major depression, the peripheral level of EPA and DHA decrease and EPA is useful for its treatment. An improved habituation to a new environment, and to make animals less stressed and more manageable is also reported to be an effect of flaxseed (Azevedo *et al*., 2011). Lignin has anticarcinogenic activity, it has been reported that flax seeds improve health and also exhibit chemoprotective properties not only in human but also in animals.

Present study is designed to investigate the effects of oral administration of flaxseeds on different behaviors and biochemical effects like depression, anxiety, locomotor activity, and cholesterol and glucose level.

Materials and Methods

Locally bred male albino Wister rats of weight as given in each experiment were housed individually under 12 h light dark cycles and room temperature (22 ± 2°C) should be same throughout the experiment. Rats have free access to the standard rodent diet and clean tap water for at least 3-4 days before experimentation. All experiments were performed according to a protocol defined by Local Animal Care Committee.

Drug Preparation: Flax seeds 200mg/kg/mL dissolved in distilled water was given orally to experimental animals. Flax seeds were purchased locally.

Behavioral Methods:

1. Open Field Activity: The assessment of locomotors activity and exploration in a novel environment as it may be altered by drug administration was done by using open field apparatus. The test consists of measuring the activity of rats in an open novel space, from which escape is prevented by a surrounding wall (Haleem and Batool, 1996) The apparatus used in this study consisted of square area 76 cm x 76 cm. Walls of this apparatus is opaque and height of walls is 42 cm. 25 equal squares are drawn on the floor of the apparatus. Testing was performed in a quiet room under white light as described by (Kennett and Dickinson 1985). To determine activity, animals taken out from their home cages were placed in the center square of the apparatus (one at a time). The numbers of square crossed by rats were scored for 5 minutes.

2. Elevated plus maze activity: The apparatus for the plus maze consists of the four arms, which are equal in size. The two opposite arms are open while the two others are closed. The length of the each arm is 50 cm and width is 10 cm. The central area of 5 cm^2 joins 10 cm arms. The walls of closed arm are 40 cm high. The maze is elevated from the ground at the height of 60 cm. To determine the activity of the rat, it was placed in the center of the maze. The time spent in the open arm of the maze was monitored for 5 min.

3. Forced swimming test: The forced swim test apparatus comprised of a glass tank having height of 56 cm and width of 30cm, which contained water at the height of 22 cmand temperature of 25 º C. In this glass tank animals were individually forced to swim for 6 minutes. The height of water was selected so that animal was prevented from touching the bottom of the glass tank and also to prevent its escape from the glass tank. The FST test is commonly used as standard pharmacological model for evaluating depression like symptoms in rats (Porsolt *et al*., 1978).

When the rats are placed in an inescapable chamber which is filled with water then the development of the state of immobility reflects the cessation of persistent escape directed behavior. In the test session animal's swimming behavior was monitored which can be defined as movement throughout the swim chamber (glass tank). The immobility time was monitored. The animal is considered immobile when it makes no further attempts to escape and only tries to keep its head above the water.

4. Water maze test: The effects on spatial memory were examined by determining the performance of rats in a water maze (WM) apparatus. Dimensions of the WM are same as described by them. The WM apparatus used in this study consisted of a rectangular tank (90 cm x 60 cm)that is made up of transparent glass. Tank was filled with room temperature clean tap water; to the depth of 18 cm. Powdered milk was added in the water of the tank so that the platform cannot be seen and this wooden platform (16 cm x 16 cm) was hidden 2 cm below the surface of water in a fixed location. First the rats were trained for a cut off time of 2 minutes and in this session each rat was introduced in the water filled tank facing the wall of tank and they were given 3minutes so that they can locate and climb on the platform. The rat was allowed to stay on the platform for few seconds. If they failed in finding the platform in the training session, it was gently guided onto the platform. Learning acquisition (LA) was determined immediately after training and STM and LTM of rats were tested 1 h and 24 h respectively after training. LA was monitored by recording the initial latency (IL) and STM and LTM were determined by recording the retention latency (RL). IL is the time taken by each rat to relocate the hidden platform immediately after training whereas RL is the time taken by each rat to locate the hidden platform 1h and 24 h after training. The cut off time for each session was 3 minutes.

Biochemical Estimation: Biochemical estimation is done by standard procedure.

Statistical Analysis: The results are presented as mean ± SD for n=6 animals in each group. The statistical significant differences were evaluated by Student's t test and values of $p<0.01$ were taken as significant differences.

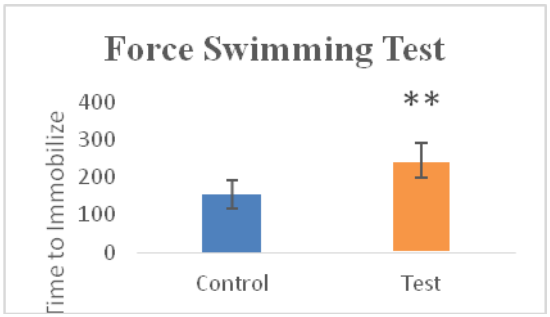

Fig. 1. Effect of Flax Seed administration (200mg/kg) on deression like symptoms in rats. Values are means ± SD (n=12). Significant difference by student's t-test: *P<0.01 compared with controls rats.

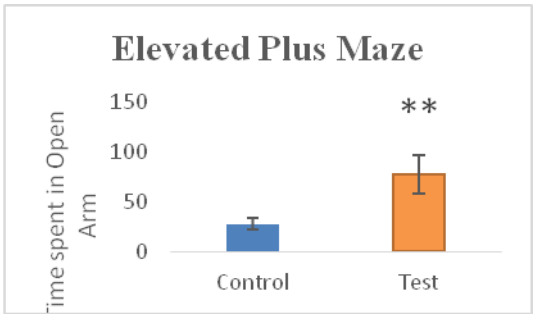

Fig. 2. Effect of Flax Seed administration (200mg/kg) on anxiety in rats. Values are means ± SD (n=12). Significant difference by student's t-test: *P<0.01 compared with controls rats.

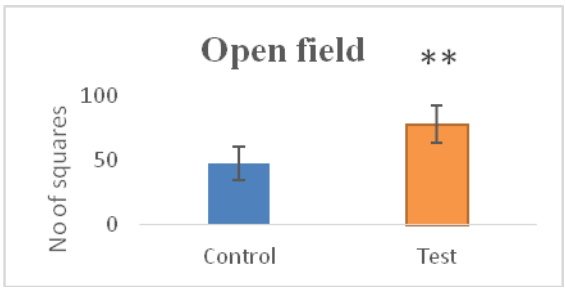

Fig. 3. Effect of Flax Seed administration (200mg/kg) on locomoter activity in rats. Values are means ± SD (n=12). Significant difference by student's t-test: *P<0.01 compared with controls rats.

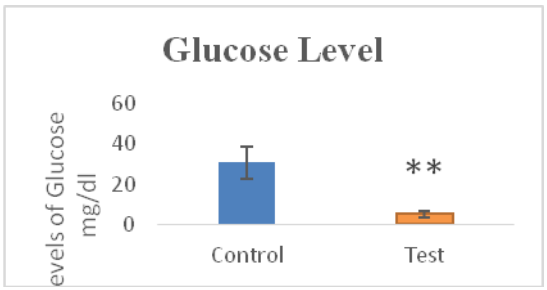

Fig. 4. Effect of Flax Seed administration (200mg/kg) on plasma glucose levels in rats. Values are means ± SD (n=12). Significant difference by student's t-test: *P<0.01 compared with controls rats.

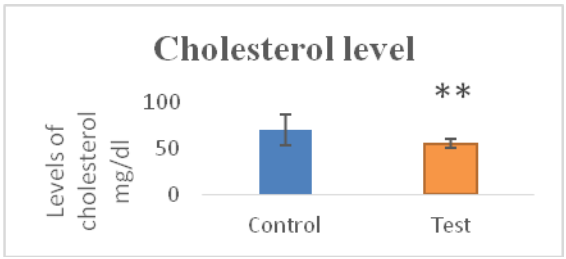

Fig. 5. Effect of Flax Seed administration (200mg/kg) on plasma Cholesterol levels in rats. Values are means ± SD (n=12). Significant difference by student's t-test: *P<0.01 compared with controls rats.

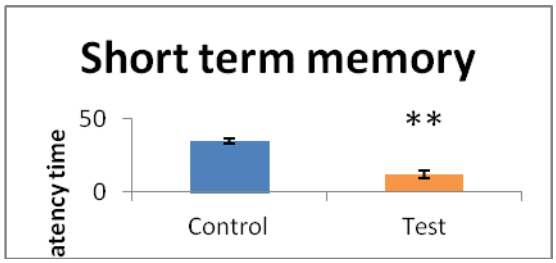

Fig. 6. Effect of Flax Seed administration (200mg/kg) on STM in rats. Values are means ± SD (n=12). Significant difference by student's t-test: *P<0.01 compared with controls rats.

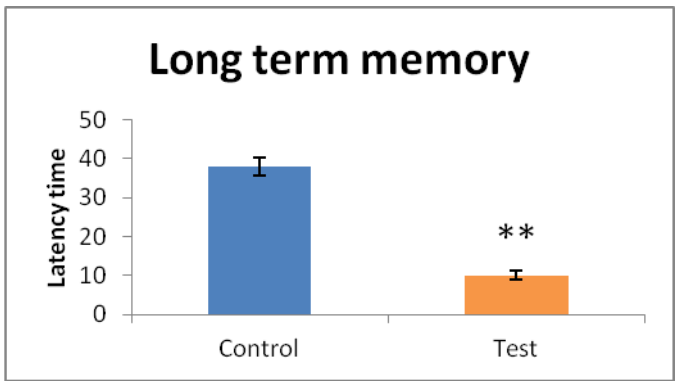

Fig. 7. Effect of Flax Seed administration (200mg/kg) on LTM in rats. Values are means ± SD (n=12). Significant difference by student's t-test: *P<0.01 compared with controls rats.

Results

Figure 1 shows the effect of flaxseeds (*Linum usitatissimum*) at doses of 200mg/kg/mL on depression in rats using forced swimming test. Data analyzed by student's t-test exhibited significant treatment effect of flaxseeds ($t=3.6920$, df: 10, $p<0.01$) on depression like symptoms in rats. The results indicate that immobility time of flaxseeds treated rats were significantly decreased ($p<0.01$) as compared to control rats.

Figure 2 Shows the effect of long term oral flaxseeds administration at doses of 200mg/kg/mL on anxiety in rats using plus maze apparatus. Data analyzed by student's t-test revealed a significant treatment effect of flaxseeds administration ($t= 6.1143$, df: 10, $p<0.01$) on anxiety. The results indicate that flaxseeds treated rats exhibited significantly decrease ($p<0.01$) in anxiety like symptoms as compare to control rats.

Figure 3 shows the effect of long term flaxseeds administration at doses of 200mg/kg/mL on locomotors activity in rats using open field apparatus. Data analyzed by student's t-test revealed a significant treatment effect of flaxseeds administration ($t=3.7874$, df: 10, $p<0.01$) on locomotors activity.

Figure 4 shows the effect of long term flaxseeds administration at doses 200mg/kg/mL on glucose level in rats. Data analyzed by student's t-test revealed significant treatment effect of flaxseeds administration ($t=3.2238$, df: 10, $p< 0.01$) on glucose level. The result indicate that flaxseeds treated rats exhibited significant decrease in ($p<0.01$) glucose level as compare to control rats.

Figure 5 shows the effect of long term flaxseeds administration at doses 200mg/kg/mL on cholesterol level in rats. Data analyzed by student's t-test revealed significant treatment effect of flaxseeds administration ($t=7.8200$, df: 10, $p<0.01$) on cholesterol level.

Figure 6 shows the effect of long term flaxseeds administration at doses 200mg/kg/mL on Short term memory using water maze apparatus in rats. Data analyzed by student's t-test revealed significant treatment effect of flaxseeds administration ($t=18.982$, df: 10, $p<0.01$) on cholesterol level.

Figure 7 shows the effect of long term flaxseeds administration at doses 200mg/kg/mL on Long term memory water maze appara in rats. Data analyzed by student's t-test revealed significant treatment effect of flaxseeds administration ($t=26.9015$, df: 10, $p<0.01$) on cholesterol level.

Discussion

Natural products and their derivatives have historically been a source of pharmaceutical leads and therapeutic drugs. It is already documented that several natural products contain such components which are biologically active and also have some protective action. Flaxseeds is considered to be an essential functional food ingredient as it contain high amount of alpha linolenic acid, lignins and fibers (Martinchik *et al*.,2012). Previously it was reported that consumption of flax seeds protect against prostate cancer, breast cancer, colon cancer and it reduced risk of heart disease and diabetes (Steven and Ehlrich, 2013). Present study was therefore designed to investigate pharmacological potential of selected brown flaxseeds in rats**.** In this study, oral administration of *Linum usitatissimum* at doses of 200mg/kg/mL for 28 days significantly decreased depression like symptoms, decreased anxiety, improve memory and reduced plasma glucose and cholesterol level.

Flaxseeds contain good quality protein and plenty of dietary fiber together with lignans as a phytohormone and omega-3 fatty acid such as α-linolenic acid. Moreover flexseed is also considered to be a good source of phenolic compounds and thus has role in reduction of certain diseases such as diabetes mellitus, cancer and atherosclerosis. In the present study, decrease in depression like symptoms were observed following flax seeds administration in rat. Flaxseeds are rich in EPA and DHA that may be involved in decreased depression like symptoms observed in flax treated rats. Previously it was reported that alteration in monoamines levels following flaxseeds consumption contributed to its neuroprotective effects. Role of monoamines and serotonin in the reduction of depression is well established (Heninger *et al*; 1996). Present study suggest increased monoamines levels following flaxseeds administration play an important role in decreasing depression like symptoms.

For the assessment of memory functions we used water maze test. In water maze test we observed STM after 60minutes of training session and LTM after 24hours. Previous studies showed that hippocampal docosahexaenoic acid content correlated with better spatial memory performance whereas arachidonic acid content correlated with longer time in solving the task. By the water maze test short term memory and long term memory was assessed. Results showed that both STM and LTM was found to be increased in flaxseeds treated rats as the latencies to find the hidden platform was decreased as compared to control rats.

Previously, it was reported that omega-3 and lignan complex isolated from flaxseed reduced the extent of hypercholesterolemia atherosclerosis and this effect was associated with marked decreases in oxidative stress, serum total cholesterol, LDL-C and risk ratio, and elevation of serum HDL-C (Prasad, 2005). Present study also reported decrease in cholesterol levels following oral administration of flaxseeds for 28 days. Flaxseeds contain a limited amount of carbohydrate and soluble fiber and other components of flaxseed fractions could potentially affect insulin secretion and its mechanisms of action in maintaining plasma glucose homeostasis (Cunnane *et*

al., 1993; Jenkins *et al*., 1999). In the present study significant decrease in blood glucose levels were observed following administration of flaxseeds. It is therefore suggested in the present study that flaxseeds could be used as drug for the treatment of anxiety, diabetes, atherosclerosis, memory disorders and depression.

The present study concluded beneficial effects of flaxseeds on memory, depression, anxiety and diabetes and suggests that these effects may be produced due to alteration in monoamines levels. Further studies are needed to investigate the exact neurochemical mechanism following flaxseeds administration.

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