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RESEARCH ARTICLE

POTENTIAL OF FLAXSEED IN THE REDUCTION OF LDL IN HYPERCHOLESTEROLEMIC RATS

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ABSTRACT

Effect of flaxseeds supplementation on serum lipid profile of *hypercholesteromia* induced animals was studied. Flaxseeds were processed (roasted), grinded and administered at dose of 250grams and 500 grams as low dose and high dose. Administration of roasted Flaxseed powder of 250 gm (low dose) and 500 grams (high dose) for 42 days produces significant (P<0.001) reduction of serum LDL-cholesterol and in body weight reduction in *hyperlipidemia* induced rats. The present study confirmed that roasted Flaxseeds powder incorporated would be considered as effective agent for lipid lowering purposes.

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INTRODUCTION

Hypercholesterolemia and Hyperlipidemia is one of the major risk factor for the development of cardiovascular diseases (Makni *et al.,* 2008). Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) Cholesterol and triglycerides are the major lipids in the blood. People with diabetes and impaired glucose tolerance (IGT) are at a risk of having too much LDL cholesterol in their blood, putting them at a high risk of developing heart disease and circulation problems. Increase in levels of LDL- cholesterol and Triglycerides are usually treated with a combination of healthy eating and increasing physical activity. The doctor prescribes medication if high level persists (International Diabetes Institute, 2006). Increased blood cholesterol level is considered to be a risk factor for coronary heart disease. Dietary and pharmacologic reduction in total and LDL cholesterol decreases the risk of coronary diseases and dietary intervention is the desirable approach. Many investigators have reported that soluble dietary fibre has the ability to lower plasma cholesterol concentration in experimental animals (Cho *et al.,* 2007; Rideout *et al.,* 2008). The Food and Drug Administration (FDA) announced a qualified health claim stating a connection between omega-3 fatty acid and a reduced risk of coronary heart disease based on sufficient scientific studies documenting a positive correlation to coronary and cardiovascular health (Meyer *et al.,* 2003). An increased serum LDL levels have been implicated as important risk factors for the development of coronary artery disease. Increased serum LDL cholesterol is highly related with the incidence of coronary heart disease (CHD). Similarly, there is an inverse association between HDL cholesterol and CHD. Intervention with a variety of drugs such as statins, fibrates, bile acid resins, and niacin will decrease LDL cholesterol and subsequent CHD risk. The National Cholesterol Education Program (NCEP) recommends that all adults over the age of 20 be screened for CHD risk with measurement of total cholesterol (TC), LDL cholesterol, HDL cholesterol, and triglyceride (TG) concentrations, while treatment decisions are largely based on LDL cholesterol concentrations (Grundy *et al.,* 2004). Flaxseeds are the best source of various bioactive components such as lignans, nutrients including protein, soluble and insoluble dietary fiber as well as omega 3 fatty acids (Hussain, 2004). Hence, the present study was undertaken to process and incorporated the roasted flaxseeds powder in diet of rats to the hyperlipidemic rats to assess its therapeutic activity.

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MATERIALS AND METHODS

Collection of raw materials

Flaxseed used was the small brown genus *linum* flax and was purchased from Local market of Lucknow City.

Primary treatment of raw materials

The cleaning of flaxseed was performed manually to remove damaged seeds, dust particles, seeds of other grains/crops and other impurities such as metals and weeds. The flaxseed grains were roasted in the household microwave oven for 2.5 minutes with 480 W output under the operating frequency of 2450 MHz.

Animals and experimental design

Animals and Maintenance: Male albino rats were selected for the study. They were of the same age and weight (180 – 200 gm).The rats were housed in polycarbonated clean cages under a 12 /12 h normal light/dark cycle. The animals were fed with standard diet and water *ad libitum*. After keeping in the laboratory condition for a week for acclimatization the experiment was initiated. The study protocol was approved by Institutional Ethical Committee, Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Registration no. 1802/GO/ Re/S/15/CPCSEA of Faculty of Zoology, Banaras Hindu University, Varanasi.

Preparation of High fat Diet for Inducing Hypercholesterolemia

For the preparation of high fat diet 5 raw eggs were boiled and 30 grams of egg yolk was separated and mixed with 75gm of wheat flour. 30 gram of butter was added with the egg yolk and wheat flour mixture and water was added to the mixture to make pellets and dried in laboratory oven at 40°C for overnight. The food prepared was kept in the refrigerator below 20°C to prevent spoilage.

Induction of Hypercholesterolemia in Rats

The high fat diet prepared was given to the rats for the induction of cholesterol in rats. The high fat diet was distributed equally among test groups for a period of 30 days and the rats of each group were sacrificed at 31st day after overnight fasting for confirmation of hypercholesterolemia.

Experimental Design

In this group total 46 rats were taken and 36 rats were fed with the high cholesterol diet and after feeding period of 30 days 6 rats were sacrificed for confirmation of hypercholesterolemia. Further they were divided into following groups-

Group I: The rats were given normal diet and water *ad libitum*.
Group II: The rats were given high fat diet and water *ad libitum*.
Group III: There were 20 rats taken in this group. They were also fed with high cholesterol and fat rich diet and water *ad libitum*. The group was further divided into two subgroups (n=10) in each group as low flaxseed group (given 250 mg of flaxseed/kg body weight/rat/day) and high flaxseed group (given 500 mg of flaxseed/kg body weight/rat/day) for a period of 42 days.

Biochemical analysis

Body weights were recorded biweekly and at the end of the stipulated period, the animals were kept for overnight fasting and sacrificed. The blood was collected from heart. About 2-3 ml of blood sample was collected and centrifuged at 2500 rpm for 25 minutes to separate serum. The serum was stored at -20°C until the analysis. From the collected blood serum, the biochemical marker such as Low Density Lipoprotein (LDL) was determined by using ENZOPAK reagent kit.

Statistical analysis

Statistical analysis was done by SPSS Version 20. Results were expressed as means±SD and the difference between the groups were tested by one-way analysis of variance (ANOVA) and the significance level was calculated using Tukey HSD test. The $p<0.001$ were considered as statistically very highly significant.

RESULTS

Administration of High fat diet results in a significant increase in blood LDL cholesterol level and also increases the body weight.

Table 1. Effect of Flaxseed on body weight in hypercholesterolemic rats

S.No.	Time interval	Negative control (n=10)		Positive control (n=10)		Low dose (n=10)		High dose (n=10)		ANOVA	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	F	'p'
1.	Before	190.00	9.97	188.00	10.52	188.50	8.11	188.80	8.57	0.083	0.969
2.	14th day	221.30	6.90	239.40	10.67	240.00	7.89	240.00	7.89	11.985	<0.001
3.	28th day	236.00	8.27	255.50	9.94	219.80	9.64	188.70	9.07	93.344	<0.001
4.	42nd day	251.70	7.69	272.00	11.16	202.40	8.41	140.20	8.07	431.71	<0.001

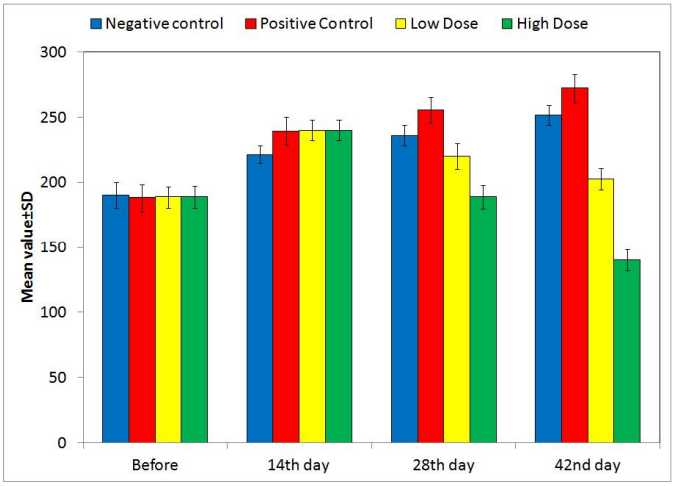
Table 2. Effect of Flaxseed on LDL Cholesterol in hypercholesterolemic rats

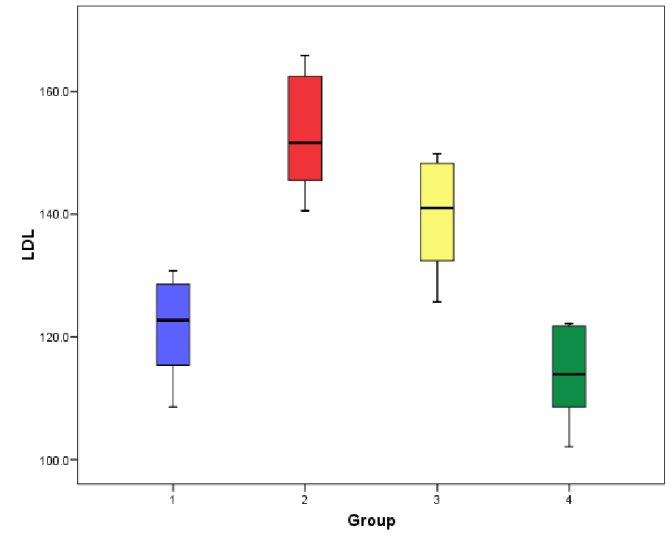
S.No.	Comparison	Mean Difference	SE	'p'
1.	Negative Control vs Positive Control	-31.82	3.64	<0.001
2.	Negative Control vs Low dose	-18.74	3.64	<0.001
3.	Negative Control vs high dose	6.81	3.64	0.259
4.	Positive Control vs Low dose	13.08	3.64	0.005
5.	Positive Control vs high dose	38.63	3.64	<0.001
6.	Low dose vs high dose	25.55	3.64	<0.001

p<0.001 represents Very Highly Significant

However, after the treatment of hyperlipidemic rats with 250mg/kg b.w and 500mg/kg b.w of flaxseed for 42 days, the blood LDL Cholesterol level significantly decreases and body

weight also reduced compared with the levels of untreated diabetic rats as shown in Table 1 & Table 2. Administration of flaxseed results in the reduction of LDL cholesterol as well as body weight.





Box plot showing dispersion of LDL levels in different study groups

DISCUSSION

Before intervention mean body weight ranged from 188.00±10.52 g (Positive control) to 190.00±9.97 g (Negative control), however, the difference among groups was not significant statistically (p=0.969). At 14th day after intervention, mean body weight among different groups ranged from 221.30±6.90 g (Negative control) to 240.00±7.89 g (high and low dose groups). Mean body weight of positive control rats was 239.40±10.67 g. Statistically, intergroup difference was significant (p<0.001). At 28th day after intervention, mean body weight among different groups ranged from 188.70±9.07 g (high dose) to 55.50±9.94 g (Positive control). Mean body weight of negative control and low dose rats was 236.00±8.27 and 219.80±9.64 g respectively. Statistically, intergroup difference was significant (p<0.001). At 42nd day after intervention, mean body weight among different groups ranged from 140.20±8.07 g (high dose) to 272.00±11.16 g (Positive control). Mean body weight of negative control and low dose rats was 251.70±7.69 and 202.40±8.41 g respectively. Statistically, intergroup difference was significant (p<0.001).

On 14th day after intervention, negative control group had significantly lower mean value as compared to all the other groups. The order of body weight at this interval was as follows:

Negative Control > High dose ~ Low Dose ~ Positive Control

On 28th day after intervention, all the between group differences were significant. The order of body weight at this interval was as follows:

Positive Control > Negative control > Low Dose > High dose

On between group comparison of LDL levels mean difference was found to be maximum between Positive controls and High dose and minimum between Negative control and high dose groups. All the between group differences except that between negative controls and high dose groups were significant statistically (p<0.001). On the basis of above evaluation, the following order of blood LDL levels were observed:

Positive Control > Low Dose > High Dose ~ Negative Control

Conclusion

The supplementation of *Flaxseed* significantly controlled the hyperlipidemic condition including the body weight reduction. In the present study, roasted flaxseed powder incorporated in rats diet were significantly reduced serum LDL-cholesterol level.

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