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EFFICACY OF FLAXSEED IN THE REDUCTION OF BLOOD CHOLESTEROL IN HYPERCHOLESTEROLEMIA RATS

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ABSTRACT

From time immemorial traditional medicines have been utilized by human society for improving the health care. In this study, anti hyperlipidemic effect of flaxseed was studied. The present study was conducted on white albino rats. The rats were fed with high fat diet for the induction of hypercholesterolemia for 30 days. The hypercholesterolemic rats were administered with roasted Flaxseed powder of 250 gm (low dose) and 500 grams (high dose). Supplementation of flaxseed for 42 days produces significant reduction of serum Total-cholesterol and in body weight reduction in hypercholesterolemia induced rats. Flaxseeds were administered at dose of 250grams and 500 grams as low dose and high dose. After treatment of flaxseed for 42 days it is confirmed that Flaxseed powder incorporated in the diet is considered as effective agent for reduction of cholesterol.

INTRODUCTION

Cardiovascular diseases are the major contributor to the global burden of disease among the noncommunicable diseases. The major risk factors responsible for cardiovascular diseases are hypertension, hypercholesterolemia, diabetics and obesity (Kearney *et al.,* 2005). Increase in lipid level contributes towards the occurence of atherosclerosis and coronary heart diseases (CHD). Lipid lowering drugs, mostly statins and fibric acid derivatives have been widely used to manage the elevated levels of various forms of lipids in hyperlipidemia patients. Due to its serious complications, these drugs have to be used safely or avoided when possible (Muscari, 2002). In recent years, the development of lipid lowering drug or formulation from natural source has gained importance. Increased blood cholesterol level is considered to be a risk factor for coronary heart disease. Dietary and pharmacologic reduction in total cholesterol decreases the risk of coronary diseases and dietary intervention is the desirable approach.

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Many investigators have reported that soluble dietary fibre has the ability to lower plasma cholesterol concentration in experimental animals (Cho *et al.,* 2007). 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 (Grundy, 2004). An increased serum cholesterol levels have been implicated as important risk factors for the development of cardiovascular disease. Flaxseed consumption in various forms as a food ingredient and for its medical properties dates from 5000 BC since its cultivation (Oomah, 1998). It is therefore not surprising that flaxseed is the most prominent oilseed studied to date as a functional food, since it is a leading source of omega-3 fatty acid, α-linoleic acid (ALA) [52% of the total fatty acid] and of phenolic compounds known as lignans (>500µg/g). (Oomah, 2000) Flaxseeds are the best source of various nutrients including protein, soluble and insoluble dietary fiber as well as omega 3 fatty acids (Hussain *et al.,* 2004). Hence, the present study was undertaken to process and incorporate the roasted flaxseeds powder in diet of rats to the hypercholesterolemic rats to assess its therapeutic activity.

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 total cholesterol 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 cholesterol level and also increases the body weight. However, after the treatment of hyperlipidemic rats with 250mg/kg b.w and 500mg/kg b.w of flaxseed for 42 days, the blood 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 cholesterol as well as body weight.

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

SN	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

$p<0.001$ represents Very Highly Significant

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).

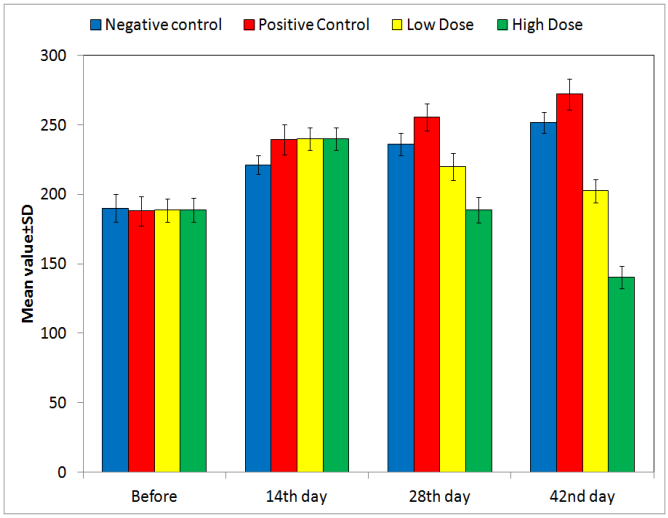

Fig.1. Intergroup comparison of body weight in different groups for rats included in Experiment

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

SN	Comparison	Mean Difference	SE	'p'
1.	Negative Control vs Positive Control	-131.37	6.33	<0.001
2.	Negative Control vs Low dose	-85.11	6.33	<0.001
3.	Negative Control vs high dose	-59.74	6.33	<0.001
4.	Positive Control vs Low dose	46.26	6.33	<0.001
5.	Positive Control vs high dose	71.63	6.33	<0.001
6.	Low dose vs high dose	25.37	6.33	0.002

p<0.001 represents Very Highly Significant

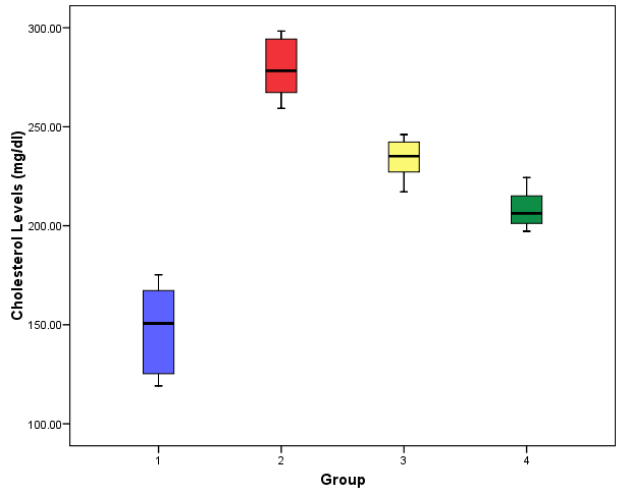

Fig. 2. Box plot showing dispersion of cholesterol levels in different study groups

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

Positive Control > Low Dose > High Dose > Negative Control

Conclusion

The supplementation of *Flaxseed* significantly controlled the hypercholesterolemia condition including the body weight reduction. In the present study, roasted flaxseed powder incorporated in rats diet were significantly reduced serum Total-cholesterol level. The administration of flaxseed revealed maximum protective effect at a dose of 500 mg/kg in comparison with 250 mg/kg body weight. The administration of flaxseed to the hypercholesterolemic rats significantly reduced total cholesterol.

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