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Anti-atherogenic effect of flaxseed (*Linum usitatissimum*) administration with a high cholesterol diet and its effect on hyperlipidemia in female Wistar rats.

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Abstract

Background: Cardiovascular disease (CVD) persists to be the world's leading reason for mortality and morbidity. The major type of CVD is coronary artery disease (CAD). CAD is the obstruction of coronary arteries frequently instigated by arteriosclerosis (plaque buildup in the coronary artery, which hampers blood flow to the myocardial muscles). This research was conducted to examine the effects of oral intake of flaxseed (*Linum usitatissimum*) powder on hyperlipidemia and atherosclerosis (induced through high-fat diet) in female Wistar rats. The pharmacological effects of flaxseed are diverse. It has an antiarrhythmic effect, anti-arthritic effect, antilipidemic, atherosclerosis, anti-inflammatory, antioxidant activity effect on the body.

Methodology: A total of 32 female Wistar rats (200 g) were divided equally into four groups. Group I (n=8) rats kept as control and given normal rat chow diet, Group II rats kept as positive control for induction of hypercholesterolemia and atherosclerosis by addition of saturated edible fat to the standard diet (high cholesterol diet - HCD), Group III rats fed with flaxseed powder at 7.5 g/kg of rat/day in the standard rat chow diet and kept as flaxseed control, and Group IV rats supplemented with flaxseed at 7.5 g/kg of rat/day along with HCD and maintained for 15 days.

Results: Group II rats showed higher lipid profile and glucose level. Flaxseed supplementation, along with a high cholesterol diet, drastically restored the serum levels of triglyceride (TG), total cholesterol (TC) decreasing frequency of CAD. Histologic investigations showed preserved myocardial walls with minor changes in flaxseed treated rats, whereas increased lymphoid aggregation with inflammation in high fat + flaxseed treated.

Conclusion: The biochemical assays and histological results showed the moderate cardio-protective activity of flaxseed administration for 15 days along with a high-fat diet was found to moderately reduce the CVD risk in rats.

Keywords

Linum Usitatissimum, Hyperlipidemia, Coronary Artery Disease, Arteriosclerosis.

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Introduction

Coronary artery disease is a disorder of coronary arteries which is chronic and multifactorial, develop silently, with no symptoms till the plaque restricts the blood flow[1]. World Health Organization (WHO) estimated 16.7 million deaths are caused annually worldwide due to cardiovascular disease[2]. According to research, in Pakistan, CVD is the largest non-communicable killer[1].

Linum usitatissimum, commonly known as the flaxseed, is known as one of the oldest crops cultivated. In *Ayurveda* and traditional Chinese medicine, flaxseeds are used to remedy cough, skin, and gastrointestinal disorders[3]. The herb possesses anti-cancerous, antilipidemic, atherosclerosis, anti-inflammatory, antioxidant properties[4]. Active constituents that flaxseed endures, omega-3-fatty acid (ω-3 FA); alpha-linolenic acid (ALA), soluble and insoluble fibers, phytoestrogenic lignans (secoisolariciresinol diglycoside-SDG)[4-6] proteins and a range of antioxidants, oleic acid and linoleic acid[4,5,7]. Flaxseed SDG is efficient in lowering hypercholesterolemic atherosclerosis by reducing oxidative stress and reducing serum levels of high-density lipoprotein cholesterol (HDL-C) in the early phase[8].

Eicosanoids originated from ω-3-F.A, in flaxseed principally progresses heart function by decreasing cholesterol level[9,10]. Due to the wide use of herbal medicine and its therapeutic effects, there is growing attention and a need to assess the mechanisms of action of herbal products carefully.

This rising understanding of herbal products attracts attention to holding fewer side effects accompanied by multiple medicinal properties. Therefore, this research intended to scientifically demonstrate the cardioprotective nature of orally administered flaxseed powder against hyperlipidemic induced arteriosclerosis and coronary artery disease in female Wistar rats.

Methodology

Plant Material

Linum usitatissimum Flaxseed were bought from the local marketplace of Karachi, Pakistan. The seeds were rinsed and dried overnight. The dried seed was crushed into powder form with the help of an electronic crusher. The grind was kept in a clean, airtight container and kept at room temperature.

Animals Housing

Female Wistar rat (Rattus norvegicus) of 15-20 weeks old and with a mean bodyweight of 150-200 g were bought from the International Center for Chemical and Biological Sciences, University of Karachi. Rats were acclimatized for 1 week inside the animal house of the Department of Physiology (University of Karachi) before the research was conducted. Rats were kept in a well ventilated and temperature-controlled room 25-28°C and 14/10 h light/dark cycles, with an unlimited diet approach (rat chow diet) and water in their cages. Body weights and other physical conditions were carefully examined throughout the study.

Ethical Guideline

This study was conducted according to the standards issued by the National Institute of Health.

Study Design

32 rats were distributed into four experimental groups as follows:

Group-I: Rats received a normal rat chow diet throughout the experimental period.

Group-II: Received high-fat diet (saturated fat/100 g of daily diet) for 15 days.

Group-III: treated with flaxseed powder 7.5 g/kg of rat/day in the rat chow diet for 15 days.

Group-IV: Received flaxseed powder 7.5 g/kg per day with a high-fat diet for 15 days.

On the 16th day, all animals were sacrificed. The cardiac piercing technique was utilized, and heparin-coated tubes were used for acquiring plasma for analysis; serum was also set apart. The heart was removed and made free of connective tissues and blood, followed by desiccation and

weighing. Lastly, the tissue was stored in a freezer at -70 ºC.

Biochemical Analysis

Plasma TC, TG, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) was estimated using Global chemical reagent package.

Histopathological

Heart tissue was fixed and submerged using formalin and then turned in paraffin block, respectively. Sections of thickness four µm were stained with Hematoxylin/Eosin stain and examined under the microscope. Histopathological changes in tissue sections were ranked with a scale from 0 to 4 (Zero means no distinguishable, 1-slight localized impairment, 2-mild localized impairment, 3-moderate localized impairment and 4-sever according to the seriousness of structural alternations.

Statistical Analysis

The results are shown as mean ± SEM (Standard error mean). All four groups were analyzed for statistically significant variations using one-way ANOVA, and significance was known at $p<0.05$, $p<0.01$ & $p<0.005$. Tukey's HSD test and Dunnett's test were utilized to assess tested groups' results with controls and to minimalize the occurrence of type I error.

Results

The mean body weight of group I (control group) of Wistar rats on a normal rat chow diet was 129.33± 8.491 g. Group II fed with a high-fat diet for 15 days has non-significant ($p<0.05$; $p=0.00$) average weight compared to Group I because the animals gain weight and, hence, the average body weight of Group II was 212.16±5.51 g.

Group III was fed with flaxseed powder of 7.5 g/kg of rat/day in the standard rat chow diet and kept as flaxseed control has average body weight 229.25±16.13 g. In comparison, the bodyweight of Group IV was 197±3.32 g, which was significant ($p>0.05$, $p=0.08$) when compared to group III.

Table 1: Comparison of Body Weight, in Control (Group I), Hyperlipidemic (Group II), flaxseed treated (Group III) and flaxseed + hyperlipidemic diet (Group IV).

Parameter	Group I	Group II	Group III	Group IV
Body weight (g)	129.33±8.49	212.16±5.51***	229.25±16.13$^{NS/***}$	197±3.32 $^{*/***}$

Numerical values are presented as mean ± SD. The significant difference amongst various groups are figured out through t-test, *p<0.05, **p<0.01, ***p<0.005, NS non-significant, contrasted with hyperlipidemic group/ with control.

In the lipid profile of group II that was on a high cholesterol diet; a non-significant ($p>0.05$) increase in TG, TC, LDL and VLDL were observed, whereas a highly significant ($p<0.01$) increase in HDL seen as compared to control group I. In group III all the parameters show non-significant ($P>0.05$) increase in values when compared to group I; TG ($p>0.05$), TC ($p>0.05$), HDL ($p>0.05$), VLDL ($p>0.05$) and LDL ($p>0.05$). In group IV, except for HDL, all the values show a decline when evaluated with group II results; HDL shows a non-significant increase ($p<0.05$) TC, LDL decrease non significantly ($p>0.05$), whereas TG and VLDL showed a very significant decrease ($p<0.01$).

Table 2: Comparison of lipid profile in all groups

Parameter	Group I	Group II	Group III	Group IV
TC (mg/dl)	100.43±3.58	161.43±27.12^{NS}	130.92±16.86 $^{NS/NS}$	143.65±19.69$^{NS/NS}$
TG (mg/dl)	88.61±3.41	131.05±17.99^{NS}	87.04±6.38$^{*/NS}$	71.693±8.08$^{**/NS}$
HDL (mg/dl)	33.22±0.94	24.45±1.16***	34.33±4.80$^{NS/NS}$	27.136±3.36$^{NS/NS}$
VLDL (mg/dl)	17.72±0.68	26.20±3.59^{NS}	17.41±1.27$^{NS/*}$	14.339±1.61$^{**/NS}$

LDL (mg/dl)	49.48±2.27	110.77±27.05^{NS}	79.17±18.35$^{NS/NS}$	102.18±20.66$^{NS/*}$

Values are represented as Mean± SEM. TC: Total Cholesterol, TG: Triglyceride, HDL: High-Density Lipoprotein, VLDL: Very Low-Density Lipoprotein, LDL: Low-Density Lipoprotein. The significant difference amongst various groups are figured out through t-test. *p<0.05, **p<0.01, ***p<0.005, NS-Non-Significant, contrasted with hyperlipidemic group/with control.

Histological findings in the current study are according to the biochemical findings. Scoring of the heart's morphological conclusions are explained and reviewed in Control, Hyperlipidemic, Flaxseed treated and Flaxseed + high fat treated rats (Table 3). Any altered cardiomyocytic histological changes were not present in the control group's heart tissues (Figure 1).

Group III (Flaxseed treated) hear tissue sections from group III represent increased inflammation and lymphocytic aggregation in myocardial walls. Rats who received only flaxseed with rat chew diet displayed preserved cardiac wall histology (Figure 3). Group IV (Flaxseed + high-fat diet) this tissue sections from group IV displayed increased lymphoid aggregation with inflammation. Flaxseed controls these changes in this group (Figure 4).

Table 3: Histopathological features in Control, Hyperlipidemic, Flaxseed treated and Flaxseed + hyperlipidemic diet.

Organ appearance	Control	Hyperlipidemic	Flaxseed + Hyperlipidemic	Flaxseed Treated
Color	Brownish Red	Brownish Red	Brownish Red	Brownish Red
Texture	Smooth	Smooth	Smooth	Smooth
Inflammation	-	+4	+2	+1
Lymphoid aggregation	-	+2	+1	-
Foam cells	-	-	-	-

Scale: 0: no distinguishable, +1: slight localized impairment, +2: mild localized impairment, +3: moderate localized impairment and +4: severe localized impairment.

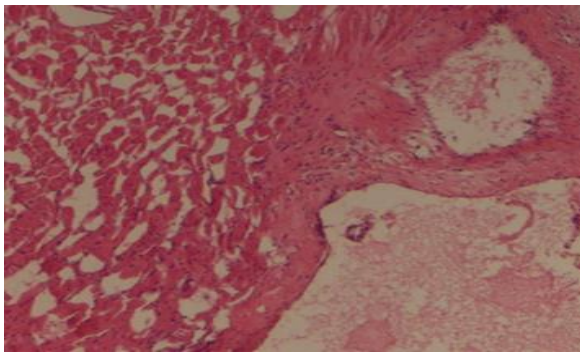

Figure 1: Histopathological features of Control rats.

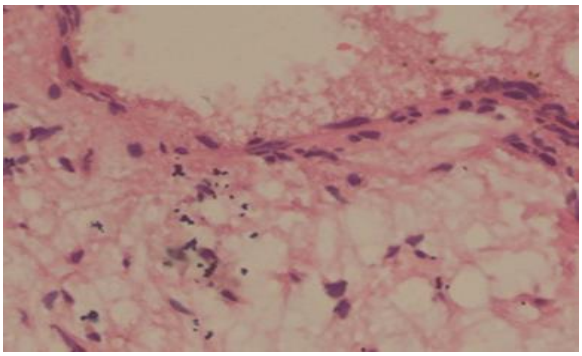

Figure 2: Histopathological features of Hyperlipidemic rats.

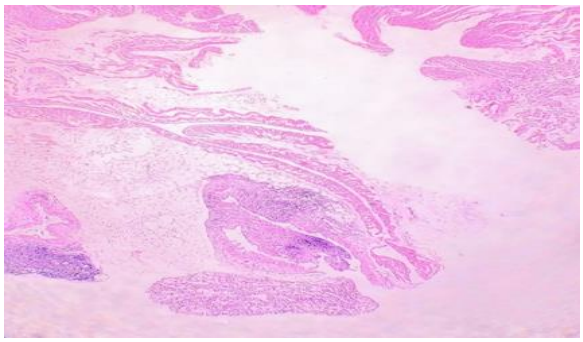

Figure 3: Histopathological features of flaxseed treated rat.

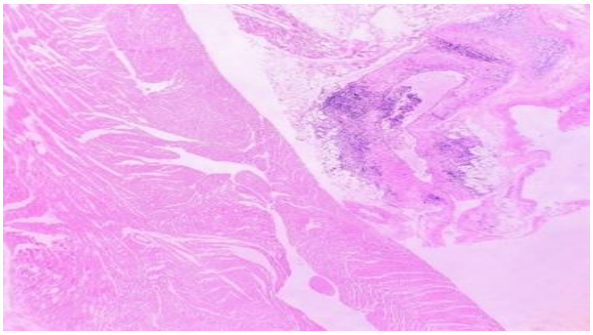

Figure 4: Histopathological features of flaxseed + high fat diet treated rats.

Discussion

Functional foods are considered superior in treating numerous chronic diseases, primarily due to their decreased side effects. *Linum Usitatissimum* is a rich source of oil, protein and dietary fiber in addition to an excellent resource of the phytochemical antioxidant, lignin[6]. Flaxseed is rich in Alpha-linolenic acid and linoleic acid[6].

Throughout the experimental phase, no atypical clinical symptoms were witnessed in Groups II and IV animals during the research period. Overweight and lethargy were seen in Group II, fed with a high-fat diet. Mild obesity was detected in Group IV, in which flaxseed powder was administered with a high-fat diet. Research denoted that an HFD raised the levels of serum cholesterol, which is coherent with former findings[11,12].

In this research, we observed that *Linum Usitatissimum* reduce triglyceride more effectively than total cholesterol. Hokanson and Austin reported that raised plasma TG levels were related to increased frequency of coronary artery diseases[13]. Elevated triglyceride levels attribute the deposition of small dense LDL that initiates atherosclerosis in group II[14]. Therefore, a drop of elevated plasma TG by *Linum Usitatissimum* seems to amplify the breakdown of TG in group III and group IV. In the current analysis, *Linum Usitatissimum* treatment increased serum High-Density Lipoprotein, a positive antihyperlipidemic effect of the herb[15]. It is stated that there is an independent relationship between HDL-C levels in blood and CVD risk. Malloy and Kan have stated that HDL levels have an independent relation with and cardiovascular disease risk[16]. HDL is characterized as good cholesterol since it aids the mobilization of TG and TC from plasma to hepatocytes, place it is catabolized then eliminated in the form of bile acids[16].

LDL and Atherogenic index of plasma (AIP) are factors to access the possibility of atherosclerosis[6]. The increase of the LDL Group II hyperlipidemia model of rats denoted the increased risk of CAD. Flaxseed lowers these parameters, therefore decrease the chances of CAD. Results indicated that flaxseed could reduce serum TG, TC, VLDL when taken with a normal diet and with a high-fat diet. Herb was less helpful in decreasing LDL.

Linum Usitatissimum is a rich source of oil, protein and dietary fiber in addition to an excellent resource of the phytochemical antioxidant, lignin[6]. Rhee et al. (2011) examined the consequence of flaxseed supplementation on overweight glucose-intolerant patients. They established that linseed (Secoisolariciresinol Diglucoside) functional ingredient acquires key antioxidant effects by stopping DNA scissions and lipid peroxidation and reducing reactive oxygen species. Antioxidants are registered in reducing inflammatory reactions, insulin resistance, and diabetes development[17].

Anila and Vijayalakshmi found out that phytochemical examination of linseed displays the existence of flavonoids and phenolics. They are known to be potent antioxidants and control

numerous enzyme systems' actions due to their interaction with several biomolecules. In reality, flavonoids and polyphenols display various pharmacological activities, containing hypolipidemic and anti-atherogenic effect[18]. Therefore, the hypolipidemic action of oral admiration of *Linum Usitatissimum* maybe credits to flavonoid in it along with interdependent action Secoisolariciresinol diglucoside[19].

Conclusion

This study has highlighted the bioactivities of flaxseed administered for 15 days in experimental models of hypercholesterinemia. We hypothesized that whole flaxseeds would recover cardiovascular changes by balancing the level of proatherogenic parameters. Significant changes might be acquired with prolonged administration of such doses of flaxseed. Regular consumption of flaxseed can be used to limit primary cases of coronary artery diseases; however, more detailed experimental studies are suggested for its use in diagnosed patients.

Conflicts of Interest

None.

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