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EFFECT OF FLAXSEED AND PUMPKIN SEEDS MIXTURE ON HYPERCHOLESTEROLEMIA

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Abstract: Flax and pumpkin seeds are rich sources of unsaturated fatty acids, antioxidants and fibers, known to have anti-atherogenic properties. Hypercholesterolemia is a state characterized by the elevated level of cholesterol in the blood. This research was designed to study the effect of flax and pumpkin seeds powder mixture on hypercholesterolemia and body weight. Thirty male albino rats were divided into three groups: a control group, a CD-chol group (control diet+ cholesterol) fed with 1.5% cholesterol and FP-chol group (flaxseed and pumpkin seed powder + cholesterol) fed with 1.5% cholesterol. Flax and pumpkin seed powder mixed at proportion of (5/1) (omega-3 and omega-6). Blood samples were collected to examine lipid profile and body weight was also measured. Thus the date was subjected to analysis of variance. Our results suggested that flax and pumpkin seed mixture has hypocholesterolemic effects which were probably mediated by polyunsaturated fatty acids (omega-3 and omega-6) present in seed mixture.

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INTRODUCTION

Hypercholesterolemia is the presence of high levels of cholesterol in the blood. It is not a disease but a metabolic disorder that can be secondary to many diseases. Hypercholesterolemia is one of the major causes of atherosclerosis. Although there are many causes, hypercholesterolemia is the permissive factor that allows other risk factors to appear. The concentration of plasma cholesterol should be regulated to avoid the risk of cardiovascular diseases (1). It has been noted that diet plays vital role in the regulation of cholesterol homeostasis. Now a days modified diets are used as medicine for hypercholesterolemia which reduces the risk of cardiovascular diseases (2).The search for new drugs capable of reducing or to regulate serum cholesterol and triacylglycerol levesl has attained importance over the years (3).

Recent studies have demonstrated that omega-3 and omega-6 fatty acids are the richest source of alpha linolenic acid which has anti-atherogenic properties by decreasing plasma cholesterol and triglyceride levels. The absorption and metabolism of alpha-linolenic acid (Omega-3) fatty acids are similar to those of linoleic acid (omega-6) fatty acids (4). The balance required in the diet between omega-3 and omega-6 fatty acids is important due to their competitive nature and their different biological roles to ensure the conversion of alpha linolenic acid to Eicosapentaenoic and docosahexaenoic acids (5). Irregular lipid metabolism is a major cause of dyslipidemia which in turn is a risk factor for cardiovascular diseases (6).

Flaxseeds and pumpkin seeds have long been used as a traditional food all over the world. Flaxseed (*linum usitatissimi*) also known as linseed contain 51-55% alpha-linolenic acid. Cardiovascular diseases, renal disorders and hypercholesteremia may be prevented by alpha-linolenic acid in flaxseeds (7). The alpha- linolenic acid which is a (omega-3 fatty acid) is a precursor of Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (8). Pumpkin

seeds of Cucurbitaceae family provide many health benefits (9). They are rich source of polyunsaturated fatty acids and antioxidants such as vitamin E and zinc (10). Pumpkin seeds contain 40.4-55.6% of linoleic acid which is an omega-6 fatty acid (11-12).

Flax and pumpkin seeds are also good sources of fiber. Fiber diet assists to avert or treat hyperlipidemia, gastrointestinal problems, blood pressure, obesity, diabetes, cancers and cardiovascular diseases (13). Vegetarians have very low risk of developing cancer, cardiovascular diseases, gastrointestinal disorders, weight gain and hyperlipidemia than non-vegetarians. Hyperlipidemia occurs due to elevated cholesterol level that leads to atherosclerosis (14). Serum cholesterol concentration is influenced by a number of dietary factors including dietary cholesterol, fat and dietary fibers (15).

Both medicine and diet help in reducing total cholesterol level but diet is considered as fundamental tool in treating hyperlipidemia (16). Moreover, numerous studies have demonstrated that high level of HDL-C are associated with a lower incidence of cardiovascular diseases (17). This study has been designed to suggest that flax and pumpkin seed mixture has significant anti-atherogenic activity when administered to hypercholesterolemic rats (18).

MATERIALS AND METHODS

Area of Research:

The research trial was carried out at National Institute of Food Science and Technology, University of Agriculture Faisalabad.

A. Sample Collection:

The present study was conducted on 30 male rats. Samples were divided into three groups on the basis of diet given to them. Group (1) G1: control rats; was fed normal diet. Group (2) G2: hypercholesterolemic rats (CD-chol) was fed balanced diet as well as diet high in cholesterol at a dose level of 15g/100 g diet; Group (3) G3: hypercholesterolemic diet supplemented with flax/pumpkin seed mixture (FP-chol. Flax and pumpkin seeds were purchased from local market of Faisalabad. Seeds were crushed at ambient temperature and stored at 4°C prior to use. Mixture of flax and pumpkin seeds rich in omega-3 and omega-6 fatty acids prepared. The ratio of flax and pumpkin seeds i.e. (omega-3/ omega-6 fatty acids) were 5/1 as recommended by the WHO and according to Makni 2010 (19). In the diet of rats Cholesterol was given in the form of purified melted butter and cholesterol rich food items.

(Control Group (A) (CDon normal routine feed	Normal routine rat feed 0-30 days
Treated Group (B) CD-(CHOL On high cholesterol diet 15g/100g	Normal routine rat feed + 15g cholesterol/100g, 0-30 days
Treated Group (C) (FP-(CHOL On Flax and pumpkin seeds powder mixture	Normal routine rat feed + 30g powder + 15g cholesterol/100g, 0-30 days

B. Proximate Analysis

Proximate analysis of flaxseed and pumpkin seed powder for protein, ash, moisture, fat and crude fiber, was done by using the method given by AOAC (2006).

C. Sampling

Data of each 30 samples was taken. Blood samples were collected to examine lipid profile. Their body weight was measured before and after trial period.

D. Serum Lipid Profile

The serum lipid profile with special reference to cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides (TG) was carried out at the end of 4 weeks after giving diet. The further detail is described here:-

1. Cholesterol

Cholesterol level of each rat was measured by

HOD-PAP method (20).

2. Low density & high density lipoproteins (LDL & HDL):

Low density lipoprotein (LDL) was measured byfollowing the procedure of McNamara et al. (1990).High density lipoprotein (HDL) was determined by HDL Cholesterol Precipitant method (21).

3. Triglycerides: The triglycerides of each rat were measured by liquid triglycerides (GPO-PAP) method (22).

E. Statistical Analysis:

For the interpretation of results data collected was subjected to statistical analysis using analysis of various techniques with completely randomized design. Means of treatment were compared byDucan's new multiple range test(23).

RESULT AND DISCUSSION

Table1: Proximate composition of flaxseed and pumpkin seed

Proximate analysis	(%) Quantity
Moisture	7.40
Crude fiber	9.34
Crude protein	3.2
Ash	2.67
Crude fat	0.90

Table 2: Mean + SE of body weight (g) before treatment of mixture of flaxseed and pumpkin seeds powder on hypercholesterolemic rats

Treatments	Mean ± SE		Minimum		Maximum	
	Before	After	Before	After	Before	After
Group 1	255.77 ± 15.32	263.85 ± 14.07	191.00	205.70	345.00	352.60
Group 2	263.70 ± 18.01	291.05 ± 15.93	209.00	241.66	375.00	380.00
Group 3	265.73 ± 15.05	240.66 ± 11.35	208.63	168.43	365.98	285.73
Overall Mean	261.73 ± 9.05	265.19 ± 8.64	191.00	168.43	375.00	380.00

Group 1: Control fats; fed on normal diet

Group 2: Hypercholesterolemic rats (CD-chol) fed with the balanced diet as well as diet high in cholesterol at a dose level of 15g/100 g diet

Group 3: Hypercholesterolemic diet supplemented with flax/pumpkin seed mixture (FP-chol)

Table 3: Mean + SE serum Cholestrol (mg/dL) after treatment with mixture of flaxseed and pumpkin seed powder in hypercholesterolemic rats.

Treatments	Means ± SE	Minimum	Maximum
Group 1	57.60 ± 1.77 ^{B}	48.00	68.00
Group 2	73.40 ± 4.21 ^{A}	52.00	97.00
Group 3	59.60 ± 2.20 ^{B}	50.00	71.00
Overall Mean	63.53 ± 2.09	48.00	97.00

AB show significantly different at P<0.01

Table 4: Mean + Serum LDL (mg/dL) after treatment with mixture of flaxseed and pumpkin seeds powder in hypercholesterolemic rats

Treatments	Means ± SE	Minimum	Maximum
Group 1	31.60 ± 1.59 [B]	25.00	43.00
Group 2	48.60 ± 3.26 [A]	32.00	61.00
Group 3	36.20 ± 1.62 [B]	30.00	46.00
Overall Mean	38.80 ± 1.85	25.00	61.00

AB shows significantly different at P<0.01

Table 5: Mean + SE serum HDL (mg/dL) after treatment with mixture of flaxseed and pumpkin seed powder in hypercholesterolemic rats.

Treatment	Mean ± SE	Minimum	Maximum
Group 1	36.30 ± 2.81[A]	20.00	55.00
Group 2	26.40 ± 2.76 [B]	16.00	42.00
Group 3	36.40 ± 2.20 [A]	23.00	45.00
Overall Mean	33.03 ± 1.69	16.00	55.00

AB shows significantly different at P<0.05

Table 6: Mean + SE of serum triglycerides (mg/dL) after treatment with mixture of flaxseed and pumpkin seeds powder in hypercholesterolemic rats

Treatment	Mean ± SE	Minimum	Maximum
Group 1	46.70 ± 3.27 [B]	31.00	64.00
Group 2	63.70 ± 5.83[A]	49.00	112.00
Group 3	50.20± 1.79 [AB]	44.00	60.00
Overall Mean	53.53 ± 2.61	31.00	112.00

AB shows significantly different at P<0.05

DISCUSSION

Proximate composition of powder mixture indicated that it contains protein (3.2%), fiber (9.34%), ash (2.67%), moisture (7.40%) and fat (0.90%). These results are comparable withprevious conclusions (24), it was reported that flaxseed powder generally contained 5.28% moisture, 4.18% protein, 1.92% ash, 1.22% crudefat, 8.80% fiber. At the end of 4th week, weightin group 1 (CD), group 2 (CD-chol) and group 3(CD-chol) was increased from 205 to 352g, 209 to 380 and 168 to 285 g respectively. This shows thatdiet rich in cholesterol when supplemented with flax and pumpkin seeds mixture powder has theleast weight gaining effects as compared to other diets (25). The results of present study are alsosimilar to Adam 2002 who found less weight gain in rats fed on whole wheat flour (5.2g/rat/day) (26,27). Wu et al (2004) reported weight reduction in Sprague Dawley rats fed with different levels offlaxseed up to 52%.

The comparison mean of cholesterol was significantly increased 73.40 + 4.21 mg/dL in group 2 (hypercholesterolemic rats (CD-chol) fed with the balanced diet as well as diet highin cholesterol at a dose level of 15g/100g diet) followed by 59.60 + 2.20 mg/dL in group 3 (hypercholesterolemic diet supplemented with flax/pumpkin seed mixture (FP-chol) and 57.60 + 1.77 mg/dL in group-1 (control rats; fed on normal diet). The overall cholesterol mean was found 63.53 + 2.09 mg/dL. Maximum cholesterol was observed in rats fed with high cholesterol diet without supplementation of flax and pumpkin

seeds in the diet, followed by group 2 which wasfed on high fat diet as well as flax and pumpkinseeds powder mixture. The decline in hepaticcholesterol levels in FP-chol group indicated the possible influence of relatively higher fiber content of seed mixture. In fact dietary fibers are known to interfere with cholesterol absorption and enterohepatic bile circulation and resulted in depletion of hepatic cholesterol pools (28). In addition, diets rich in fibers are known to reduce serum cholesterol levels by inhibition of hepaticlipogenesis (29).

The results of the present study are in line withthe findings of several other researchers who reported reduction in LDL cholesterol due to intake of flax and pumpkin seeds (30). Vigaya Lakshmi et al (2009) reported 14.7% reduction in serum LDL concentration of subjects consuming 40g flaxseed/day (31). Kumar et al (2014) studied the effect of flaxseed and date palm leaves extract and concluded that LDL level was reduced byfeeding rats on diets having these extracts (32). Jangele et al (2014) found that supplementation of dietary flaxseed restored the elevated level of LDL in hyperglycemic rats. The results are also supported by research work of Goyal 2014 (33) who reported that HDL level increase in rats fed with 20% raw flaxseed while it increased maximum in group fed with 30% flaxseed. But Chen et al (1984) also concluded that high cholesterol diet increases the HDL cholesterol of Sprague Dawley rats fed these fibers (34). Brant et al (2012) illustrated the effect of flaxseed anddate palm leaves extract on improving HDL levelin normal and diabetic rats (35).

Our results showed that flax and pumpkin seed mixture rich in PUFAs (poly unsaturated fatty acids) had a strong hypertriglyceridemic and hypocholesterolemic effects in plasma of rats with a reduction of plasma LDL-C levels and an increase in HDL-C levels. Furthermore, the atherogenic index markedly decreased causing a significant reduction in LDL/HDL ratio in FP-chol group. The increase in HDL-C ratio is one of the most important criteria of anti-hypercholesterolemic agent.

In the present study rats fed with high cholesteroldiet along with the supplementation of flaxseeds and pumpkin seeds resulted in reduction of triglycerides level in hypercholesterolemic rats.

CONCLUSION

The study suggests that flax and pumpkin seed mixture has significant anti-atherogenic activity when administered to hypercholesterolemic rats. The hypocholesterolemic effects of flax and pumpkin seeds mixture may be due to the presence of omega-3 and omega-6 fatty acids and fibers.

REFERENCES

1. Link JJ, Rohatgi A, de Lemos JA. HDL cholesterol: physiology, pathophysiology, and management. Current problems in cardiology. 2007;32(5):268-314.
2. Sirtori CR, Galli C, Anderson JW, Arnoldi A. Nutritional and nutraceutical approaches to dyslipidemia and atherosclerosis prevention: focus on dietary proteins. Atherosclerosis. 2009;203(1):8-17.
3. Dietschy JM. Dietary fatty acids and the regulation of plasma low density lipoprotein cholesterol concentrations. The Journal of nutrition.1998;128(2):444S-8S.
4. Simopoulos AP. The importance of the omega-6/ omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Experimental biology and medicine. 2008;233(6):674-88.
5. Mazza M, Pomponi M, Janiri L, Bria P, Mazza S. Omega-3 fatty acids and antioxidants in neurological and psychiatric diseases: an overview. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2007;31(1):12-26.
6. Bray GA. Medical consequences of obesity. The Journal of Clinical Endocrinology & Metabolism. 2004;89(6):2583-9.
7. Pagano AE. Whole grains and the gluten-free diet. Practical Gastroenterology. 2006;30(10):66.
8. Brenna JT, Salem N, Sinclair AJ, Cunnane SC. α-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. Prostaglandins, leukotrienes and essential fatty acids. 2009;80(2):85-91.
9. Stevenson DG, Eller FJ, Wang L, Jane J-L, Wang T, Inglett GE. Oil and tocopherol content and composition of pumpkin seed oil in 12 cultivars. Journal of agricultural and food chemistry. 2007;55(10):4005-13.
10. Shahidi F. Natural antioxidants: chemistry, health effects, and applications: The American Oil Chemists Society; 1997.
11. Barakat LA, Mahmoud RH. The antiatherogenic, renal

protective and immunomodulatory effects of purslane, pumpkin and flax seeds on hypercholesterolemic rats. North American journal of medical sciences. 2011;3(9):411.
12. Ristic-Medic D, Perunicic-Pekovic G, Rasic-Milutinovic Z, Takic M, Popovic T, Arsic A, et al. Effects of dietary milled seed mixture on fatty acid status and inflammatory markers in patients on hemodialysis. The Scientific World Journal. 2014;2014.
13. Caligiuri SP, Edel AL, Aliani M, Pierce GN. Flaxseed for hypertension: implications for blood pressure regulation. Current hypertension reports. 2014;16(12):499.
14. McEvoy CT, Temple N, Woodside JV. Vegetarian diets, low-meat diets and health: a review. Public health nutrition. 2012;15(12):2287-94.
15. Health UDo, Services H. Healthy People 2000: National Health Promotion and Disease Prevention Objectives- Nutrition Priority Area. Nutrition Today. 1990;25(6):29- 39.
16. Stone NJ, Robinson JG, Lichtenstein AH, Merz CNB, Blum CB, Eckel RH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Journal of the American College of Cardiology. 2014;63(25 Part B):2889-934.
17. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al. Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. hypertension. 2003;42(6):1206-52.
18. Makni M, Fetoui H, Gargouri NK, Garoui EM, Zeghal N. Antidiabetic effect of flax and pumpkin seed mixture powder: effect on hyperlipidemia and antioxidant status in alloxan diabetic rats. Journal of Diabetes and its Complications. 2011;25(5):339-45.
19. Makni M, Sefi M, Fetoui H, Garoui EM, Gargouri NK, Boudawara T, et al. Flax and Pumpkin seeds mixture ameliorates diabetic nephropathy in rats. Food and Chemical Toxicology. 2010;48(8):2407-12.
20. Johnson I, Gee J, Brown J. A Comparison of Rice Bran, Wheat Bran and Cellulose as Sources of Dietary Fibreinthe Rat. Food Sciences and Nutrition. 1989;42(3):153- 63.
21. Assmann G, Cullen P, Schulte H. The Münster Heart Study (PROCAM). Results of follow-up at 8 years. European heart journal. 1998;19:A2-11.
22. Bernard Y, Bonnes M, Giuliani M, Amoni F. A Cross- Cultural Approach to the Study of Domestic Space.Istituto di Psicologia del CNR, Rome. 1982.
23. Duncan DB. Multiple range tests for correlated and heteroscedastic means. Biometrics. 1957;13(2):164-76.
24. Berman KH, Harrigan GG, Riordan SG, Nemeth MA, Hanson C, Smith M, et al. Compositions of seed, forage,and processed fractions from insect-protected soybeanMON 87701 are equivalent to those of conventional soybean. Journal of agricultural and food chemistry. 2009;57(23):11360-9.
25. Makni M, Fetoui H, Gargouri N, Garoui EM, Jaber H, Makni J, et al. Hypolipidemic and hepatoprotective effects of flax and pumpkin seed mixture rich in ω-3 and ω-6 fatty acids in hypercholesterolemic rats. Food and Chemical Toxicology. 2008;46(12):3714-20.
26. Adam A, Crespy V, Levrat-Verny M-A, Leenhardt F, Leuillet M, Demigné C, et al. The bioavailability of ferulic acid is governed primarily by the food matrix rather thanits metabolism in intestine and liver in rats. The Journalof nutrition. 2002;132(7):1962-8.
27. Wu L-Y, Juan C-C, Ho L-T, Hsu Y-P, Hwang LS. Effect of green tea supplementation on insulin sensitivity in Sprague– Dawley rats. Journal of agricultural and foodchemistry. 2004;52(3):643-8.
28. Gunness P, Gidley MJ. Mechanisms underlying the cholesterol-lowering properties of soluble dietary fibre polysaccharides. Food & function. 2010;1(2):149-55.
29. Theuwissen E, Mensink RP. Water-soluble dietary fibers and cardiovascular disease. Physiology & behavior. 2008;94(2):285-92.
30. Vijayalakshmi V. Effect of flaxseed and samai supplementation on lipid profile of hyperlipidemic patients: Acharya NG Ranga Agricultural University Rajendranagar, Hyderabad; 2009.
31. Kumar A, Kumar P, Kumar V, Kumar M. Traditional uses of wetland medicinal plant Acorus calamus: review and perspectives. Int J Referred Online Res. 2014;17:37-67.
32. Jangale NM, Devarshi PP, Dubal AA, Ghule AE, KoppikarSJ, Bodhankar SL, et al. Dietary flaxseed oil and fish oil modulates expression of antioxidant and inflammatory genes with alleviation of protein glycation status and inflammation in liver of streptozotocin–nicotinamide induced diabetic rats. Food chemistry. 2013;141(1):187-95.
33. Goyal A, Sharma V, Upadhyay N, Gill S, Sihag M. Flax and flaxseed oil: an ancient medicine & modern functional food. Journal of food science and technology.2014;51(9):1633-53.
34. Chen W-JL, Anderson JW, Jennings D. Propionate maymediate the hypocholesterolemic effects of certain soluble plant fibers in cholesterol-fed rats. Proceedings of the society for experimental biology and medicine. 1984;175(2):215-8.
35. Brant LHC, Cardozo LFMdF, Velarde LGC, Boaventura GT. Impact of flaxseed intake upon metabolic syndrome indicators in female Wistar rats. Acta cirurgica brasileira. 2012;27(8):537-43.