International Journal of Pharma Medicine and Biological Sciences

ISSN 2278 – 5221 www.ijpmbs.com
Vol. 1, No. 2, October 2012

Research Paper

FATTY ACID COMPOSITION AND HYPOLIPIDEMIC EFFECT OF ROASTED FLAXSEED POWDER

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Effect of flaxseeds supplementation on serum lipid profile of selected hypercholesteremic patients was studied. Flaxseeds were processed (roasted) and analysed for its nutritional and fatty acid profile and incorporated into chapattis (15gms) and evaluated organoleptically. Administration of roasted Flaxseed powder of 15 gm for 4 weeks produces significant (P<0.001) reduction of serum total cholesterol, triglyceride, LDL-cholesterol and increase serum HDL cholesterol in hyperlipidemic group. On the other hand, changes of lipid profile in hypercholesteremic patients without roasted Flaxseeds incorporated were not significant (P<0.001). The present study suggests that roasted Flaxseeds powder incorporated would be considered as effective agent for lipid lowering purposes.

Keywords: Flaxseed, Roasted, Hyperlipidemia

INTRODUCTION

Hyperlipidemia is the current medical as well social problem, leading to increasing morbidity and mortality. The major risk factors of hyperlipidemia are associated with atherosclerosis which predisposes ischemic heart disease and cerebrovascular disease (Brown and Goldstein, 1990). Most patients who present with hyperlipidemia have a polygenic predisposition to raised blood lipids aggravated by dietary or lifestyle indiscretion. Consumption of dietary omega-3 fatty acids for health benefits has been extensively studied by the medical, scientific, and nonscientific communities; in recent years a vast amount of literature has been published on their findings. Omega-3 fatty acids have significant positive effects on health and general wellbeing (Siddiqui and Ruxton, 2004; Holub, 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 (Meyer *et al.,* 2003).

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Flaxseeds are the best source of lignans, nutrients including protein, soluble and insoluble dietary fiber as well as omega 3 fatty acids (Shahzad Hussain, 2009). Omega 3 fatty acids play their role in reducing the risk of cardiovascular diseases (Horrobin and Manku, 1990; Morris, 2004). Ground flaxseed is high in omega 3 fatty acids which have been shown to reduce hypertension, cholesterol and triglyceride level (Oomah and Mazza, 1998) and improving heart functions due to eicosanoides derived from omega 3 fatty acids (Simopoulos, 1999; Lucas *et al.,* 2002; Jenkins *et al.,* 1999; Arjmandi *et al.,* 1997; Bierenbaum *et al.,* 1993). The flaxseed fiber is also considered to reduce the blood glucose and cholesterol levels by delaying and reducing their absorption in the body (Shen *et al.,* 1998). Hence, the present study was undertaken to process and incorporated the roasted flaxseeds powder into chapattis and supplemented to the hyperlipidemic subjects to assess its therapeutic activity.

MATERIALS AND METHODS

Processing of Flaxseeds

Flaxseeds were selected as a source of linolenic acid. The procured flaxseeds were cleaned thoroughly to remove adhering of dust and foreign matters and roasted in Iron vessel. Then ground into powder and packed in polythene bags and stored until required.

Nutritional analysis

The nutritional properties such as moisture and total ash were determined using BIS method. The carbohydrate and fibre were assessed using AOAC method. The assessment of protein by Lowry's method and fat by Soxhlet method has been done. The calcium and Iron were determined using NIN method. All the estimations were done in triplicates. GC-MS analysis of the fatty acid was carried out after methylation. GC-MS analysis was performed with GC Clarus 500 Perkin Elmer equipment. Compounds were separated on Elite-5MS capillary column (5% diphenyl /95% Dimethyl poly siloxane), 30 × 0.2 5mm × 0.25 μm df). Oven temperature was programmed as follows: isothermal temperature at 110ºC for 2 min., then increased to 200ºC at the rate of 10ºC/min., then increased up to 280ºC at the rate of 5ºC/min. held for 9 min. Ionization of the sample components was performed in the EI mode (70 eV). The carrier gas flow rate was 1ml/min, and 3 μl of sample was injected. The detector was Mass detector turbo mass gold-Perkin Elmer. The total running time for GC was 36 min., and software Turbomass 5.2 was used in this GC-MS study.

Preparation of Chapatti

Chapattis were prepared, using 85gms of wheat flour and 15gms of roasted flaxseeds powder for making dough. The procedure for preparation was given below

Flowchart for the preparation of chapattis

Whole wheat flour

↓

Roasted flaxseed powder

↓

Add water and salt

↓

Made into dough

↓

Divided into balls

↓

Thin it in roll board and pin

↓

Heat the tawa and cook till it was done

Sensory Analysis

The sensory attributes including appearance, colour, flavour, taste, texture and overall acceptability were evaluated by a trained 10 member panel. The Nine-point hedonic scale was used to evaluate the degree of liking and disliking for preference of the chapattis.

Supplementation Study

Forty hyperlipidemia patients with the range of Serum total cholesterol between 250-275mg/dl, Triglycerides between 150-300mg/dl and Serum LDL cholesterol between 125-200mg/dl of either sex, aged 40-50 years were selected for the study from Thanjavur Medical College Hospital, Thanjavur. Patients having history of coexisting liver, kidney or thyroid disorders, etc. were not included in the study. Counselling of the patients about the study was done and informed consents were taken from the patients. The study was approved by the Institutional ethical committee. The fasting blood samples of 5 ml were collected to estimate the lipid profile.

The patients were divided into two groups: one group received only their usual anti-hyperlipidimic treatment (control group n=20) and another group received roasted flaxseed powder incorporated chapattis with their usual treatment (experimental group n=20). Patients were advised to come after overnight fasting and blood samples were collected in early morning. The control group on the day 1, blood sample was taken as baseline record and advised to continue their usual hyperlipidemic treatment (i.e. drug or diet control plus exercise). In experimental group on day 1, blood sample was taken as baseline record and advised to continue their usual hyperlipidimic treatment (i.e. drug or diet control plus exercise). They were advised to consume 15 gm of roasted flaxseeds powder incorporated in 8 5gm of wheat flour for the preparation of chapattis during breakfast or dinner as per their convenience for the period of 30 days. Keen follow up was done for the entire study period. All the parameters of lipid profile i.e., serum total cholesterol was estimated according to Zlatkis *et al.* (1954), LDL-cholesterol, triacylglyceride by Autoenzyme triglyceride kit and HDL-cholesterol by phospho-tungstate/ magnesium method were done at the end of the 30th day. The changes on the lipid levels of each group before and after supplementation were absorbed and statistically analysed using paired T-test.

All values expressed as mean in mg/dl ± standard deviation. Statistical significance of difference between the base line serum lipid level for control group and experimental group was performed.

RESULTS AND DISCUSSION

Nutritional Properties of Roasted Flaxseed Powder

The nutrient content of Roasted flaxseed powder in table 3 was reported as moisture (4.37%), Carbohydrate (27.60g), Protein (25.58g), Fibre (2.62g), Calcium (297.25mg), Iron (14.01mg) and Vitamin C (500mg) respectively (Table 1).

Table 1: Nutritional Properties of Roasted Flaxseed Powder

Nutritional Properties	Quantity
Moisture	4.37%
Carbohydrate	27.60g
Protein	25.58g
Fibre	2.62g
Calcium	297.25mg
Iron	14.01mg
Vitamin C	500mg

The earlier study conducted by Hussain (2004) in which he has found significant improvement in the proximate composition (ash, fat, protein and fiber) of full fat flaxseed flour supplemented with whole wheat flour. Daun (2003) reported about 28% dietary fiber in flaxseeds.

The fatty acid of roasted flaxseeds powder analyzed using GC-MS were shown in Table 2 and Figure 1. The identification of fatty acid compounds were based on peak area and molecular formula. Roasting improves the unsaturated fatty acid content (Table 2 and Figure 1).

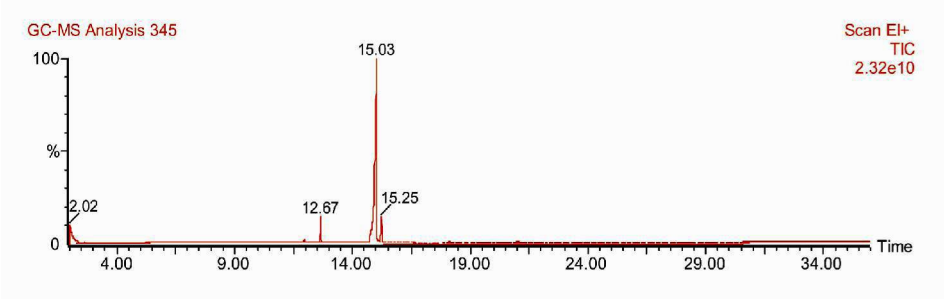
The maximum peak area represented in the roasted flaxseeds (Table 2 and Figure 1) was 9, 12, 15-Octadecatrienoic acid, methyl ester (93.74%) ($C_{19}H_{32}O_2$) with retention time of 14.98. This compound is called as Alpha-Linolenic acid. Alpha-linolenic acid is a kind of omega-3 fatty acid found in plants. Linolenic acid, an *n*-3 fatty acid is a member of the group of Essential Fatty Acids (EFAs), because they cannot be produced within the body and must be acquired through diet. Linolenic acid is the most abundant unsaturated component of several seeds and oils particularly flaxseeds and its oil. In recent years there has been considerable interest in the beneficial physiological effects of the omega-3 fatty acids (Zatonski, et al, 2008). Dietary á-linolenic acid has been assessed for its role in cardiovascular health. Clinical benefits have been concluded that modest dietary consumption of α-linolenic acid (2 to 3 g per day) will help in the primary and secondary prevention of coronary heart disease (Mozaffarian, 2005).

Nikolic *et al*., (2008) reported that fatty acid profile of wheat flour can be improved by its supplementation with rice flour because wheat flour is deficient in linolenic acid. The results of present study were in line with the findings of Gambus *et al.,* (2004) who found that addition of

Table 2: Components Identified in Roasted Flaxseed Powder (GC MS Study)

No	RT	Name of the compound	Molecular formula	Peak Area %
1	12.66	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	5.97
2	14.98	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	$C_{19}H_{32}O_2$	93.74
3	15.22	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	0.07
4	17.29	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)	$C_{20}H_{34}O_2$	0.06
5	17.52	9-Octadecanoic acid, ethyl ester, (Z)	$C_{19}H_{36}O_3$	0.16

Figure 1: Components Identified in Roasted Flaxseed Powder (GC MS study)



10-13% flaxseeds in bread resulted about 800-1000 time enhancement of linolenic acid content of bread as compare to control bread produced from non flaxseed supplemented flour.

Organoleptic Evaluation of the Chapattis

The developed chapattis were found to be acceptable with the mean overall acceptability scores of 7.5±1.2 by the selected panel members.

Supplementary Impact of Roasted Flaxseed Powder

The mean serum total cholesterol, HDL-cholesterol, LDL-cholesterol level and triglyceride of control group on the first day and after 4 weeks (day 30) was compared with serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride level of experimental group on the first day and after 4 weeks (day 30). The results were shown in Table 3.

The roasted flaxseed powder incorporated chappathis significantly ($p < 0.001$) reduced serum total cholesterol, serum HDL-cholesterol, serum LDL-cholesterol and serum triacylglyceride level in hyperlipidemic patients. No significant changes found in serum HDL-cholesterol, serum LDL-cholesterol and serum triacylglyceride level in control group. But 0.05% level of significance was seen in serum total cholesterol level for control group who were under normal medications.

DISCUSSION

The present study has been undertaken to demonstrate the effect of roasted Flaxseeds powder on lipid profile in hyper-lipidemic patients. In this study parameter of lipid profile was done for all hyperlipidemic patients. Estimation of lipid profile was done in all the patients after 4 weeks.

Table 3: Serum Lipid Profile In Patients With Hyperlipidemia Treated With and Without Roasted Flaxseed Powder Supplementation

Days	With roasted flaxseed powder	Without roasted flaxseed powder
Total Cholesterol (mg/dl)		
Day 1	241.60±22.55	225.70±22.69
Day 30	223.65±22.97**	220.00±25.82*
HDL (mg/dl)		
Day 1	41.35±11.17	42.10±12.95
Day 30	45.45±8.65**	41.40±7.12^{ns}
LDL (mg/dl)		
Day 1	153.10±20.86	138.90±22.38
Day 30	141.80±18.69**	133.90±22.48^{ns}
Triglycerides (mg/dl)		
Day 1	207.00±58.53	200.90±50.811
Day 30	186.35±49.54**	97.90±49.55^{ns}

Note: **means 1% level of significance; ^{ns} means not significant.

No significant changes were observed in all the parameters of lipid profile in control group. But significant changes were observed in serum total cholesterol, LDL-cholesterol, serum HDL-cholesterol and tri-acylglyceride level in experimental group.

The hypocholesterolemic effects of linolenic acid have been reported in both animals and humans (Chan *et al.,* 1991). Garg *et al.* (1989) demonstrated that feeding an linolenic acid-rich diet to rats lowered serum cholesterol levels more effectively than a diet rich in linoleic acid. Ratnayake *et al.* (1992) showed that a 20% and higher flaxseed diet given for 90 days in rats decreased serum total cholesterol. In studies of hypercholesterolemia rabbits. Prasad (1997) showed that dietary flaxseed reduced the total and LDL cholesterol and prevented hypercho-lestero-lemia atherosclerosis. Cholesterol feeding in rabbits caused a significant increase in the circulating total cholesterol, LDL-cholesterol, VLDLcholesterol, and also in the ratios of total cholesterol: HDL-cholesterol and LDLcholesterol: HDL-cholesterol. These results were consistent with earlier reports (Prasad, 2005; Vijaimohan *et al.,* 2006) which have clearly established a correlation between dietary lipids and serum lipid profile.

Consuming moderate doses of ground flaxseeds (1-4 tbsp, 1 tbsp = 7 g) per day can modestly reduce circulating Total Cholestrol (6%–11%) and LDL cholesterol (9%–18%) levels, and can lower various markers associated with atherosclerotic cardiovascular disease in humans. Evidence to date suggests that the dietary fibre and (or) lignan content of flaxseeds provide the hypocholesterolemic action (Chantal *et al.,* 2009). The positive influence of Flaxilip Capsule observed on the hypercholesterolemia and cholesterol related cardiac disorders. Flaxilip Capsule was helpful in providing significant change in elevated lipid profile (Shubhangi, 2011).

It is well documented that elevated total cholesterol and low density lipoprotien cholesterol (LDLc) levels promote atherosclerosis and cardiovascular complications (Dominiczak, 1998). Supplementation of cholesterol in diet rapidly results in a marked increase in the production of cholesteryl ester rich-VLDL by the liver and intestine (Demacker *et al.,* 1991). Elevated serum triglycerides are considered as independent risk factor for cardiovascular disease (Asia Pacific Cohort Studies Collaboration, 2004). Similar observations were made by number of workers, demonstrated hypolipidemic effect of fenugreek powder in experimental animals like rabbit, rat, etc (Al-Habori *et al.,* 1998).

CONCLUSION

In the present study, roasted flaxseed powder incorporated chapattis were significantly reduced serum total cholesterol, serum LDL-cholesterol, serum HDL-cholesterol and triglyceride level. So, it can be suggested that roasted flaxseed powder incorporated chapattis can be used for lipid lowering purposes.

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