EFFECT OF FLAXSEED AND SAMAI SUPPLEMENTATION ON LIPID PROFILE OF HYPERLIPIDEMIC PATIENTS

By

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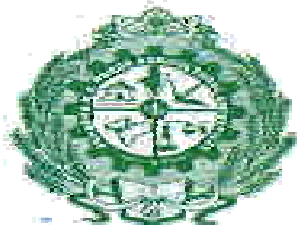

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CERTIFICATE

Ms. JOSNA.B has satisfactorily prosecuted the course of research and that the thesis entitled **"EFFECT OF FLAXSEED AND SAMAI SUPPLEMENTATION ON LIPID PROFILE OF HYPERLIPIDEMIC PATIENTS"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.

Date: 14/10/2009 **(Dr. V. VIJAYALAKSHMI)**

Place: Hyderabad Major Advisor

CERTIFICATE

This is to certify that the thesis entitled **"EFFECT OF FLAXSEED AND SAMAI SUPPLEMENTATION ON LIPID PROFILE OF HYPERLIPIDEMIC PATIENTS"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF HOME SCIENCE** of Acharya N. G. Ranga Agricultural University, Hyderabad, is the record of the bonafide research work carried out by **Miss. JOSNA. B** under my guidance and supervision. The Student Advisory Committee has approved the subject of the thesis.

No part of the thesis has been submitted by the student for any other degree or diploma or has been published. The published part has been fully acknowledged. All the assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

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ABSTRACT

Coronary Heart Disease (CHD) is the major killer in the world today accounting for over 80% death worldwide. Elevated total and LDL cholesterol level in the blood are major risk factors for CHD. Diet is the primary and most potent treatment for hyperlipidemia.

Flaxseed (*Linum usitatissimum*) is basically used for production of industrial linseed oil. It is not widely used as an edible grain in many countries. Whereas, samai (*Panicum summatrense*) or little millet is considered as minor millet which is generally used as a bird feed. Flaxseed is the richest source of α-linolenic acid and n-3 fatty acid and also contains other components like dietary fiber, mucilage, lignans and phenolic compounds. Samai also has beneficial nutrients like protein, high amounts of fiber, B-complex vitamins, essential amino acids minerals and phytochemicals including phytate. The nutrient components of flaxseed and samai are potentially beneficial for maintaining good health and reducing incidence of various diseases like coronary heart diseases, cancer, rheumatoid arthritis and hypertension. They have also shown hypocholesterolemic effects. However, the health benefits of flaxseed and samai are not fully utilized because the consumption of flaxseed and samai are low due to lack of knowledge of the consumers and limited availability in the market.

Since, the existing generation is interested and consume more of bakery products, the present study was designed to incorporate flaxseed and samai in baked products where both are rich in fiber and useful in lowering cholesterol levels and to determine the effect of supplementation on lipid profile of the selected hyperlipidemic patients.

Bakery products viz., bread, muffin and masala biscuit were developed by incorporating flaxseed at 10, 20 and 40 per cent levels. In all the products a combination of 50 per cent refined flour and 50 per cent of samai flour were used as the base. Muffins and masala biscuits were evaluated by sensory evaluation trials. Bread was excluded due to its poor attributes and poor end product. Among different levels of flaxseed incorporated muffins and masala biscuits, 20 and 40 per cent flaxseed incorporated masala biscuits obtained high overall acceptability scores. Therefore these two products were selected for supplementation for a period of two months.

A total of 21 subjects with total cholesterol above 155 mg/dl, age within the range of 35-50 year who are non-diabetic and not using lipid altering medications were selected from the staff of Acharya N. G. Ranga Agricultural University, both at Rajendranagar and College of Home Science, Saifabad, Hyderabad. Subjects were randomly assigned to three groups i.e. experimental group I, experimental group II and control group (n=7). Experimental group I and experimental group II were given 20 and 40 per cent flaxseed incorporated biscuits providing 8 g and 16 g flaxseed/day respectively. Control group was given low fat commercially available marie biscuits. The effect of supplementation was assessed by analysis of lipid profile initial (0 day), during (30^{th} day) and after (60^{th} day) the supplementation.

On analyzing the plasma lipid profile, it was observed that consequent to supplementation, a significant decrease was observed only in plasma total cholesterol (3.2%) and plasma LDL cholesterol (4.36%) of experimental group I and plasma total cholesterol (11.4%), plasma LDL cholesterol (6.79%) and plasma VLDL cholesterol (13.09%) of experimental group II after two months of supplementation. A slight reduction was observed in plasma HDL cholesterol levels of all the groups i.e. both experimental group I and experimental group II (1.8%) and control group (3.1%) after two months of supplementation.

Hence, from the above study it can be concluded that in view of hypocholesterolemic effect of flaxseed and samai, it can be advised for daily consumption to hypercholesterolemic as well as normal people in order to reduce the risk of cardio-vascular disease.

CHAPTER I

INTRODUCTION

Coronary heart disease (CHD) is the major cause of death in most of the developed countries and its prevalence is rapidly increasing in developing countries. Over the past 40 years, the prevalence of coronary heart disease in urban India has increased by a factor of six to eight, to about 10 per cent among persons 35 to 64 years of age (Reddy, 2004).

Hyperlipidemia refers to an elevation of plasma or serum lipid concentration such as cholesterol, phospholipids and triglycerides and it is proved to be a risk factor for the development of coronary heart disease (Danielle et al. 2002). Diet is the primary and most potent treatment for most persons with dyslipidemia. Most individuals can decrease their serum cholesterol by 10-20 per cent through adaptation of high fiber, low fat diet with inclusion of monounsaturated fatty acids (MUFA), polyunsaturated fatty acid (PUFA) and vegetable protein in the diet.

Flaxseed (or linseed, *Linum usitatissimum*) is an ancient crop used for fibre (linen), oil (linseed oil) and food. It is grown in most regions of the world – North and South America, Europe, Asia, Africa and Australia. Canada is both the largest producer (2.27 million metric tons) and the only significant exporter of flaxseed. Substantial production is also found in China and India (Westcott and Muir, 2000).

In India, flaxseed is grown all over the country, except in Kerela, Chennai, Delhi, Manipur, Tripura and Andaman and Nicobar islands. Uttar Pradesh and Madhya Pradesh together account for nearly two – thirds of total production.

In economic climate, when farmers have to reduce their production of cereals, flaxseed offers a very interesting alternative due to its usefulness for a variety of purposes (Liljedahl and Degerman, 1993). Flaxseed is mainly used for the production of industrial linseed oil which is used in the manufacture of paints and varnishes. It is not widely recognized as an edible grain in many countries. However, in North America, flaxseed has been accepted at low levels as a component of some brands of cereals in speciality breads and as a seed dressing on buns (Ratnayake et al. 1992). In India, linseed oil has limited use as cooking and frying oil, used in combination with mustard, groundnut and coconut oils. It can be hydrogenated for edible purposes (Salunkhe et al. 1992).

The importance of flaxseed consumption is mainly related to its high content of polyunsaturated fatty acids (PUFA). It has the highest proportion of the n-3 fatty acids, that comes to about 50-55 per cent of the total fatty acids present in flaxseed (Chen et al. 1994).

Recent developments in the area of dietary fats have led to the discovery of abundance of long chain n-3 PUFA in fish (Ghafoorunissa and Indu, 1993). Fish oils are rich source of n-3 PUFA like α-Linolenic Acid (ALA), Eicosapentanoic Acid (EPA) and Docosahexaenoic Acid (DHA) and so, fish oil can provide a multitude of health benefits. However, encapsulated fish oil is not suited for lifetime daily use. So, increasing the fish intake seems to be an ideal way to improve n-3 fatty acid status. But, the recommendations to increase fish intake has not been effective (Mantzioris et al. 2000). The reason is that the availability of fish cannot sustain the proposed daily intake of n-3 fatty acids (at least 500 mg). Also, marine products are not ubiquitous in the culinary

tradition of many countries, and many people do not like the taste of fish (Crawford et al. 2000).

Thus, the potential for world's population at large to increase its fish consumption is limited and increased intake of plant sources of n-3 fatty acids is much greater (Allman et al. 1995). Due to aforesaid reasons, there has been an increasing need to identify plant sources which can provide n-3 PUFA (Poly Unsaturated Fatty Acid).

Linoleic acid (LA), 18 : 2n-6 is the principal PUFA in oils from plant seeds and is universally found in the vegetable kingdom. In the plant chloroplasts, linoleic acid is desaturated to give α- linolenic acid (ALA), but mammals cannot convert linoleic acid to α-linolenic acid, because the desaturase enzyme responsible for this conversion is found only in plants (Calder, 2001). These two fatty acids cannot be made by mammals and have to be provided through diet, hence they are termed as Essential Fatty Acids (EFAs). Plant tissues and plant oils tend to be rich sources of linoleic acid and α-linolenic acid. However, the richest source of α-linolenic acid is flaxseed; other good sources of α-linolenic acid are maize, sunflower, safflower, soybean oil and rapeseed (Cunnane et al. 1993).

Flaxseed is slowly gaining importance and seems to have several medicinal properties. It contains a variety of other components like dietary fibre, mucilage, lignans and phenolic compounds, which render it potentially beneficial for maintaining good health and reducing the incidence of various diseases especially coronary heart disorders (Deckere et al. 1998; Ezaki et al. 1999; Mantzioris et al. 2000). It has been shown to inhibit growth of prostate cancer and breast cancer (Connolly et al. 1997; Chajes et al. 2000). It can relieve the symptoms of rheumatoid arthritis (Darshan et al. 1991; Kremer,

1996; Volker and Garg, 1996; Mantzioris et al. 2000; Calder, 2001). It lowers high blood pressure in hypertensive patients (Deckere et al. 1998; Bemelmans et al. 2000). It has been shown to delay the loss of immunological functions (Calder et al. 2001; Deckere et al. 2001). It also favours for normal fetal brain and visual development (Lewis et al. 2000; Jorgensen et al. 1999).

There is evidence that whole flaxseed lowers serum cholesterol in both normal and hyperlipidemic subjects (Bierenbaum et al. 1993; Cunnane et al. 1995). This hypolipidemic effect of flaxseed consumption can be better utilized along with its other health benefits provided that flaxseed is consumed as a part of some foods in the traditional daily menu. In America and Canada, flaxseed bread is a commonly available consumer product containing about 7 per cent ground flaxseed by weight. Other baked products such as muffins and cookies are also popular. The promotion of a variety of flaxseed incorporated traditional recipes can be a small, yet effective method for improving the health of the community.

Mutual supplementation of cereals with legume/oil seed has been advocated by several workers for complementing amino acids. Hence, further improvement of nutritive value of the products can be done by the addition of millet instead of refined flour with flaxseed (oilseed).

Samai (*Panicum sumatrense Roth ex Roem. & Schult*.) or **Little Millet** (*Panicum sumatrense,* Syn.: *Panicum miliare auct. non Lam*.) is a species of millet in the family Poaceae. Samai is a relative of Proso millet and grown throughout India but is of little importance elsewhere and has received very little attention from plant breeders as a crop source.

Samai are grown in harsh environments where other crops cannot grow or yield poorly. Samai grow well on poorly fertilized and dry soils and fit well in hot climates with short rainfall periods and cool climates with warm summers. The plants need good drainage, have a low moisture requirement and do not do well in waterlogged soils. Samai are grown with limited water resources by a multitude of small farmers in many countries, usually without application of fertilizers or other inputs. The plant varies in size between 30-90cm and its oblong panicle varies in length between14 to 40 cm. The seeds of little millet are much smaller than proso millet. Consumed by disadvantaged groups, they are often referred to as "coarse grain" or "poor people's crops".

Little millet is highly nutritious, non-glutinous and like buckwheat and quinoa, is not an acid forming food so is soothing and easy to digest. In fact, it is considered as one of the least allergic and most digestible grains available. It is considered to be a warming grain which will help to heat the body in cold or rainy seasons, therefore consumed more in rainy and winter seasons.

Samai has a mild sweet taste nut-like flavor and contains a myriad of beneficial nutrients with nearly 15% protein, high amounts of fiber, B-complex vitamins including niacin, thiamin, and riboflavin, the essential amino acid methionine, a lipid lecithin and vitamin E. It is particularly high in minerals like iron, magnesium, phosphorus, and potassium. The seeds are also rich in phytochemicals, including phytic acid, believed to lower cholesterol, and associated with lowering cancer incidence (Ashutosh Dubey and Verma, 2009)

Hence, the present study was planned to design products with a combination of flaxseed and samai where both are rich in fibre and useful in lowering cholesterol levels.

Since, the existing generation is interested in and consumes more of bakery products, the present study was designed to incorporate flaxseed and samai in baked products and to determine the effect of supplementation on lipid profile of the selected hyperlipidemic patients.

The objectives of the present study are as follows:

General objective:

To develop Flaxseed (*Linum usitatissimum*) incorporated low fat baked products with samai (*Panicum sumatrense Roth ex Roem. & Schult.*) and study the effect of supplementation on hyperlipidemic patients.

Specific objectives:

- To standardize low fat baked products with different levels of flaxseed and samai incorporation.
- To study the acceptability of products by a trained panel of judges.
- To supplement the most acceptable two different levels of incorporated products to hyperlipidemic patients for a period of two months.
- To study the impact of level of supplementation on the lipid profile of subjects before, during and after supplementation.

CHAPTER II

REVIEW OF LITERATURE

The flaxseed (*Linum usitatissimum*) also known as linseed, is an ancient traditional crop grown in several countries for fibre (linen), oil (linseed oil) and food.

Samai or little millet (*Panicum sumatrense*) is widely cultivated as minor millet throughout India as a food for both human and birds.

There are very few studies on samai (little millet) but many studies have been conducted to prove the health benefits of flaxseed consumption. A comprehensive review of these studies is presented under the following heads :

2.1 Therapeutic benefits of flaxseed

2.2 Role of millet in health and disease

2.3 Role of fibre in cardiovascular disease

2.1 THERAPEUTIC BENEFITS OF FLAXSEED

Flaxseed seems to have several medicinal properties and is slowly gaining importance in therapeutic use. The nutrient composition of flaxseed which has therapeutic benefits is shown in table 1 and 2 (Nagraj and Reddy, 1997).

Flaxseed with its essential fatty acid and antioxidant content gained therapeutic importance in relieving several physiological disorders of the body. Fatty acid composition of flaxseed oil is shown in table 3(Nagraj and Reddy, 1997). The essential fatty acids omega-3 and omega-6 in flaxseed are converted in the body to prostaglandins (a hormone like substance), which regulate the steroid production, hormone synthesis,

blood clotting ability, nerve transmission and water retention. Prostaglandins also play a role in relieving inflammation, pain and pressure in joints, blood vessels and eyes (Budwig 2000).

Table 1: Nutrient composition of flaxseed (percentage)

Constituents	Percentage/Amount
Moisture	6.5-10
Protein	20-24
Oil	37-42
Carbohydrates	15-29
Crude fibre	5-9
Ash	2-4

Table 2: Mineral and vitamin content of flaxseed

Constituent	Amount/100 g
Calcium	0.17 g
Magnesium	0.37 g
Iron	2.7 g
Carotene	30 µg
Thiamine	0.23 mg
Riboflavin	0.07 mg
Niacin	1.0 mg
Choline	0.99-1.18 mg
Vitamin E	21.7 mg

Table 3: Fatty acid composition of flaxseed oil (mg/g)

Fatty acid	Amount (mg/g)
Super unsaturated fatty acids	
Alpha linolenic fatty acid	550 mg
Polyunsaturated fatty acids	
Linoleic fatty acid	170 mg
Monounsaturated fatty acid	
Oleic acid	180 mg
Saturated fatty acids	
Palmitic acid	60 mg
Stearic acid	40 mg

A study in USA on flaxseed revealed that an antibiotic substance linamine found in flaxseeds could cure diseases against which there is no medical treatment in man and animals. A flaxseed placed in the eye usually removes a cinder (Gill, 1987).

Budwig (2000) conducted extensive research by supplementing a mixture of two table spoons of flaxseed oil mixed with one quarter cup of low fat cottage cheese for a longer period showed impressive results with respect to:

- Anti-tumor activity inhibiting cancer cell growth.
- Normalize blood pressure, reduce cholesterol levels and preventing atherosclerosis.

- Boost the immune system, relieving symptoms of rheumatoid arthritis and asthma.
- Stimulates brown fat cells and increase metabolic rate making it easier to burn fat.
- Ease weight loss in obesity and improving the function of the anabolic hormone, insulin.
- Accelerate healing of sprains, strengthen finger and toe nails and improving eye sight and odor perception.

2.1.1 Role of flaxseed in cardiovascular disease

Dietary saturated fat and cholesterol contribute to cardiovascular disease. n-3 fatty acids act to prevent heart disease through a variety of actions. They

- ✓ act as prostaglandins and leukotriene precursors
- ✓ have anti-inflammatory properties.
- ✓ inhibit synthesis of cytokines and mitogens
- ✓ stimulate endothelial-derived nitric oxide.
- ✓ have hypolipidemic properties
- ✓ recover atherosclerosis (Connor, 2000).

Epidemiological data shows that elevated blood cholesterol, especially low-density lipoprotein (LDL) cholesterol is the major risk factor in the development of Coronary heart disease (Kannel et al, 1971).

The liver not only removes triglyceride and chylomicrons from the blood, it also synthesizes and packs triglyceride into VLDL particles and releases them back into the blood circulation. Like chylomicrons, VLDL particles contain mostly triglycerides. Some

of the VLDL particles lose triglyceride in the blood and become cholesterol rich LDL particles (Dennis Lee, 2001).

Kritchevsky et al. (1991) reported that supplementation of high levels of flaxseed (up to 20%) reduced cholesterol deposition in liver in a study conducted on rats.

Ratnayake et al. (1992) studied the nutritional effect of flaxseed by feeding weanling rats with diet containing ground linseed at 0, 10, 20 and 40 per cent levels for a period of 90 days. Results showed that serum triglycerides, total cholesterol and LDL cholesterol concentrations were significantly lower in rats fed with 20% and 40% flaxseed diets as compared to control group fed with flaxseed free diet.

Bierenbaum et al. (1993) studied the effect of supplementation of three slices of flaxseed containing bread i.e. 15 g ground flaxseed for a period of 3 months on serum lipid levels in 15 hyperlipidemic subjects on long term intake of vitamin E (800 IU/day). A highly significant drop in serum cholesterol (18 mg/dl) and LDL- cholesterol (19 mg/dl) levels was observed. HDL cholesterol levels did not change with flaxseed consumption.

DeLorgeril et al. (1994) reported that in a secondary prevention trial of myocardial infarction, patients consuming an experimental diet with higher ALA content experienced a significant reduction in death due to cardiovascular disease.

Cunnane et al. (1995) studied the lipid profile in healthy young adults by supplementing 50 g flaxseed per day for 4 weeks. During that period α-linolenate increased significantly in adipose tissue and plasma n-3 polyunsaturated fatty acids were increased in plasma lipids significantly. Plasma LDL reduced by 8%. It was concluded

that the traditional flaxseed had modest beneficial effect on several indices of nutritional status without compromising antioxidant status.

Ingsam et al. (1995) reported that 15% dietary flaxseed and flax oil attenuated the decline in renal function and reduced glomerular injury with favorable effects on blood pressure, plasma lipids and urinary prostaglandins in rats.

Allman et al. (1995) suggested that consumption of ALA rich oils may offer protective effects against cardiovascular disease over linoleic acid rich oils via their ability to decrease the tendency of platelets to aggregate.

Brandle et al. (1997) studied the effect of garlic and linseed oil on prolongation of life span in hypertensive rats by dietary interventions. The results showed that the systolic blood pressure measured during the compensatory stage was significantly lowered by both linseed oil and garlic and their combinations.

Prasad (1997) reported that flaxseed feeding has protective effect against various risk factors of cardiovascular diseases including hypercholesterolemia related heart attack and strokes and aortic atherosclerosis.

Arjmandi et al. (1998) reported that 38 g flaxseed supplemented in the form of bread and muffins to postmenopausal women for a period of 6 weeks significantly lowered LDL cholesterol (14.7%).

In a study conducted at National Institute of Nutrition, Hyderabad, India (1998), 50 g flaxseed in the form of roti was fed to five volunteers for 36 days. It was found that there was a significant reduction in plasma cholesterol and LDL cholesterol after flaxseed consumption.

Deckere et al. (1998) proposed that an increase in arterial compliance by dietary ALA, which reduce the workload of heart, might contribute to a reduced risk in coronary heart diseases by ALA.

Jenkins et al. (1999) studied the health aspects of partially defatted flaxseed on serum lipids of hyperlipidemic subjects, and found that 3 weeks of flaxseed supplementation significantly reduced total cholesterol (5.5 ± 1.2%) and LDL cholesterol (9.7 ± 1.8%).

Hu et al. (1999) studied the association between dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease and reported that a higher intake of alpha-linolenic acid is protective against fatal IHD. Higher consumption of foods such as oil-based salad dressing that provide polyunsaturated fats, including alpha-linolenic acid, may reduce the risk of fatal IHD.

Talom et al. (1999) studied the effect of high flaxseed diet containing 52 to 60 per cent flaxseed oil on blood pressure and mesenteric arterial blood in hypertensive rats. The results suggested that high flaxseed diet improved the endothelial vasorelaxant function in rats through pressure independent mechanisms.

Barowicz et al. (2000) studied the hypocholesteromic effect of fat feed in the diets of growing pigs. They observed that total cholesterol in the blood stream decreased from 98.6 mg% in control group to 88.5 mg% in the group supplemented with 15% calcium salts of linseed oil fatty acids.

Bemelmans et al. (2000) investigated the association of dietary intake of ALA and LA with coronary heart disease risk factors. They suggested that in a CHD high risk

population with LA rich diet, diastolic blood pressure may be decreased by replacing LA in the diet with ALA.

Meeta Maheshwari (2002) studied the effect of supplementation of flaxseed incorporated laddu for a period of 2 months on serum lipid levels of 25 (normolipidemic and hyperlipidemic) subjects. Results showed a significant drop in serum lipid profile.

Sam et al. (2003) studied the beneficial effects of soy protein and flaxseed meal on hypertriglyceridemia and liver steatosis associated with obesity and diabetes. The hypotriglyceridemic and hypocholesterolemic effects of flaxseed meal may have important therapeutic implications in patients with hypertriglyceridemia and hypercholesterolemia and deserve further study in humans with these disorders. Flaxseed meal supplementation may provide a new therapeutic strategy to reduce hypertriglyceridemia and fatty liver.

Elina Lipilina and Vijay Ganji (2008) determined the optimum amount of ground flaxseed substitution for flour in bakery products and evaluated the effect of flaxseed on sensory and nutritional qualities of those bakery products using a consumer panel.

Anagha et al. (2008) investigated the extent to which the daily incorporation of approximately 30 g of flaxseed, a rich source of lignans, omega-3 fatty acids, and fiber, for a period of 3 months into the diet of Native American postmenopausal women positively affected their lipid profiles. The study showed that Native American postmenopausal women had benefited from regular consumption of flaxseed by reducing the risk of cardiovascular disease as seen by lowered LDL-C and total cholesterol levels.

Le Anne et al. (2008) studied the effects on markers of cardiovascular risk in hypercholesterolemic adults by supplementing 40 g per day of ground flaxseed

containing baked products or matching wheat bran products for 10 weeks while following a low fat, low cholesterol diet and results showed the decrease in cardiovascular risk.

The consumption of flaxseed is associated with a reduction in total cholesterol, including the LDL cholesterol and triglycerides. Study after study has shown a positive response to eating ground flax seeds daily. Eating low fat foods, increasing exercise, limiting the salt, sugar and eating flax seed daily are a few ways that can win the battle against high cholesterol. Flax helps to reduce clotting time and thereby reduces the chance for heart attacks and strokes. Regular intake of flax protects against arrhythmias and helps keep the arteries clear and pliable.

2.1.2 Role of flaxseed in health and other disease

With increasing awareness of the relation between diet and health, consumers are seeking foods and food supplements that have beneficial effect on their personal health.

Traditional varieties of flaxseed are rich in α–linolenic acid, soluble fibre mucilage and mammalian lignan precursors. In this section, the role of flaxseed in various disease conditions other than cardiovascular disease like cancer, immune function, diabetes, rheumatoid arthritis, renal diseases, pregnancy/fertility and obesity are discussed.

2.1.2.1 Flaxseed and Cancer

The incidence of breast cancer is lower in population groups where the diet is vegetarian or low in fat and high in dietary fibre (Taioli et al. 1991). The cancer protective effects of these diets are presumably due to mammalian lignans. These substances are produced by the bacteria in the colon of humans and animals from precursors in high fibre foods. Flaxseed is the richest source of mammalian lignan

precursors (Thompson et al. 1991). The primary lignan found in flaxseed is Secoisolariciresinol Diglycoside (SD), which is converted by the intestinal microflora to Enterodiol (ED) and Enterolactone (EL).

Mammalian lignans ED and EL have been hypothesized to modulate hormone related cancers, such as breast cancer, because of their structural similarity to the estrogens, estradiol and diethylstilbestrol and the weak estrogen/antiestrogen tamoxifen, which is widely used for breast cancer treatment (Nayfield et al, 1991).

Serraino and Thompson (1991) reported that flaxseed supplementation at 5 per cent level decreased the initiation and promotion of mammary carcinogenesis in rats.

Thompson et al. (1996a) reported that flaxseed oil and flaxseed (in a dose independent manner) reduced the growth of established tumours at an advanced stage of carcinogenesis whereas mammalian lignan precursor SD exerted the greatest inhibitory effect on the development of new tumours.

In another study, Thompson et al. (1996b) isolated SD from flaxseed and tested it for effects of mammary tumourigenesis in rats fed on high fat (20%) diet. The results showed that SD has an antitumour effect when provided at the early promotion stage of tumourigenesis and may contribute to the health benefits of high fibre foods.

Nesbitt et al.(1999) reported that mammalian lignan production from flaxseed precursors is not affected by processing of flaxseed in the form of muffin or bread.

Tou and Thompson (1999) reported that exposure to 5-10% flaxseed for lifetime or gestation and lactation induced structural changes in the rat mammary gland that could potentially reduce mammary cancer risk.

Lilian et al. (2005) studied the effects of dietary flaxseed on tumor biological markers and urinary lignan excretion in postmenopausal patients with newly diagnosed breast cancer by supplementing 25 g of flaxseed containing muffins from the time of diagnosis and found that dietary flaxseed has the potential to reduce tumor growth in patients with breast cancer.

Studies have shown flaxseed to inhibit the growth of human estrogen-dependent breast cancer. The lignans in flaxseed are phyto-estrogens, plant chemicals that mimick the effects of estrogen in the body by increasing the production of a compound known as sex hormone-binding globulin, or SHBG. This protein regulates estrogen levels by displacing excess estrogen from cells in the body. It is believed that displacing estrogen in this manner might help prevent those cancers that depend on estrogen, such as breast cancer, from starting and developing. Ground flaxseed appears to be effective in preventing prostate cancer. In addition to the phytoestrogen effect, flaxseed lingnans bind to male hormone receptors and promote the elimination of testosterone (Meeta maheshwari, 2002).

2.1.2.2 Role of flaxseed in diabetes

Cunnane et al. (1993) reported that flaxseed was hypoglycemic in two test meal situations, one involving flaxseed flour incorporated into a test bread compared with standard white bread, and the other involving flaxseed mucilage, combined with glucose compared with glucose alone. In both the cases, they observed that lowering of post – meal change in blood glucose was about 27%.

Cunnane et al. (1995) reported that when compared with the control muffin, consumption of flaxseed muffin for breakfast during 4 weeks did not significantly affect the peak blood glucose concentration. They also found that there was no significant difference between test group and control group in either fasting blood glucose concentrations, peak blood glucose or total area under curve during the oral glucose tolerance test performed on the subjects.

McManus et al. (1996) compared the effects of linseed oil with fish oil supplementation in subjects with Type II diabetes. They reported that neither linseed oil nor fish oil significantly affected glycemic control.

Gerster (1997) reported that in Type II diabetic patients, 3 months supplementation with fish oil at 35 mg EPA + DHA per kg body weight led to significant reduction in triglyceride values whereas an equal amount of ALA in flaxseed oil had no effect. They found that glycemic control was not influenced by either oil.

Pan et al. (2007) studied the effect of a flaxseed-derived lignan supplement on glycemic control, lipid profile and insulin sensitivity in type II diabetic patients. They concluded that daily lignan supplementation resulted in modest, yet statistically significant improvements in glycemic control in type II diabetic patients without apparently affecting fasting glucose, lipid profile and insulin sensitivity.

Eating flaxseed reduces blood sugar levels after a meal and increases insulin levels because of its high content of soluble fiber. Indeed, flaxseed carbohydrate (what remains after the oil is removed) shows a beneficial effect. Flaxseed has been shown to improve insulin sensitivity. An interesting, yet unproven, potential benefit may be the

prevention of type I and type II diabetes; in animal models, flaxseed has been shown to delay the onset of the disease.

2.1.2.3 Role of flaxseed in rheumatoid arthritis

Kelley et al. (1991) reported that diet containing flaxseed oil suppressed some of the indices of cell mediated immunity without affecting any of the indices of humoral immunity. They suggested that if such suppressive effects can be attained in individuals with autoimmune diseases or chronic inflammations, such diets may be useful in the management of diseases like arthritis, lupus and allergies.

Mantzioris et al. (1994) suggested that flaxseed oil used as a component of daily food preparation can result in potentially beneficial fatty acid alterations. This could be useful in inflammatory conditions like rheumatoid arthritis, psoriasis and ulcerative colitis, either in conjunction with or without fish oil capsules.

Volker and Grag (1996) conducted studies on rheumatoid arthritis patients, giving 6.4 g DHA+EPA per day. All studies reported improvements, including reduced duration of morning stiffness, reduced number of tender or swollen joints, reduced joint pain, reduced time to fatigue and increased grip strength.

Mantzioris et al. (2000) suggested that n-3 fatty acid supplementation of the diet has a beneficial effect in patients with rheumatoid arthritis.

Flax is high in Omega 3 essential fatty acid which is a good news for people who suffer from inflammatory disorders, including rheumatoid arthritis. Health experts recommend eating foods high in Omega 3's for people suffering from rheumatoid arthritis. It is the inflammation within the joints that cause severe pain associated with

arthritis. Daily consumption of flaxseed or flax oil reduces inflammatory responses by as much as 30%.

2.1.2.4 Flaxseed and Immune function

Kumar et al. (1992) examined the effects of n-3 and n-6 fatty acids on lymphocyte proliferation and their ability to produce Tumour Necrosis Factor (TNF) and interleukin-2 (IL-2) *in vitro*. They reported that α–linolenic acid exhibited least cytotoxicity to lymphocytes with maximum inhibitory action on TNF with lesser effect on IL-2 production.

Hubbard et al. (1994) studied the effect of different oils on tumouricidal activity and eicosanoid production in marine macrophages of mice. The results revealed that the mice fed on linseed oil and fish oil produced significantly lower levels of both prostaglandins and leukotriene C4 when compared with macrophages of mice fed safflower oil.

Levander and Ager (1995) studied the antimalarial effect of flaxseed oil when fed to vitamin E deficient mice at 12.5 per cent flaxseed oil to basal diet. It was observed that the highly unsaturated fat in flaxseed oil exerted sufficient oxidative stress in vitamin E deficient mice to inhibit the growth of malarial parasite.

Caughey et al. (1996) examined the effect of a flaxseed oil based diet on TNF-α and IL-1 β synthesis in healthy men. They reported that use of flaxseed oil in domestic food preparation for four weeks inhibited TNF-α and IL-1 β production by about 30%.

Byleveld et al. (1999) conducted experiments to determine the effect of diets containing fish oils, linseed oil or beef tallow (control diet) and different doses and routes

of immunization on immunity against influenza virus in mice. They reported that immunized mice fed with linseed oil cleared more virus than non immunized control rats regardless of immunization route.

Calder (2001) reported that high levels of either ALA or EPA/DHA reduce chemotaxis of neutrophils, monocytes, T lymphocytes and T lymphocyte proliferation. They concluded that the immunological effects of large amounts of n-3 PUFA suggest that they might be helpful in therapies for diseases characterized by immune dysfunction.

Research has found that eating flax daily favorably affects immunity, the body's ability to defend itself successfully against bacteria and viruses. Two components of flax, lignans and ALA (alpha-linolenic acid), have been found to affect immune cells and compounds that control immune reaction.

2.1.2.5 Flaxseed and renal diseases

Ingram et al. (1995) reported that 15 per cent dietary flaxseed and flax oil attenuated the decline in renal function and reduced glomerular injury with favourable effects on blood pressure, plasma lipids and urinary prostaglandins in rats.

William et al. (1995) studied flaxseed as a potential treatment for lupus nephritis. They concluded that 30 g flaxseed/day was well tolerated and conferred benefit in terms of renal function as well as inflammatory and atherogenic mechanisms important in the pathogenesis of lupus nephritis.

Deckere et al. (1998) reported that n-3 PUFA have beneficial effect in renal transplantation by reducing complications.

Westcott and Muir (2000) investigated role of flaxseed in both immune and non-immune models of renal injury. They concluded that in both models, flaxseed was beneficial in slowing the decline of renal functioning. They also demonstrated that SD present in flaxseed delays onset of proteinuria and decreases renal injury.

Manuel et al. (2003) conducted a study to determine whether changing the source of protein intake from animal protein, casein, to plant protein in the form of either soy protein concentrate or flaxseed protein in the diet has a different impact on renal function and nephropathy. They concluded that dietary protein substitution with flaxseed meal reduces proteinuria and glomerular and tubulointerstitial lesions in obese SHR/N-cp rats and that flaxseed meal is more effective than soy protein in reducing proteinuria and renal histologic abnormalities in this model. The reduction in proteinuria and renal injury was independent of the amount of protein intake and glycemic control.

Secoisolariciresinol diglycoside (SD), protein and n-3 fatty acid content in flaxseed helps in reducing proteinuria and renal injury.

2.1.2.6 Role of flaxseed in obesity

Many investigators have reported lack of weight gain by study participants in the face of greater energy consumption during the flaxseed supplementation period (Pan and Storlien, 1993; Ishida et al. 1995; McManus et al. 1996**;** Thompson et al. 1996; Yuan et al. 1999; Jenkins et al. 1999).

This lack of weight gain may be explained by a higher rate of lipid oxidation, by lower food efficiency or by greater energy expenditure as suggested by Macdiarmid et al. (1996).

Roth Maier et al. (1998) reported that feeding diets with whole and ground flaxseed to broiler chicken slightly reduced weight gain by 7% compared with those fed on control diet.

Similarly, Schumann et al. (2000) reported that feeding diets containing 100 g/kg ground flaxseed, 40 g/kg flax oil or 100 g/kg dry n-3 to laying hens reduced weight gain and significantly reduced hepatic fat content compared to feeding control diet with animal and vegetable oil as a fat source.

Soluble fiber along with phytochemicals and n-3 fatty acids helps in burning excess cholesterol from the body and helps in reducing weight.

2.2 ROLE OF MILLET IN CARDIOVASCULAR DISEASE

Even if the present generation has ventured into the healthful world of whole grain foods, millet may be one that everyone hasn't tried. Millet, tiny seed-like grain used to make bird seed has a variety of health properties that make it quite worthy of human attention. Millet was once an important source of food for the Chinese, but has been slower to be embraced by American culture. This whole grain adds additional taste and texture to recipes, but it can also be enjoyed as a "nutty" tasting cereal all on its own. Millet is one of the four gluten-free grain-like seeds on the Body Ecology program. Some of the key reasons millet is part of your healthy Body Ecology diet is because it:

- Does not feed pathogenic yeast (Candida),
- Acts as a prebiotic to feed important microflora in your inner ecosystem
- Provides serotonin to calm and soothe your moods.
- Helps hydrate your colon to keep you regular.

- Is alkaline.
- Is easily digested (Body ecology, 2007).

Millet is full of nutrients which body needs, such as:

- Magnesium
- Calcium
- Manganese
- Tryptophan
- Phoshorus
- Fiber
- B vitamins
- Antioxidants

And that's not all. Many studies have been done on millet nutrition to identify its benefits for human health. Here are some of the findings:

- Magnesium in millet can help reduce the affects of migraine and heart attack.
- Niacin (vitamin B3) in millet can help lower cholesterol.
- Phosphorus in millet helps with fat metabolism, body tissue repair and creating energy (phosphorus is an essential component of adenosine triphosphate or ATP, a precursor to energy in your body)
- Millet can help lower risk of type 2 diabetes.
- Fiber from whole grains has been shown to protect against breast cancer.

- Whole grains have been shown to protect against childhood asthma (Body ecology, 2007).

Prashant et al. (2005) investigated the beneficial role of a millet-based diet in protecting against oxidative stress and maintaining glucose levels in vivo in type II diabetes. Whole grain flour of finger millet and Korean millet (KM) were incorporated at 55% by weight in the basal diet fed to alloxan-induced diabetic rats over a period of 28 days. Blood glucose, cholesterol, enzymatic and nonenzymatic antioxidants, lipid peroxides in blood plasma, and glycation of tail tendon collagen were measured. The rats fed the Korean millet (KM)-enriched diet showed a greater reduction in blood glucose (42%) and cholesterol (27%) than those fed the finger millet (36% and 13%).

Choi et al. (2005) examined the effects of intake of Korean foxtail millet protein (FMP) on plasma levels of lipid, glucose, insulin, and adiponectin in genetically type 2 diabetic KK-Ay mice and concluded that when mice were fed a normal FMP diet or a high-fat-high-sucrose diet containing FMP for 3 weeks, in both experiments plasma concentrations of HDL-cholesterol and adiponectin increased remarkably in comparison with a casein diet group, whereas concentrations of insulin decreased greatly and that of plasma glucose was comparable to that in the casein diet group. Considering the role of adiponectin, insulin, and HDL-cholesterol in diabetes, atherosclerosis, and obesity, it appears likely that FMP may improve insulin sensitivity and cholesterol metabolism through an increase in adiponectin concentration.

Naoyuki Nishizawa (2006) examined the effect of feeding of proso-millet protein concentrate (PMPC) for 21 days on plasma levels of HDL cholesterol, HDL subfractions and lecithin: cholesterol acyltransferase (LCAT) activities in rats and from the result they

concluded that PMPC could have a beneficial effect on cardiovascular disease because HDL2 subfractions may have the more strongly protective effect against the risk of coronary heart disease.

Diousse and Gaiano (2007) evaluated prospectively the association between breakfast cereal intake and incident HF among 21 376 participants of the Physicians' Health Study I and concluded that a higher intake of whole grain breakfast cereals is associated with a lower risk of HF.

Kyung-Ok et al. (2008) investigated the effect of dietary Korean proso-millet protein concentrate (PMP) on glycemic responses, plasma lipid levels, and the plasma level and gene expression of adiponectin in obese type II diabetic mice under normal and high-fat feeding conditions. The findings were that the feeding of PMP clearly elevated plasma high-density lipoprotein cholesterol (HDL cholesterol) and adiponectin levels and brought about effective reduction in the levels of glucose and insulin in mice under high-fat diet conditions as compared with a control diet.

Nishizawa et al. (2009) investigated the effects of dietary Japanese millet on diabetic mice and concluded that feeding of a high-fat diet containing Japanese millet protein concentrate (JMP, 20% protein) to type II diabetic mice for 3 weeks significantly increased plasma levels of adiponectin and HDL cholesterol and decreased the levels of glucose and triglyceride as compared to control.

Millet is a grain that should also be included in the list of heart-healthy choices because of its status as a good source of magnesium. Magnesium has been shown in studies to reduce the severity of asthma and to reduce the frequency of migraine attacks. Magnesium has also been shown to lower high blood pressure and reduce the risk of

heart attack, especially in people with atherosclerosis or diabetic heart disease. Niacin (vitamin B3) can be of help in lowering high cholesterol. Phytonutrient abundant in millet are plant lignans, which are converted by friendly flora in our intestines into mammalian lignans, including one called enterolactone that is thought to protect against heart disease (Anderson et al, 2000).

2.3 ROLE OF FIBRE IN CARDIOVASCULAR DISEASE

The National Cholesterol Education Programme (NCEP) guidelines have formed the basis internationally for treatment of hyperlipidemia (NCEP, 1993). Dietary guidelines emphasized to reduce saturated fat, cholesterol and body weight. So an important question was therefore what dietary additions to the current lipid lowering strategy can be used to enhance the effectiveness of low saturated fat diets.

The possible health benefits of dietary fibre in reducing the risk of chronic diseases have been hypothesized since the ground breaking observations of Bakhit et al, 1994.

Soluble fibre and vegetable proteins have been shown independently to reduce serum cholesterol. A combination of soluble fibre and a moderate intake of vegetable protein with low saturated fat diet reduced both LDL-cholesterol and the ratio of LDL-C: HDL-C (Jenkins et al. 1999).

Bell et al. (1990) examined cholesterol lowering effect of soluble fibre breakfast cereals in fifty eight male patients with mild to moderate hypercholesterolemia. Patients followed a step 1 diet for a minimum of 6 weeks; then randomly assigned to groups incorporating either corn flakes or one of the two soluble fibre cereals (Pectin or psyllium

enriched) in the diet for additional 6 weeks. They found that during the only diet phase, total cholesterol dropped by 38%. During cereal-plus diet phase, total and LDL-cholesterol values of pectin enriched group dropped by an additional 2.1% and 3.9% respectively but 5.9% and 5.7% respectively in psyllium group. During cereal plus diet phase, no significant effect on HDL-cholesterol, triglyceride or body weights were found within or between the groups.

Arjmandi et al. (1992) studied whether soluble fibre and cholesterol influence in vivo hepatic and intestinal cholesterol biosynthesis. Ninety six male Sprague-Dawley rats were randomly assigned to eight dietary treatments. Rats were fed with ad libitum access, diets containing 10% dietary fibre as cellulose (control), pectin, psyllium or oat bran with or without 0.3% cholesterol for 3 weeks. Pectin and psyllium were found to reduce liver and serum total cholesterol significantly whereas oat bran was ineffective. Hepatic sterol synthesis was significantly greater in soluble fibre groups than cellulose, but the effect on intestinal sterol synthesis was less pronounced.

Fernandez et al. (1992) reported that prickly pear pectin reversed LDL-receptor suppression induced by a hypercholesterolemic diet in guinea pigs fed with either a diet containing 15 g/100 g lard and 0.25 g/100 g cholesterol or the low cholesterol diet in which cellulose was partially replaced (2.5 g/100 g) by prickly pear pectin. They found 46% and 64% reduction in hepatic free and esterified cholesterol in prickly pear pectin diet. A marked reduction of 33% in LDL- cholesterol was also noticed during that diet phase. Their data indicated an increase in apo B/E receptor expression was a major metabolic response by which intake of prickly pear pectin decreased plasma LDL concentrations.

Hood and Sidhu (1992) studied the effect of guar gum and tocotrienols on cholesterol metabolism on the Japanese quail. Their result indicated that both guar gum and tocotrienols are effective in reducing blood cholesterol of young quails but ineffective in case of matured quail.

Abbey et al. (1993) fed male rats with non-polysaccharides pectin, methylcellulose or guar gum with corn oil or with 60% of corn oil replaced fish oil. They found lower value of plasma cholesterol in case of pectin group. LDL-receptor activity was higher in case of rats fed with pectin + fish oil or pectin + fish oil + cholesterol than rats fed with pectin only.

Gallaher et al. (1993) experimented the effect of viscosity and fermentability on plasma and liver cholesterol levels. Hamsters were fed with 0.12% cholesterol and 5% fibre as high viscosity hydroxypropyl methyl cellulose (HV-HPMC), low viscosity hydroxypropyl methyl cellulose (LV-HPMC), high viscosity guar gum (HV-GG) or low viscosity guar gum (LV-GG). Their study showed that greater viscosity of intestinal contents was strongly associated with cholesterol reduction but contribution of fibre fermentation remained uncertain. HV-HPMC group had highest plasma cholesterol reduction than LV-HPMC and HV-GG groups.

Jenkins et al. (1993) studied the effect of very high intake of fibre in diets low in saturated fat and cholesterol on blood lipids. They studied forty three volunteers with hyperlipidemia in a cross over study involving two four-month dietary periods. The two metabolic diets containing foods in either soluble or insoluble fibre and were separated by a two month NCEP step-2 diet. The metabolic diets were low in saturated fat and cholesterol, high in carbohydrate (≥60% of total calories) and very high in fibre (>24

g/1000 kcal). Their results indicated that blood lipids fell to their lowest levels by 9th week. When diet with soluble fibre was compared with diet with insoluble fibre, the subjects total cholesterol, LDL, HDL levels were found to be lower by a mean of 4.9±0.9 per cent, 4.8±1.3% and increase by 3.4±1.3% respectively and excretion of fecal bile acid was also increased.

Anderson et al. (1994) observed that ten different dietary fibres have significantly different effects on serum and liver lipids by feeding male Sprague-Dawley rats with diet containing 10g cholesterol + 2g cholic acid/kg diet + 60g fibre/kg diet. They reported that rats fed with psyllium had the lowest serum and liver cholesterol than others. Values for serum and liver cholesterol were similar for rats fed insoluble rich fibres (corn bran, cellulose and wheat bran). Their observations indicated that feeding soluble fibre rich diet produced lower serum and liver cholesterol than commonly available sources of insoluble fibre.

Fernandez et al. (1994) reported that citrus pectin and cholesterol interact to regulate hepatic cholesterol homeostasis and lipoprotein metabolism. Guinea pigs were fed with increasing concentrations of citrus pectin with low or high cholesterol. They found that with high cholesterol diet, plasma LDL-cholesterol reduced in a dose response manner by 29%, 30% and 67% with 7.5%, 10% and 12.5% citrus pectin intake respectively.

Fernandez et al. (1995) reported that intake of 12.5% guar gum significantly altered endogenous cholesterol metabolism by decreasing hepatic cholesterol pools, altering hepatic cholesterol homeostasis and reducing plasma LDL concentrations.

Fernandez (1995) investigated the LDL-cholesterol lowering effects of pectin, guar gum and psyllium. He found that intake of pectin, guar gum or psyllium with high cholesterol diets results in faster plasma LDL fraction catabolic rate (FCR) with no effect on LDL apo B flux rates or pool size, suggesting that fibre reduced LDL-cholesterol without decreasing the number of LDL particles. Cholesterol absorption was reduced by 30% with pectin diet.

Lia et al. (1995) investigated the effect of oat β-glucan on bile acid excretion and barley fraction on cholesterol excretion in ten ileostomy subjects. They found that oat β-glucan (3.8 g β-glucan/d) increased bile acid excretion and fibre rich barley fraction increased cholesterol excretion.

Shane and Walker (1995) reported that corn bran supplementation of a low fat controlled diet lowers serum lipids in men with hypercholesterolemia. Their results indicated that low fat controlled diet significantly lowered all serum parameters except HDL-cholesterol whereas corn fibre supplementation resulted in additional lowering of serum total cholesterol, triglycerides, VLDL-cholesterol and LDL-cholesterol.

Pirjo et al. (1996) conducted a study on intake of dietary fiber and risk of coronary heart disease in a cohort of Finnish men. Results suggested that independent of other risk factors, greater intake of foods rich in fiber can substantially reduce the risk of coronary heart disease, and particularly death due to coronary heart disease, in middle-aged, smoking men.

National Institute of Nutrition (1998) reported that fibre is more effective in reducing serum cholesterol levels ranging from 10 to 24 per cent. It is less effective in subjects whose intake of fat is high, especially saturated fat. Replacement of SFA with

carbohydrates from grains, vegetables, legumes and fruits reduces total and LDL-C, with only a minor effect on HDL-C and triacylglycerols and modest weight reduction (Turley *et al*., 1998).

David et al. (1999) examined the role of fiber consumption and its association with insulin levels, weight gain, and other CVD risk factors compared with other major dietary components. Results showed that fiber consumption lowered insulin levels, weight gain, and other CVD risk factors more strongly than did total or saturated fat consumption. High-fiber diets may protect against obesity and CVD by lowering insulin levels.

Liu et al. (2002) conducted a study to examine the hypothesis that higher intake of dietary fiber is inversely related to the risk of cardiovascular disease (CVD) and myocardial infarction (MI) in a large prospective cohort of women and concluded that a higher intake of dietary fiber was associated with a lower risk of CVD and MI, although the association was not statistically significant after further adjusting for multiple confounding factors.

Mark et al. (2004) conducted a study to estimate the association between dietary fiber intake and the risk of coronary heart disease and found that consumption of dietary fiber from cereals and fruits is inversely associated with risk of coronary heart disease.

Estruch et al. (2009) studied the effects of dietary fibre intake on risk factors for cardiovascular disease in subjects at high risk and concluded that increasing dietary fibre intake with natural foods is associated with reductions in classical and novel cardiovascular risk factors in a high-risk cohort.

Flaxseed and samai contain soluble fiber that has been associated with increased diet quality and decreased risk of cardiovascular disease. Soluble or viscous fibers

modestly reduce LDL cholesterol beyond levels achieved by a diet low in saturated and trans fatty acids and cholesterol alone.

CHAPTER III

MATERIALS AND METHODS

The present study was carried out in two parts.

1. Standardization of low fat baked products with different levels of incorporation with flaxseed and samai and
2. Study the impact of supplementation on the lipid profile of subjects before, during and after supplementation.

The details of the methodology followed in the study are presented under the following heads :

3.1 Procurement of Flaxseed and Samai.

3.2 Product Standardization.

3.3 Testing product acceptability.

3.4 Selection of subjects and supplementation.

3.5 Assessment of lipid profile before, during and after supplementation.

3.6 Statistical analysis.

3.1 PROCUREMENT OF FLAXSEED AND SAMAI

Flaxseed and samai were procured in bulk from whole sale market, Hyderabad. Flaxseed and samai thus obtained were cleaned by sieving and hand-picking to remove all dirt/foreign material. Plate 1 and 2 shows whole raw flaxseed and samai.

3.2 PRODUCT STANDARDIZATION

Some common bakery products were selected and prepared by incorporating ground flaxseed at 10, 20 and 40 percent levels. Fifty per cent of refined flour and fifty

Plate 1 : Whole raw flaxseed

Plate 2 : whole raw samai

Plate 3 : Ground flaxseed

Plate 4 : Samai flour

per cent of samai flour was taken as a base flour (table 4) since millet contains more of fibre and other nutrients which are beneficial than a refined flour i.e., maida. Plate 3 and 4 depicts the ground flaxseed and samai flour.

Products selected for experiment were bread, muffin and masala biscuit. Control product selected was the low fat marie biscuits available in the market.

Bakery products were initially prepared using standard recipes and later on low fat products were standardised using the same standard recipes.

Table 4 : Composition of flour

	Base flour (gm)		**Flaxseed (gm)**
	Refined flour (gm)	**Samai flour (gm)**	
Standard products	100	–	–
Experimental products			
10 %	45	45	10
20 %	40	40	20
40 %	30	30	40

Bread was excluded for its poor end product due to poor attributes. Among masala biscuits and muffins, two levels of masala biscuits were accepted viz., 40 and 20 percent respectively. Owing to subject's preference and ease of consumption 40 and 20 per cent levels of masala biscuits were selected for supplementation.

3.3 TESTING PRODUCT ACCEPTABILITY

A score card was developed in order to judge various sensory attributes viz., colour, flavour, texture, taste and overall acceptability of the products, with numerical scores assigned for each attribute (Peryam and Piligrim, 1957).

A panel of 21 trained judges had selected the most acceptable product using five point Hedonic scale to score each attribute with highest score of 5 and lowest score of 1 assigned to the most preferred and the least preferred characteristic respectively at two levels of incorporation of flaxseed.

The score card used for sensory evaluation is presented in appendix 1.

Recipes and cost of the products selected for supplementation is presented in appendix 2.

3.4 SELECTION OF SUBJECTS AND SUPPLEMENTATION

The present study was carried out with the cooperation of the staff of Acharya N. G. Ranga Agricultural University, both at Rajendranagar and College of Home Science, Hyderabad.

All the willing staff aged between 35-50 years were screened for total cholesterol out of which 21 subjects with total cholesterol above 155 mg/dl were selected for supplementation with prior consent of the subjects. Subjects who were at a risk of hyperlipidemia and hyperlipidemic subjects who were not on any drugs and free from other diseases were selected. Subjects with total cholesterol above 155 mg/dl (border level) were selected because subjects with plasma cholesterol above 200 mg/dl were on drugs due to which flaxseed and samai effect cannot be predicted due to drug interaction.

Based on the plasma total cholesterol levels the subjects (n=21) were randomly divided into three groups. One group was selected for supplementation with 20 per cent flaxseed incorporated biscuits, second group with 40 per cent flaxseed incorporated

biscuits and third group was selected as a control group.

Plate 5 : Masala biscuit with 20 per cent flaxseed

Plate 6 : Masala biscuit with 40 per cent flaxseed

Two biscuits, weighing 20 g each were given daily to each subject (Total 40 g) of two experimental groups, thus providing 8.0 g (Plate 5) and 16.0 g (Plate 6) flaxseed to Group I and Group II respectively for a period of two months. Small quantities of flaxseed was selected since high concentration of flaxseed cannot be consumed regularly and to avoid side effects from over dose. 40 g of low fat marie biscuit available in market was given to control group subjects.

Two biscuits were provided everyday to subjects and were allowed to eat at any time of the day.

3.5 ASSESSMENT OF LIPID PROFILE BEFORE, DURING AND AFTER SUPPLEMENTATION

Effect of supplementation of flaxseed and samai incorporated masala biscuits providing 20 and 40 per cent levels of flaxseed was assessed by analysis of total cholesterol initially and lipid profile at 30 and 60 days i.e., before, during and after supplementation.

3.5.1 Biochemical parameters

3.5.1.1 Collection of blood samples

Fasting blood sample (3 ml intravenous blood) was drawn from each subject by a trained technician into EDTS added tubes (Plate 7). Plasma was separated by centrifuging the blood samples (2000 – 2500 rpm, 10 minutes) within an hour of collection and transferred into plastic storage vials and stored at 2 - 8^{o}C until further analysis.

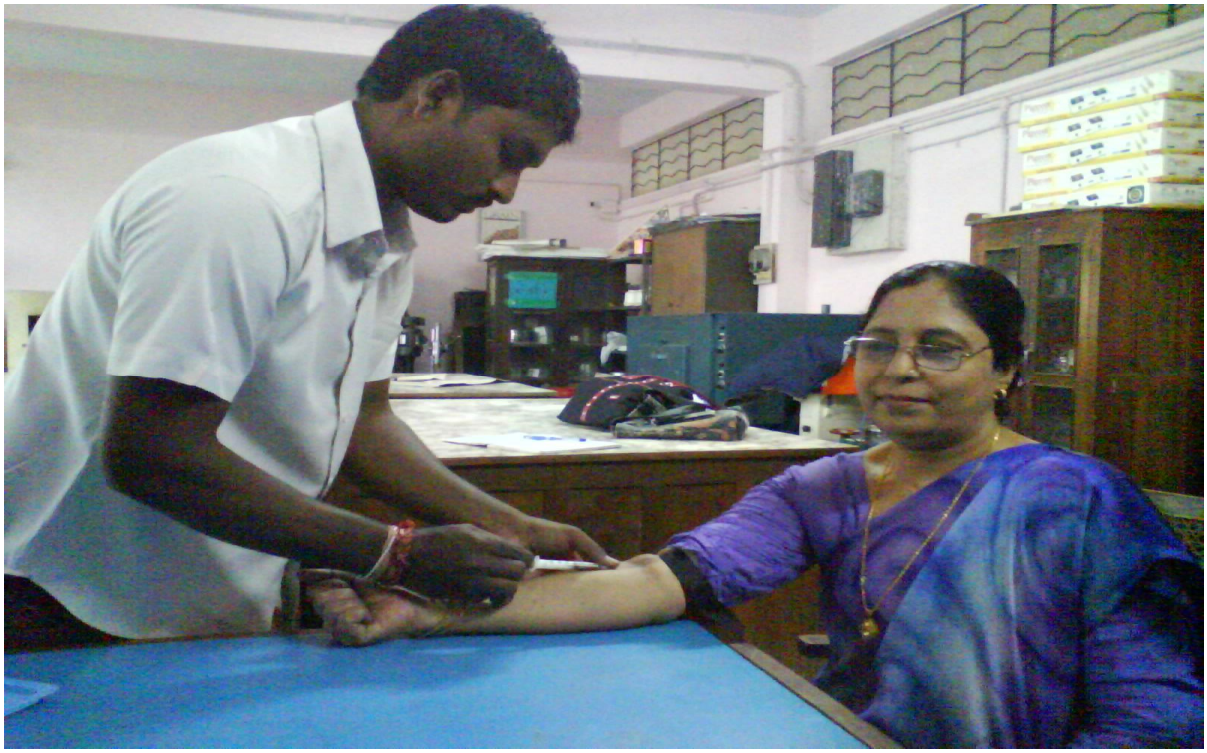

Plate 7 : Drawing of blood sample

Plate 8 : Administration of experimental biscuits

3.5.1.2 Analysis of plasma lipid profile

Initially subjects were screened based on total cholesterol level. Further plasma samples obtained from each subject during(after 30 days of supplementation) and after the supplementation were analysed for lipid profile which includes total cholesterol, HDL – cholesterol, LDL – cholesterol, VLDL cholesterol and triglyceride levels. Details of estimation are given in appendix 3.

Total Cholesterol : Total cholesterol was estimated by using cholesterol kit (enzymatic method) by the method given by Wybenga and Pillegi (1970).

HDL Cholesterol : HDL cholesterol was estimated by using HDL cholesterol kit using the method of Wybenga and Pillegi, 1970 (PTA precipitation and enzymatic method).

Triglyceride : Triglyceride was estimated using triglyceride kit by glycerol phosphate oxidase (GPO) method of Fossati and Lorenzo (1982).

LDL Cholesterol : LDL cholesterol was calculated using Friedwald's formula (Friedwald et al. 1972).

LDL (mg%) = Total cholesterol – HDL cholesterol – (Triglycerides/5)

VLDL Cholesterol : VLDL cholesterol was calculated using the formula

VLDL (mg%) = Total cholesterol – HDL cholesterol – LDL cholesterol

3.6 STATISTICAL ANALYSIS

The data was tabulated and subjected to statistical analysis at the end of the study for mean, standard deviation, paired t-test and ANOVA. Results were expressed as mean ± SD.

CHAPTER IV

RESULTS AND DISCUSSION

The present study was undertaken to develop low fat baked products incorporated with samai and flaxseed at different levels and to evaluate the effect of supplementation of selected product on serum lipid level of hypercholesterolemic subjects. The product selected was masala biscuit incorporated with 20 and 40 per cent flaxseed. Twenty one hypercholesterolemic subjects including borderline (>155 mg/dl) hypercholesterolemic were selected randomly and assigned to control and two experimental groups. First group of seven subjects were given 20 per cent flaxseed incorporated biscuits and second group of seven were given 40 per cent flaxseed incorporated biscuits and other seven were given control low fat marie biscuits.

The products developed were subjected to sensory evaluation.

The supplementation was carried out for a period of two months. Plasma lipid profile i.e. total cholesterol, triglyceride, HDL cholesterol, VLDL cholesterol and LDL cholesterol were analyzed before (0 day), during (after 30 days) and after supplementation (after 60 days). Results pertaining to the study conducted are presented under the following heads.

4.1 Standardization and nutritive value of the products developed.

4.2 Sensory evaluation of the products.

4.3 Screening of the subjects for supplementation.

4.4 Effect of supplementation on lipid profile of hypercholesterolemic subjects.

4.1 STANDARDIZATION AND NUTRITIVE VALUE OF THE PRODUCTS DEVELOPED

In the present study, the products like bread, muffin and biscuit were prepared. In all the products a combination of 50 per cent refined flour and 50 per cent of samai flour were used as the base. Flaxseed was incorporated at 10, 20 and 40 per cent levels.

Initially the products were prepared using a standard recipe and later on low fat products were standardized.

4.1.1 Bread

Initially bread was prepared from a standard recipe. Then experimental products were prepared by incorporating 10, 20 and 40 per cent flaxseed flour in base flour. The nutritive value of standard recipe and experimental recipe is presented in table 4.

Table 5 : Nutrient composition of standard bread and experimental bread/100 g.

Nutrient composition	Standard bread	Experimental bread		
		10 % flaxseed	20 % flaxseed	40 % flaxseed
CHO (g)	75.90	68.34	64.14	55.83
Protein (g)	11.00	10.45	11.54	13.73
Fat (g)	12.90	18.23	21.66	28.52
Crude fiber (g)	0.30	4.03	4.12	4.29
Energy (K.Cal)	464	479	497	534

Table 5. indicates that the CHO (carbohydrate) content of standard bread was high i.e. 75.9 g than that of experimental breads with 10 per cent, 20 per cent and 40 per cent flaxseed flour i.e. 68.34 g, 64.14 g and 55.83 g respectively. The protein content of

standard bread was 11.0 g and experimental bread at 10 per cent, 20 per cent and 40 per cent flaxseed flour had 10.45 g, 11.54 g and 13.73 g respectively.

Fat and crude fiber content of standard bread was lower i.e. 12.90 g fat and 0.30 g fiber than experimental breads i.e. 18.23 g (10%), 21.66 g (20%) and 28.52 g (40%) of fat and 4.03 g (10%), 4.12 g (20%) and 4.29 g (40%) of crude fiber. The energy content of standard bread was lower i.e. 464 KCal than experimental breads i.e. 479 KCal, 497 KCal and 534 KCal in 10, 20 and 40 per cent flaxseed flour respectively.

4.1.2 Muffin

Nutrient composition of standard muffin and experimental muffin are presented in table 6.

Table 6 : Nutrient composition of standard muffin and experimental muffin/100 g.

Nutrient composition	Standard muffin	Experimental muffins		
		10 % flaxseed	20 % flaxseed	40 % flaxseed
CHO (g)	166.40	116.34	112.14	103.83
Protein (g)	24.0o	10.45	11.54	13.73
Fat (g)	82.38	46.23	49.66	56.52
Crude fiber (g)	0.30	4.03	4.12	4.29
Energy (K.Cal)	1494	928	946	983

From the above table it is revealed that standard muffin has higher content of CHO (carbohydrate), protein, fat and energy and very little crude fiber than experimental muffins.

The carbohydrate content of standard muffin were higher i.e. 166.40 g than experimental muffins i.e. 116.34 g (10%), 112.14 g (20%) and 103.83 g (40%). The protein and fat content of standard muffin are higher i.e. 24.00 g protein and 82.38 g fat than experimental muffins i.e. 10.45 g (10%), 11.54 g (20%) and 13.73 g (40%) of protein and 46.23 g (10%), 49.66 g (20%) and 56.52 g (40%) of fat.

Standard muffin contained very little crude fiber i.e. 0.30 g whereas the experimental muffins i.e. 4.03 g (10%), 4.12 g (20%) and 4.29 g (40%) were similar to experimental bread. The energy content of standard muffin was higher i.e. 1494 KCal than experimental muffins i.e. 928 KCal (10%), 946 KCal (20%) and 983 KCal (40%).

4.1.3 Masala Biscuit

Among different varieties of biscuits, masala biscuit was selected since it contains medium amounts of salt and sugar which does not have much effect on blood glucose and blood pressure and can be consumed daily because of spices added for taste. The nutrient composition of standard biscuit and experimental biscuit are presented in table 7.

Table 7 : Nutrient composition of standard biscuit and experimental biscuit/100 g

Nutrient composition	Standard masala biscuit	Experimental masala biscuits		
		10 % flaxseed	20 % flaxseed	40 % flaxseed
CHO (g)	94.39	86.83	82.63	74.32
Protein (g)	11.25	10.70	11.80	14.00
Fat (g)	41.06	26.4	29.82	36.68
Crude fiber (g)	0.52	4.26	4.34	4.51
Energy (K.Cal)	792	627	646	683

The above table shows that the carbohydrate and fat content of standard biscuit was higher i.e. 94.39 g carbohydrate and 41.06 g fat than experimental biscuits i.e. 86.83 g (10%), 82.63 g (20%) and 74.32 g (40%) of carbohydrate and 26.40 g (10%), 29.82 g (20%) and 36.68 g (40%) of fat.

Protein content of standard biscuit is 11.25 g and experimental biscuits 10.70 g (10%), 11.80 g (20%) and 14.00 g (40%). Standard biscuit contained very little crude fiber i.e. 0.52 g whereas experimental biscuits had 4.26 g (10%), 4.34 g (20%) and 4.51 g (40%) similar to experimental bread and muffin. The energy content of standard biscuit was higher i.e. 792 KCal than experimental biscuits i.e. 627 KCal (10%), 646 KCal (20%) and 683 KCal (40%). Therefore it shows that the experimental biscuits had moderate protein, higher fiber and lower fat and energy than the standard recipe which is advantageous for the hypercholesterolemic patients.

Nutrient composition of all the products revealed that standard recipes had higher content of carbohydrate, fat and energy with very low fiber content as compared to experimental recipes. This is due to substitution of half quantity of base flour with samai flour, incorporation of flaxseed and lower quantity of fat and sugar.

In experimental products as there was increase in flaxseed incorporation i.e. from 10 per cent to 20 per cent and then to 40 per cent, there was a gradual increase in protein and crude fiber.

4.2 SENSORY EVALUATION OF THE PRODUCTS

All the experimental products developed except bread were evaluated for various sensory characteristics by a selected panel of trained judges. Bread was discarded because of poor end product quality.

The mean scores of sensory evaluation of experimental products developed are presented in table 8.

The results revealed that highest scores were obtained for T3 followed by T2 i.e. among masala biscuits and muffins, 40 and 20 per cent of flaxseed incorporated masala biscuits were accepted. Though an overall significant difference ($P<0.05$ and $P<0.01$) was found in all sensory characteristics for all products, significant difference was not observed among each of the sensory characteristics for individual products.

Table 8 : Sensory mean scores obtained for experimental products.

Characteristics	Masala Biscuits			Muffins			CD (Critical difference)	F-Ratio
	T1	T2	T3	T4	T5	T6		
Color	3.3 ± 0.80^{ab}	3.8 ± 0.74^{a}	3.7 ± 0.64^{a}	3.0 ± 0.83^{bc}	2.7 ± 0.98^{c}	2.1 ± 0.85^{d}	0.5	13.20
Flavor	3.2 ± 0.81^{b}	3.6 ± 0.69^{ab}	3.7 ± 0.66^{a}	2.7 ± 0.47^{cd}	2.7 ± 0.75^{d}	2.2 ± 0.77^{e}	0.4	13.66
Texture	3.2 ± 0.75^{b}	3.7 ± 0.73^{a}	3.9 ± 0.67^{a}	2.7 ± 0.66^{cde}	2.6 ± 0.83^{de}	2.3 ± 0.91^{e}	0.47	14.4
Taste	3.1 ± 0.55^{bc}	3.9 ± 0.55^{a}	3.8 ± 0.37^{a}	2.9 ± 0.67^{cd}	2.6 ± 0.83^{de}	2.3 ± 0.85^{e}	0.4	21.12
Overall acceptability	3.0 ± 0.73^{bc}	3.7 ± 0.49^{a}	4.0 ± 0.60^{a}	2.8 ± 0.72^{cd}	2.5 ± 0.69^{de}	2.3 ± 0.64^{e}	0.4	20.88

Values are mean ± SD. Values with different superscripts are significantly different at 5% level.
T1 and T4 - 10% GF + 45% SF + 45% RF (10% GF + 90% BF)
T2 and T5 - 20% GF + 40% SF + 40% RF (20% GF + 80% BF)
T3 and T6 - 40% GF + 30% SF + 30% RF (40% GF + 60% BF)
Note:
GF – ground flaxseed; SF – samai flour; RF – refined flour; BF – base flour.

A significant difference was found between two accepted products (T2 and T3) and T4, T5 and T6 in all the sensory characteristics. In flavor, significant difference was found only among T1 and T3 and not between T1 and T2. But a significant difference was found in other sensory characteristics i.e. texture, taste and overall acceptability among accepted products and T1.

Among all the experimental products 20 and 40 per cent flaxseed incorporated biscuits i.e. T2 and T3 obtained highest score for appearance, flavor, texture, taste and overall acceptability. Therefore these two products were selected for supplementation.

4.3 SCREENING OF THE SUBJECTS FOR SUPPLEMENTATION

Selection of the subjects for the present study was carried out amongst the staff of Acharya N. G. Ranga Agricultural University, Rajendranagar and College of Home Science, Saifabad, Hyderabad by screening them for their total cholesterol levels.

Screening was done to select the subjects with total cholesterol above 155 mg/dl, age within the range of 35-50 year who were free from diabetes and not using lipid altering medications.

With brief information of the study, thirty six subjects were screened for total cholesterol. Among thirty six subjects, 21 subjects had total cholesterol above 155 mg/dl. Further with willingness and interest of subjects, twenty one subjects were selected for supplementation. Informed consent was obtained from them for the study.

Subjects were randomly assigned to three groups i.e. control, experimental group I and experimental group II (n=7). Experimental group I was given 20 per cent flaxseed incorporated biscuits and experimental group II was given 40 per cent flaxseed incorporated biscuits. Forty gram biscuits i.e. 2 biscuits each weighing 20 g were given

daily and subjects were allowed to consume at any time of the day for a period of two months. Control group was given 40 g low fat marie biscuits i.e. 4 biscuits. Nutrient composition of biscuits are presented in table 9.

Table 9 : Nutrient composition of control and experimental biscuits/40g

Nutrient composition	Control biscuit	Experimental biscuit of group I	Experimental biscuit of group II
CHO (g)	15.40	33.05	29.73
Protein (g)	1.60	4.72	5.60
Fat (g)	2.10	11.93	14.67
Crude fiber (g)	-	1.74	1.80
Energy (KCal)	87	258	273

4.4 EFFECT OF SUPPLEMENTATION ON LIPID PROFILE OF HYPERCHOLESTEROLEMIC SUBJECTS

Initially only total cholesterol was estimated, lipid profile i.e. TC (total cholesterol), HDL(high density lipoprotein), LDL(low density lipoprotein), VLDL(very low density lipoprotein) and TG(triglyceride) were estimated during and after supplementation period.

4.4.1 Total Cholesterol

The table 10. shows the difference in total cholesterol levels of experimental groups and control group at different time periods.

The mean values of plasma total cholesterol levels in control and experimental groups during and after the supplementation are shown in fig. 1.

Table 10: Effect of supplementation with 20 % and 40 % flaxseed incorporated biscuits on total serum cholesterol levels of subjects.

Subjects	Experimental group I *					Experimental group II *					
Period of supplementation	Initial (mg/dl) P_1	During (mg/dl) P_2	% difference after 1 month	After (mg/dl) P_3	% difference at the end of experimental period	Initial (mg/dl) P_1	During (mg/dl) P_2	% difference after 1 month	After (mg/dl) P_3	% difference at the end of experimental period	Initial (mg/dl) P_1
1	159	156	1.9	152	4.4	189	174	8.0	159	15.9	172
2	157	166	-5.7	154	1.9	188	172	8.5	166	11.7	179
3	178	176	1.1	174	2.2	172	158	8.1	152	11.6	162
4	168	166	1.1	156	7.1	205	182	11.2	178	13.2	205
5	176	188	-5.6	182	-3.4	176	169	4.0	160	9.1	182
6	184	180	2.2	173	6.0	168	152	9.5	159	5.4	158
7	176	172	2.3	169	4.0	178	164	7.9	157	11.8	198
Mean ± SD	171.14 ± 10.14	172.0 ± 10.52	-0.5 %	165.71 ± 11.67	3.2 %	182.29 ± 12.66	167.29 ± 10.14	8.2 %	161.57 ± 8.34	11.4 %	179.43 ± 17.43

'*' indicates significant difference at 5 % level ($P<0.05$)

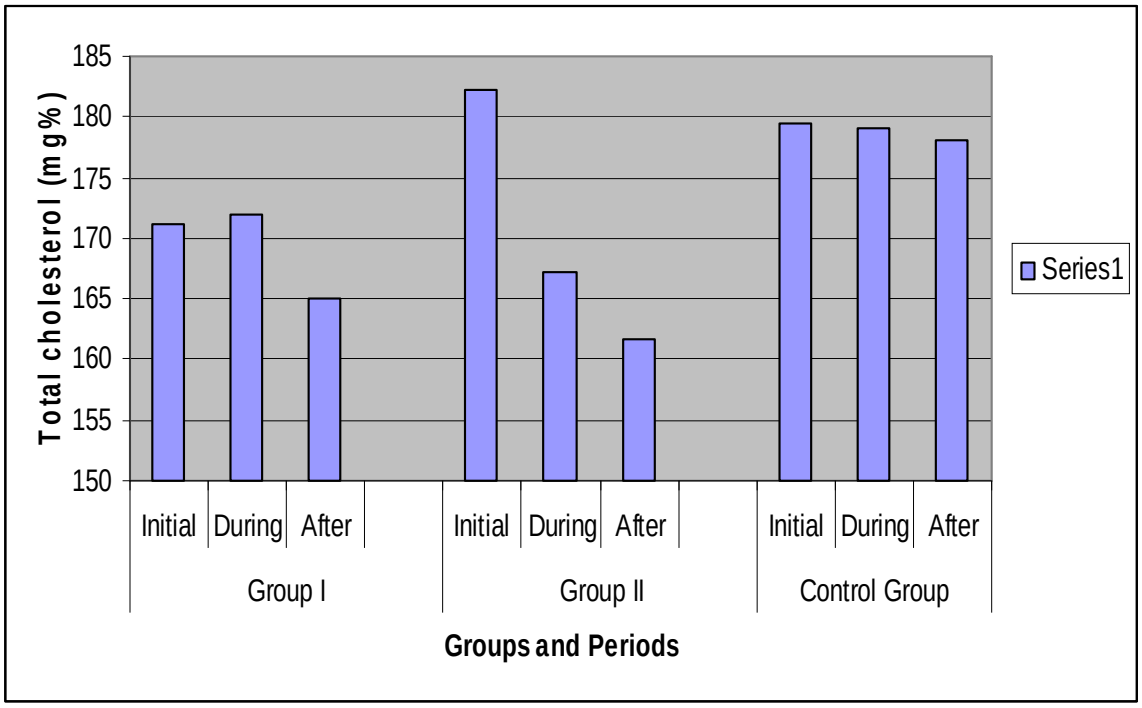

Fig. 1: Mean plasma total cholesterol (mg%) levels initial, during and after supplementation

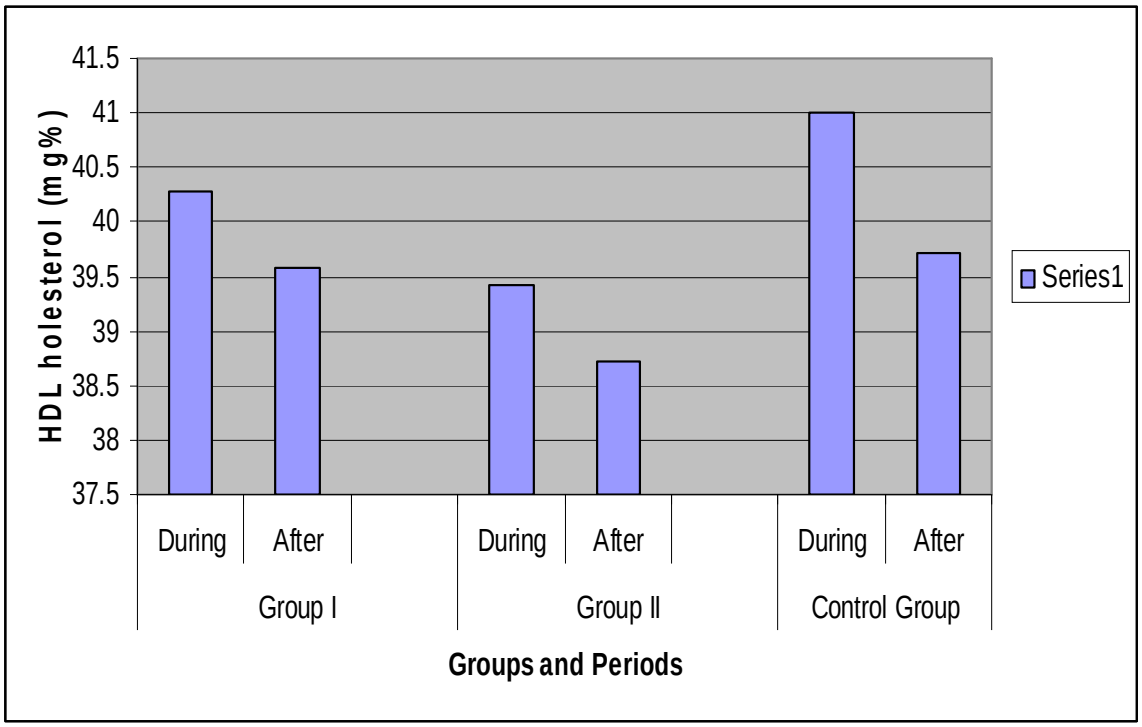

Fig. 2: Mean plasma HDL cholesterol (mg/dl) levels during and after supplementation

In experimental group I, a slight increase in total cholesterol level was observed after 30 days i.e. 0.5 per cent. This increase was due to stress and high fat non-vegetarian food consumption from three subjects. These subjects were counseled to reduce stress and fat consumption. After 60 days a significant decrease ($P<0.05$) of 3.2 per cent from initial cholesterol level was observed. The mean initial value of 171.14 mg/dl increased slightly ($P>0.05$) to 172.0 mg/dl after 30 days but reduced further significantly ($P<0.05$) to 165.71 mg/dl after 60 days of supplementation.

In experimental group II, a significant decrease ($P<0.05$) of 8.2 and 11.4 per cent from initial cholesterol level was observed after 30 and 60 days of supplementation respectively. The mean initial value of 182.29 mg/dl reduced significantly ($P<0.05$) to 167.29 mg/dl after 30 days and reduced further significantly ($P<0.05$) to 161.57 mg/dl at the end of supplementation. Whereas in case of control group only slight reduction of 0.2 and 0.7 per cent of the initial cholesterol level was observed after 30 and 60 days of supplementation respectively. In control group slight decrease in the mean value from 179.43 mg/dl to 179.14 mg/dl after 30 days and 178.14 mg/dl after 60 days of supplementation was observed. A significant difference was found after 30 and 60 days of supplementation in both experimental groups but not in control group.

The reason for the reduction of total cholesterol levels could be the presence of alpha linolenic acid (ALA) in flaxseed or an effect of dietary flaxseed on reducing the intake of saturated fat. Flaxseed mucilage might also be hypocholesterolemic as observed with other soluble fibres (Jenkins et al. 1987). The reduction in total cholesterol observed in the present study may be related to the lipid changes observed with other viscous fibre sources due to greater fecal losses of bile acid and primary bile acid synthesis.

Phytic acid present in flaxseed (23-33 g of phytic acid per kg of meal) is a natural plant inositol hexaphosphate commonly found in seeds and represents the principal form of stored phosphate. Phytic acid has been reported to have hypocholesterolemic effects (Thompson, 1994).

The results of present study revealed that supplementation of 8 and 16 g flaxseed/day for a period of two months to subjects with total cholesterol above 155 mg/dl caused an overall reduction of 3.2 per cent (experimental group I) and 11.4 per cent (experimental group II) respectively in the plasma total cholesterol concentration.

Jenkins et al. (1999) studied the health aspects of partially defatted flaxseed on serum lipid of hyperlipidemic subjects, and found that 3 weeks of flaxseed supplementation significantly reduced total cholesterol (5.5 ± 1.2%).

4.4.2 HDL Cholesterol

Plasma HDL cholesterol values of the subjects during and at the end i.e. after one month and two month are presented in table 11.

The mean values of plasma HDL cholesterol levels in control and experimental groups during and after the supplementation are shown in fig. 2.

HDL cholesterol level decreased by about 1.8 per cent in experimental groups I and II and 3.1 per cent in control group. However Table 11 and fig. 2 clearly depict that flaxseed supplementation showed no significant change ($P<0.05$) in HDL cholesterol levels in any of the experimental groups and control group.

Similar findings have been reported in other studies. One of the similar study conducted by Bierenbaum et al. (1993) on the effect of supplementation of three slices of flaxseed containing bread i.e. 15 g ground flaxseed for a period of 3 months on serum

Table 11: Plasma HDL cholesterol levels of subjects during and after supplementation

Subjects	Experimental group I			Experimental group II			
	During (mg/dl)	After (mg/dl)	% difference	During (mg/dl)	After (mg/dl)	% difference	Durin (mg/d
1	33	32	-3.03	37	32	-13.5	46
2	44	41	-6.82	40	41	2.5	44
3	41	41	0	40	38	-5.0	37
4	36	38	5.56	48	44	-8.33	32
5	42	42	0	37	34	-8.11	53
6	46	41	-10.87	36	38	5.66	34
7	40	42	5.0	38	34	-10.53	41
Mean ± SD	40.29 ± 4.50	39.57 ± 3.40	-1.8 %	39.43 ± 4.08	38.71 ± 4.27	-1.8 %	41.0 ± 7.3

'-' indicates decrease in HDL cholesterol level

lipid levels in 15 hyperlipidemic subjects on long term intake of vitamin E. A highly significant drop in serum cholesterol (18 mg/dl) and LDL- cholesterol (19 mg/dl) levels was observed. HDL cholesterol levels did not change with flaxseed consumption.

4.4.3 LDL Cholesterol

Table 12. shows the difference in LDL cholesterol levels of experimental groups and control group at different time periods i.e. during (after one month) and after (after two month) supplementation.

Fig.3. depicts the mean values of plasma LDL cholesterol levels of both experimental groups and control group during and after supplementation.

In LDL cholesterol level, significant decrease ($P<0.05$) of 4.36 and 6.79 per cent was observed from 30^{th} day to last day of supplementation in experimental group I and II respectively. The mean LDL cholesterol level on 30^{th} day, 104.71 mg/dl, significantly ($P<0.05$) reduced to 100.14 mg/dl after 60 days of supplementation in experimental group I. Similarly there was a significant decrease ($P<0.05$) from 105.14 mg/dl to 98.0 mg/dl in the mean LDL cholesterol levels of experimental group II.

In control group only a slight increase was observed in LDL cholesterol of 0.72 per cent from 30^{th} day to last day of supplementation (60^{th} day). The mean value of 99.43 mg/dl after 30 days slightly decreased to 98.71 mg/dl after 60 days of supplementation.

As mentioned earlier, flaxseed is a rich source of mammalian lignan precursors. Lignans modulate the activities of cholesterol -7-α-hydroxylase and acyl CoA cholesterol transferase. Because the former enzyme is involved in the synthesis of bile acids from cholesterol, lignans may also lead to altered cholesterol catabolism and could thereby

Table 12: Plasma LDL cholesterol levels of subjects during and after supplementation

Subjects	Experimental group I *			Experimental group II *			
	During (mg/dl)	After (mg/dl)	% difference	During (mg/dl)	After (mg/dl)	% difference	Durin (mg/d
1	100	95	5.0	113	102	9.74	98
2	96	88	8.33	105	96	8.57	103
3	107	105	1.87	97	94	3.09	90
4	102	101	0.98	125	109	12.8	113
5	117	111	5.13	109	100	8.26	99
6	107	102	4.67	91	97	-6.59	97
7	104	99	4.81	96	88	8.33	96
Mean ± SD	104.71 ± 6.68	100.14 ± 7.31	4.36 %	105.14 ± 11.7	98.0 ± 6.61	6.79 %	99.43 ± 7.14

'*' indicates significant difference at 5 % level ($P<0.05$)
'-' indicates increase in LDL cholesterol level

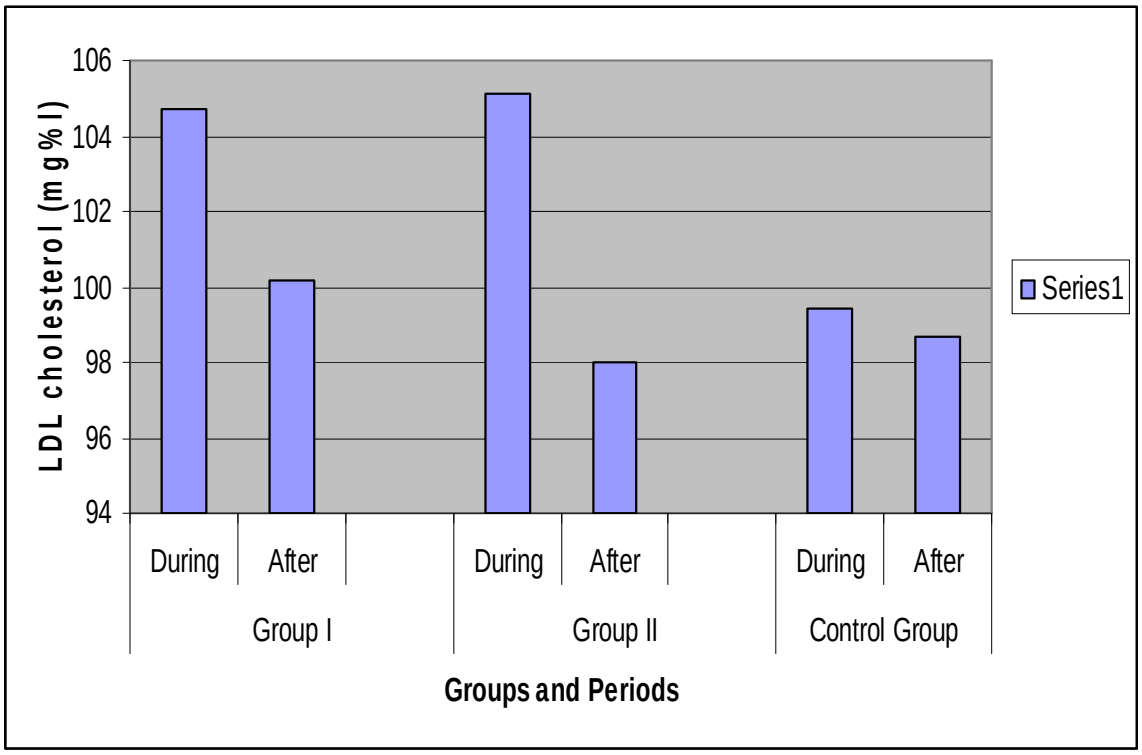

Fig. 3: Mean plasma LDL cholesterol (mg%) levels during and after supplementation

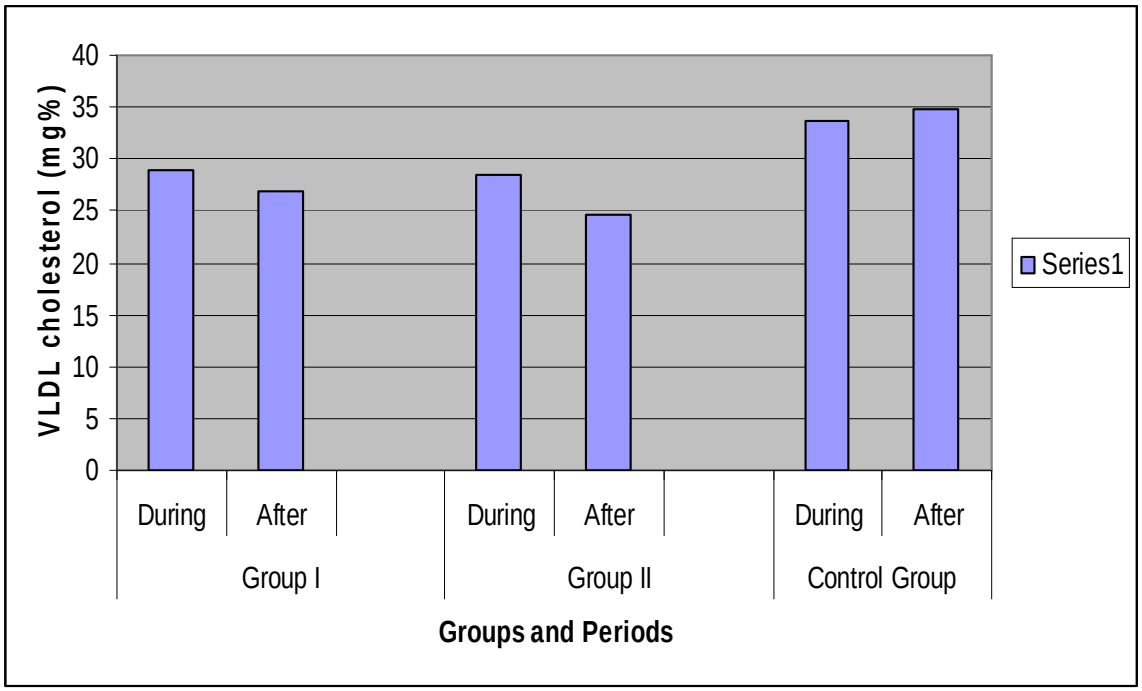

Fig. 4: Mean plasma VLDL cholesterol (mg%) levels during and after supplementation

contribute to the modest flaxseed- induced reduction in plasma LDL cholesterol reported here. The reduction in plasma LDL cholesterol may have been achieved in part through increased fecal excretion of bile salts (Thompson et al. 1991).

The flaxseed mucilage may also have a role in hypolipidemic effect due to physico-chemical properties like water-holding capacity, apparent solubility, binding ability, degradability and particle size. The hypocholesterolemic effects of flaxseed gum may also be due to the high production of short-chain fatty acids from fermentation in the colon (Glore et al 1994).

Cunnane et al. (1994) studied the lipid profile in healthy young adults by supplementing 50 g flaxseed per day for 4 weeks. During that period α-linolenate increased significantly in adipose tissue and plasma n-3 polyunsaturated fatty acids increased significantly. Plasma LDL reduced by 8%. It was concluded that the traditional flaxseed had modest beneficial effect on several indices of nutritional status without compromising antioxidant status.

Arjmandi et al. (1998) reported that 38 g flaxseed supplemented in the form of bread and muffins to postmenopausal women for a period of 6 weeks significantly lowered LDL cholesterol (14.7%).

These studies revealed that increasing the flaxseed per cent had increased the LDL cholesterol reduction.

4.4.4 VLDL Cholesterol

The intermittent and final values of plasma VLDL cholesterol concentration of subjects are presented in table 13.

Table 13: Plasma VLDL cholesterol levels of subjects during and after supplementation

Subjects	Experimental group I			Experimental group II *			
	During (mg/dl)	After (mg/dl)	% difference	During (mg/dl)	After (mg/dl)	% difference	Durin (mg/d
1	26	25	3.85	39	25	35.9	28
2	26	25	3.85	27	25	7.4	32
3	28	28	0	24	24	0	35
4	31	26	16.13	32	24	25.0	43
5	29	29	0	27	26	3.7	30
6	31	26	16.13	25	24	4.0	27
7	31	30	3.23	25	25	0	41
Mean ± SD	28.86 ± 2.27	27.00 ± 2.0	6.45 %	28.43 ± 5.35	24.71 ± 0.76	13.09 %	33.7 ± 6.2

'*' indicates significant difference at 5 % level ($P<0.05$)
'-' indicates increase in VLDL cholesterol level

The mean values of plasma VLDL cholesterol levels in different groups during and after supplementation are illustrated in fig. 4.

With regard to plasma VLDL cholesterol level table 13. reveals that a significant difference ($P<0.05$) was observed only in experimental group II but not in experimental group I and control group. There was a reduction of 6.45 per cent in plasma VLDL cholesterol levels with mean value reduction from 28.86 mg/dl to 27.00 mg/dl in experimental group I but significant difference was not found. A higher per cent reduction of 13.09 per ecnt was observed in VLDL cholesterol levels of experimental group II with mean value reduction from 28.43 mg/dl to 24.71 mg/dl than other two groups. Whereas in control group no decrease in VLDL cholesterol level was seen inturn 3.41 per cent increase in VLDL cholesterol level was observed.

This observation is consistent with findings reported by other researchers. Among these one of the study conducted by Meeta Maheshwari (2002) on the effect of supplementation of flaxseed incorporated laddu for a period of 2 months on serum lipid levels of 25 (normolipidemic and hyperlipidemic) subjects also showed a significant drop in serum lipid profile.

4.4.5 Triglyceride

The values of plasma triglyceride concentration of the subjects during and after supplementation are presented in table 14.

Fig. 5. depicts the mean plasma triglyceride values of subjects in different groups during and after supplementation.

Table 14. reveals that there was no significant difference ($P<0.05$) in any of the groups. But when tested for critical difference and mean difference, significant difference

Table 14 : Plasma triglyceride levels of subjects during and after supplementation

Subjects	Experimental group I			Experimental group II			
	During (mg/dl)	After (mg/dl)	% difference	During (mg/dl)	After (mg/dl)	% difference	Durin (mg/d
1	132	128	3.03	169	126	25.44	142
2	134	126	5.97	136	127	6.62	164
3	144	142	1.39	134	122	8.96	175
4	136	132	2.94	159	121	23.9	217
5	147	146	0.68	146	132	9.59	152
6	156	129	17.31	128	122	4.69	135
7	154	152	1.3	126	124	1.59	149
Mean ± SD	143.29 ± 9.64	136.43 ± 10.16	4.79 %	142.57 ± 16.25	124.86 ± 3.85	12.42 %	162.(± 27.6

‘-’ indicates increase in triglyceride level

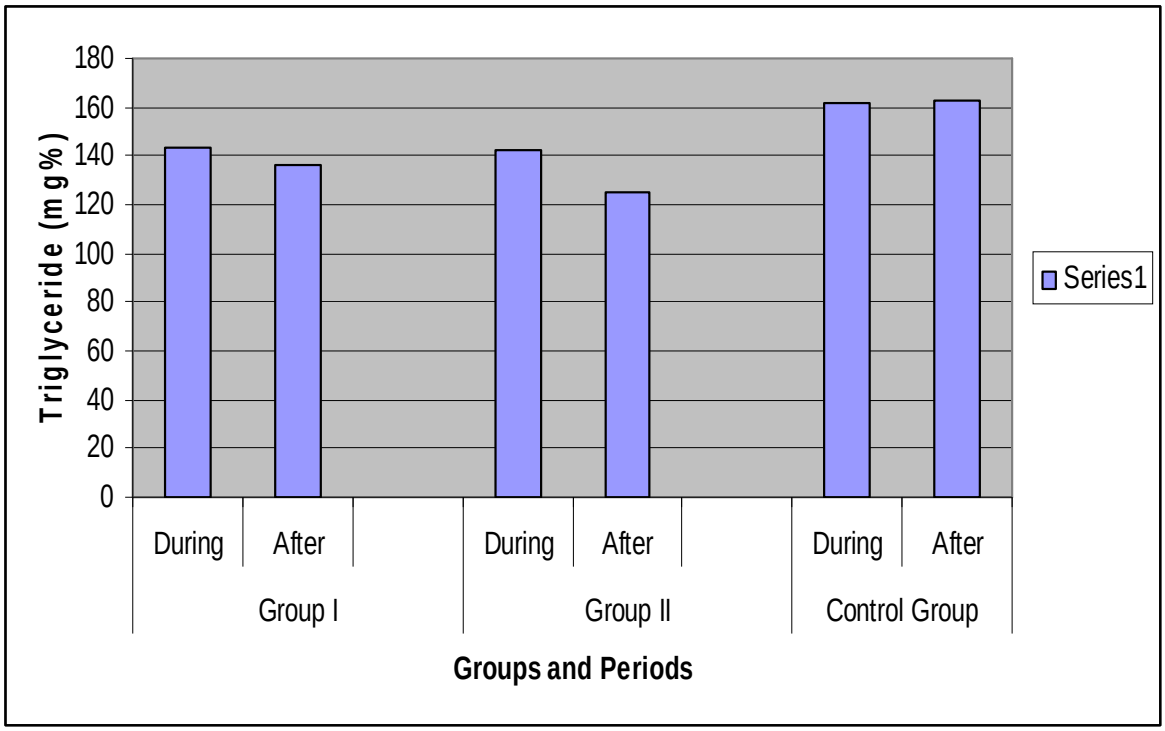

Fig. 5: Mean plasma triglyceride (mg%) levels during and after supplementation

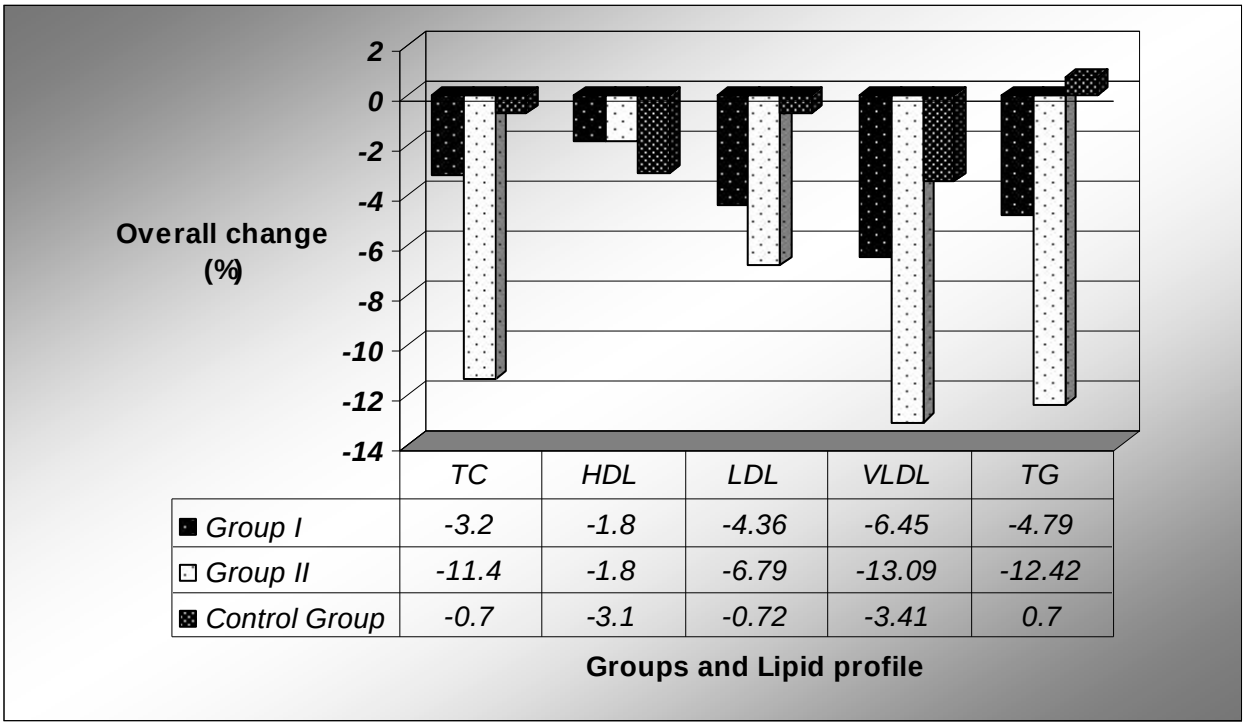

Fig. 6: Overall percentage change in lipid profile after supplementation

was observed in the serum triglyceride levels in both the experimental groups. The per cent decrease after 60 days from 30th day of supplementation was 4.79 and 12.42 per cent in experimental group I and experimental group II respectively.

The mean plasma triglyceride value on 30th day i.e. 143.29 mg/dl decreased to 136.43 mg/dl in experimental group I and from 142.57 mg/dl decreased to 124.86 mg/dl in experimental group II.

In control group, the mean value after 30 days of supplementation 162.0 mg/dl increased slightly to 163.14 mg/dl with 0.7 per cent after 60 days of supplementation.

The lack of effect of flaxseed on triglyceride levels has been observed by Blonk, Mulder and other researchers. Blonk et al. (1990) reported that low doses of fish oil significantly reduced plasma triglyceride concentration.

It may be suggested that DHA (Docosahexaenoic Acid) and EPA (Eicosapentanoic Acid) present in fish oil are responsible for hypotriglyceridemic effect. α-linolenic acid ingested through diet gets converted to its longer chain metabolites i.e. DHA and EPA, but the conversion is a highly complex process that can be modified by numerous dietary factors. The ratio of polyunsaturated to saturated fatty acids may be of particular importance. The lack of hypotriglyceridemic effect of flaxseed observed in the present study may be explained by insufficient conversion of α-linolenic acid to its long-chain metabolites.

The difference in overall percentage of lipid profile after supplementation is depicted in fig. 6. The results of the present study show that the 20 per cent flaxseed incorporated biscuits supplementation had significant impact ($P<0.05$) on total cholesterol and LDL cholesterol. However slight reduction was observed in HDL

cholesterol, VLDL cholesterol and triglyceride concentrations. Whereas in 40 per cent flaxseed incorporated biscuits supplementation had significant impact ($P<0.05$) on total cholesterol, LDL cholesterol, VLDL cholesterol and triglyceride concentrations but a slight reduction in HDL cholesterol (NIN-National Institute of Nutrition 1998). Control group had no significant decrease ($P<0.05$) in the lipid profile.

Similar result was found by Ratnayake et al. in 1992. They studied the nutritional effect of flaxseed by feeding weanling rats with diet containing ground linseed at 0, 10, 20 and 40 per cent levels for a period of 90 days. Results showed that serum triglycerides, total cholesterol and LDL cholesterol concentrations were significantly lower in rats fed with 20% and 40% flaxseed diets as compared to control group with flaxseed free diet.

CHAPTER V

SUMMARY AND CONCLUSIONS

Coronary heart disease is a major health problem worldwide. Hyperlipidemia is elevation in plasma LDL cholesterol, triglycerides and decreased HDL cholesterol. The elevated level of cholesterol in the blood is a major risk factor for coronary heart disease. Numerous studies have shown that inclusion of complex carbohydrates, soy protein and soluble fibre with low fat reduced the elevated plasma cholesterol levels to normal range.

Clinical trials have demonstrated conclusively that lowering serum/plasma cholesterol levels will decrease morbidity and mortality from coronary heart disease (CHD) in patients with established coronary heart disease. Treatment of elevated LDL cholesterol in patients with prior coronary heart disease and/or other atherosclerotic disease is known as secondary prevention, whereas clinical management of patients without coronary heart disease is known as primary prevention. Diet therapy remains the first line of treatment of high blood cholesterol and drug therapy is reserved for patients who are considered to be at high risk for coronary heart disease.

`Flaxseed and samai are the foods with phytochemical factors that provide demonstrated physiological benefit or reduce the risk of chronic disease, above and beyond their basic nutritional functions.

Hence the present study was carried out to develop and evaluate the acceptability of various flaxseed and samai incorporated at different levels in low fat baked products and to study the effect of supplementation on lipid profile of the hyperlipidemic subjects.

Flaxseed and samai were procured from local wholesale market, cleaned and powdered to incorporate into various low fat baked products. The recipes selected for

product development were bread, muffin and masala biscuit. Products were developed by incorporating flaxseed at 10, 20 and 40 per cent levels. In all the products, a combination of 50 per cent refined flour and remaining 50 per cent of samai flour were used as the base. Sensory evaluation was done for muffin and masala biscuit. Bread was excluded due to its poor end product. As per the results of sensory evaluation masala biscuit with 40 and 20 per cent level of flaxseed incorporation was selected for supplementation.

For supplementation study, 21 subjects aged between 35-50 years with total cholesterol above 155 mg/dl were selected. Subjects were randomly assigned to control group (n=7), experimental group I (n=7) and experimental group II (n=7) for a period of two months.

Supplementation was in the form of two biscuits (total 40 g) providing 8 g flaxseed/day, which provided 11.93 g total fat, 33.05 g carbohydrate, 4.72 g protein and 1.74 g fiber for experimental group I. For experimental group II, 40 g biscuits (2 biscuits) provide 16 g flaxseed/day, which provided 14.67 g total fat, 29.73 g carbohydrate, 5.6 g protein and 1.8 g fiber. Whereas control group was supplemented with 40 g of low fat marie biscuits which provided 2.1 g total fat, 15.4 g carbohydrate, 1.6 g protein and no fiber.

Plasma total cholesterol was assessed at three intervals i.e., initial, during and after supplementation. HDL cholesterol, LDL cholesterol, VLDL and TG were assessed at 2 intervals i.e., during and after supplementation.

A significant reduction of 3.2 and 11.4 per cent was found in total cholesterol of experimental group I and group II respectively at the end of experimental period.

Whereas in case of control group only a slight reduction of 0.7 per cent was found after two months of supplementation.

A significant reduction of 4.36 and 6.79 percent was found in plasma LDL cholesterol level of experimental group I and II respectively at the end of the experimental period from second month of experimental period. A slight reduction of 0.72 percent was found in control group.

A significant reduction of 13.09 per cent was found in VLDL cholesterol level of experimental group II at the end of experimental period from second month of experiment. In experimental group I, a slight reduction of 6.45 per cent was found. Whereas in control group, there was a slight increase in VLDL cholesterol level i.e., 3.41 per cent.

A non significant difference was observed in HDL cholesterol and triglyceride levels in all the groups. Instead of increase there was a slight decrease 0f 1.8 per cent in HDL cholesterol level of both the experimental groups and 3.1 per cent decrease in control group. A slight decrease of about 4.79 and 12.42 per cent was observed in triglyceride levels of experimental group I and II respectively. Whereas in control group, a slight increase of 0.7 per cent was found after supplementation period from second month of supplementation period.

Analysis of plasma lipid profile revealed that 20 per cent flaxseed supplementation caused a significant overall reduction in total cholesterol and LDL cholesterol. Forty per cent flaxseed incorporation caused a significant overall reduction in total cholesterol, LDL cholesterol and VLDL cholesterol, whereas control group had no significant reduction in any component of the lipid profile.

Dietary fats containing different fatty acids alter the extent and character of post-prandial lipid metabolism. Chronic feeding of polyunsaturated fatty acids (PUFA) reduce post-prandial lipemia produced by a meal containing other fatty acids. Long chain n-3 PUFA like α–linolenic acid (ALA), eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) are present in abundance in fish, but increasing the consumption of fish/fish oil is not a suitable option for a large section of population owing to vegetarian dietary habits. Among the plant sources of n-3 PUFA, flaxseed is the richest source of α-linolenic acid. After ingestion, α-linolenic acid can undergo consecutive chain elongation and desaturation to yield other long chain n-3 PUFA like EPA and DHA.

Flaxseed and samai millet contain other beneficial components like dietary fiber mucilage, lignans and phenolic compounds, which impart many health benefits. It has been postulated that soluble fiber binds to bile acids in the intestinal lumen, which results in a reduced bile acid pool circulating back to the liver. This binding action stimulates production of more bile acids from cholesterol that is either made endogenously or captured from the circulation. Soluble fibers are fermented in the large bowel by colonic bacteria which results in the production of the short chain fatty acids (SCFAs) – acetate, propionate and butyrate. These SCFAs are absorbed through the portal vein, inhibiting hepatic cholesterol synthesis by limiting the action of HMG-COA reductase (the rate limiting enzyme required for cholesterol biosynthesis) or by increasing catabolism of LDL-cholesterol. Soluble fiber also delays gastric emptying, thereby reducing a post prandial serum concentration which reduces hepatic cholesterol production through mediation of HMG-COA reductase.

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APPENDIX – I
SCORE CARD FOR THE SENSORY EVALUATION OF THE PRODUCTS DEVELOPED

Name of the Evaluator :
Date :
Time :

Please evaluate the given samples for following attributes using guidelines

- Rinse your mouth in between evaluating each sample
- Use five point scale

Sl.No.	Attributes	Sample Number					
		1	2	3	4	5	6
	COLOUR/APPEARANCE						
5	Highly appealing						
4	Moderately appealing						
3	Appealing						
2	Slightly appealing						
1	Not appealing						
	FLAVOUR						
5	Very pleasant						
4	Pleasant						
3	Fair						
2	Poor						
1	Very poor						
	TEXTURE						
5	Very good						
4	Good						
3	Fair						
2	Poor						
1	Very poor						
	TASTE						
5	Very good						
4	Good						
3	Fair						
2	Poor						
1	Very poor						
	OVERALL ACCEPTABILITY						
5	Highly acceptable						
4	Moderately acceptable						
3	Acceptable						
2	Slightly acceptable						
1	Not acceptable						

Suggestions / Comments

Signature of the evaluator

APPENDIX – II

COST AND METHOD OF PREPARATION OF PRODUCTS SELECTED FOR SUPPLEMENTATION

20% AND 40% FLAXSEED INCORPORATED MASALA BISCUIT

20% FLAXSEED INCORPORATED MASALA BISCUIT	40% FLAXSEED INCORPORATED MASALA BISCUIT
Ingredients	**Ingredients**
Refined flour - 40 g	Refined flour - 30 g
Sami flour - 40 g	Sami flour - 30 g
Ground flaxseed - 20 g	Ground flaxseed - 40 g
Sugar - 10 g	Sugar - 10 g
Fat - 10 g	Fat - 10 g
Ammonia - 0.8 g	Ammonia - 0.8 g
Baking powder - 0.5 g	Baking powder - 0.5 g
Salt - 1.0 g	Salt - 1.0 g
Zeera powder - 0.6 g	Zeera powder - 0.6 g
Green chillies - 0.7 g	Green chillies - 0.7 g
Curry leaves - 1.0 g	Curry leaves - 1.0 g
Cost : 2/- per day (2 biscuits)	**Cost : 1.70/- per day (2 biscuits)**

Method

- Fat was creamed and rubbed into the flour which is sieved.
- Ground flaxseed was added to this and again rubbed properly.
- A solution of sugar, salt, ammonia and baking powder was made in 25 ml of water. This was slowly mixed into the flour and fat mixture, spices were added and soft but stiff dough was made.
- Dough was then rolled out into 1/8” thickness and cut with a biscuit cutter.
- Biscuits were pricked and baked at 190ºF for 15 minutes in preheated oven.

APPENDIX – III

ESTIMATION OF TOTAL CHOLESTEROL, HDL CHOLESTEROL AND TRIGLYCERIDES

1. ESTIMATION OF TOTAL CHOLESTEROL

Principle

Cholesterol esterase (CHE) hydrolyzes cholesterol ester. Free cholesterol is oxidized by the cholesterol (CHOLESTEROL) to cholest-4en-3-one and hydrogen peroxide. Hydrogen peroxide formed reacts with 4 aminoantipyrine and phenol in presence of peroxidase (POD) to produce pink coloured quinoneimine dye. The intensity of colour produced is proportional to the cholesterol concentration.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{Cholest} - 4 \text{ en-3-one} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + H_2O$$

Reagents

Enzyme reagent

Distilled water

Standard

Plasma sample

Reagent preparation

Buffer solution was brought to room temperature. One vial of enzyme reagent was reconstituted with 20 ml buffer solution.

Procedure

All the above reagent were pipetted into clean dry test tubes labeled blank (B), standard (S) and test (T).

	Blank (B)	Standard (S)	Test (T)
Enzyme reagent	1.0 ml	1.0 ml	1.0 ml
Distilled water	10 µl	--	--
Standard	--	10 µl	--
Plasma sample	--	--	10 µl

The above constituents were mixed well and incubated at 37ºC for 5 minutes. Absorbance of test (T) and standard (S) and blank (B) was measured on a spectrophotometer at 505 nm (Hg 546 mm).

Calculations

$$\text{Cholesterol in mg \%} = \frac{\text{A of (T)}}{\text{A of (S)}} \times 200$$

Where, A = absorbance

T = test sample

S = standard

2. ESTIMATION Of HDL CHOLESTEROL

Principle

The VLDL and LDL fractions of plasma sample are precipitated using PTA and then HDL in the supernatant is separated by centrifugation and measured for its cholesterol content. The enzyme cholesterol ester hydrolase (CHE) hydrolyzes the ester

cholesterol. Then cholesterol is oxidized by cholesterol oxidase (CHO) to choleest-4-n-3-one hydrogen peroxide. Hydrogen peroxide in presence of enzyme peroxidase (POD) reacts with 4-amino antipyrine and phenol to produce a red coloured complex, whose absorbance is proportional to HDL cholesterol concentration.

Plasma (HDL)+Precipitating reagent →Precipitate (VLDL & LDL)+Supernatant (HDL)

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{Cholest} - 4 \text{ en-3-one} + H_2O$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{Phenol} \longrightarrow \text{Quinoneimine dye} + H_2O$$

Reagents

Precipitating reagent

Enzyme reagent

Buffer solution

HDL cholesterol standard

Reagent preparation

One vial of enzyme reagent was reconstituted with 10 ml of buffer solution.

Procedure

It involves two steps.

Step I : Preparation of VLDL and LDL :

The following constituents were pipetted into a clean dry centrifuge tube.

Plasma	-	0.1 ml
Precipitating reagent	-	0.1 ml

These were mixed well and allowed to stand at room temperature for 5 minutes. Centrifugation was done at 2000-3000 rpm for 10 minutes to get a clean supernatant.

Step II : Assay of HDL cholesterol

The following constituents were pipetted into clean dry test tubes labeled blank (B), standard (S) and test (T).

	Blank (B)	Standard (S)	Test (T)
Enzyme reagent	1.0 ml	1.0 ml	1.0 ml
Distilled water	50 µl	--	--
HDL - standard	--	50 µl	--
Plasma sample	--	--	50 µl

All the above reagents were mixed well and incubated at 37ºC for 5 minutes. Absorbance of test (T) and standard (S) was measured on a spectrometer at 505 nm (Hg 546 mm).

Calculations

$$\text{HDL Cholesterol in mg \%} = \frac{\text{A of (T)}}{\text{A of (S)}} \times 100$$

Where, A = absorbance

T = test sample

S = standard

3. ESTIMATION OF TRIGLYCERIDES

Principle

Triglycerides in the sample are hydrolyzed by microbial lipase to glycerol and free fatty acid. Glycerol is phosphorylated by ATP (Adenosine – 5 Triphosphate) to

glycerol – 3 phosphate in reaction catalyzed by glycerol kinase. Glycerol - 3 – phosphate is oxidized to dihydroxy acetone phosphate (DAP) in a reaction catalyzed by enzyme glycerol phosphate oxidase (GPO).

In this reaction, hydrogen peroxide is produced in equimolecular concentrations to the level of triglycerides present in the sample. H_2O_2 reacts with 4-amino antipyrine and 3, 5-dicloro-2-hydroxy benzene sulphonic acid (DHBS) in a reaction catalyzed by peroxidase (HPOD). The result of this oxidative coupling is red quinoneimine dye.

The absorbance of this dye in solution is proportional to the concentration of triglycerides in the sample. the series of reactions involved in the assay is :

$$\text{Triglyceride} \xrightarrow{\text{Lipase}} \text{Glycerol + free fatty acid}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{Glycerol kinase}} \text{G-3P + ADP}$$

$$\text{G 3 P + } O_2 \xrightarrow{\text{GPO}} \text{DAP + } H_2O_2$$

$$2H_2O + 4\ \text{DAP + DHBS} \xrightarrow{\text{HPOD}} \text{Red quinoneimine dye + } 4H_2O$$

Reagents

Working reagent

Plasma sample

Standard

Procedure

Above reagents were pipetted into clean dry test tubes labeled blank (B), standard (S) and test (T).

	Blank (B)	Standard (S)	Test (T)
Working reagent	1.0 ml	1.0 ml	1.0 ml
Standard	--	10 µl	--
Plasma sample	--	--	10 µl

All the above constituents were mixed well and incubated at 37ºC for 10 minutes. Absorbance of test (T) and standard (S) was measured against blank (B) on a spectrophotometer at 520 nm.

Calculation

$$\text{Cholesterol in mg \%} = \frac{\text{A of (T)}}{\text{A of (S)}} \times 200$$

Where, A = absorbance

T = test sample

S = standard