3

METHODOLOGY

In the present context, the study entitled **"Hypolipidemic effect of high fiber and omega 3 rich foods"** was carried through clinical trials. The main aim of the present study was to evaluate the nutritional status of the subjects and lifestyle changes in their day to day activities. Their lifestyles and dietary habits have been the subject of innumerable epidemiological and clinical investigations. The investigation consists of the following steps:

Phase I

A. Selection of the locale
B. Selection of the hyperlipidemic subjects
C. Formulation of the tool and collection of data
    a. Demographic profile of the selected subjects
    b. Collection of details on dietary and lifestyle pattern, personal and family history of hyperlipidemics
D. Assessment of nutritional status of the selected subjects
    a. Anthropometric measurements
        i. Measurement of height
        ii. Measurement of weight
        iii. Body Mass Index
        iv. Measurement of Waist and Hip circumference
        v. Waist to Hip Ratio
    b. Dietary survey – Individual food and nutrient intake
    c. Biochemical parameters – Analysis of serum lipid profile

Phase II

A. Selection of the ingredients for the supplements
B. Formulation and evaluation of the supplements
    a. Formulation and organoleptic evaluation of the supplements
    b. Composition and cost of the selected supplements

c. Nutrient content of the selected supplements

C. Analysis of the supplements

Phase III

A. Human feeding trials.

a. Orientation to the selected subjects regarding the supplements

Phase IV

A. Evaluation of the effect of intervention among the selected subjects

B. Consolidation, analysis and interpretation of the data

PHASE I

A. Selection of the locale

A larger study in rural Andhra Pradesh, reported that age-adjusted annual mortality rates of 200-250/100 000 while studies in urbanized Kerala and Mumbai have reported very high cardiovascular mortality with age-adjusted rates approaching 500/100 000 for men and 250/100 000 for women. These rates are almost twice that of United States (Soman *et al.,* 2011). Based on the above study the investigator had selected Hyderabad city, capital of Andhra Pradesh which is a cosmopolitan city and has all kinds of people from various region and were found to have more prevalence of hyperlipidemics which is suitable to conduct the present study.

The investigator has selected the venue as Hyderabad city in such way for easy accessibility, communication with the target groups and availability of adequate number of hyperlipidemics. “Care Hospital” located in the heart of Hyderabad city had the advantage of a heterogeneous population with people from all economic groups and occupations. The subjects who are visiting the hospital are from all over the country. The hospital was selected for the easy accessibility and the subjects were periodically visiting the hospital for their check-ups. The Care Hospital, Hyderabad had well equipped laboratory with all modern and sophisticated instruments for the analysis of blood parameters. The hospital authorities - Doctors, Dietician and Nursing staff extended their hand to help and

support the investigator throughout the study period. The subjects were very co-operative and gave their consent to act as a participant in the present study.

B. Selection of the hyperlipidemic subjects

The whole group from which the sample has been drawn is technically known as universe or population and the group actually selected for the study is known as sample. It can be regarded as representative of the entire population (Gupta, 2010 and Kothari, 2011).

The 1000 subjects selected for the present study were in the 35-75 years age group, had a total serum cholesterol level of ≥200mg/dl and were outpatients at the "Care Hospital, Hyderabad. "Out of 1000 subjects, 150 subjects were chosen as sub-sample for the in-depth study. The subjects were in the 40-60 years age group and had a total cholesterol level of ≥240 mg/dl

The inclusion and exclusion criteria used for the selection of the 150 hyperlipidemics for human feeding trials were as follows:

Inclusion criteria

- Age group between 40-60 years
- Total Cholesterol level ≥240 mg/dl
- Participation in regular health check-ups in the hospital
- Free of other medical care complications
- Status as outpatient
- Flexibility to modify their lifestyle
- Willing to give written consent to participate in the intervention study and consume the supplements regularly for a period of three months

Exclusion criteria

- Age > 60 years
- Presence of medical complications and disorders

- Taking too many medicines
- Excessive high cholesterol levels defined as >300 mg/dl
- Inpatients and patients who are in need of immediate medical attention
- Not visiting the hospital periodically for regular health check-ups
- No interest in lifestyle modification
- Not willing to participate in the human feeding trials

- **Study design**

The research design of the present study was shown in the following page (Figure 1) as a flow chart. The intervention study was carried out for 90 days.

C. Formulation of the tool and collection of data

Questionnaires are a popular means of collecting data. A questionnaire consists of a number of questions printed or typed in a definite order on a form or a set of forms (Gupta, 2010 and Kothari, 2011).

a. Demographic profile of the selected subjects

Questionnaire was formulated and used to obtain information regarding the age, sex, educational status and nature of occupation, activity pattern, type of family and income level of the family (Appendix – I).

The demographic profile was collected from 1000 hyperlipidemic subjects.

b. Collection of details on dietary and lifestyle pattern, personal and family history of hyperlipidemics

Dietary information was collected on meal pattern, food habits, food consumption pattern, type of oil used for cooking and knowledge about cholesterol lowering foods. The lifestyle pattern such as exercise, smoking and alcohol consumption was also collected by using a questionnaire. The details related to the hyperlipidemia condition, medical, personal and family history, frequency of monitoring, type of treatment, symptoms, complications and awareness regarding the disorder were also recorded.

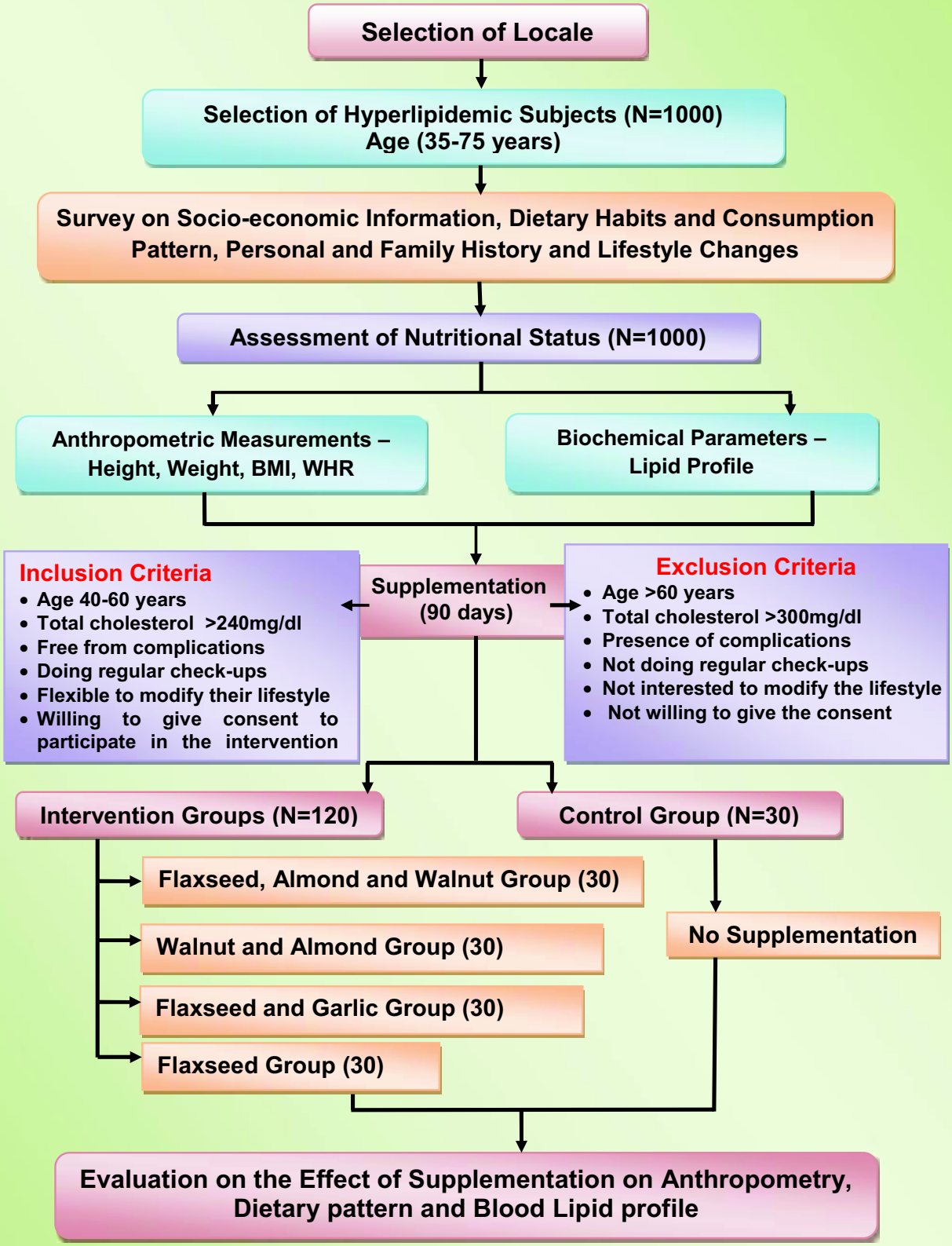


FIGURE 1 - STUDY DESIGN

D. Assessment of nutritional status of the selected subjects

Nutritional assessment is an in-depth evaluation of both objective and subjective data related to an individual's food and nutrient intake (Jelliffe, 1966). Anthropometry is the means by which body composition can be assessed in living people. The results reflect both health and nutritional status and can be used to predict health and survival. These approaches are for the most part relatively non-invasive methods that assess the size or body composition of an individual. For adults, body weight and height are used to evaluate overall nutritional status and to classify individuals as healthy or non-healthy weights (NHANES, 2013).

Once the data on an individual is collected and organized, the practitioner can assess and evaluate the nutritional status of that subject. The assessment leads to a plan of care or intervention, designed to help the individual either maintain the assessed status or attain a healthier status.

The data for a nutritional assessment falls into the following categories: Anthropometric, Biochemical and Dietary pattern.

a. Anthropometric measurements

The term anthropometric refers to comparative measurements of the body. Anthropometrics are the objective measurements of body muscle and fat. They are used to compare individuals and other factors like socio-economic status, lifestyle etc., and risk factors that are involved in causing the problems like cardiovascular diseases. Weight and height are the most frequently used anthropometric measurements. Hence, height and weight of the subjects were recorded (Ray and Iqbal, 2011).

i. Measurement of height

Standing height is the measurement of the maximum distance from the floor to the highest point on the head, when the subject is facing directly ahead. Shoes should be off, feet together and arms by the sides. Heels, buttocks and upper back should also be in contact with the wall when the measurement is made. Height measurement can vary throughout the day, usually being higher in the morning,

so to ensure reliability height should be measured at the same time of day (Ray and Iqbal, 2011).

Height in centimetres was recorded for all the selected 1000 subjects in the hospital itself using a stadiometer .

ii. Measurement of weight

Weight that is believed to be maximally healthful for a person, based chiefly on height but modified by factors such as gender, age, build and degree of muscular development. Measuring body mass can be valuable for monitoring body fat or muscle mass changes, or for monitoring hydration level.

To record the body weight measurement the person stands with minimal movement with hands by their side, minimum clothing and without shoes. Weight was recorded to the nearest 0.1 kg using the digital electronic weighing machine. For reliability, weight is recorded in the morning (12 hours since eating). Body weight can be affected by fluid in the bladder (weigh after voiding the bladder). Other factors to consider are the amount of food recently eaten, hydration level, the amount of waste recently expelled from the body, recent exercise and clothing (Ray and Iqbal, 2011).

For all the selected 1000 subjects, measurement of weight in kilograms were recorded using an electronic digital balance in the hospital and compared with the standard values.

iii. Body Mass Index

According to World Health Organization (WHO, 2004), Body Mass Index (BMI) is a simple index of weight for height that is commonly used to classify underweight, over weight and obesity in adults. The body mass index (Quetelet index) was calculated by dividing the individual's weight in kilogram by the square of his or her height in meters (Garrow and Webster, 1985). This was calculated to find out the grade of obesity of the selected subjects (Ray and Iqbal, 2011).

For all the selected 1000 subjects BMI was calculated by dividing the measurement of weight in kgs and height in m^2. These values were computed and classified in different grades of obesity given in Table II and compared with the standard values.

TABLE II

CUT OFF VALUES FOR DIFFERENT GRADES OF OBESITY

BMI Values (Kg / m^2)*	Classification
< 18.5	Underweight
18.5 – 24.99	Normal
25.0 – 29.99	Pre obese / Overweight
30.0 – 32.49	Mild obese class I
32.50 – 34.99	Moderate obese class I
35.00 – 37.49	Mild obese class II
37.50 – 39.99	Moderate obese class II
≥ 40.0	Obese III

** Adapted from WHO, 2004 and ICMR, 2010*

iv. Measurement of waist and hip circumference

According to the National Institutes of Health, a high Waist Circumference (WC) is associated with an increased risk for cardiovascular disease, hypertension, dyslipidemia and Type II diabetes when the BMI is between 25 and 34.9. (BMI greater than 25 is considered overweight and a BMI greater than 30 is considered obese).

Waist Circumference can be useful for those people categorized as normal or overweight in terms of BMI. (For example, an athlete with increased muscle mass may have a BMI greater than 25 - making him or her overweight on the BMI scale - but a waist circumference measurement would most likely indicate that he or she is, in fact, not overweight). Changes in waist circumference over time can indicate an increase or decrease in abdominal fat. Increased abdominal fat is associated with an increased risk of heart disease.

- **Waist circumference**

In the recent past, particularly with increasing incidence of degenerative diseases, considering the significance of abdominal adiposity in diet related chronic diseases, waist and hip circumferences are used to evaluate the abdominal adiposity in subjects. The subjects were asked to stand erect with weight evenly balanced on both feet, which are placed about 25-30 cms apart. The level of the lowest rib margin was marked. The iliac crest in the mid-axillary line was felt and a mark was made. The measuring tape (non-stretchable and non-elastic fibre glass) was passed around the waist horizontally midway between the lowest rib margin and iliac crest and the circumference in cms was measured up to the nearest mm. The observer was made to sit on a stool in front of the subject while taking the measurement. All the adult males with waist circumference of ≥102 cm and females with ≥80 cm were identified as having abdominal obesity (WHO, 2008 and ICMR, 2010).

- **Hip circumference**

To measure the circumference of the hip area, as a measure of the underlying hip structure, musculature and adipose tissue when combined with the measure of abdominal girth, the Waist to Hip Ratio (WHR) has been shown to be related to the risk of coronary heart disease (Welborn *et al.,* 2003).

For measuring the hip circumference, a fibre glass tape was placed horizontally over the buttocks and the circumference was measured at the point yielding the maximum circumference in cms up to the nearest mm (WHO, 2008 ICMR, 2010). Waist circumference should be taken at the narrowest circumference between ribs and hips.

v. Waist to Hip ratio

Measurement of waist and hip circumference was done and the ratios were calculated and compared with the standard value. All the adult males with waist hip ratio of ≥0.95 and women with ≥0.80 will be identified as obesity (WHO, 2008 ICMR, 2010). Waist hip ratio was calculated for the selected subjects and compared with the standard.

For all the selected 1000 subjects WHR were computed by dividing subject's waist circumference in cms by hip circumference in cms and compared with the desirable values suggested by ICMR (2010).

b. Dietary survey – Individual food and nutrient intake

Dietary survey constitutes an essential part of any complete study of nutritional status providing essential information on nutrient intake, sources of nutrients, food habits and attitudes. Diet survey in a community is usually done by weighment method or recalling method. Since earlier investigators have evidenced enough to prove the accuracy of both the methods to be similar, the recall method was selected, as it is less time consuming to collect the information regarding their consumption pattern. The 24-hour recall method is based on an in-depth interview conducted by a trained interviewer. The interviewer solicits detailed information about everything the subject had eaten the previous day over the past 24-hr period (Godhia, 2011). Diet survey was done by the 24-hour recall method for 25 subjects involved in the clinical trial.

The cooked amount of each food item consumed by the subject was then converted back into raw foodstuff using the formula given below. These raw quantities of various food stuffs consumed through different food preparations were calculated and summed up so as to obtain the total consumption of a particular food item during the past 24-hour period.

$$R_i = \frac{T_R}{T_C} \times C_I$$

Where R_i = Raw amount of a particular foodstuff consumed by the individual from a given preparation

T_R = Total raw quantity of the foodstuff used in that preparation

C_I = Intake of the cooked amount of that preparation

T_C = Total cooked quantity of the food prepared

From the raw equivalence of the food calculated, nutrient intake of the subjects were computed for the experimental (N-15) and control group (N-10) using the Nutritive value of Indian Foods, (2012) and compared with the recommended allowances given in the Dietary Guidelines for Indians – A Manual by ICMR (2010).

c. Biochemical parameters – Analysis of serum lipid profile

The lipid profile is a blood test done to assess the status of fat metabolism in the body and is important in determining the risk of coronary heart disease. This includes measuring lipids (fats) and its derivatives known as lipoproteins. Lipoproteins are compounds containing fat and proteins and include free cholesterol, cholesterol esters, triglycerides, phospholipids and Apo proteins. They are tests that have been shown to be good indicators. The lipid profile typically includes:

- Total Cholesterol (TC)
- High Density Lipoprotein Cholesterol (HDL-C)-often called as good cholesterol
- Low Density Lipoprotein Cholesterol (LDL-C)-often called as bad cholesterol
- Triglycerides
- Very Low Density Lipoprotein Cholesterol (VLDL-C)

a. Collection of blood sample

Blood sample is obtained by inserting a needle into a vein in the arm. Sometimes a drop of blood is collected by puncturing the skin on a fingertip. This finger stick sample is typically used when a lipid profile is being measured on a portable testing device, for example, at a health fair. The subject needs to fast for 9-12 hours before having blood drawn; only water is permitted.

From each subject, five ml of blood sample was collected from the antecubital vein and were taken preferably before breakfast to avoid the influence of food digestion on blood composition. Care was taken to avoid hemolysis, during the collection of blood for serum separation. Blood was allowed to clot for nearly

three hours, at room temperature. Then the clot was centrifuged and supernatant serum was separated and stored in well stoppered bottles, in a freezer and used for the analysis of lipids.

b. Analysis of blood sample for serum lipid profile

The lipid profile is used to determine the risk of heart disease and to guide the health care provider in deciding what treatment may be best. The results of the lipid profile are considered along with other known risk factors of heart disease to develop a plan of treatment and follow-up. Depending on the results and other risk factors, treatment options may involve lifestyle changes such as diet and exercise or lipid-lowering medications such as statins.

For the analysis of serum lipid profile the following methods were used

Total Cholesterol – CHOD-PAP method

HDL Cholesterol – CHOD-PAP method

Triglycerides – Enzymatic colorimetric test – GPO-PAP

VLDL Cholesterol – $\frac{\text{Triglycerides}}{5}$ where 5 is a constant factor

LDL Cholesterol – Total Cholesterol – (HDL Cholesterol + VLDL Cholesterol)

Estimation of Total Cholesterol (TC) and High Density Lipoprotein Cholesterol (HDL-C) was done using the enzymatic method suggested by Friedwald (1972). In this procedure, serum low density lipoprotein and very low density lipoprotein were selectively precipitated by μg++ ions and phosphotungstate and removed by centrifugation. Cholesterol associated with HDL fractions remaining in the solution was carefully estimated by enzymatic method.

Triglycerides were estimated using the enzymatic method, suggested by Friedwald (1972). The intensity of the colour developed is directly proportional to the triglyceride concentration and was estimated photometrically at 540nm.

The Low Density Lipoprotein (LDL) cholesterol values were calculated from high density lipoprotein cholesterol, total cholesterol and VLDL cholesterol values using the following formula.

LDL Cholesterol = Total Cholesterol – (HDL Cholesterol + VLDL Cholesterol)

For all the selected 1000 hyperlipidemics the above mentioned blood profile values were recorded from the hospital record at the start of the study period for the screening of the hyperlipidemics.

Before the start of the human feeding trials the subjects were screened for their lipid profile (Total Cholesterol, LDL Cholesterol, HDL Cholesterol, VLDL Cholesterol and Triglycerides) using above mentioned procedures. Subjects having total cholesterol level >240 mg/dl were included for the in-depth study. Serum lipid profile of one hundred and fifty hyperlipidemics subjects (experimental, N=120 and control, N=30) were analysed before and after the supplementation period using the standard method to find out the effect of supplementation.

PHASE II

Today's unhealthy daily living behaviours are tomorrow's risk factors. These risk factors exert a steadily rising effect on the risk of disease and interact with each other to increase the overall risk. As food based approaches are gaining importance, diet plays an important role in many of the lifestyle diseases especially cardiovascular, it is more important to emphasize on this in every individual's life. The most improvement comes with adding small amounts of nuts - an ounce, or about three to four tablespoons, five or more times a week and are packed with full of omega-3 fatty acids and fibre rich foods in a day-to-day life.

A. Selection of the ingredients for the supplements

Animal fats are complex mixtures of triglycerides, with lesser amounts of phospholipids and cholesterol. As a consequence, all foods rich in animal fat contain cholesterol to varying extents. Cholesterol is not present in plant-based food sources unless it has been added during the food preparation. However, plant products such as flaxseeds and peanuts contain cholesterol-like compounds called phytosterols, which are suggested to help to lower serum cholesterol levels.

So in the present study the investigator used the plant based foods such as flaxseeds, walnuts and almonds (Plate I). Though they are costly, they are excellent sources of dietary fibre and omega-3 essential fatty acids. People are unaware of nuts and its beneficial effects to the humans. Nuts have powerful antioxidant properties by fighting harmful free radicals. They have several benefits like anti-inflammation, regulation of fat metabolism, hormonal imbalance and central nervous system, supports immune system, better cognitive function, reduces the risk of cancer, diabetes apart from heart disease. Foods like almonds, walnuts, flaxseeds and garlic are used for the formulation and preparation of the supplementation. The benefits and facts about the selected foods are given below.

- **Flaxseeds**

  Botanical name: *Linum usitatissimum*

  Native: Eastern Mediterranean to India

  Active constituents: Lignan (antioxidant), alpha linolenic acid, dietary fibre.

  Main Action: It controls cholesterol levels and blood pressure.

  Functions: Promotes bone health; reduce some menopausal symptoms; has anti-inflammatory benefits; regulates fat metabolism; hormones and central nervous systems.

  Relevant information: Intake of flaxseeds has also been shown to decrease the ratio of LDL to HDL cholesterol in several human studies and

Walnut

Flaxseed

Almond

Major Ingredients

Pepper

Minor Ingredients

Garlic

Cumin seed

Redchillie

PLATE I

INGREDIENTS

to increase the level of Apo lipoprotein A1, which is the major protein found in HDL cholesterol (the "good" cholesterol). This HDL related benefit may be partly due to the simple fiber content of flaxseeds, since 2 tablespoons of ground flaxseed provide about 4 g of dietary fiber (Aliani *et al.,* 2011). In another work by Kristensen *et al.,* (2012), he showed that Flax drink lowered fasting total cholesterol and LDL cholesterol by 12 and 15 per cent respectively, whereas Flax bread produced a reduction of only 7 and 9 per cent respectively.

- **Walnuts**

  Botanical name: *Juglans regia*

  Native: Southern California coast

  Active constituents: Ellagic acid and melatonin (antioxidants), fats - vegetable and monosaturated fats.

  Main Action: Lowers serum cholesterol, LDL cholesterol, prevents clotting of arteries.

  Functions: Supports immune system, anti-cancer properties, provides satiety with fewer calories, weight reduction.

  Relevant information: A handful of walnuts contain almost twice as much antioxidant as an equivalent amount of any other commonly consumed nut (ACS, 2011). During short term trials, the Harvard scientists found that walnut-rich diet consumption decreased total and low density lipoprotein cholesterol (Banel and Hu, 2009).

- **Almonds**

  Botanical name: *Prunus dulcis*

  Native: Mediterranean climate region of the Middle East

Active constituents: Natural free radical (antioxidant), monounsaturated fats and complete protein.

Main Action: Helps to reduce serum cholesterol, LDL and blood glucose and increases HDL cholesterol.

Functions: Weight loss; lowers risk of gallstones; satiates hunger; prevents constipation; maintains healthy blood vessels and nervous system; boosts blood circulation; strengthens bones; muscles and organs.

Relevant information: Numerous studies reported that there is a 10 per cent reduction in bad cholesterol (LDLs) without harming the levels of good cholesterol (HDLs) for study participants consuming almonds versus those who don't. The 10 per cent reduction in LDL cholesterol is achieved by aiming to eat around 73 g of almonds per day (i.e. half cup, around 400 calories). One dose-response study of almonds showed a 5 per cent reduction in LDL cholesterol per 1/4 cup of almonds and 10 per cent for a 1/2 cup (Jenkins *et al.,* 2002). In addition to their cholesterol lowering effects, almonds ability to reduce heart disease risk may also be partly due to the antioxidant action of vitamin-E found in it, as well as to the LDL lowering effect of almonds monounsaturated fats (LDL is the form of cholesterol that has been linked to atherosclerosis and heart disease). When almonds are substituted for more traditional fats in human feeding trials, LDL cholesterol can be reduced from 8 to 12 per cent (Kelly and Sabate, 2006 and WHFoods: Almonds, 2012).

- **Garlic**

  Botanical name: *Allium sativum*

  Native: Central Asia

  Active constituents*:* Thiosulfinates (allicin), sulfoxides (alliin), dithiins (ajoene) are responsible for its characteristically pungent odour and act as an antioxidant, manganese, vitamin-B6 and vitamin-C.

Main Action: Diminishes blood clots in atherosclerosis; reduces cholesterol; blood pressure and glucose levels; acts as a flavouring agent in food preparation.

Functions: Antibacterial; antiviral and antifungal activity improves iron metabolism.

Relevant information: Cholesterol lowering was observed in the studies where the subjects consumed about one and half gram or one gram of garlic per day. Rana *et al.,* (2006) opines that the garlic lowered total cholesterol and triglyceride levels by up to 20 mg/dl in human. LDL cholesterol levels were very modestly lowered whereas HDL cholesterol was not affected by the administration of garlic. The cholesterol lowering abilities of garlic appear to be dose dependent (Gorinstein *et al.*, 2006). The benefits of garlic were more pronounced when used as a long term treatment and in individuals who started treatment with higher total cholesterol levels. Garlic did not appear to have any significant effects on other lipid levels, including HDL and LDL (Zeng *et al.,* 2012).

- **Pepper**

  Botanical name: *Piper nigrum*

  Native: South India

  Active constituents: Lignans, alkaloids, flavonoids, aromatic compounds and amides.

  Main Action: Helps for digestion; relieves gas; treat food poisoning; stomach chills; cholera, dysentery and vomiting caused by hypothermia; acts as flavouring agent.

  Functions: Anti-cancer; natural antioxidant and anti-inflammatory properties; immune enhancer; promotes intestinal health; improves digestion.

Relevant information: Saturated fats in the food are one of the main causes of oxidative stress and black pepper minimizes it. The high levels of cholesterol and triglycerides associated with oxidative stress inhibit the efficacy of important antioxidants like glutathione, superoxide dismutase, catalase, glutathione peroxidase, vitamin-C and vitamin-E. Black pepper actually maintains and enhances the levels and efficacy of important antioxidant compounds (Meghwal and Goswami, 2012). A tablespoon of black pepper contains 0.1 g each of saturated and unsaturated fats and is enough pepper to season a family-size portion of a relatively spicy dish. Between the low fat content and the balance of saturated and unsaturated fat, black pepper is unlikely to directly stimulate the body to change its cholesterol count (USDA, 2011).

- **Cumin Seed**

Botanical name: *Cuminum cyminum*.

Native: East Mediterranean to India

Active constituents: Apigenin and luteolin (antioxidants)

Main Action: Used during indigestion and menstrual problems

Functions: Anti-cancer; antiseptic; anti-fungal and anti-viral properties; boosts immune system; helps in healing

Relevant information: Cuminum cyminum is widely used as a spice in many countries. Treatment of streptozotocin-diabetic rats with CC and glibenclamide for 28 days caused a reduction in blood glucose, glycosylated hemoglobin, creatinine, blood urea nitrogen and improved serum insulin and glycogen (liver and skeletal muscle) content when compared to diabetic control rats (Jagtap and Patil, 2010). Cumin's anti-glycation properties proved useful in another study, in which diabetic rats were able to stave off cataracts after oral

dosing with cumin powder (Kumar *et al.,* 2009). Another study found that cumin extract reduced total cholesterol, triglycerides and pancreatic inflammatory markers in diabetic rats. It also prevented excessive weight loss. Again, it beat out glibenclamide (Dhandapani *et al.*, 2002).

All the above listed nuts and spices are selected to formulate the supplementation. These nuts are rich in dietary fibre, contain antioxidant properties and are highly nutritious with bundle of health benefits.

B. Formulation and evaluation of the supplements

a. Formulation and organoleptic evaluation of the supplements

The major ingredients like almonds, walnuts and flaxseeds were used in the preparation of the product along with the minor ingredients like pepper, cumin seed, red chilli and garlic, which were used to enhance the taste and flavour of the product. These major and minor ingredients are used for the formulation and preparation of different variations. The composition and combination of the foods selected are given in Table III.

The ingredients used in various combinations were roasted and powdered and tried out in the Foods laboratory, Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

In the first combination, with flaxseed alone four variations were done with different combinations by just altering the quantity of flaxseed.

In the flaxseed and garlic combination, various proportions of flaxseed and garlic were formulated. The higher the quantity of flaxseed the more the dietary fibre present in it. As dietary fibre is the natural characteristic of flaxseed, it has a chance of indigestion in some individuals, leading to constipation. In the case of garlic, as the quantity increases it gives the pungent flavour which may not be palatable to any particular individual. Based on these criteria variation IV was

selected in which both flaxseed and garlic are used in such a way that is more palatable and acceptable by the individual.

TABLE III

DIFFERENT COMBINATIONS OF THE SELECTED FOODS

Combination	Ingredients (g)				
	Flaxseed	Almond	Walnut	Cumin seed	Garlic
Flaxseed					
Variation I	25	-	-	3	-
Variation II	35	-	-	3	-
Variation III	40	-	-	3	-
Variation IV	45	-	-	3	-
Flaxseed and garlic					
Variation I	30	-	-	5	15
Variation II	35	-	-	5	10
Variation III	40	-	-	5	5
Variation IV	45	-	-	3	3
Flaxseed, almond and walnut					
Variation I	30	10	10	3	-
Variation II	35	10	10	3	-
Variation III	40	10	10	3	-
Variation IV	45	10	10	3	-
Almond and walnut					
Variation I	-	25	25	-	-
Variation II	-	20	20	-	-
Variation III	-	15	15	-	-
Variation IV	-	10	10	-	-

In the third combination - flaxseed with almond and walnut - four variations were carried out with different proportions of flaxseed, almond and walnut, other ingredients like cumin seeds, redchilli, pepper corn were used in the same quantity. Among these variations, every variation has its own flavour, taste and texture. Variation III and IV had a high proportion of almond and walnut and has given a nutty taste and the texture is little soggy. Variation I was appropriate with a proper blend of nutty flavour and is more of a powder in texture and more palatable and acceptable.

In the last combination - almond and walnut - different proportions of almond and walnut were used. From the variation I to III the quantity was more and variation IV was adequate in quantity and also cost effective. It also gives fullness and more calories along with desirable fat. So the variation IV was selected for the supplementation which gives satiety to some extent and was easily affordable as healthy foods by the subjects and was highly accepted.

Sensory evaluation is a multidisciplinary science. It is a measure of sensory characteristics like sight, smell, taste, touch, hearing and acceptability of food products. Thus, quality of food is judged in terms of appearance, colour, taste, texture, flavour and overall acceptability (Chandrasekhar, 2002).

After the formulation of the foods, the different powders prepared in various combinations were evaluated by sensory evaluation with the help of semi-trained individuals of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, using score card with five point numerical scoring (Srilakshmi, 2011).

The powders that got the maximum score in the organoleptic evaluation are trustworthy results for the best acceptable product for further use. The scores obtained by different powders are given in Table IV.

From Table IV, it is evident that in variation IV of the flaxseed combination got the highest score of 26.80, flaxseed and garlic combination of variation IV has

the highest score of 26.94. In the flaxseed, almond and walnut combination, variation I has the highest score of 27.12.

TABLE IV

ORGANOLEPTIC EVALUATION OF THE SUPPLEMENTS (MAX. SCORE-30)

Combinations	Criteria						Total
	Appearance	Colour	Texture	Flavour	Taste	Overall Acceptability	
Flaxseed							
Variation I	4.32	4.12	4.19	4.26	4.31	4.15	25.35
Variation II	4.12	4.36	4.16	3.87	3.89	3.70	24.10
Variation III	4.72	4.35	4.56	3.50	3.78	4.21	25.12
Variation IV	**4.53**	**4.44**	**4.44**	**4.58**	**4.39**	**4.42**	**26.80**
Flaxseed and garlic							
Variation I	3.52	3.65	3.85	3.75	4.10	4.25	23.12
Variation II	4.52	4.25	4.32	4.20	4.12	4.15	25.56
Variation III	3.16	3.56	3.49	4.10	3.89	3.95	22.15
Variation IV	**4.54**	**4.50**	**4.45**	**4.60**	**4.45**	**4.40**	**26.94**
Flaxseed, almond and walnut							
Variation I	**4.64**	**4.61**	**4.44**	**4.50**	**4.47**	**4.46**	**27.12**
Variation II	4.50	4.42	4.39	4.33	4.44	4.42	26.50
Variation III	4.56	4.42	4.42	4.22	4.39	4.39	26.40
Variation IV	4.58	4.53	4.33	4.50	4.42	4.39	26.75

b. Composition and cost of the selected supplements

The consumable capacity by the individual was also carried out using various quantities of the prepared powders. The quantities used are 20 g, 25 g and 30 g. Of all these quantities 20 g was accepted and appreciated by the semi-trained individuals for daily consumption.

The composition of the powders selected for 20 g was shown in Table V. The use of ingredients in the preparation of the powder in different combinations is as given below.

TABLE V

COMPOSITION, QUANTITY AND COST OF THE FOODS SELECTED FOR THE INTERVENTION (20g)

S.No	Composition	Ingredients (g)					Total cost (₹)
		Flax seed	Almond	Walnut	Garlic	Cumin seed	
I	**Flaxseed, almond and walnut**	10	4.4	4.4	-	1.2	10.00
II	**Almond and walnut**	-	10	10	-	-	15.00
III	**Flaxseed and garlic**	17.6	-	-	1.2	1.2	3.50
IV	**Flaxseed**	18.8	-	-	-	1.2	4.00

Flaxseed, almond and walnut

Flaxseeds were roasted nicely until they are crackled. Red chilli, pepper corn and cumin seed were also roasted; almond and walnut were added while grinding. All the ingredients along with salt were finely ground into a powder (Plate II a).

Almond and walnut

Walnut (10 g) and almonds (10 g) were used as such for the supplementation (Plate II b).

Flaxseeds and garlic

Flaxseed, garlic, pepper corn, cumin seed and red chilli are used and roasted. All the ingredients along with salt are ground into powder (Plate II c).

Flaxseed

Flaxseed, pepper corn, cumin seed and red chilli were roasted individually. All these ingredients are then powdered by adding salt (Plate II d and Plate III).

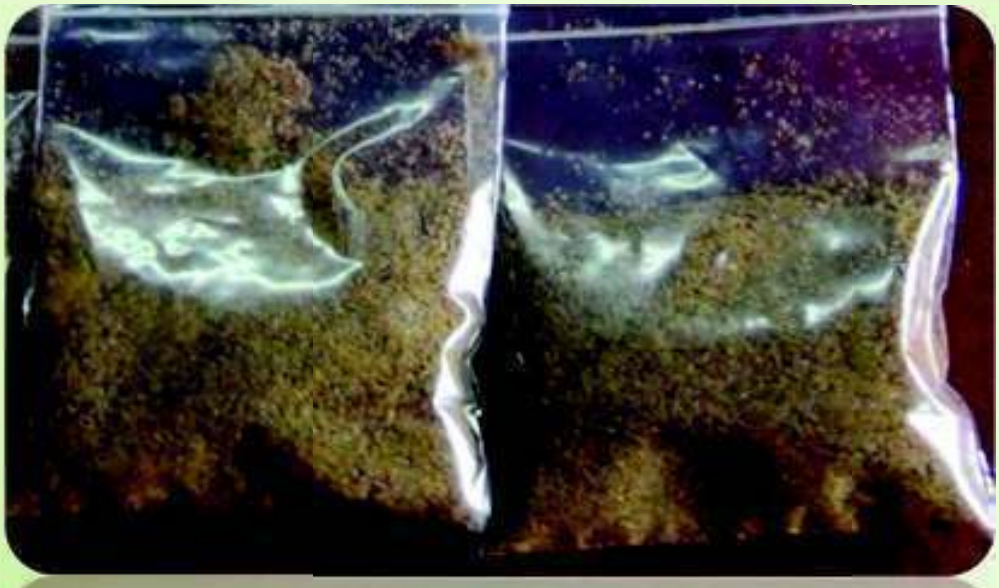
Flaxseed+Almond+Walnut (FAW)

PLATE II

INGREDIENTS USED FOR THE SUPPLEMENTS (a)

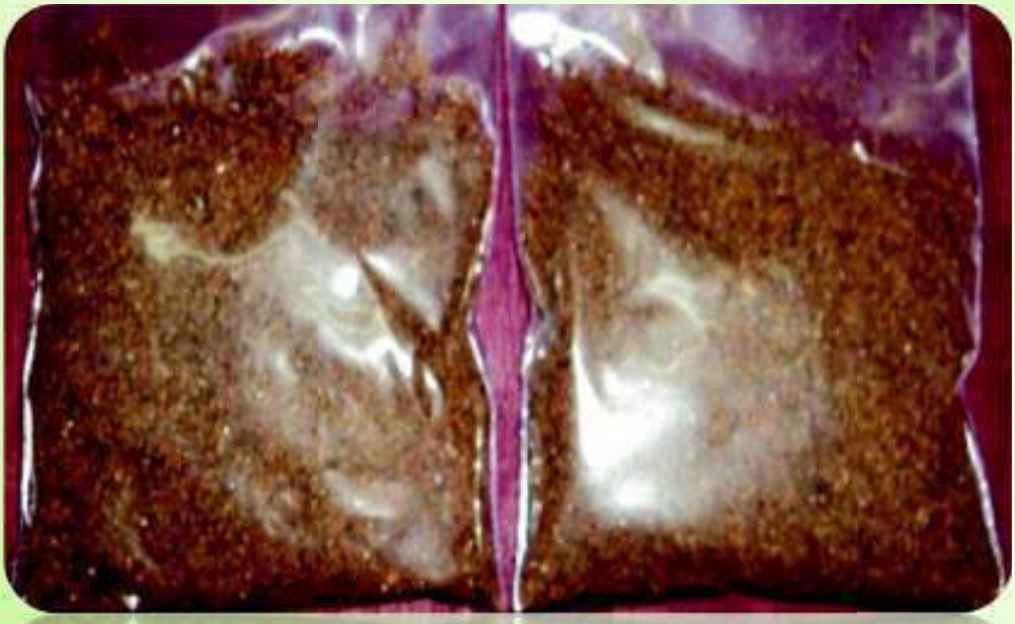
Almond + Walnut (AW)

INGREDIENTS USED FOR THE SUPPLEMENTS (b)



Flaxseed+Garlic (FG)

INGREDIENTS USED FOR THE SUPPLEMENTS (c)



Flaxseed (FS)

INGREDIENTS USED FOR THE SUPPLEMENTS (d)

Roasting

Grinding

Packing

PLATE III

PREPARATION OF THE SUPPLEMENTS

The cost of the supplements was also calculated and depicted in Table IV. As per the cost is concerned, FG combination has the lower price of ₹3.50, FS combination is ₹4.00, FAW combination is ₹10 and the highest is the AW combination which cost ₹15 per 20 g of the supplements.

The cost of all the supplements was very feasible, affordable and are within the reach of all income groups.

c. Nutrient content of the selected supplements

The nutrient content for the supplements are calculated using Nutritive value of Indian foods (2012) and are given in the Table VI.

TABLE VI

NUTRITIVE VALUE OF THE SELECTED SUPPLEMENTS (20g)

Intervention groups	Energy (Kcal)	Protein (g)	Fat (g)	Carbohydrates (g)	Dietary fibre (g)	Omega-3 fatty acids (g)
Flaxseed, almond and walnut (FAW)	116.3	3.7	9.8	4.3	3.9	3.2
Almond and walnut (AW)	134.2	3.5	12.2	2.1	1.7	0.9
Flaxseed and garlic (FG)	99.3	3.5	7.6	5.9	5.2	4.0
Flaxseed (FS)	103.9	3.7	8.1	5.9	5.5	4.0

Flaxseed, Almond and Walnut (FAW) combination has 116.3 k.cal of energy, 3.7 g of protein, 9.8 g fat, 4.3 g carbohydrate, 3.9 g dietary fibre and 3.2 g omega-3 fatty acids per 20 g.

Almond and Walnut (AW) combination has the highest nutritive value of 134.2 k.cal of energy, 3.5 g of protein, 12.2 g fat, 2.1 g carbohydrate, 1.7 g dietary fibre and 0.9 g omega-3 fatty acids per 20 g.

When compare with the other combinations the lowest nutritive value is the Flaxseed and Garlic (FG) combination which supplies99.3 k.cal of energy, 3.5 g of protein, 7.6 g fat, 5.9 g carbohydrate, 5.2 g dietary fibre and 4.0 g omega-3 fatty acids per 20 g.

The nutrient content of the Flaxseed (FS) combination has the high nutritive value of 103.9 k.cal of energy, 3.7 g of protein, 8.1 g fat, 5.9 g carbohydrate, 5.5 g dietary fibre and 4.0 g omega-3 fatty acids per 20 g.

Omega-3 fatty acids are high in the FAW combination and low in calories when compared with the other combinations.

C. Analysis of the supplements

The selected three supplements namely

- Flaxseed, almond and walnut (FAW)
- Flaxseed and garlic (FG) and
- Flaxseed (FS)

were analysed for dietary fiber, fat, active constituents and anti-nutritional factors to verify its suitability for human feeding trials, in the Stanes Herbal Division and Phyto-Pharma Testing Lab, Coimbatore using standard procedures and just to cross check it was also analysed in the SGS India Pvt Ltd, Chennai, both the labs are NABL accredited.

Phase III

A. Human feeding trials

After analysing the selected supplements for various parameters (dietary fiber, dietary fat, active constituents and anti-nutritional factors) and found suitable for human feeding trials, the study was initiated. Ethical clearance was obtained by the Institutional Human Ethical Committee of Avinashilingam University, Coimbatore (HEC.2011.30). Written consent was obtained from the selected hyperlipidemics before initiation of the dietary intervention.

The selected 150 hyperlipidemic subjects were divided into five groups of which four are experimental groups (N=30 each per group) and one control group (N=30) as given in Table VII. They were asked to consume the supplement as per the instructions given to them.

TABLE VII

GROUPING OF SUBJECTS FOR INTERVENTION

Intervention groups	Number of subjects	Supplementation	Quantity (g)
FAW	30	Flaxseed, almond and walnut	20
AW	30	Almond and walnut	20
FG	30	Flaxseed and garlic	20
FS	30	Flaxseed	20
CG	30	No supplementation	-

a. Orientation to the selected subjects regarding the intervention

All the subjects were educated about the supplement, its health benefits, usage and storage capacities. They were also given information on

- After taking the consent from the selected subjects, supplements were given.
- Blood samples are collected from the subjects for the analysis of lipid levels before and after the intervention period.
- The supplement was packed in an air tight zip-lock covers and instructed that the subjects, to consume one packet each per day. The supplement was distributed at an interval of fifteen days in the hospital itself.
- Storage capacities – The details about storage capability of the supplement was provided to the subjects for proper utilization of the supplement.

PLATE IV

ORIENTATION AND DISTRIBUTION OF THE SUPPLEMENTS

The powder (20 g) packets were distributed to the selected hyperlipidemic subjects and were instructed to visit the hospital every fortnight to receive the same. The selected hyperlipidemic subjects received the packets for a period of three months (Plate IV).

Phase IV

A. Evaluation of the effect of intervention among the selected subjects.

The effect of supplementation was evaluated after a period of three months, the anthropometric measurements, diet recall and complete serum lipid profile (Total Cholesterol, LDL-cholesterol, HDL-cholesterol, Triglyceride, VLDL) of the experimental and control groups were recorded before and after the intervention period (Appendix – II and XI).

B. Consolidation, analysis and interpretation of the data.

The data collected were systematically consolidated and statistically analysed by using IBM SPSS Statistics software (version 20) for arriving at the results of the effect of supplementation on complete lipid profile levels among the selected hyperlipidemics and the findings are presented, discussed and concluded in the next chapter results and discussion.