CHAPTER 3

METHODOLOGY

The present study was planned to determine the effect of Soya and Caralluma on anthropometric measurements, lipid profile, blood pressure, nutrient intake and physical discomforts of obese post-menopausal women subjects. The methodology is discussed under the following headings:

3.1 Statement of the problem
3.2 Hypotheses
3.3 Description of variables
3.4 Research design
3.5 Sampling procedure
3.6 Duration of the study
3.7 Tools used for the study
3.8 Collection of data
3.9 Processing and analysis of data

3.1 STATEMENT OF THE PROBLEM

Reducing body weight and lowering lipid levels by various intervention programmes meant for weight reduction calls for special effort. But reduction by appropriate supplements is found to be an effortless yet effective technique. Therefore supplementation of obese post-menopausal women with Soya (high protein source) as TVP granules and Caralluma (indigenous dietary supplement) in the form of a capsule was attempted, to suppress the appetite and thereby cause a reduction in body weight, plasma lipid levels, blood pressure and nutrient intake, with an improvement in physical discomforts.
3.2 HYPOTHESES

1. There would be a significant difference in body weight, BMI, waist circumference, hip circumference, waist to hip ratio (WHR), mid upper arm circumference (MUAC) measured initially and after 30, 60 and 90 days of supplementation with Soya in obese post-menopausal women subjects.

2. There would be a significant difference in serum triglyceride, total cholesterol, HDL, LDL, VLDL, TC/HDL ratio and LDL/HDL ratio levels analysed initially and after ninety days of supplementation with Soya in obese post-menopausal women subjects.

3. There would be a significant difference between systolic pressure and diastolic pressure measurements assessed initially and after ninety days of supplementation with Soya among the subjects.

4. There would be a significant difference between energy, protein, fat and carbohydrate intake of subjects determined initially and at the end of supplementation with Soya.

5. There would be a significant difference between the physical discomforts of obese women with respect to sweating, exhaustion, breathlessness, fatigue, inability to climb stairs, pain in joints, lethargic at work, gastritis, constipation, flatulence and indigestion, observed initially and at the end of ninety days of supplementation with Soya.

6. There would be a significant difference in body weight, BMI, waist circumference, hip circumference, waist to hip ratio (WHR) and mid upper arm circumference (MUAC) measured initially and after 30, 60 and 90 days of supplementation with Caralluma in obese post-menopausal women subjects.

7. There would be a significant difference in serum triglyceride, total cholesterol, HDL, LDL, VLDL, TC/HDL ratio and LDL/HDL ratio levels analysed initially and after ninety days of supplementation with Caralluma in obese post-menopausal women subjects.
8. There would be a significant difference between systolic pressure and
diastolic pressure values assessed initially and after ninety days of
supplementation with Caralluma among the subjects.

9. There would be a significant difference between energy, protein, fat and
carbohydrate intake of subjects determined initially and at the end of
supplementation with Caralluma.

10. There would be a significant difference between the physical discomforts
of obese women with respect to sweating, exhaustion, breathlessness,
fatigue, inability to climb stairs, pain in joints, lethargic at work, gastritis,
constipation, flatulence and indigestion, observed initially and at the end
of ninety days of supplementation with Caralluma.

11. There would be no significant difference between the anthropometric
measurements like weight, BMI, waist circumference, hip circumference,
WHR ratio and MUAC obtained initially and after 30, 60 and 90 days
without any supplementation in the control group.

12. There would be no significant difference between the lipid profile
measurements such as triglyceride, LDL, VLDL, HDL, total cholesterol
and risk factor ratio levels such as TC/HDL ratio and LDL/HDL ratio
taken initially and after ninety days without any supplementation in the
control group.

13. There would be no significant difference between systolic and diastolic
pressure taken initially and after ninety days without any
supplementation in the control group.

14. There would be no significant difference between energy, protein, fat and
carbohydrate intake of subjects initially and after ninety days, without
any supplementation in the control group.

15. There would be no significant difference between the physical
discomforts of obese women with respect to sweating, exhaustion,
breathlessness, fatigue, inability to climb stairs, pain in joints, lethargic at
work, gastritis, constipation, flatulence and indigestion, taken initially and after ninety days without any supplementation in the control group.

16. There would be no significant difference between the anthropometric measurements like weight, BMI, waist circumference, hip circumference, WHR ratio and MUAC obtained initially before the supplementation period for the Soya group and those obtained for the control group.

17. There would be a significant difference in the anthropometric measurements such as weight, BMI, waist circumference, hip circumference, WHR ratio and MUAC taken at the end of 30, 60 and 90 days supplementation with Soya for the experimental group and those taken for the control group after 30, 60 and 90 days without supplementation.

18. There would be no significant difference between the lipid profile measurements such as triglyceride, LDL, VLDL, HDL, total cholesterol and risk factor ratio levels such as TC/HDL ratio and LDL/HDL ratio taken initially before the supplementation period for the Soya group and those obtained for the control group.

19. There would be a significant difference in the lipid profile measurements such as triglyceride, LDL, VLDL, HDL, total cholesterol and risk factor ratio levels such as TC/HDL ratio and LDL/HDL ratio taken at the end of supplementation with Soya for the experimental group and those taken for the control group after ninety days without supplementation.

20. There would be no significant difference between systolic and diastolic pressure taken initially before the supplementation period for the Soya group and those obtained for the control group.

21. There would be a significant difference in systolic and diastolic pressure taken at the end of supplementation with Soya for the experimental group and those taken for the control group after ninety days without supplementation.
22. There would be no significant difference between energy, protein, fat and carbohydrate intake of subjects initially before the supplementation period for the Soya group and those obtained for the control group.

23. There would be a significant difference in energy, protein, fat and carbohydrate intake of subjects taken at the end of supplementation with Soya for the experimental group and those taken for the control group after ninety days without supplementation.

24. There would be no significant difference between the physical discomforts of obese women with respect to sweating, exhaustion, breathlessness, fatigue, inability to climb stairs, pain in joints, lethargic at work, gastritis, constipation, flatulence and indigestion, taken initially before the supplementation period for the Soya group and those obtained for the control group.

25. There would be a significant difference in the physical discomforts of obese women with respect to sweating, exhaustion, breathlessness, fatigue, inability to climb stairs, pain in joints, lethargic at work, gastritis, constipation, flatulence and indigestion taken at the end of supplementation with Soya for the experimental group and those taken for the control group after ninety days without supplementation.

26. There would be no significant difference between the anthropometric measurements like weight, BMI, waist circumference, hip circumference, WHR ratio and MUAC obtained initially before the supplementation period for the Caralluma group and those obtained for the control group.

27. There would be a significant difference in the anthropometric measurements such as weight, BMI, waist circumference, hip circumference, WHR ratio and MUAC taken at the end of 30, 60, 90 days supplementation with Caralluma for the experimental group and those taken for the control group after 30, 60 and 90 days without supplementation.
28. There would be no significant difference between the lipid profile measurements such as triglyceride, LDL, VLDL, HDL, total cholesterol and risk factor ratio levels such as TC/HDL ratio and LDL/HDL ratio taken initially before the supplementation period for the Caralluma group and those obtained for the control group.

29. There would be a significant difference in the lipid profile measurements such as triglyceride, LDL, VLDL, HDL, total cholesterol and risk factor ratio levels such as TC/HDL ratio and LDL/HDL ratio taken at the end of supplementation with Caralluma for the experimental group and those taken for the control group after ninety days without supplementation.

30. There would be no significant difference between systolic and diastolic pressure taken initially before the supplementation period for the Caralluma group and those obtained for the control group.

31. There would be a significant difference in systolic and diastolic pressure taken at the end of supplementation with Caralluma for the experimental group and those taken for the control group after ninety days without supplementation.

32. There would be no significant difference between energy, protein, fat and carbohydrate intake of subjects initially before the supplementation period for the Caralluma group and those obtained for the control group.

33. There would be a significant difference in energy, protein, fat and carbohydrate intake of subjects taken at the end of supplementation with Caralluma for the experimental group and those taken for the control group after ninety days without supplementation.

34. There would be no significant difference between the physical discomforts of obese women with respect to sweating, exhaustion, breathlessness, inability to climb stairs, pain in joints, lethargy at work, gastritis, constipation, flatulence and indigestion taken initially before the supplementation period for the Caralluma group and those obtained for the control group.
35. There would be a significant difference in the physical discomforts of obese women with respect to sweating, exhaustion, breathlessness, fatigue, inability to climb stairs, pain in joints, lethargic at work, gastritis, constipation, flatulence and indigestion taken at the end of supplementation with Caralluma for the experimental group and those taken for the control group after ninety days without supplementation.

36. There would be no significant difference between the anthropometric measurements like weight, BMI, waist circumference, hip circumference, WHR ratio and MUAC obtained initially before the supplementation period for the Soya group and those obtained for the Caralluma group.

37. There would be a significant difference between the anthropometric measurements like weight, BMI, waist circumference, hip circumference, WHR ratio and MUAC obtained at the end of supplementation period for the Soya group and those obtained for the Caralluma group after ninety days of supplementation.

38. There would be no significant difference in the lipid profile measurements such as triglyceride, LDL, VLDL, HDL, total cholesterol and risk factor ratio levels such as TC/HDL ratio and LDL/HDL ratio taken initially for Caralluma and Soya group.

39. There would be a significant difference in the lipid profile measurements such as triglyceride, LDL, VLDL, HDL, total cholesterol and risk factor ratio levels such as TC/HDL ratio and LDL/HDL ratio taken at the end of supplementation with Caralluma group and those taken for the Soya group after ninety days of supplementation.

40. There would be no significant difference in systolic and diastolic pressure taken initially for Soya and Caralluma group.

41. There would be a significant difference in systolic and diastolic pressure taken at the end of supplementation with Soya and those taken for the Caralluma group after ninety days of supplementation.
42. There would be no significant difference in energy, protein, fat and carbohydrate intake of subjects taken initially for Caralluma and Soya group.

43. There would be a significant difference in energy, protein, fat and carbohydrate intake of subjects taken at the end of supplementation with Caralluma and those taken for the Soya group, after ninety days of supplementation.

44. There would be no significant difference in the physical discomforts of obese women with respect to sweating, exhaustion, breathlessness, fatigue, inability to climb stairs, pain in joints, lethargic at work, gastritis, constipation, flatulence and indigestion taken initially for Soya and Caralluma group.

45. There would be a significant difference in the physical discomforts of obese women with respect to sweating, exhaustion, breathlessness, fatigue, inability to climb stairs, pain in joints, lethargic at work, gastritis, constipation, flatulence and indigestion taken at the end of supplementation with Soya and those taken for the Caralluma group after ninety days of supplementation.

46. There would be a significant correlation within anthropometric measurements like body weight, BMI, MUAC, WHR, waist circumference and hip circumference among Soya supplemented group.

47. There would be a significant correlation within anthropometric measurements like body weight, BMI, MUAC, WHR, waist circumference and hip circumference among Caralluma supplemented group.

48. There would be a significant correlation within lipid profile assessments such as TC, LDL, HDL, VLDL, TC/HDL ratio, LDL/HDL ratio and triglycerides among Soya supplemented group.
49. There would be a significant correlation within lipid profile assessments such as TC, LDL, HDL, VLDL, TC/HDL ratio, LDL/HDL ratio and triglycerides among Caralluma supplemented group.

50. There would be a significant correlation between anthropometric measurements like body weight, BMI, MUAC, WHR, waist circumference, hip circumference and lipid profile measurements such as triglyceride, total cholesterol, LDL, HDL, VLDL, TC/HDL ratio and LDL/HDL ratio, blood pressure values such as, systolic and diastolic pressure, nutrient intake like energy, protein, fat and carbohydrate intake among the Soya supplementation group, in the post intervention phase.

51. There would be a significant correlation between anthropometric measurements like body weight, BMI, MUAC, WHR, waist circumference, hip circumference and lipid profile measurements such as triglyceride, total cholesterol, LDL, HDL, VLDL, TC/HDL ratio and LDL/HDL ratio, blood pressure values such as, systolic and diastolic pressure, nutrient intake like energy, protein, fat and carbohydrate intake among the Caralluma supplementation group, in the post intervention phase.

3.3 DESCRIPTION OF VARIABLES

The description of the variables related to the present study” Effect of Soya and Caralluma on obese post-menopausal women” is presented as follows:

A. Independent variables

The variables, which act as a stimulus, are independent variables (Kothari, 2004). The factors which will influence the responses of obese post-menopausal women have been categorized as independent variables.

a. Soya

Soyabean contains 30-50% protein. Soy protein is therefore a high quality, complete protein. These are either isolated (or) textured soy protein (Singh, 2002). It is operationalised in the form of textured vegetable protein granules called as soya
granules which has natural golden yellow colour, with uniform shape and acceptable flavours, packed in polythene packets of about 40 g each.

b. Caralluma

It is an edible, succulent cactus grown wild all over India and is part of the daily diets of several native populations (Preuss, 2007). It is operationalised in the form of an extract [Caralluma fimbriata extract (CFE)], as a fine powder, which was filled in gelatin capsules (zero size; approved pharmaceutical grade) of 500 mg each and was packed in polypropylene containers.

B. Dependent variables

The dependent variable is the response variable, the event following the observed behavior (Kothari, 2004). The following variables are the responses to the characteristics of the samples and hence are termed as dependent variable (Kothari, 2004).

i). Anthropometric measurements

Body weight

Weight is the simplest measurement of growth and nutritional status (Swaminathan, 1985). Weight was operationalised as the exact body weight measured with minimal clothes, footwear and marked in an erect standing posture with head, abdomen and legs in the same plane, using standard weighing machine, by Jeliffe method (1966). It was measured in Kilograms.

Mid upper arm circumference (MUAC)

It is operationally defined as the measure of fullest circumference of the upper arm at the midpoint, between the olecranon process of the ulna and the acromion process of the scapula, with the hands lying parallel to the body (Hall et al, 2007), using a stretch resistant tape by Harisson method (1988). It is measured in centimeters. MUAC is proposed as another important indicator of obesity, and is also reported to closely reflect body fat tissue (Macizioglo et al, 2010).
**Waist circumference**

Waist circumference is operationally defined as the measure of the fullest distance around the abdomen at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest, using a stretch resistant tape by WHO method (2008). It is measured in centimeters. Waist circumference is one of the most practical tools to assess abdominal fat for chronic disease risk and during weight loss treatment (Health Ministry of India, 2008).

**Hip circumference**

Hip circumference is operationally defined as the measure at the level of maximum posterior extension of the buttocks (AIHW, 1997), using a stretch resistant tape by WHO method (2008). It is measured in centimeters.

**Waist to hip ratio (WHR)**

The waist to hip ratio was calculated from waist and hip circumference measurements using the formula,

\[
\text{WHR} = \frac{\text{Waist circumference}}{\text{Hip circumference}}
\]

(Williams et al, 1990).

**Body mass index (BMI)**

Body mass index (BMI) is calculated by dividing the weight by the height squared; its unit is therefore Kg/m$^2$, among the subjects. BMI range of 25-29.9 was used for the intervention study. BMI remains the most commonly used approach for public health studies (Nisha, 2006).

**ii). Lipid profile**

**Total cholesterol (TC)**

A fat-related compound, cholesterol is synthesized by animal bodies and found in our diet only in animal food sources. Elevated blood cholesterol has been shown to be a primary key to the development of atherosclerosis (Mensink et al, 2003). It is operationally defined as the amount of total cholesterol present in serum.
of the subjects, estimated by CHOD-PAP enzymatic photometric method, which is measured in mg/dl.

**Total Triglycerides (TG)**

They circulate throughout your body with the help of proteins that transport the lipids (lipoproteins) (ICSI, 2010). High triglycerides are often a sign of other conditions that increase the risk of heart disease and stroke as well, including obesity and metabolic syndrome (Arsenault et al, 2009). It is operationally defined as the amount of total triglycerides present in serum of the obese post-menopausal subjects, estimated by Trinder method, which is measured in mg/dl.

**Very low density lipoprotein (VLDL)**

These are lipoproteins which transports endogenous triglycerides (Mahan, 2004). These are operationalised as the level of lipoproteins present in serum of the subjects, which are calculated from the amount of serum triglycerides present in them using the formula, TG (triglyceride)/5. It is measured in mg/dl.

**Low density lipoprotein (LDL)**

These are lipoproteins which transports major portion of cholesterol in the body (Mahan, 2004). It is operationally defined as the amount of low density lipoprotein present in the serum of the respondents, calculated using the Friedwald formula. It is measured in mg/dl.

**High density lipoprotein (HDL)**

These are lipoproteins involved in reverse cholesterol transport (Mahan, 2004). HDL is operationally defined as the level of HDL present in the serum of the subjects, assessed using Immuno FS method. It is measured in mg/dl.

**Total cholesterol/HDL ratio and LDL/HDL ratio**

Two frequently used risk factors assessment ratios are the TC/HDL ratio and the LDL/HDL ratio. If TC/HDL ratio of 4.4 is assigned a CHD risk ratio of 1.0, a TC/HDL ratio of 3.4 cuts CHD risk in half whereas a ratio of 9.6 doubles the ratio. An even stronger correlation is noted for the LDL/HDL
ratio (Grundy, 1990). They are operationally defined as the level of risk ratios computed among the subjects, using the following formulas:

\[
\frac{TC}{HDL} = \frac{\text{Total cholesterol}}{\text{High density lipoprotein}}
\]

\[
\frac{LDL}{HDL} = \frac{\text{Low density lipoprotein}}{\text{High density lipoprotein}}
\]

iii). Blood pressure measurements

**Systolic blood pressure**

Systolic pressure was defined as the maximum pressure during systole. It undergoes considerable fluctuations. Excitements, exercise, meals etc, increase it, while sleep, rest etc, diminish it (Chatterjee, 1999). It is operationally defined as the measure of the first of the consecutive Korotkoff sounds that appear, after the subjects had relaxed for 5 minutes, by auscultatory method using sphygmomanometer. It is expressed in mm/Hg (Chaudhuri, 1993).

**Diastolic blood pressure**

Diastolic pressure was defined as the minimum pressure during diastole. It is the measure of peripheral resistance. Increase of diastolic pressure indicates that the heart is approaching towards its failure (Chatterjee, 1999). It is operationalised as the point of disappearance of sound observed at the end of all the consecutive phases of Korotkoff sounds in auscultatory method of assessing blood pressure, using sphygmomanometer. It is expressed in mm/Hg (Chaudhuri, 1993).

iv). Nutrient intake analysis

**Energy**

It is operationally defined as the energy or calorific value of foods consumed by the subjects depending on the quantity of carbohydrates, fats and proteins present in them. The energy value of foods is computed using Nutritive value of Indian foods (Gopalan, 1986). It is expressed in terms of kilocalories (Kcal) or mega joules (MJ) (Swaminathan, 1985).
Protein

Protein is defined as the most abundant nitrogen containing compound in the diet of the subjects. It is measured in grams (Gibney et al., 2009). It is also computed using Nutritive value of Indian foods (Gopalan, 1986), based on per 100 grams of food consumed.

Fat

Fat is operationally defined as the amount of both visible and invisible fats consumed by the subjects, which is essential to supply more calories without increasing the bulk of the food. (Rama Rao et al., 1994). It is computed using Nutritive value of Indian foods (Gopalan, 1986), based on per 100 grams of food consumed. It is measured in grams.

Carbohydrate

Carbohydrates are the single most abundant and economic sources of food energy in the human diet, constituting 40-80% of total energy intake in different populations (Gibney, 2009). It is calculated from the amount of food consumed by the subjects using Nutritive value of Indian foods (Gopalan, 1986). It is measured in grams.

v. Assessment of physical discomforts

Sweating

It is operationally defined as a common occurrence among obese postmenopausal women, typically experienced as a feeling of intense heat with sweating and may typically last from two to thirty minutes for each occurrence (NCCAM, 2008).

Joint pain

As woman approaches menopause, typically between the ages of 45 and 55, her body goes through drastic hormonal fluctuations which may affect the bone health and hence she will often experience joint pain (NWHIC, 2007). It is operationally defined as pain in joints with severe intensity, preventing the subjects from stretching (or) folding their joints.
Fatigue (physical fatigue)

It is operationally defined as decreased muscle performance and functional capacity among post-menopausal women, due to change in ovarian hormone status (Sipila, 2003).

Exhaustion

It is defined as a state of extreme loss of physical and mental abilities caused by fatigue or illness (Mosby, 2009). Feeling of exhaustion is most likely linked to other symptoms of menopause like hot flashes that inevitably drain your energy (NIH, 2011).

Breathlessness

It is operationally defined as shortness of breath observed among the subjects due to an increased amount of fat accumulation in the chest wall and abdomen, which limits respiratory expansion reducing lung volume (Feldman, 2000).

Inability to climb stairs

It is defined as difficulty in climbing stairs without holding any support observed among obese post-menopausal women due to breathlessness and also due to complications such as flat feet and pain in legs (Swaminathan, 1985).

Lethargic at work

Lethargy is operationally defined as the state of sluggishness, drowsy dullness or apathy feeling observed among the subjects. Around menopause, some women experience changing moods, decreasing ability to cope with normal life stresses, forgetfulness, and a lessening of self esteem. As these changes occur, tiredness and lethargy may reduce a woman's capacity to carry out normal life routines. (Barclay, 2010).

Constipation

It is defined as the infrequent passage of hard stools, difficulty in evacuation observed in subjects. Menopausal constipation generally occurs due to the slowing of the gastrointestinal system and heavy demands put on the liver. Eating while stressed, overeating or eating too many unhealthy foods may result in constipation (NWHIC, 2007).
Flatulence

Flatulence is operationally defined as abdominal discomfort with bloating and belching, experienced by the subjects. It is caused by an excess of gas in the stomach or intestines. It can be painful also. It affects about 25 percent of menopausal women, with more than 69 percent reporting an increase in gas and bloating (Lund, 2008).

Indigestion

Indigestion, known as "upset stomach" or "dyspepsia", means hard or difficult digestion. It is operationalised as regurgitation of stomach contents back into the oesophagus due to gastroesophageal reflux disease (GERD), which is a complication associated with obesity (Nisha, 2006).

Gastritis

Gastritis occurs when the lining of the stomach becomes inflamed or swollen (Lee and Feldman, 2010). Low stomach acidity and increased infection by Helicobacter pylori (H. pylori) (Morihara et al, 2001) one of the most common chronic bacterial infections of humans are recognized as a major cause of gastritis, gastric ulcer disease, gastric carcinoma and B-cell gastric lymphoma (Young, 2001). It is operationally defined as a condition characterized by chronic or recurrent pain in the upper abdomen, upper abdominal fullness. It is accompanied by bloating and belching.

3.4 RESEARCH DESIGN

Research design is an arrangement of conditions of data in a manner that aims to combine relevance to the research purposes with economy in procedure. (Gupta, 2001). The research design is the conceptual structure within which research is conducted. It constitutes the blue print for the collection, measurement and analysis of data (Kothari, 2004).

The present research work was carried on human subjects, hence an ethical committee, comprising of the following members, a lawyer, a doctor, a staff of an NGO, a subject expert and the supervisor, was formed. Based on the recommendations, a consent letter was framed. A copy of the consent letter and the
ethical committee approval letter is given in Appendix I. After getting the consent of the participants the research work was carried out.

The study was conducted in three phases,

Phase I- General survey using Ex-post facto research design.

Phase II – Development of Caralluma extract capsule and procurement of soya granules

Phase III- Intervention study using Experimental research design.

Phase I

General survey

Ex-post facto research design was adopted in the first phase. Ex-post facto research is systematic empirical enquiry in which the scientist does not have direct control of independent variables because their manifestations have already occurred (or) because they are inherently not manipulated (Gupta, 2001).

Using this design, general information, case history, information regarding menstruation, indulgence in weight reducing activities, psychological factors influencing food intake, dietary habits, frequency of consumption of foods, physical discomforts, complications of obesity, nutrient intake and energy expenditure pattern of obese post-menopausal women were studied.

Phase II

Development of Caralluma extract capsule and procurement of soya granules

The aerial parts of the fresh herbage of Caralluma fimbriata (50kg) (Plate 1) was collected and cleaned thoroughly. Its identity was confirmed with the help of pharmacognosist of Centre for Traditional Medicine and Research (CTMR). The herbage was then chopped into small pieces of 2 inches and it was dried under direct sunlight, to bone dry condition. The dried herbage (5kg) (Plate 2) was then pulverized into a coarse powder using heavy duty mixer (all its contact parts are made of stainless steel). The coarse powder was soaked (overnight soaking) in a solvent of hydroalcohol containing 10 litres of ethyl alcohol and 10 litres of distilled
Plate 1: Fresh Plant of Caralluma fimbriata

Plate 2: Dried herbage of Caralluma fimbriata
water, in the ratio of 50:50. The mixture was warmed to 40°C in a water bath and the extract was filtered using vacuum filter. The filtrate so obtained was concentrated in a rotary vacuum flash evaporator, until it becomes a thick paste. The paste was then dried under vacuum using vacuum desiccator and powdered into a fine powder using heavy duty mixer. The fine powder obtained was filled in hard gelatin capsules (zero size; approved pharmaceutical grade), using manual capsule filling machine. Each capsule contains 500mg of Caralluma fimbriata extract. The capsules were filled and given in polypropylene containers to the subjects. Physico-chemical properties of Caralluma was analysed in CTMR. A copy of the physico-chemical properties of Caralluma is given in Appendix- VIII.

Soya granules (TVP granules) were obtained from the market (Sakthi Soyas) Physico-chemical properties and nutritional facts of Soya analysed in Sakthi Soyas Private Limited, is given in Appendix- VIII.

Phase III

Intervention study

The second phase was experimental in nature. A pre-test post test experimental design was used to determine the effect of Soya and Caralluma on anthropometric measurements, lipid profile, blood pressure, nutrient intake and physical discomforts of selected obese post-menopausal women subjects.

In the pre test-post test design, pre-test was administered before the application of experimental and control treatments and post-test was administered at the end of the treatment period (Best and Kahn, 1995).

Obese post-menopausal women subjects, whose menstrual cycle was stopped for more than five years, and whose BMI was within 25-29.9 range were selected as sub sample for the experimental study. The subjects were randomly assigned to experimental groups (Soya and Caralluma group) and control group.

Fig

Research design- Intervention

Experimental and control group ——— Pre-test – Treatment – Post-test design
Pre-test ——— Post - test
90 days
Both the groups were subjected to Pre-test, initially. The subjects of the experimental groups were given dietary supplements (one group received soya and another group received caralluma) and control group were not given any supplement, their impact on anthropometric measurements (were determined at the end of 30, 60 and 90 days), lipid profile, blood pressure, nutrient intake and physical discomforts were assessed at the end of supplementation period (90 days) (Post-test).

3.5 SAMPLING PROCEDURE

PHASE I

General survey

Initially one thousand three hundred and fifty post-menopausal women were approached and out of them, women who were found to be obese (BMI>25) and those who were able to give all the required information were selected for the present study. Due to reasons such as unwillingness, and were either of ideal body weight or underweight, only six hundred subjects were taken as the final sample. The study was conducted in Chennai. The sample for the study was selected from different parts of Chennai city, by random sampling technique.

PHASE III

Intervention study

Purposive sampling technique was used to select the subjects (n=150) for the experimental study, from the sample (n=600) of the general survey, based on the inclusion criteria of the study. The sub-sample selected (n=150) had a BMI within the range 25-29.9 to ensure uniformity in degree of obesity and were post-menopausal in status for more than five years. The sub-sample selected (n=150) were randomly assigned into three groups, comprising of 50 subjects in each group.

**Soya Group:** The subjects were given Soya supplement, 40g each, as TVP granules, as evening snack.

**Caralluma Group:** The subjects were given Caralluma supplement, 2 capsules (for one subject) of 500mg [Caralluma fimbriata extract(CFE)] each per day, one capsule, 30 minutes before breakfast and another 30 minutes before lunch.

**Control Group:** The subjects were treated as control group without any supplementation.

The experimental study was carried out for a period of 90 days.
A written consent was obtained from all the subjects (n=150) of intervention study, after explaining them the objectives of the study. They were requested to extend their full cooperation to carry out the analysis of the experimental research. It was confirmed that the experimental subjects in phase III were willing to take the dietary supplement (n=50 for Soya and another n=50 for Caralluma) for a period of 90 days.

**Criteria for selection of the subjects for intervention study**

Obese post-menopausal women subjects were selected (n=150) (after obtaining their willingness to pursue the study) for the experimental research using the following inclusion and exclusion criteria,

**Inclusion criteria,**

1) With BMI in the range of 25 – 29.9  
2) In their post-menopausal stage (whose menstrual cycle was stopped for more than five years).

**Exclusion criteria**

Exclude those subjects,

1) With complications such as diabetes, hypoglycemia, thyroid disorders, atherosclerosis, coronary artery disease.  
2) Who indulged in any form of weight reducing activities such as using weight reduction pills, meal replacements, surgical procedure or starvation diet for losing weight.

**Pilot study**

Few obese post-menopausal women subjects who fulfilled both exclusion and inclusion criteria of the intervention study were selected (apart from phase III subjects) from phase I and were given Soya (n=13) and Caralluma (n=13) supplement, few were not given any supplement (n=13), for a period of 45 days, the subjects were explained about the way it need to be consumed before supplementation. The compliance of the subjects towards the supplement and any problems aroused while supplementation (in the way of administration and while assessing) were analysed and rectified.
**Experimental Design of the Study**

Selection of post-menopausal women (n = 1350)  
(Phase I)

Selection of obese post-menopausal women  
with a BMI > 25  (n= 600)

Base line investigation

Administer the interview schedule to determine personal history, dietary habits, medical history, energy expenditure pattern, frequency of food intake.

Assess height, Weight (Wt) and BMI.

Diet survey by 24 hour dietary recall for 3 days.

Selection of the representative sample  
(n = 150) with BMI 25 – 29.9 and post-menopausal for more than 5 years

Product Preparation  
(Phase II)

Soya granules bought from market

Caralluma capsules were developed

Supplementation programme  
for a period of 90 days

Parameters studied:
Initially, 30, 60 days – Wt, BMI, HC, WC, WHR, MUAC
Initially – Lipid profile
Initially – Nutrient intake
Initially – Blood pressure
Initially – Physical discomforts

Control group (n=50)  
without supplementation

Soya group (n= 50)  
40gms of soya granules per day

Caralluma group (n= 50)  
2 capsules (500 mg of CFE each) / day

Assessment of parameters after 90 days of supplementation

Wt, WHR, BMI, MUAC, WC, HC

Lipid Profile

Nutrient intake

BP

Physical discomforts
3.6 DURATION OF THE STUDY

The study extended over a period of six months for general survey and ninety days for experimental study between the years 2008 and 2010.

3.7 TOOLS USED FOR THE STUDY

The tools used for data collection are,

1) Interview schedule - to obtain general information, case history, information regarding menstruation, weight reduction activities, psychological factors influencing food intake, frequency of consumption of foods, dietary habits, energy expenditure pattern, physical discomforts, nutrient intake and complications of the subjects.

2) Anthropometric assessments - height, weight, mid upper arm circumference, waist circumference, hip circumference, waist to hip ratio (WHR) and Body mass index (BMI) of the subjects were assessed.

3) Blood pressure measurements - Systolic blood pressure and diastolic blood pressure of the subjects were measured.

4) Biochemical tests - Total cholesterol (TC), Triglycerides, Very low density lipoprotein (VLDL), Low density lipoprotein (LDL), High density lipoprotein (HDL), Total cholesterol/HDL ratio, LDL/HDL ratio of the subjects were analysed.

5) Diet survey - Intake of nutrients like energy, protein, fat and carbohydrate among the subjects were assessed using 24 hour dietary recall method for three days.

1. Interview Schedule

An interview schedule was prepared and administered to elicit information from all subjects initially, before selection of subject for intervention study. General information pertaining to their age, educational qualification, occupation, socio-economic status, family history, information regarding menstruation, psychological
factors related to obesity, dietary habits, physical discomforts, complications associated with obesity, energy expenditure pattern and frequency of food intake of the subjects were obtained (n=600). Queries related to physical discomforts of the subjects were re-administered again to the subjects of the experimental study (n=150) from the same Interview schedule at the end of post-intervention period. A copy of the Interview schedule is presented in the Appendix II.

Reliability and validity of the tool

The interview schedule was pre-tested on 150 obese post-menopausal women not included in the study, before it was administered to the subjects. From the pre-tested interview schedule, ambiguous and unstructured questions were rectified and reframed. The modified interview schedule was then used in the study.

2. Anthropometric Measurements

All the measurements were obtained under standard conditions and with utmost care.

a) Height measurements

Height was measured using a stadiometer. The subject (adult obese women) was required to stand bare foot and stand on the platform of the stadiometer with heels together, arms hanging and ensured that the neck, shoulders, buttocks, heels were in the same plane and perpendicular to the ground. The head piece was lowered and made to contact the top of the head. The measurement was directly read from the scale of the stadiometer. The height was recorded nearer to 0.1 cm, in upright standing posture (Jeliffe, 1966).

b) Weight measurements

The weight was assessed by using standard weighing machine. The subject was made to stand on the centre of the scale without touching anything else and with minimum clothing and the weight was recorded to the nearest 0.5 kg. The measurements were preferably taken under basal conditions before taking a full meal (Jeliffe, 1966).
c) **Mid-upper arm circumference**

The mid upper arm circumference (MUAC) was measured with the subject standing erect. The subject's elbow was flexed to 90° and the midpoint between the tip of acromion and olecranon process was located. The tape was placed around the arm at the midpoint, with the arm relaxed and elbow extended and the circumference was recorded to the nearest 0.1 cm (Harrison et al, 1988).

d) **Waist circumference**

Waist circumference should be measured at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest, using a stretch resistant tape that provides a constant 100 g tension. The subject should stand with feet close together, arms at the side and body weight evenly distributed, and should wear little clothing. The subject should be relaxed, and the measurements should be taken at the end of a normal expiration. Each measurement should be repeated twice; if the measurements are within 1 cm of one another, the average should be calculated. If the difference between the two measurements exceeds 1 cm, the measurement should be repeated (WHO, 2008b).

e) **Hip circumference**

Hip circumference should be measured around the widest portion of the buttocks, with the tape parallel to the floor. The subject should stand with feet close together, arms at the side and body weight evenly distributed, and should wear little clothing. The subject should be relaxed, and the measurements should be taken at the end of a normal expiration. Each measurement should be repeated twice; if the measurements are within 1 cm of one another, the average should be calculated. If the difference between the two measurements exceeds 1 cm, the measurement should be repeated. The hip circumference measurement should be taken around the widest portion of the buttocks (WHO, 2008b).
f) **Body mass index (BMI)**

The body mass index was calculated from height and body weight measurements using the formula,

\[
\text{BMI} = \frac{\text{Weight (kg)}}{\text{Height}^2 (\text{m})}.
\]

It is a reasonable indication of the nutritional status of adults and used as an indicator of health risk.

g) **Waist to hip ratio (WHR)**

The waist to hip ratio was calculated from waist and hip circumference measurements using the formula,

\[
\text{WHR} = \frac{\text{Waist circumference}}{\text{Hip circumference}}.
\]

A schedule was prepared to collect anthropometric data. A copy of the schedule is appended with the thesis (Appendix IV).

3. **Blood pressure assessment**

The systolic and diastolic pressure of each subject was recorded with two calibrated mercury sphygmomanometer, using auscultatory method. The subjects were seated with uncrossed legs and rested for at least 5 minutes. The instrument is kept at the level of the heart and the cuff is tied round the upper arm, at least 2.5 cm above the elbow. The heart, sphygmomanometer and the arm should be at same horizontal plane. The bell of the stethoscope is placed on the brachial artery near the elbow. Pressure is raised to 200 mm Hg and then gradually released. Deflation is started at a rate of 2-3 mm/sec, neither more nor less. At first there is no sound heard by the stethoscope (“graveyard silence”); after the deflation has reached a particular stage, a ‘tap’ sound is heard and this marks the SBP, the reading at the side scale is noted, deflation continued till all sounds disappear. Between the tap sound and the
reappearance of silence a series of sounds, called Korotkov sounds, are heard as follows:

Phase I- the appearance of the tap sound. This marks the Systolic blood pressure.

Phase II- the sound take up “murmuring” quality.

Phase III- the sound become very loud and ‘gonging’ in quality. Sound is most intense in this phase.

Phase-IV- the sound suddenly becomes ‘muffled’.

Phase-V- all sounds disappear. This marks the diastolic blood pressure (Chaudhuri, 1993).

A schedule was prepared to collect blood pressure measurements. A copy of the schedule is appended with the thesis (Appendix V).

4. **Biochemical Tests**

Ten milliliter of blood was drawn from each subject before and after supplementation, for the various hematological tests.

The following estimations were made,


2. Serum triglyceride – Trinder method.

3. Plasma lipoproteins

   i) HDL - Immuno FS method.

   ii) VLDL – calculated using the following formula,

   \[
   \frac{\text{TG}}{5}
   \]

   iii) LDL - calculated using the following formula,

   \[
   \text{TC}-(\text{HDL+VLDL}) \quad \text{(Friedwald formula)}
   \]
iv) TC/HDL ratio – calculated using the formula,

\[
\frac{TC}{HDL}
\]

v) LDL/HDL ratio – calculated using the formula,

\[
\frac{LDL}{HDL}
\]

Lipid profile analysis was done in Sharp lab services, Chennai. A copy of the procedures is given in Appendix VI.

5. **Diet survey**

Three day, 24 hour dietary recall (oral questionnaire method) was used to elicit information regarding the daily consumption of calories, protein, fat and carbohydrate. This is a useful method in carrying out a diet survey for a large number of people in a short time.

Inquiries are made retrospectively about the nature and quantity of foods eaten during the previous 24, 48 and 72 hours. The data that is collected have to be translated into mean intake (grams) of food in terms of cereals, pulses, vegetables, fruits, milk, meat, fish, eggs, fats and sugars. (Bourne, 1971 and Taskar et al, 1967). The mean intake of nutrients of the subjects was calculated using the food composition tables given in “Nutritive value of Indian foods” (Gopalan, 1986) and compared with recommended dietary allowances and requirements, given by Indian council of medical research (2010). A copy of the dietary recall schedule is presented in the appendix (Appendix III).

3.8 **COLLECTION OF DATA**

**Phase I**

Administration of the Interview schedule, and 24 hour dietary recall

Post-menopausal women subjects (n=1350) from different parts of Chennai city were approached and out of them only 600 obese post-menopausal women who
were obese (BMI >25, assessed using height and weight measurements of post-menopausal women subjects) and who were found to give all the required information were selected as the subjects for the present study. The women were contacted in their homes, offices, fitness centres, clinics and self-help groups. The data was collected by the investigator with the help of printed materials in the form of Interview schedule and 24 hour dietary recall schedule. The purpose of the study was clearly explained to the respondents. Six hundred subjects were subjected to the Interview schedule and 24 hour dietary recall schedule (for 3 consecutive days) individually by the investigator. The questions were presented in simple and comprehensive manner, while the answers obtained were recorded immediately on the schedule. The respondents were asked to be candid and honest in their answering. Height, weight and BMI assessments were carried out. Based on body mass index and the information gathered, 150 subjects who satisfied the criteria for selection were chosen for the experimental study.

Phase III

Orientation of the subjects

The investigator selected the subjects for experimental study based on the criteria for the selection. The subjects chosen were briefed about the nature and the importance of the intervention study. The investigator was responsible for the motivation of the subjects. This included imparting a better understanding of the supplement, inculcating a positive attitude towards it and emphasizing the importance of regular consumption of the supplement and the way it has to be administered like for Soya consumption, how it has to be consumed (without adding any other flavouring substances, oil, and without any processing like cooking, it has to be taken in the same form as it is given) and how much it has to be consumed, (40g/day) and for Caralluma, how many capsules to be consumed (twice daily) and when (30 minutes before having their breakfast and lunch) were taught to the subjects.

Distribution of the supplement

The amount of Soya (Plate 3) to be taken by each subject per day is 40g (as TVP granules, as in the form in which it is available in the market), it was given in polythene packets (containing 40g of TVP granules after being measured in a standard weighing machine). The total amount of Caralluma supplement (Plate 4) to be taken by each subject per day is 1g. This amount was given in the form of two
Plate 3: Soya Granules (Textured Vegetable protein)

Plate 4: Capsule containing Caralluma fimbriata extract (CFE)
capsules of 500mg each per day. The subjects were advised to take one capsule 30 minutes before breakfast and another one 30 minutes before lunch. The duration of the intervention was 90 days, during which the investigator visited the subjects fortnightly to give the supplement (Soya and Caralluma were given to the respective subjects, every fortnight) and to report adverse events, if any. The compliance of the subjects to the ingestion of the supplement was also monitored during the visit, every fortnight. The above mentioned dosage for Caralluma and Soya was determined on the basis of studies conducted by Kuriyan et al (2007), Lawrence and Choudary (2004) and Allison et al (2003), respectively.

**Assessment of selected anthropometric measurements**

The body weight and standing height of the subjects were recorded using a standard weighing machine and stadiometer respectively. The mid-upper arm circumference, hip circumference, waist circumference of the subject was assessed using a measuring tape. The assessments were made initially before supplementation (first day) and at the end of 30, 60 and 90 days of supplementation with Soya (as TVP granule), Caralluma (as capsules) and without supplementation in control group.

**Determination of systolic and diastolic blood pressure**

Systolic and diastolic blood pressure measurements were determined using sphygmomanometer, initially before supplementation (first day) and at the end of supplementation with Soya (as TVP granules), Caralluma (as capsules) and without supplementation in control group for a period of ninety days.

**Collection of blood sample for lipid profile analysis**

Ten milliliter of venous blood was drawn from ante-cubital vein after sterilizing the region with alcohol. Blood samples were drawn in the morning after 10-12 hours of fasting, using disposable syringes from each subject. The collected blood samples were allowed to stand at room temperature for 2-3 hours. After the clot has formed and the serum separated, the blood samples were centrifuged for 20 minutes at 3000 rpm. The supernatant layer of serum was transferred into clean, dry, labeled vials. The serum was stored in refrigerator for the subsequent estimation of total cholesterol, triglyceride, and HDL levels. All the estimates were carried out
within 24-48 hours after separating the serum. A copy of the experimental procedure is given in the Appendix VI. The assessments were made initially before supplementation (first day) and at the end of supplementation with Soya (as TVP granules), Caralluma (as capsules) and without supplementation in control group (ninetieth day).

Re-administration of the Interview schedule and 24 hour dietary recall

The subjects were presented with the same Interview schedule (questions related to only physical discomforts) and 24 hour dietary recall (for 3 consecutive days), which was given initially before supplementation, at the end of ninety days of supplementation with Soya and Caralluma and without supplementation in control group. This was to determine the improvements if any in the physical discomforts caused by obesity and to find the energy, protein, carbohydrate and fat intake at the end of ninety days.

Calculation of nutrient intake

The average calories, protein, fat and carbohydrate intake was calculated using food composition tables given in Nutritive value of Indian foods by Indian council of medical research (Gopalan, 1986).

3.9 PROCESSING AND ANALYSIS OF DATA

In the present study, processing of data was done to determine the efficacy of Soya and Caralluma supplement on selected anthropometric measurements, lipid profile, blood pressure, nutrient intake and physical discomforts of obese post-menopausal women. The results were tabulated and the analysis was done as follows:

3.9.1 Descriptive analysis

The information obtained pertaining to general information, case history, information regarding menstrual cycle, weight reduction regimen followed, psychological factors influencing food intake, dietary information, physical discomforts, complications associated with obesity, energy expenditure pattern, food frequency and nutrient intake of subjects (n = 600) using the Interview schedule and 24 hour dietary recall was subjected to descriptive analysis. Percentage analysis and mean were calculated.
3.9.2 Inferential analysis

Statistical package for Social Science (SPSS) was used to compute inferential analysis. The “t” test of significance was done to find out the difference in anthropometric measurements, during the four assessments, initially, at the end of 30, 60 and 90 days of supplementation. “t” test was also applied to analyse the variation in lipid profile and blood pressure levels, during the two assessments, initially and after 90 days of supplementation. The nutrient intake of the subjects (n = 150) obtained initially and at the end of 90 days were statistically analysed using “t” test of significance.

Information regarding physical discomforts of obese post-menopausal women were obtained using an interview schedule during both pre and post intervention period, which was subjected to statistical analysis. Within group analysis of physical discomforts of the subjects was done using Wilcoxon sign rank test and between group analysis of physical discomforts of obese post-menopausal women was carried out using Mann-Whitney test.

3.9.3 Relational Analysis

To study the relationship between two variables within and between groups, correlation analysis was done. Correlation coefficient matrix was used to find out the changes in anthropometric measurements during the post-intervention phase for both Soya and Caralluma group. Correlation coefficient matrix was also used to examine the variations in lipid profile parameters during post-intervention phase for the both the experimental groups. Correlation coefficient was used to determine the relationship between anthropometric measurement, lipid profile parameters, blood pressure and nutrient intake among Soya and Caralluma groups.

The statistical methods used in the present study are appended in Appendix VII.

Data were collected from the subjects and analysed statistically as indicated in the methodology, details of which are presented and discussed in the next chapter.