METHODOLOGY

The methodology adopted for the present study on **Bone Mineral Health of Women and Impact of Intervention Strategies** is presented under the following headings:

PHASE I Selection of Area, Sample and Conduct of Survey

A. Selection of Area and Sample for the Survey
B. Formulation of Interview Schedule
C. Conduct of Survey and Collection of Information on Socio-Economic, Dietary and Other Background Details

PHASE II Assessment of Nutritional and Health Status and Bone Health Profile of the Selected Women

A. Anthropometric Measurements
B. Bone Mineral Density Measurement
C. Clinical Examination and Biophysical Assessment
D. Biochemical Assessment
E. Dietary Assessment

PHASE III Formulation and Nutrient Analysis of the Supplements and Conduct of Supplementation Study

A. Selection of Ingredients and Formulation of the Supplements
B. Nutrient Analysis of the Supplements
C. Microbial testing and Cost Computation of the Supplements
D. Approval for the Research Design and Supplementation Study
E. Selection of Women for Supplementation Study
F. Conduct of Supplementation Study
G. Evaluation of the Impact of Supplementation

PHASE IV Conduct of Nutrition Education and Lifestyle Management Programme

A. Selection of Women for Nutrition Education
B. Development of Nutrition Education Materials
C. Conduct of Nutrition Education and Lifestyle Management Programme
D. Demonstration and Training on Preparation of Mixes and Recipes Using Nutri Mixes
E. Evaluation of the Impact of Nutrition Education

PHASE V Analysis of Data

PHASE I

Selection of Area, Sample and Conduct of Survey

A. Selection of Area and Sample for the Survey

The area selected for the present study was Coimbatore District which is the third largest and second most popular city in Tamilnadu, India. It is a prominent medical tourism destination in South-India. According to 2011 Census, Coimbatore has a population of 3,458,045 of which male and female account for about 1,729,297 and 1,728,748 respectively (www.census2011.co.in). The investigator selected 8 rural and 4 urban areas for the present study which included rural areas like Thudiyalur (R), Kavundampalayam (R), Vadavalli (R), Madukkarai (R), Thondamuthur (R), Palladam (R), Sulur (R), Sultanpet (R) and urban areas like Selvapuram (U), Saibaba Colony (U), Peelamedu (U) and Gandhipuram (U) (Figure 1).

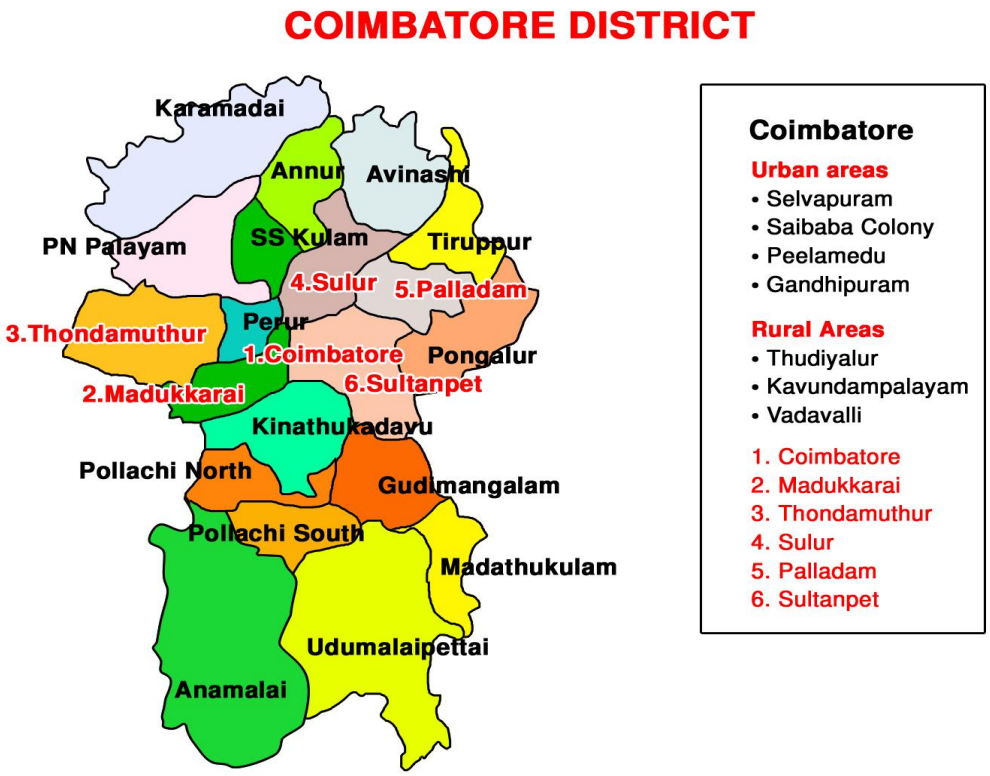
The investigator is familiar with these areas and easy availability of adequate number of women for the study formed the basis for the selection of these areas. Moreover these selected areas are convenient for conducting health camps and other activities. The investigator could establish a good rapport with the people of these areas. The investigator met the local and hospital authorities,

explained the purpose of the research and obtained permission to select the women for the present study.

Figure 1 represents the map of the selected areas in Coimbatore District.

Figure 1

Map of the selected areas of Coimbatore District for the study



Women were selected by purposive sampling method. In purposive sampling method, a desired number of sample units are selected liberally or purposely depending upon the object of the enquiry so that only the important items representing the true characteristics of the population are included in the sample (Kothari, 2007).

The investigator received help from four hospitals and three pharmacies like Global Ortho and Trauma Care hospital, Sakthi Nursing Home, Ganga Hospital, Coimbatore Bone and Joint hospital, Pharmed Medical Services, Madhuram Medicals and Thulasi Pharmacy located in Coimbatore city. The data

was collected through various Bone Health Awareness camps. Around 2230 women of 21–70 years were studied for Bone Mineral Density measurements by ultrasonic method. During these camps, the participants were interviewed about their socioeconomic back ground, nutrition history, food preference and life style habits.

Women are more prone to low bone mass conditions and are at greater risk of developing osteoporosis compared to men because of the hormonal change that can affect bone density which leads to osteoporosis (Cummings and Melton, 2002). The osteoporosis landscape in India and numerous research studies have revealed the tough fact that the occurrence of bone related disorders has raised rapidly between women of different ages due to inadequate nutrition and lifestyle changes. This is attributed to their skeletal structure, menstruation cycle, nutrition intake etc. Hence only women were chosen for the present study. Among the total population of 2230 women surveyed, around 120 women in the age group of 30-45 years with low bone mass condition, namely osteopenia were selected for the supplementation study.

Osteopenia is a condition where bone mineral density is lower than normal. It is considered to be a precursor to osteoporosis. Bone Mineral Density (BMD) is a measurement of the level of minerals in the bones that reflect density and strength. There is a greater risk in osteopenia since as the time passes, one may develop low Bone Mineral Density leading to osteoporosis. However, not every person diagnosed with osteopenia will develop osteoporosis. More specifically, osteopenia is defined as a BMD T-score between -1.0 and -2.5 (www.webmd.com).

B. Formulation of Interview Schedule

An interview schedule is a method of data collection with a set of questions to be answered by the respondents (Kothari, 2009). In the present study, an interview schedule was formulated to elicit the background information from the selected women regarding the socio economic details including age, sex,

occupation, educational status, family type, monthly income and family background. Questions regarding dietary pattern including type of diet, meal pattern, quantity of food consumption, food intake, fat and oil consumption, beverage consumption, physical activity and exercise pattern, personal habits, family history of illness, reproductive history and menstruation problems were included in the interview schedule. The formulated schedule was pretested on a sample of ten selected women and finalised (Appendix - I).

C. Conduct of Survey and Collection of Information on Socio-Economic, Dietary and Other Background Details

According to Monoarul *et al.,* (2014), a statistical formula was used to estimate the number of sample to be taken.

$n = P \times (100 - P) \times z^2 / d^2$

where,

P is the anticipated prevalence,

d is the desired precision,

z is appropriate value from normal distribution for desired confidence level.

The anticipated prevalence of low bone mass density is taken as 40 % and 95 % confidence limit was selected. Based on these factors, the sample size was estimated at a minimum of 600.

All the women subjects visiting the bone health awareness camps over a period of one year were interviewed and the details were collected. The selected women were advised to respond positively and assured that the data collected will be kept confidential. The investigator explained the purpose of the study to the selected women before conducting the survey and their cooperation was obtained. The data was collected through 30 bone health awareness camps and all the 2230 women who came for the awareness camps were selected and interviewed in convenient places and times (Plate 1).

Plate 1

Collection of Background information

PHASE II

Assessment of Nutritional and Health Status and Bone Health Profile of the Selected Women

Nutritional status is a condition of health of an individual as influenced by the intake and utilization of the nutrients. It can be determined by the correlation of information obtained through medical and diet history and through physical examination and appropriate laboratory investigation (Srilakshmi, 2012).

Nutritional assessment is the science of determining nutritional status of subjects. Assessment of nutritional status of the community is one of the first step in the formulation of public health strategy to combat malnutrition (Bamji, 2009). Data was collected in subjective and objective form relating to the subject's food intake, medicial history, life style habits etc. For effective assessment of the subject's nutritional status, a four way approach including anthropometric measurements, clinical examination, bio-chemical and dietary assessment was adopted for all the 2230 women who attended the bone health awareness camps.

A. Anthropometric Measurements

Anthropometry is the universally applicable, inexpensive quantitative technique for determining an individual's body composition by measuring, recording and analysing specific dimensions of the body such as height and weight and body circumference at the waist, hip and chest. It reveals both health

and nutritional status and also envisages performance, wellbeing and survival. Therefore it is used in various intervention programmes to monitor health and nutritional status of the selected population. Anthropometric measurements of human body reflect changes in morphological variation due to inappropriate food intake or malnutrition (Srilakshmi, 2010). Anthropometric measurements can indicate the risk or possibility of disease attack at a later stage by giving vital clues. This remains true for bone disorders also.

i. Measurement of Height

Height is a measure of an individual's health and growth as opposed by body weight and is principally a measure of skeletal body tissues, leg, pelvis, spine and skull (Jelliffe and Jelliffe, 2005). Since height is affected only by long term deprivation, it is considered as an index of chronic or long duration malnutrition (Mallikharjuna and Ramakrishna, 2009)

The height of the selected women was measured using a non-stretchable fiber glass tape fixed to the wall. Women were made to stand straight in an erect position without foot wear and feet parallel on the ground. The back, buttocks and head were held erect and the shoulders in a relaxed position. The vertical distance from feet to head was measured and recorded to the nearest 0.1cm (Plate 2).

ii. Measurement of Weight

Body weight is the total mass of an individual including muscle, fat, water and bone and it is the most widely used simplest method to assess the growth and development of an individual (NIN, 2009). Weight is a time honored basic indicator of a person's health. Increased body weight of a person creates risks like hypertension, cardiovascular disease, diabetes and increased mortality. A change in body weight is an indicator of nutritional status. Being underweight / overweight adversely influences morbidity and mortality (www.healthknowledge.org.uk).

A portable weighing balance was used to measure the weight of the selected women. During the measurement of body weight, the individuals were asked to wear minimal clothing and stand straight with bare foot and without bending on the weighting machine. The scale was zeroed before weighing and it was also calibrated regularly during every time it was used. The weights of the selected women were taken in kilograms to the nearest 0.1 kg and recorded (Plate 3).

iii. Body Mass Index

Weight is a poor indicator of fatness and obesity. Direct relation cannot be arrived between weight and health risks. Body Mass Index (BMI) serves as an effective tool to indicate obesity and under-nutrition, since it also takes into account of a person's height. Body Mass Index accounts for weight and height of an individual. BMI is the simple index of weight for height that is commonly used to classify underweight, overweight and obesity among adults (WHO, 2011).

BMI (Kg/ m^2) range*	Category
< 18.5	Under weight
18.5 to 24.9	Normal
25.0 to 29.9	Over weight
> 30	Obese

* WHO Classification (2011)

BMI is a good indicator for bone-related disorders. People with low BMI often stress their bones. Lack of muscle mass increases the loading of bone, which results in pain and damage. The BMI of the selected women was calculated using the formula: BMI = Weight (kg) / Height (m^2).

iv. Measurement of Waist Circumference

The distribution of body fat is an important factor in obesity. Excess of fat in

abdominal location lead to high risk of cardiac problems. The waist circumferences for men greater than 40 inches or 102 cm and women with a waist circumference greater than 35 inches or 88 cm are at high risk (Wellborn and Dhaliwal, 2007). According to Byers (1998) waist circumference to assess the abdominal fat accumulation is an independent predictor of disease risk. A high waist circumference with a high BMI increases disease risk (Lutz/przytulski, 2011).

Waist circumference measurements of the selected women were taken when they were standing straight and relaxed with very light clothing by keeping a fiber glass tape parallel to the floor and measuring around the waist (umbilicus). The distance around the smallest area below the ribcage and above the umbilicus (belly button) provides a risk prediction for obesity related diseases. The tape was tightened without crushing and the readings were recorded (Plate 4).

v. Measurement of Hip Circumference

Hip circumference is measured as one-third of the distance between the superior iliac spine and the patella (Davidson, 1999). The selected women were made to stand with feet close together, arms at the side and the body weight being evenly distributed. Measurements were taken at the end of a normal expiration using a fiber glass tape up to 0.1 cm accuracy at the fullest part of the hip (maximum posterior extension between the iliac crest and buttocks) keeping the tape parallel to the floor (Plate 5).

vi. Waist to Hip Ratio (WHR)

Waist to Hip Ratio indicates the proportion of fat stored in a person's body in the waist and hip zones. The body stores fat in two ways, around the middle and around the hips. People in whom, fat are stored in hips and buttocks area have less health risk than those with high fat in waist area. The waist measurement divided by hip measurement was taken as Waist to Hip Ratio (Wellborn and Dhaliwal, 2007).

Waist to Hip Ratio = Waist circumference (cm) / Hip circumference (cm)

The ratio of Waist to Hip is an indicator of Central Obesity. Adult men with waist to hip ratio greater than or equal to 0.95 and women with greater than or equal to 0.80 are considered as having central obesity (Rao and Ramakrishna, 2009). Abdominal obesity is often measured as the waist to hip ratio (WHR) which is the ratio of obesity in the upper trunk to that in the lower trunk. A Waist to Hip Ratio greater than 0.8 in women and greater than 1.0 in men indicates increased risk of problems related to obesity (Lutz/przytulski, 2011).

Plate 2

Measurement of Height

Plate 3

Measurement of Weight

Plate 4

Measurement of Waist Circumference

Plate 5

Measurement of Hip Circumference

B. Bone Mineral Density (BMD) Measurement

The commonly used biophysical method for the study of osteoporosis is the measurement of Bone Mineral Density (BMD) (Bamji *et al.,* 2004). According to AIIMS (2010), the Bone Mineral Density less than –2.5 is an indication of osteoporosis. The procedure used for measuring BMD is given in Appendix - II.

Bone Mineral Density measures the quantity of calcium in a section of the bones and is measured by modern diagnostic methods. Dual Energy X-ray Absorptiometry (DEXA) is a most precise method to measure BMD. However this device is very expensive and is not portable. New methods for measuring bone mineral density namely ultrasound and Quantitative Computer Tomography (QCT) are also developed. The ultrasound system measures BMD at the patient's crown heel. The duration of the test is about one minute. The ultrasound based equipment for testing Bone mineral density is very compact, fast, non-invasive, painless and less expensive than traditional DEXA systems. Bone densitometry also helps in assessment of a patient's risk of fracture (www.imaginis.com).

Density changes in the heel are captured by ultrasound method. Quantitative ultrasound (QUS) is a new noninvasive method for estimating bone mineral status at peripheral skeleton sites. The QUS methods provide structural information regarding bones, which is important in determining the fracture risk. The ultrasound is a traveling mechanical vibration. The mechanical and structural properties of the medium progressively alter the shape, intensity and speed of the propagating wave. The velocity of transmission and the amplitude of the ultrasound signal are influenced by the bone tissue, reflecting its density, architecture and elasticity (Baroncelli, 2008). This principle forms the basis of ultrasound BMD measurements (Plate 6).

C. Clinical Examination and Biophysical Assessment

i. Clinical Examination

Clinical Examination is a commonly used method of assessment of nutritional status of communities (Srilakshmi, 2005). It provides a number of clues about general nutritional status of the person and specific deficiency state

(Kenneth, 2008). Clinical symptoms are observations in a patient. The medical history and clinical symptoms were used together to ascertain the clinical status. These observations are different from normal functioning of the body parts. Often, it relates to an individual's health condition. The clinical symptoms examined included fatigue, loss of appetite, giddiness, muscle pain etc. Previous fractures also serve as an indicator for low-bone mass disorders. Details regarding morbidity pattern and the prevalence of existing diseases and symptoms of osteoporosis were collected from all the subjects. A trained physician conducted the clinical examination for all the 2230 women and entered in the same interview schedule form (Plate 7). Based on the clinical condition and the disease status, supplementation was planned.

Plate 6

Measurement of Bone Mineral Density (BMD) by Ultra Sound Method

Plate 7

Clinical Examination of Women

ii. Biophysical Assessment - Blood Pressure

The blood gets circulated throughout the body. The force exerted by blood against the artery walls, during its circulation is termed as blood pressure. The measurement of blood pressure is a physiological variable that indicates the condition of cardiovascular system (Venkatraman, 2009).

The blood pressure normally rises and falls throughout the day. The physician measures the blood pressure using a calibrated pressure gauge. The physician wraps an inflatable cuff with a pressure gauge around the arm of selected women seated in a chair with foot placed on the floor and in a relaxed position (Plate 8). The pressure is raised to 200 mm Hg and then reduced gradually. Then he or she listens to the pulse with a stethoscope while releasing air from the cuff. The gauge measures the pressure in the blood vessels when the heart beats (systolic) and when it rests (diastolic). Blood pressure was measured for the selected women and they were classified as per Joint National Committee (JNC, 2004) guidelines.

Category	**Blood Pressure Range ***	
	Systolic level (mm Hg)	Diastolic level (mm Hg)
Low Blood Pressure	< 120	< 80
Normal Blood Pressure	120	80
Prehypertension	120 to 139	80 to 89
Hypertension - Stage I	140 to 159	90 to 99
Hypertension - Stage II	>160	> 100

* Joint National Committee (2004)

Plate 8

Measurement of Blood Pressure

D. Bio-chemical Assessment

Laboratory tests based on blood and urine can be important indicators of nutritional status, but they are influenced by non-nutritional factors as well. Laboratory results can be altered by medications, hydration status and disease states or other metabolic processes such as stress. As with other areas of nutrition assessment, biochemical data need to be viewed as a part of the whole (Chanda, 2013).

Bio-chemical estimations help to diagnose the disease condition. Understanding the criticality of bio-chemical parameters enable the risk posed by the patient. For e.g. low level of serum calcium indicates potentially low-bone mass condition. Calcium in the bone is mobilised in order to maintain the serum calcium level. This in-turn causes bone loss. Biochemical measurements indicate the immediate past intake of nutrients through food or the changes caused by prolonged deficient intake of nutrients. The low dietary intake will result in bio-chemical warning indications, which transform into clinical problems (www.cdc.gov/nutritionreport).

Biochemical assessment is used to distinguish between deficiency, adequacy and over load of one or more nutrients (Geissler and Power, 2005). Biochemical measures of nutritional status yield objective and quantitative data and are the most sensitive indicators of nutritional status (www.nhibi.nih.gov). The selected women were informed to come in empty stomach early morning before

the breakfast for blood collection for conduct of biochemical tests. The Blood collection was done by a trained laboratory technician using standard procedures (Plate 9).

i. Blood haemoglobin

Haemoglobin is a protein found in the red blood cells containing iron. It serves as the principal transporter of molecular oxygen through blood in humans. It picks up oxygen from lungs and transfers it to the tissues where it is used by cells. A low haemoglobin count is generally defined as less than 13.5 g / dl of blood for men and less than 12 g / dl for women and referred to as anaemia which is related with many diseases (www.mayoclinic.com/health/low-haemoglobin/ mayo/83). Blood haemoglobin was estimated for all the selected women by cyanmethaemoglobin method (NIN, 1990).

ii. Total cholesterol

Total cholesterol is a rough measure of all cholesterol and triglycerides present in the human body. It is a measure of HDL and LDL cholesterol. A cholesterol value above 200 mg/dl implies higher risk for cardio-vascular diseases. Total cholesterol values listed below were used as reference (National Cholesterol Education Program, 2001).

Desirable: < 200 mg/dl

Borderline risk: 200 to 239 mg/dl

High risk: > 239 mg/dl

Total blood cholesterol among the selected osteopenic women was estimated by using enzymatic colorimeter test CHOD-PAP method proposed by Allian *et al.,* (1974).

iii. Fasting blood sugar

Blood glucose level is an indicator of diabetic condition and further heart diseases (Bamji *et al*, 2003). Fasting blood glucose level is the most common method used to measure blood glucose. This indicator represents glucose homeostasis. The desirable fasting blood sugar level is 80 to 115 mg/dl (Bamji *et*

al, 2003). Blood sugar of selected osteopenic women was tested by using enzymatic colorimeter test GOD-PAP method (Trinder, 2012). The fasting blood glucose was measured in empty stomach in the early morning after a minimum of 8 hours subsequent to intake of last meal.

iv. Postprandial blood sugar

Postprandial means “after meal”. Hence postprandial blood sugar refers to concentration of blood glucose measured after eating. Glucose concentration begins to rise after 10 minutes of meal, thereby resulting in absorption of dietary carbohydrates. The glucose level begins to peak after 1 hour from meals, and returns to pre-prandial levels within 2 to 3 hours after meal. The desirable postprandial blood sugar according to Bamji *et al.,* (2003) is 120 to 160 mg/dl. The postprandial blood glucose levels of the selected osteopenic women were estimated one and half hours after the consumption of breakfast.

v. Measurement of serum calcium

Calcium is a key nutrient and also an essential building block for lifelong bone health for the body to stay strong and healthy. Almost every cell in the body uses calcium in some way, controlling the nervous system, muscles and heart (www.helpguide.org). Around 99 per cent of calcium in the body is present in bones and the remaining one per cent is present in blood. In serum, calcium exists equally in free ionized form and also bound form with albumin. Calcium helps in enzymatic activation, muscle contraction, coagulation of blood, regulation of some hormonal secretions and cell membrane permeability (Pascal *et al.,* 2005).

Normal serum calcium level of adults range between 8.5 – 10.2 mg / dl (www.nlm.nih.gov). A high level of serum calcium is found in hyperparathyroidism, malignant tumours, osteoporosis and adrenal insufficiency. Decreased levels of serum calcium are found in hypoparathyroidism, renal failure, rickets, Vitamin D deficiency and pancreatitis. Serum calcium of the selected osteopenic women was estimated by using the calcium reagent kit intended for in vitro quantitative determination of calcium in serum. At a neutral pH the calcium forms a complex

with Arsenazo III, the colour intensity of which is directly proportional to the concentration of calcium in the sample (Baver, 1981).

E. Dietary Assessment

Dietary assessment is the set of methods that measure and estimate the food intake which can be converted into nutrient intake by means of Food Composition Table (Godhia, 2011). Diet is a vital determinant of health and nutritional status of people. Food consumption survey helps to elicit the quality and quantity of food consumed (Bamji *et al*., 2004).

A 24 hour recall method is commonly used and more appropriate for assessment of food intake of large populations than of individuals which requires less guidance than other methods and in addition memory of food quantities are recalled more accurately (Damayanthi, 2013). In the present study, a 24 hour recall method was used to elicit information on the food and nutrient intake of a subsample of 120 women (5%) (Plate 10).The food intake during early morning, breakfast, lunch and dinner was recorded for three days and the quantity of food consumed was assessed using standard measurements. From the mean food intake, nutrient intake was computed and compared with the Recommended Dietary Allowance (RDA) given by ICMR (2010).

Plate 9

Blood collection for Biochemical estimations

Plate 10

Dietary Assessment

PHASE III Formulation and Nutrient Analysis of the Supplements and Conduct of Supplementation Study

Dietary supplements combined with normal food intake have shown improvement in bone health. Diet supplements are prepared so that the targeted nutrients are made available for body synthesis. They are given in various forms like health mixes, drinks, capsules and recipes etc. The efficient distribution of diet supplement must be followed regularly. The nutrition policy must focus on improvements in usual diet and planning for nutritional supplements if required (Manders, 2006). Accordingly in the present study suitable food ingredients were selected and combined to formulate nutri mixes for supplementation to selected osteopenic women.

Selection of Ingredients and Formulation of the Supplements

A. Selection of Ingredients

i. Ragi (Eleusine Coracana)

Ragi is an important staple food among Indians especially among the lower income group. Ragi also known as finger millet ranks sixth among the cereals grown in India. It has extremely good shelf life. This can be attributed to the lower fat content in ragi and also recommended for its anti-ulcerative property.

Among the cereals and millets, finger millet has the maximum amount of calcium (344 mg%) and potassium (408 mg%). It has higher dietary fiber, minerals and sulfur containing amino acids compared to white rice, the current major staple in India. The dietary fiber content of finger millet (11.5 %) is much higher than the fiber content of brown rice, polished rice and all other millets such as foxtail, little kodo, and barnyard millet (Shobana *et al.,* 2013).

It also lowers the cholesterol and blood glucose. Nutritionally, its importance is well recognised because of its high content of calcium (0.38 %), dietary fiber (18 %) and phenolic compounds (0.3–3 %). They are also recognized for their beneficial effects on health such as anti-diabetic, anti-tumerogenic, anti-atherosclerogenic effects, and antioxidant and antimicrobial properties (Mathanghi and Sudha (2012). It also helps in weight reduction, controlling diabetes, lowering cholesterol, preventing anaemia, aiding relaxation, strengthening bones, helping digestion, improving lactation and an excellent source of protein and aminoacids and reverts skin ageing (www.thefitindian.com).

Ragi is an excellent foundation of calcium and helps consolidating bones. It is defined as an excellent source of nutrition for growing children and ageing persons. Ragi consumption helps in developing bones in growing children and maintaining the bone strength of adults. It is also instrumental in keeping ailments like osteoporosis away and can also cut down the risk of fractures (www.theresearchpedia.com). Hence the investigator selected ragi as one of the cereal component of the supplement.

ii. Soya Bean (Glycine Max)

Soybean is very important for vegetarians and vegans because it is affluent in valuable nutrients. Soya bean is known for the rich protein content and recognized as an oil seed containing several useful nutrients including protein, carbohydrate, vitamins, and minerals. Dry soybean supplies 36g protein, 19g oil, 35g carbohydrate (17 % of which is dietary fibee) and 5g minerals in 100g and numerous other components including vitamins (Liu, 1997). Soybean protein is considered to be a good alternative for animal protein (Sacks *et al.,* 2006) and their nutritional profile except sulfur amino acids (methionine and cysteine) is almost similar to that of animal protein because soybean proteins contain most of the essential amino acids required for animal and human nutrition (Hajos *et al.,* 1996).

Scientists have rated the quality of protein in soya at 74 per cent compared to meat with 90 per cent. Hence soy serves as a protein rich food for vegetarians. There is a variety of soy based foods like soya flour, soya oil and soya drinks are few among them. Recent research have shown that soy foods can improve bone mass. Soya contains five per cent of minerals (including calcium). Soy food and soybean isoflavones consumption lowered osteoporosis, improved bone health and reduced bo ne related problems as stated by Toda *et al.,* (1999) and Messina and Messina (2000). Consumption of soy foods may reduce the risk of osteoporosis and help alleviate hot flash associated with menopausal symptoms which are major health concerns for women (Persky *et al*., 2002).

Ho *et al.,* (2005) reported that 375 ml of calcium-fortified soymilk supplementation, or an equivalent of about two glasses, is among the effective strategies for bone acquisition and the optimization of peak bone mass in adolescent girls. Hence people with low bone disorders can consume soya based supplements which will help in calcium enhancement.

iii. Wheat (Triticum)

Wheat is the most extensively consumed food crop across the globe and considered as an excellent source of protein, minerals, B-group vitamins and dietary fiber (Kumar, 2011).

Globally, there is no doubt that the number of people who rely on wheat for a substantial part of their diet amounts to several billions. Wheat provides nearly 55 per cent of carbohydrate and 20 per cent of the food calories. It contains carbohydrate 78.1 per cent, protein 14.7 per cent, fat 2.1 per cent, minerals 2.1 per cent and considerable proportions of vitamins and minerals (zinc and iron). Wheat is also a good source of traces of minerals like selenium and magnesium essential to good health as stated by Adams *et al.,* (2002), Fraley (2003), Shewry *et al.,* (2006) and Topping (2007).

Wheat grains are also rich in pantothenic acid, riboflavin and some minerals, sugars etc. The bran, which consists of pericarp, testa and aleurone, is also a dietary source for fiber, potassium, phosphorus, magnesium, calcium, and niacin in small quantities. The whole wheat, which includes bran and wheat germ provides protection against diseases such as constipation, ischaemic heart disease, disease of the colon called diverticulitis, appendicitis, obesity and diabetes (Hadjivassiliou *et al.*, 2003).

Whole wheat flour is a coarse textured flour ground from the entire wheat kernel and thus contains the bran, germ and endosperm. The presence of bran reduces gluten development. Baked products made from whole-wheat flour tend to be heavier and denser than those made from white flour. In India, wheat is consumed in the form of roti, bread, biscuits and as other recipes. Each portion of wheat e.g. grain, germ, flakes etc has nutrients and the rich content of protein in wheat helps to build strong body and hence wheat was used in the preparation of the supplement.

iv. Milk Powder

Powdered milk is a manufactured dairy product prepared by evaporating milk to dryness. Milk powder is a preserved product that has a longer shelf life than liquid milk and does not require refrigeration due to its low moisture content. Powdered milk is used for food, health, nutrition and in biotechnology. This food is a good source of protein, riboflavin, vitamin B12, vitamin D, calcium and phosphorus (www.nutritiondata.self.com). Milk powder contains the complete twenty one amino acids that are the edifice blocks of proteins and are rich in soluble vitamins and minerals.

As stated by USAID the distinctive average amount of key nutrients in the unreconstituted nonfat dry milk are (by weight) 36 per cent protein, 52 per cent carbohydrates (predominantly lactose), 1.3 per cent calcium and 1.8 per cent potassium. Whole milk powder, on the other hand, include on an average 25 - 27 per cent protein, 36-38 per cent carbohydrates, 26 - 40 per cent fat, and 5 - 7 per cent ash (minerals). However, inappropriate storage conditions such as elevated relative humidity and high ambient temperature can considerably humiliate the nutritive value of milk powder (http://self.gutenberg.org/articles/dried_milk).

The National Nutrient Database reveals that a 1/4-cup serving of powdered milk provides approximately 9.5 per cent of the recommended daily amount of carbohydrates and 15 to 18.3 per cent of the recommended amount of protein. Milk powder serves as a rich source of calcium, providing nearly 12 per cent of the amount one need daily in a quarter cup serving. The Institute of Medicine recommends a consumption of 1-1.2 g of calcium daily. Powdered milk also contains phosphorus, magnesium, potassium, vitamin A, vitamin C and vitamin D (www.livestrong.com).

High calcium milk powder consumption is helpful in reducing the bone loss at the lumbar spine among healthy postmenopausal women and reducing height

loss (Chen *et al*., 2015). Lau *et al*., (2004) reported that supplementing the diet of Chinese children with milk powder was effective in enhancing bone accretion.

v. Sesame Seeds (Sesamum Indicum)

Sesame seeds add a nutty taste and a delicate, almost invisible, crunch to many Asian dishes. Sesame is an important source of phyto-nutrients such as omega-6 fatty acids, flavonoid phenolic anti-oxidants, vitamins, and dietary fiber with potential of anti-cancer as well as health promoting properties (www.nutrition-and-you.com).

Sesame seeds also contain two unique substances namely sesamin and sesamolin known to have a cholesterol lowering effect in humans and to prevent high blood pressure. Both of these were also reported to increase the hepatic mitochondrial and the peroxisomal fatty acid oxidation rate in experimental animals (Raghavan *et al*, 2010).

Sesame is rich in sulfur containing amino acids and limited in lysine and contains significant amounts of oxalic (2.5%) and phytic (5%) acids (Kapadia *et al*., 2002). Sesame seeds are an excellent source of copper and calcium and also rich in phosphorus, iron, magnesium, manganese, zinc and vitamin B1 (Hasan *et al*., 2000). Many health benefits of sesame may be attributed to its lignan especially sesamin (Jeng and Hou, 2005). Molecules of sesame seed oil maintain good cholesterol (High Density Lipoprotein, HDL) and reduce bad cholesterol (Low Density Lipoprotein, LDL) (Sirato-Yasumoto *et al*., 2001). Sesamin increases the fat burning process and decreases the storage of fat in the body (Penalvo *et al*., 2006).

vi. Oats (Avena Sativa)

Oats form a part of regular meal in European countries. They grow in cool places and in India, Kashmir produces the bulk of oats. Nowadays, it is gaining

recognition as a regular diet throughout the world. Oats is a good source of protein, minerals, and fiber. India has large number of patients with cardio-vascular diseases. Oats can reduce the risk of heart diseases. Moreover, they serve as better food to lower the cholesterol, reduce obesity, hypertension and insulin resistance (Mushtaq *et al*., 2014). Oats is a rich source of soluble fiber, well balanced proteins and several vitamins and minerals essential for human health (Esposito *et al*., 2005).

Oats is one of the minority foods that hold high levels of soluble fiber, which contains a polysaccharide known as ß- D glucan (www.flahavans.com). This prebiotic ingredient is related with improved gut health, escalating the absorption of calcium and magnesium and lowering blood glucose and cholesterol levels (www.gfc.ca.com).

Oats is the best whole grain food to prevent osteoporosis. Oats are rich in the right minerals to promote bone mineral density. Oats contain calcium, magnesium, zinc and copper. These minerals are found in the bone. In addition, the latter three minerals improve the uptake of calcium into the bones. Therefore, taking breakfasts of oatmeal can help in preventing osteoporosis. Oatmeal and oat straw are also excellent for bone health. Oat straw tea is an excellent source of silica (www.progressivehealth.com).

Silicon has a role to play in keeping bones and connective tissues healthy. It is found in many tissues in the body although how much is needed in the diet is not clear. Deficiency in humans has never been found. It has been stated that men and younger women with a high intake of silicon in the diet had denser bones. It contributes to bone mineral content and is proven to strengthen bones. Silicon is found in oats, barley and rice (National Osteoporosis Society, 2011).

vii. Flax seed (Linum usitatissimum)

Flax is a member of the genus Linum in the family Linaceae. It is a popular traditional food and remedy, as flaxseed and flaxseed oil, or linseed oil which contain alpha-linolenic acid (ALA), an omega-3 fatty acid.

Lignans are one of the major classes of phytoestrogens that help to scavenge free radicals in the body. Flaxseed is considered to be one of the best sources of lignans (0.3 g per 100g) that benefit health on regular consumption (Adlercreutz, 2007). Its high concentration of lignans, is of great choice for all women being younger, middle-aged, or older that helps to normalize the menstrual cycle, control menopause and reduce the risk of osteoporosis, cancer and heart disease. Intake of flaxseed on a daily basis results in hormonal changes that are beneficial to women of all ages. In menstruating women who consumed 10 g (about 2 tsp) of flax seed on a daily basis, considerable hormonal changes have resulted (www.womenfitness.net).

The health benefits of flax seed also include weight reduction, anti-cancer effect, reduction of the risk of cardiovascular disease, decreasing blood pressure, enhancing blood vessel tone, lessening inflammation and promoting immune system functions, dealing with autoimmune and inflammatory disorders such as rheumatoid arthritis, psoriasis and lupus. Researchers at the University of Toronto found that total blood cholesterol levels dropped by 9 per cent and LDL (bad cholesterol) decreased by 18 per cent when a group of nine healthy women included 50 g of milled flax seeds to their regular diets daily for four weeks (Andrea *et al.,* 2013). Studies conducted in postmenopausal women have reported that flaxseed supplementation (38 g/day of whole flaxseed) can lower serum LDL cholesterol (Arjmandi, *et al*, 1998), serum Total Cholesterol, LDL cholesterol, Triglycerides, apolipoprotein A_1 and apolipoprotein B (Lucas *et al*., 2002).

B. Formulation of the Supplements

Food supplementation is one of the most effective ways of improving health status and also for combating malnutrition especially deficiency diseases to reach some or all population. Formulation of supplementary foods based on low cost, locally available ingredients familiar to home makers has been one of the best strategies to improve the nutritional status of population groups.

A combination of foods, from all the food groups taken in moderate amounts makes a meal complete and adequate. Groups of foods can also be combined to treat the same illness either because they have similar and complementary effects or because they have properties that are different but work together to achieve the desired result. Complementary food supplements can be defined as food-based complements to the diet that can be mixed with or consumed in addition to the diet and the reason of which is to add nutritional value. Complementary food supplements are equivalent to food fortification in the sense that they increase the intake of essential nutrients from food (Nestel *et al.,* 2003).

Plate 11

Ingredients for Basic Mix

i. Formulation and Preparation of Nutri Mix

An ultimate food for prevention and modification of nutritional inadequacies is supposed to be of high nutritive value, acceptable, readily available at low cost, familiar to the community and have better acceptance both in good health and illness (Swaminathan, 2005).

Development of supplement using low cost, locally accessible indigenous foods recognizable to the community especially women, has been one of the strategies confirmed to be effective in improving the health status of the community (Sherleker and Udipi, 2006).

Food mixes are developed with the idea to supply nutritionally high biological value protein and also to utilize as concentrated source of specific nutrients. Health mix prepared from millets is considered as a healthy food and compositionally it has immense protein, low fat and high fiber (Ramasri *et al.,* 2014).

For nutri mix preparation, along with basic mix combinations I, II, III and IV were prepared with sesame seeds and groundnuts and acceptability tests were done. The composition of the selected ingredients in basic mix and nutri mix variations is given in Table I.

TABLE I

COMPOSITION OF INGREDIENTS FOR NUTRI MIX VARIATIONS

Ingredients	Basic mix (90g)	NMC I (100g)	NMC II (100g)	NMC III (100g)	NMC IV (100g)
Ragi flour	50 g	50 g	40 g	50 g	40 g
Soya flour	10 g	10 g	20 g	10 g	20 g
Wheat flour	10 g	10 g	10 g	10 g	10 g
Milk powder	10 g	10 g	10 g	10 g	10 g
Sesame seeds	-	10g	10g	-	-
Oats	5 g	5 g	5 g	5 g	5 g
Flax seeds	5 g	5 g	5 g	5 g	5 g
Groundnuts	-	-	-	10 g	10g
Total	**90 g**	**100 g**	**100 g**	**100 g**	**100 g**

NMC - Nutri Mix Combination

A food supplement namely basic mix was formulated consisting of ragi flour, soya flour, wheat flour, milk powder, flax seeds and oats. This supplement is rich in calcium, protein and other components that helps in absorption of calcium. The selected ingredients used in the preparation of the supplement namely basic mix is given in Plate 11. The selected ingredients namely ragi flour, soya flour, wheat flour, sesame seeds, groundnuts, flax seeds and oats were purchased from local market cleaned, dried, roasted and powdered separately. Finally all the ingredients were mixed with milk powder and blended well uniformly. The steps involved in nutri mix preparation is specified in Figure 2.

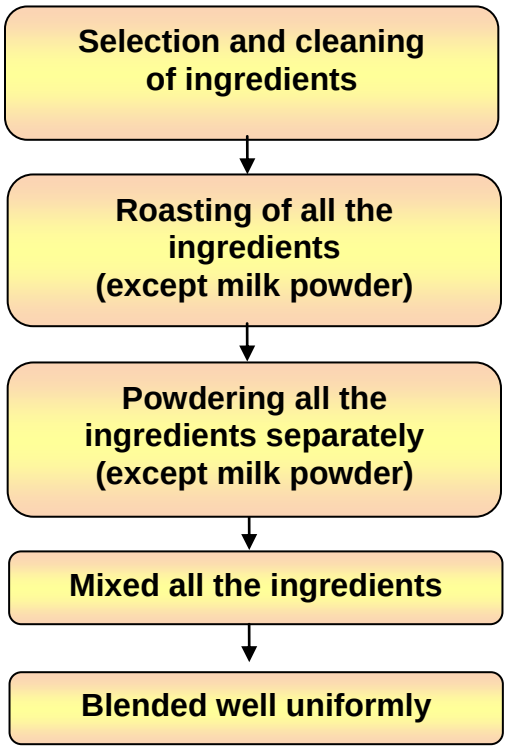


Figure 2

Steps Involved in Nutri Mix Preparation

ii. Acceptability Testing

Accordingly the nutri mix variations were standardized and evaluated for acceptability using a 9 point hedonic scale by a panel of 20 members. The

hedonic rating scale was used for the attributes like colour, appearance, texture, flavour and taste as Like extremely (9), Like very much (8), Like moderately (7), Like slightly (6), Neither like nor dislike (5), Dislike slightly (4), Dislike moderately (3), Dislike very much (2) and Dislike extremely (1) (Appendix - III). Sensory assessment is an essential and finest technique for evaluating novel products which offers quality measure and production control (Vidhya and Narain, 2010). The overall quality of foods may be packed up into uniqueness such as colour, appearance, flavor, taste and texture (Ranganna, 2004). The most accepted combinations with highest scores were nutri mix I and II which were used for supplementation.

C. Nutrient Analysis of the Supplements

Nutritional composition of supplements nutri mix I and II was analysed by standard procedures. Energy was determined by Pearson method (1991), Carbohydrate by AOAC method (2007), Fat and Protein by AOAC method (2006). For determination of fiber, calcium, iron, phosphorus, moisture and ash AOAC method (2005) was used. Vitamin B was assessed by AOAC method (2008) and Vitamin D by AOAC method (2007). Analysis were done in triplicates and the values were entered.

D. Microbial Testing and Cost Computation of the Supplements

Nutri mixes were evaluated for microbial counts like Total Bacterial Count (TBC) and Total Yeast and Mould Count. Storage was done at room temperature for a period of 3 months. The two nutri mixes were evaluated for bacterial, yeast and mould counts at the beginning and at the end of 3 months storage and the quality was evaluated.

Cost calculation will help to assess the feasibility of the supplements for preparation at household and commercial level. The cost of the nutri mixes was

computed as per the prevalent market prices of the ingredients. For calculation of cost, aspects like ingredient cost, preparation, processing and packaging cost were included.

E. Approval for the Research Design and Supplementation Study

The investigator presented the research design in the research conventions and workshops. Approval for the supplementation study was obtained from the Institution Human Ethics Committee of Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore - 641043 which comprised of doctors, lawyers, professors, management and common people. The approval number is AUW.IHEC.2013:35. The details are given in Table II. The certificates, approval and appreciations are enclosed in Appendix – IV

Table II

Details of the presentation of the research design of the study

Name of the Programme/ Seminar	Organizing Institute	Dates
Workshop on "Path Finder – Explore, Experiment, Elucidate Research : A Colloquium 2010"	Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore	22.12.2010 - 23.12.2010
All India Workshop on "Research Methodology for Research Scholars"	Pondicherry University, Puducherry	04.08.2011 - 05.08.2011
Research Convention 2011 – "Research Ethics and Post Research Perspectives"	Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore	10.08.2011 - 11.08.2011
Research Ethics and Post Research Methods	Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore	13.09.2012 - 15.09.2012
Institution Human Ethics Committee	Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore	21.02.2013

F. Selection of Women for Supplementation Study

The women for the supplementation study were selected from two different urban localities. Out of the surveyed 2230 women, 245 women were interviewed and among them 120 osteopenic women based on BMD scores less than ≥ 1.0 and serum calcium levels less than 9.0 mg / dl were selected for the study. They were divided into three groups with 40 women in each group. After screening, 80 subjects were selected for experimental groups I and II. Each group was provided with nutri mix I and II. Women in the age group of 30 to 45 years with BMI 18 to 25 kg/m^2, free from disease conditions like Asthma, High Blood Pressure, Diabetes Mellitus, Heart disease and menopause were selected for the supplementation study. Obese and severely malnourished women were not included for the study. Their doubts were cleared and written consent for participation in supplementation was obtained from them. A group of 40 women with the same criteria served as control group without supplementation.

G. Conduct of the Supplementation Study

Food supplementation is essential to overcome the nutrition gap (Soma and Santa, 2015) and it is one of the effective ways developed with the purpose and an approach of improving the health status (Raajeswari and Bhooma, 2014). Nutrient rich nutri mixes were planned in consultation with the doctors and nutritionists for supplementation to the osteopenic women to meet the daily requirements that helps in improving the calcium nutriture. The experimental groups were supplemented with 50 g of nutri mix per day for 5 months. The nutri mix was packed as individual sachets with measured quantity of 50 g pack in food grade covers for consumption and distributed to the selected women every week. The investigator prepared the nutri mix every week, visited the women groups weekly once for providing the supplements and checked the feedback (Plate 12).

During the supplementation period all the selected women were requested to follow their regular diet. Feedback on compliance, taste and any other side effects was obtained regularly. Women were asked not to waste the nutri mix supplied to them and consume them fully.

Plate 12

Distribution of the Supplement to the Selected Women

H. Evaluation of the Impact of Supplementation

At the beginning and at the end of 5 months supplementation period, anthropometric measurement and clinical examination were done for the selected women. Fasting and postprandial blood glucose, serum calcium, phosphorus, haemoglobin and cholesterol were estimated for both control and experimental group women. Bone mass was measured by ultra-sound (BMD) method. The final values were compared with the initial values to evaluate the impact of supplementation.

PHASE IV

Conduct of Nutrition Education and Life Style Management Programme

A. Selection of Women for Nutrition Education

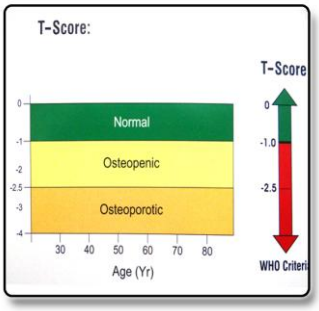
An important tool for combating malnutrition problem is creating awareness among the public using appropriate nutrition education package (www.whoindia.com). Nutrition education is a blend of educational approach, accompanied by environmental hold, planned to help voluntary adoption of food preferences and nutrition associated behaviours contributing to healthiness and well being. Among the total study population of women, 245 women were interviewed who expressed their willingness to participate in the intervention study. Out of them 125 osteopenic women were selected and divided into five groups of 25 women in each group for easy access and conduct of nutrition education and life style management programmes.

B. Development of Nutrition Education Materials

A questionnaire was formulated to elicit the information regarding the nutrition knowledge of the selected women in the management of the diet related diseases. Each question was assigned with marks and scored one if correctly answered and scored zero if wrongly answered (Appendix - V). Knowledge Attitude and Practice (KAP) scores were obtained initially from the selected women. Based on the initial knowledge scores a nutrition education programmme was developed. The content of nutrition education included topics on low cost foods, healthy recipes, role of nutrients and disease management, need for assessing bone mineral health status, identification of bone related complications and other health risk, dietary management and calcium rich supplements. Various teaching aids such as booklets, pamphlets, charts, posters, displays were developed (Plate 13).

Booklets and Pamphlets

Charts and Posters



Displays

Plate 13

Nutrition Education Materials

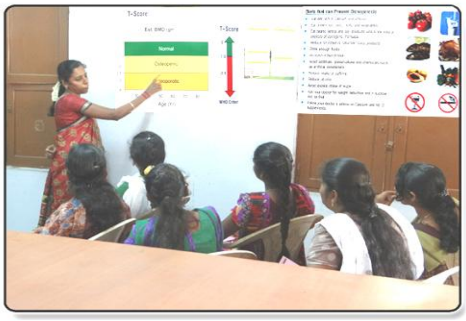
C. Conduct of Nutrition Education and Life Style Management Programme

Nutrition education is one of the most effective tools of changing the food habits of the people without affecting their sentiments (Orstead *et al.,* 1985). Nutrition education program was conducted for the selected women to sensitize them regarding the need to consume healthy and nutritious food which improves healthy living using power point presentations and demonstrations along with lecture method. Nutrition education was given in groups by gathering the selected women in a common place convenient to them for duration of 2 hours / week for a period of 3 months for 125 selected women of different localities. The selected members who could not attend the education classes at a common place were given individual counselling by the investigator at their residences. The nutrition education programmes conducted is given in Plate 14.

Demonstrations

Presentations

Lecture Method



Diet Counselling

Individual Counselling

Plate 14

Conduct of Nutrition Education

In order to help the osteopenic women from joint and other types of pains a life style management programme was planned. This included muscle strengthening exercises, weight bearing exercises, stretching exercises and physiotherapy treatment sessions by trained physiotherapist and technicians in clinics at common places suitable for the women (Plate 15).

Exercise is an important modifiable factor to recover bone accretion and lessen the risk of developing osteoporosis related weakening conditions in later life (Shweta *et al.,* 2012). Calcium intake in addition to physical activity were positively associated with bone mineralisation (Selma *et al.,* 2013). For conducting life style management programme 5 batches of 25 women in each group formed the sample for 2 hours / week.

Plate 15

Lifestyle Management Sessions for Women

D. Demonstration and Training on Preparation of Mixes and Recipes Using the Mixes

As part of entrepreneurial activity, demonstration and training on the preparation of nutri mixes were given for a group of 30 selected women. Various traditional recipes using nutri mixes were demonstrated to women in groups during their convenient times.

The recipes included simple items like Ladoos, Health Drink, Cookies (baked), Adai (shallow fat fried) and steamed items like Kollukattai, Idiyappam, Plain Pittu, Sweet Pittu, and Kolaputtu (Plate 16). The recipes were standardized and overall acceptability were evaluated.

Standardisation is important in order to assure uniform quality and quantity of the products prepared and quality is the ultimate criterion of the desirability of any food product (Srilakshmi, 1996).

SIMPLE RECIPES

Health mix

Ladoos

BAKED ITEM

Cookies

SHALLOW FAT FRIED

Adai

STEAMED ITEMS

Kollukattai

Idiyappam

Plain Pittu

Sweet Pittu

Plate 16

Recipes Developed with Nutri Mixes

With a view of popularization and commercialisation of the nutri mixes, cookies were prepared using the nutri mix powders. Bakery products are popular items among all age groups. Cookies or biscuits form the most popular item because of their sensory character, convenience, ready to eat nature and extensive shelf life. Cookies are an excellent product to provide additional nutrients (Sujitha and Thirumani, 2013).

For cookies preparation, the ingredients in different proportions were tried out and the most accepted ratio was used. The ingredients were measured, mixed together to make a dough consistency, then flattened into half inch thickness by rolling and made into small cookies by cutting with biscuit cutters. Placed into greased tray and baked. The steps involved in cookie preparation is given in Plate 17. They were packed in two sachets convenient for daily consumption. Home makers were motivated to prepare nutri mix cookies on a large scale, fix prices and market the products as an entrepreneurial activity. Marketing of the nutri mix powder and cookies was done with the support of the hospital authorities and pharmacies.

Weighing of ingredients

Mixing of ingredients

Kneading and Dough preparation

Rolling the dough

Cutting with biscuit cutter

Placing in the greased tray

Diced cookies before baking

Baked Cookies

Plate 17

Steps Involved in Cookie Preparation

E. Evaluation of the Impact of Nutrition Education

The impact of nutrition education was evaluated using the same questionnaire. The change in KAP scores after nutrition education on various aspects was evaluated comparing the scores before and after nutrition education. The feedback on life style management was also obtained.

PHASE V

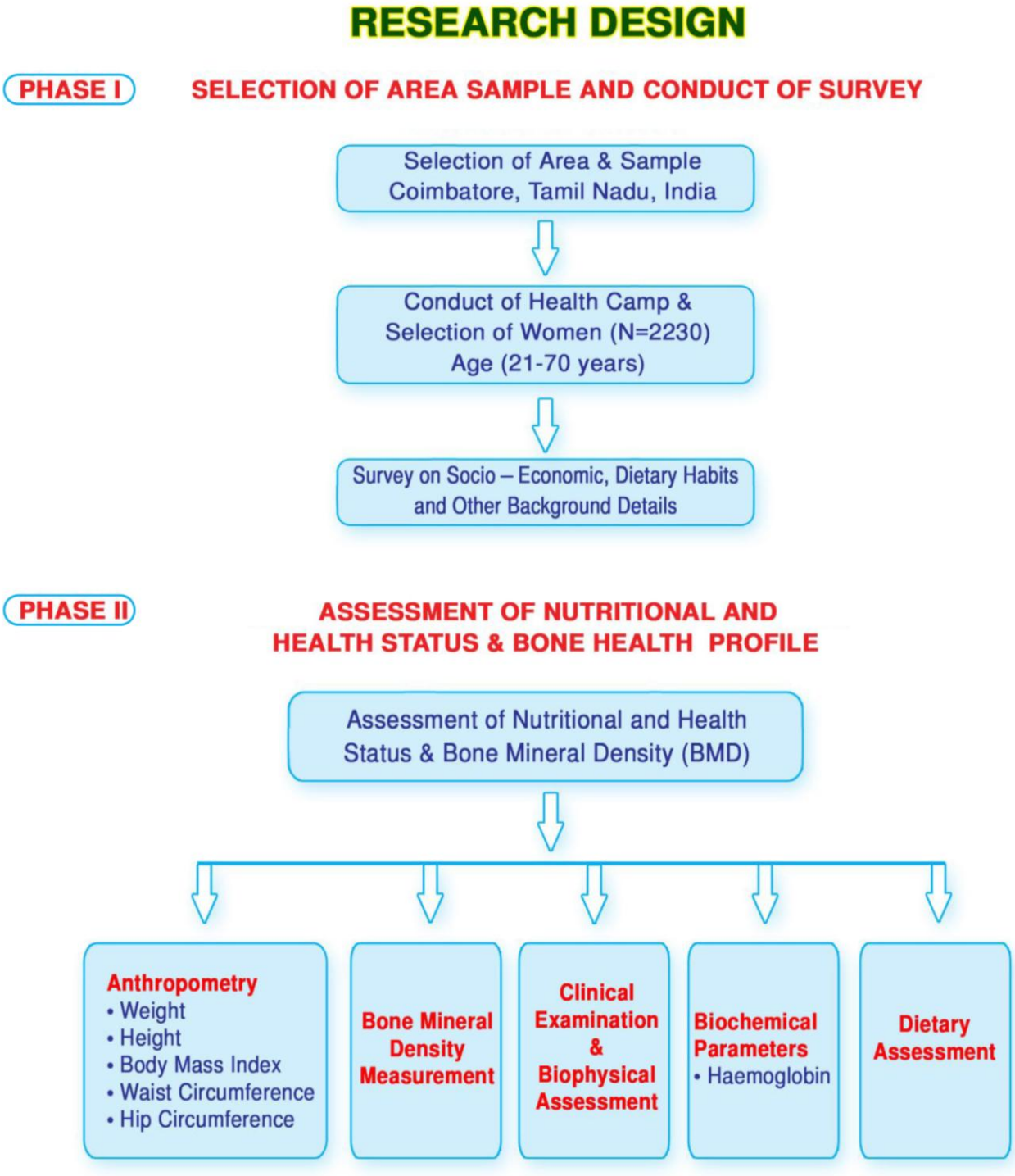
Analysis of Data

The data collected was tabulated systematically, consolidated and statistically analysed for arriving at the results of the effect of intervention strategies among the selected women.

All the data obtained in the present investigation were subjected to statistical analysis using SPSS 17.0 version. Student 't' test and two way ANOVA were selected based on the relevant parameters. The significance level was set at 5% ($p<0.05$) and 1% ($p<0.01$) level. Mean, standard deviation, test of significance, correlation analysis were used as tools for interpreting the data.

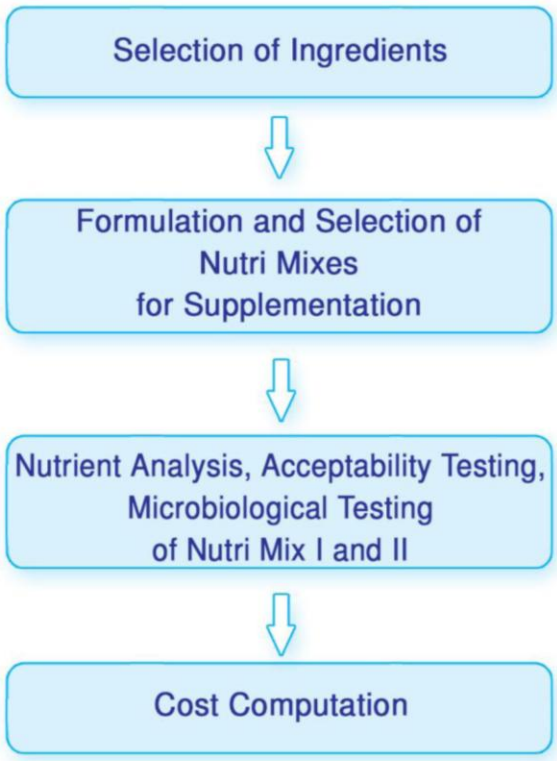
The Research Design of the present study is presented in Figure 3.

Figure 3

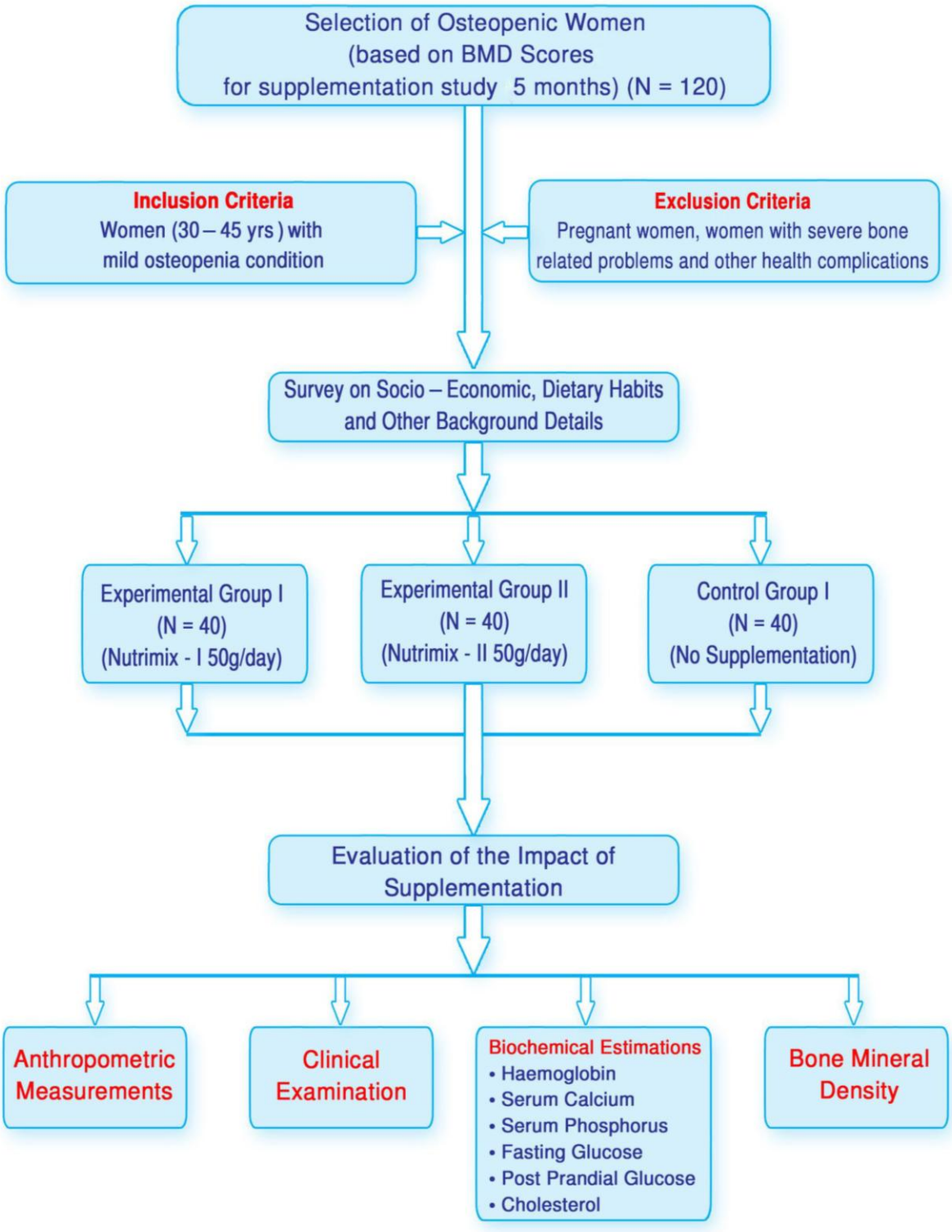

RESEARCH DESIGN
PHASE I
SELECTION OF AREA SAMPLE AND CONDUCT OF SURVEY
Selection of Area & Sample
Coimbatore, Tamil Nadu, India
Conduct of Health Camp &
Selection of Women (N=2230)
Age (21-70 years)
Survey on Socio – Economic, Dietary Habits
and Other Background Details
PHASE II
ASSESSMENT OF NUTRITIONAL AND
HEALTH STATUS & BONE HEALTH PROFILE
Assessment of Nutritional and Health
Status & Bone Mineral Density (BMD)
Anthropometry
• Weight
• Height
• Body Mass Index
• Waist Circumference
• Hip Circumference
Bone Mineral Density Measurement
Clinical Examination & Biophysical Assessment
Biochemical Parameters
• Haemoglobin
Dietary Assessment

PHASE III

A) FORMULATION AND NUTRIENT ANALYSIS OF THE SUPPLEMENTS

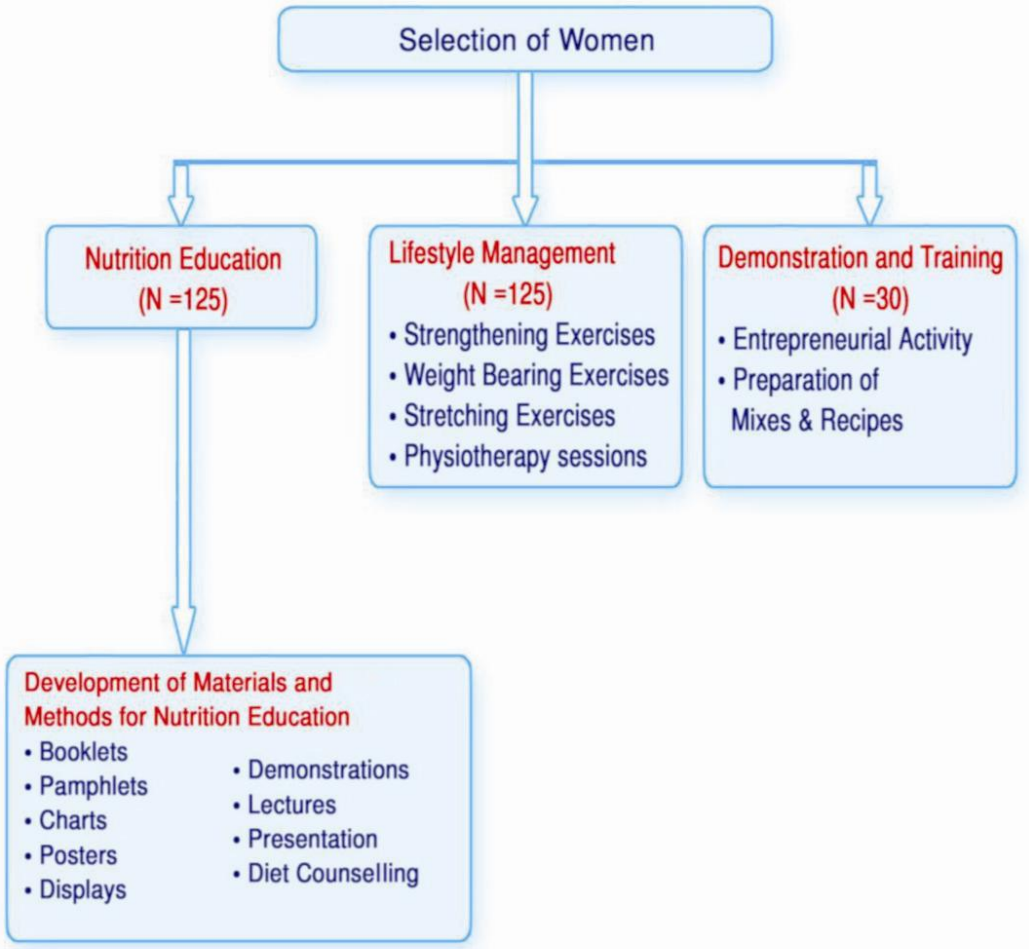


B) CONDUCT OF SUPPLEMENTATION STUDY



PHASE IV

CONDUCT OF NUTRITION EDUCATION & LIFESTYLE MANAGEMENT PROGRAMME



PHASE V **ANALYSIS OF DATA**