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METHODOLOGY

The research design pertaining to the study **"Effect of Functional Foods and Lifestyle Modification Strategies on Hypertensive Men and Women in Coimbatore, Tamil Nadu"** is presented under the following titles.

A. Phase I - Selection and grouping of hypertensives.

a. Selection of locale

b. Screening and grouping of hypertensives

c. Selection of tools for the conduct of the study

d. Assessment of nutritional and health status

1. Anthropometric measurements
2. Food and nutrient Intake
3. Blood pressure level

e. Analysis of biochemical parameters

1. Blood glucose
2. Lipid profile
3. Serum sodium
4. Serum potassium

B. Phase II – Formulation and evaluation of supplement

a. Selection of functional foods

b. Formulation and selection of supplement

c. Nutrient content of the supplement

C. Phase III – Dietary intervention

D. Phase IV – Lifestyle intervention

E. Phase V – Evaluation and analysis of data

A. Phase I : Selection and Grouping of hypertensives

a. Selection of locale

Hypertension epidemiological studies from India in the last twenty years have shown that prevalence of hypertension (diagnosed by systolic BP ≥140 mm Hg and/or diastolic BP ≥90 mm Hg) in urban locations has stabilized to about 25–30% but it has increased in rural populations from 15 to 25 per cent. This urban–rural convergence of hypertension in India is due to rapid urbanization of rural populations with consequent changes in lifestyles (sedentariness, high intake of dietary salt, sugar and fat) and increase in body weight. Hypertension prevention, screening and control, policies and programs, need to be widely implemented in India, especially in rural populations (Gupta, 2015).

Tamil Nadu is one among the most urbanized largest state in India. The recently released 2011 censes date on urban agglomerations showed that, in the last decade, about half of the increase in urban population had occurred in four urban agglomerations. Coimbatore was one among the four places (Srivathsan, 2013). It also had considerable rural population and more agricultural lands. Hence Coimbatore district was selected for the present study.

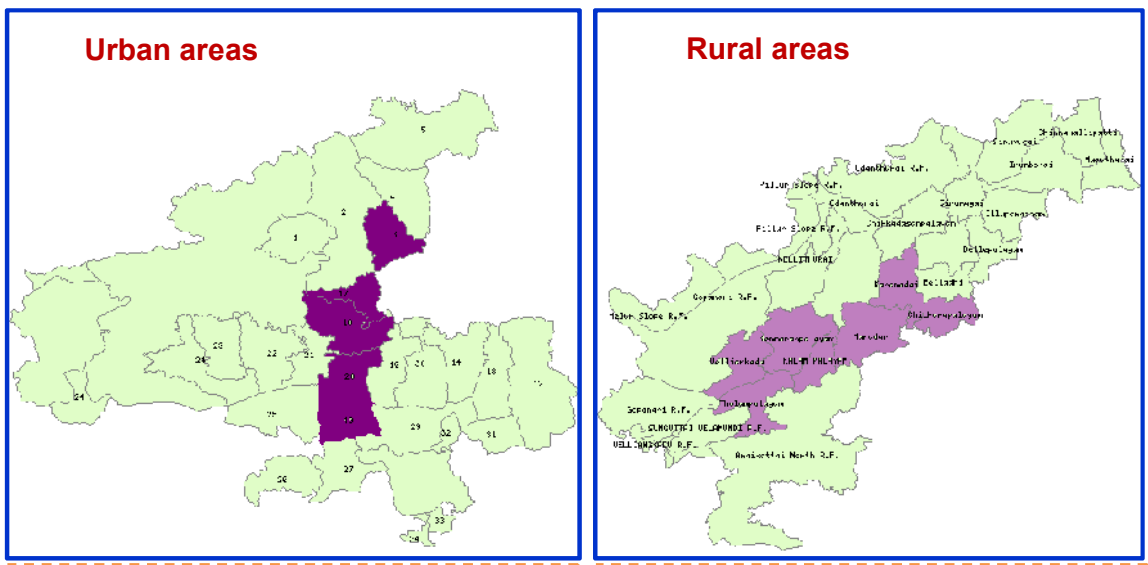
For the census of India 2011, the definition of urban areas was the all places with a municipality, corporation, cantonment board or notified town area committee etc and all other places which satisfied criteria like a minimum population of 5,000 and at least 75 per cent of the male main working population engaged in non-agricultural pursuits and a density of population of at least 400 person per square Kilometer. Cities and towns were part of urban settlements.

The National Sample Survey Organisation (NSSO) defined ‘rural’ as an area with a population density of up to 400 per square kilometer, villages with clear surveyed boundaries but no municipal board, minimum of 75 per cent of male working population involved in agriculture and allied activities (NSSO, 2010).

Based on the above criteria, urban areas and rural areas form Coimbatore district were selected for the study. Coimbatore district was divided into 10 taluks. From the ten taluks, Coimbatore north taluk which had more than 7 major industries with greater part of people engaged in non agricultural activities and its adjacent Mettupalayam taluk in which greater part of people engaged in agricultural and allied activities were selected for the study.

In Coimbatore north taluk, census towns namely Kavundampalyam, Thudiyalur, Kurudamplayam, Narasimanaicken palayam, Periyanaicken palayam were selected for the study for urban areas. Coimbatore north taluk had shown a considerable increase in urban population over the last decade. Urban agglomeration exposed people to adverse nutritional environment, inactivity and stress which were also responsible for epidemiological and nutrition transition (Issac, 2014). Mettupalyam Taluk had more agriculture lands and considerable rural population. Villages namely Tholampalayam, Vellinakadu, Karamadai, Kemmarampalayam, Chickaram palayam, Kalampalayam were selected for the study. Figure -1 illustrates the selected locale of the study.

FIGURE - 1 SELECTED LOCALE OF THE STUDY



- **Kavundampalayam**
- **Thudiyalur**
- **Narasimanaicken palayam**
- **Periyanaicken palayam**
- **Kurudampalayam**

- **Karamadai**
- **Marudur**
- **Veliiankadu**
- **Tholampalayam**
- **Kemmarampalayam**
- **Chikrampalayam**
- **Kalampalayam**

b. Selection and grouping of the hypertensives

Multistage sampling technique was used to select the subjects for this study. Multi-stage sampling is a further development of the principle of cluster sampling. Multistage sampling refers to sampling plans where the sampling was carried out in stages using smaller and smaller sampling units at each stage. In a two-stage sampling design, a sample of primary units was selected and then a sample of secondary units was selected within each primary unit (Kothari.,2004).

In first stage, the subjects in the age group of 25-55 years were included from selected urban and rural areas and in the second stage hypertensives in the age group of 25-55 years were indentified and included from rural and urban areas for in-depth study. According to Anchala *et al.,* (2014), in India 29.8 per cent were hypertensive, among them 25 per cent rural and 42 per cent urban Indians are aware of their hypertensive status. Hence, health camps were organized in selected rural and urban areas to identify the hypertensives. The community health camps were organized at the selected villages with the help of local panchayat leaders, Self Help Group (SHG) member leaders and youth volunteers from the areas. The health camps were conducted at a centre place of each selected area, so as to have population representation from in and around each selected area. The health camps were conducted with the support of a medical practitioner and a group of medical assistants and health care personnel.

Plate - I Health Camps at selected areas

Male and female subjects in the age group of 25-55 years were included for the health camp. Blood pressure was measured for men and women using standard sphygmomanometer with the help of a medical practitioner. Use of good blood pressure measurement technique was essential to the accurate diagnosis of resistant hypertension (Ogedegbe *et al*., 2010). The subject was asked to sit quietly in a chair with his or her back supported for 5 minutes before taking the measurements. Equipment with correct cuff size with the air bladder encircling at least 80 per cent of the arm and supporting the arm at heart level during the cuff measurement was used to measure the blood pressure. A minimum of 3 readings were taken at intervals of at least 2-3 minutes and the average of 3 readings were taken to represent the subject's blood pressure. The blood pressure was measured in both the arms and the arm with the higher pressures was generally used to make further measurements.

About 985 and 829 male and female were available for the health camps in the selected rural and urban areas. Nine hundred and eighty five rural included 468 men and 517 women and the urban 829 comprised of 397 men and 432 women.

1. Prevalence of hypertension

Among the randomly included urban and rural men and women, prevalence of hypertension was analysed. The following Table - I states the prevalence of hypertension in rural and urban areas.

TABLE – I PREVALENCE OF HYPERTENSION IN RURAL AND URBAN AREAS

Subjects	Number Screened	Hypertensives	
		Number	Per cent
Rural	**985**	**256**	**26**
Urban	**829**	**257**	**31**

The Table-I clearly says that total prevalence of hypertension was found to be 26 per cent in 985 rural and 31 per cent in 829 urban subjects.

2. Sex wise distribution of hypertension

About 513 hypertensives were identified in both rural and urban areas and the sex wise distribution of hypertensives is presented in Table -II.

TABLE - II SEX WISE DISTRIBUTION OF HYPERTENSIVES

Area	Subjects	Number screened	Hypertensives	
			Number	Per cent
Rural	Men	468	127	27
	Women	517	129	25
	Total	**985**	**256**	**26**
Urban	Men	397	128	32
	Women	432	129	30
	Total	**829**	**257**	**31**

Nine hundred and eighty five rural subjects included 468 male and 517 female and the urban 829 comprised of 397 male and 432 female. Out of 985 rural, 27 per cent men and 25 per cent women were found to be hypertensives and among 829 urban, 32 per cent men and 30 per cent women were observed as hypertensives. Even though the prevalence was low in rural areas in both sexes when compared to urban areas, it was not far below. It was also showing increasing trend on par with urban areas.

3. Detected and known cases of hypertension

Table - III and Figure – 2 illustrate the percentage of detected and known cases of hypertension in rural and urban areas.

TABLE – III DETECTED AND KNOWN CASES OF HYPERTENSION

Subjects	Men		Women		Total	
	Number	Per cent	Number	Per cent	Number	Per cent
Rural						
Known cases	61	48	28	22	89	35
Detected	66	52	101	78	167	65
Total	**127**	**100**	**129**	**100**	**256**	**100**
Urban						
Known cases	80	63	47	36	127	49
Detected	48	37	82	64	130	51
Total	**128**	**100**	**129**	**100**	**257**	**100**

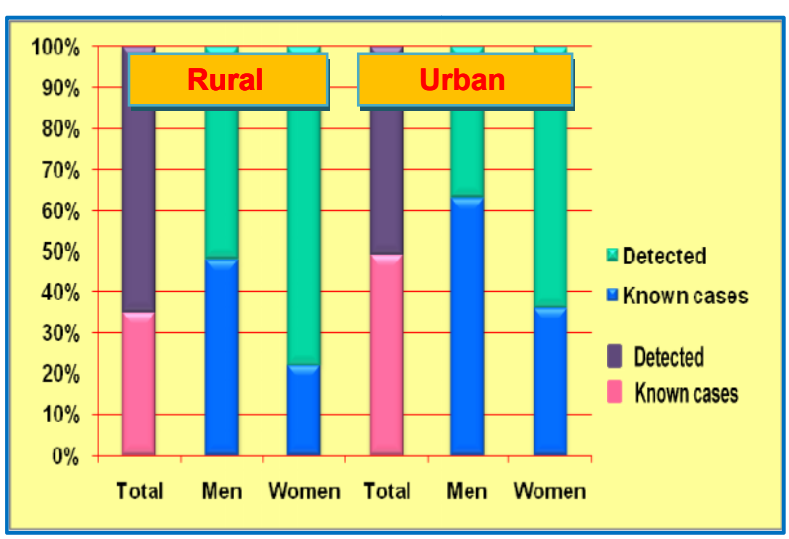


FIGURE -2 DETECTED AND KNOWN CASES OF HYPERTENSION

Table -III and Figure-2 state that among the hypertensives, 65 per cent of rural and 51 per cent of urban were detected through health camp and 35 per cent of rural and 49 per cent of urban were known cases. The present study results obviously overlapped the results of the Perspective Urban Rural Epidemiology (PURE) study which reported 30.7 per cent prevalence of hypertension in India and among them hypertensive awareness (40.4 per cent) was very low (Chow *et al*.,2013).

A total of 513 identified hypertensives comprised of 256 hypertensives from rural areas (127 men and 129 women) and 257 hypertensives from urban areas (128 men and 129 women) in the age group of 25-55 years were included for further study.

c. Selection of tools for the conduct of the study

A well structured questionnaire was formulated and validated to elicit the demographic profile and associated etiological factors of hypertension among the selected hypertensives. According to Kothari (2004), a questionnaire consists of a number of questions printed or typed in a definite order on a form or set of forms. This method of collecting data is free from the bias of the interviewer and answers are in respondent's own words. This method is low cost even when the universe is large and is widely spread geographically.

In the present study, the questionnaire was divided into five parts to elicit the information regarding socio economic status, dietary pattern, lifestyle pattern, family and personal history, psycho social and environmental factor associated with hypertension. Five hundred and thirteen selected hypertensives were approached personally and the questionnaire was explained and distributed to elicit relevant data.

The background information such as name, sex, age, residing area, number of family members, family type, marital status, educational qualification, occupation, monthly income were collected.

Food and dietary pattern of the subjects were recorded including the consumption frequency and quantity of cereals, pulses, vegetables, fruits, processed foods, ready to eat packed foods, fast foods, bakery products, carbonated drinks, fried foods and eating meal out. The type of cooking oil preferred and amount of salt used were also observed.

To draw the information regarding the family and personal medical history details collected on duration of hypertension (if known), presence of other lifestyle disease namely cardiovascular disease, diabetes mellitus etc, family history of hypertension, diabetic mellitus, obesity, cardiovascular diseases, stroke, history of antihypertensive therapy if used and its effects were recorded.

The lifestyle patterns like history of tobacco use, smoking, alcohol intake, physical exercise pattern were also observed. Emotional nature of the individual also elicited using the questionnaire.

Global Physical Activity Questionnaire (GPAQ) was formulated by WHO for physical activity surveillance in countries. It was used to collect information on physical activity participation in three settings (or domains) as well as sedentary behavoiour comprising of 16 questions. The domains were activity at work, travel to and from places and recreational activities. METs (Metabolic Equivalents) were commonly used to express the intensity of physical activities and were also used for the analysis of GPAQ data.

MET was the ratio of a person's working metabolic rate relative to the resting metabolic rate. One MET is defined as the energy cost of sitting quietly and is equivalent to a caloric consumption of 1 kcal/kg/hr. It was estimated that, compared to sitting quietly, a person's caloric consumption was four times as high when being moderately active and eight times as high when being vigorously active.

Overall energy expenditure of individual was calculated by assigning four METs to the time spent in moderate activities and 8 METs to the time spent in vigorous activities. Hence total time spent on physical activity during a typical week, the number of days as well as the intensity of physical activity were recorded under each domain.

WHO recommendation on physical activity for health is 150 minutes of moderate-intensity physical activity or 75 minutes of vigorous –intensity physical activity of an equivalent combination of moderate and vigorous intensity physical activity achieving at least 600 MET-minutes. . Based on cummulative MET score of all the domains and sub domains, the physical activity was further classified based on MET-minutes into three groups as: Inactive/low (<600 met-minutes), active (600 -1200 met-minutes) and highly active (>1200 met-minutes). (WHO, 2009 and WHO, 2010).

d. Assessment of Nutritional and Health status

1. Anthropometric measurements

Anthropometry was the universally applicable, inexpensive quantitative techniques for determining an individual's body fat composition by measuring, recording, and analyzing specific dimensions of the body, such as height, weight and bodily circumference at the waist, hip, and chest. It reflected both health and nutritional status and also predicted performance, health and survival. Hence, it was used in various intervention programmes to monitor health and nutritional status of the selected population.

Anthropometric measurements namely height, weight, waist and hip circumferences were recorded and Body Mass Index (BMI), Waist Hip Ratio (WHR) and Waist Height Ratio (WHtR) were computed for all the 513 selected hypertensives.

i. Body weight

Body weight measured the total weight of an individual, including muscle, fat, water and bone. Changes in body weight were an important indicator of nutritional status; being underweight or overweight adversely influences mortality and morbidity. Gupta and Gupta (2010) stated that a substantial and consistent body of evidence from observational studies and clinical trials showed that weight was directly associated with blood pressure.

Body weight of 513 subjects was measured using a portable weighing machine. The individual was requested to stand still on the platform of the weighing machine with the body weight evenly distributed between both the feet. Light indoor clothing was allowed to be worn, but the footwear was removed when the weight was measured. The scale was zeroed before weighing and it was also calibrated regularly during the study.

ii. Height

Height was measured for all the selected 513 hypertensives by using a portable stadiometer. The persons, whose height has to be measured, stood in an

erect position without any foot wear and they were also asked to put their feet together and to look straight. The subjects were asked to take a deep breath and stand tall to aid the straightening of the spine. Shoulders should be relaxed. The movable headboard was then gently lowered until it touched the crown of the head.

The height measurement was taken at maximum inspiration with the examiner's eyes level with the headboard to avoid parallax errors. Height was recorded in centimeter and up to the nearest millimeter.

iii. Body Mass Index (BMI)

Increasing BMI is clearly associated with a higher risk of elevated blood pressure and other cardiovascular disease risk factors, and increased mortality (Shihab *et al*., 2012). THE BMI was widely used to predict the overweight and obesity. The body mass index was calculated as weight (in kg) divided by height (in m^2). The selected hypertensives were classified based on World Health Organisation (WHO, 2004) Asian classification of BMI as given below.

Asian classification of BMI*

BMI (Kg / m^2)	Classification
< 18.5	Underweight
18.5 – 22.9	Normal range
≥23	Over weight
23.0 – 24.9	At risk of obesity
25.0 – 29.9	Obese I
≥ 30.0	Obese II

***WHO, (2004)**

iv. Waist Hip Ratio (WHR)

The medical risks due to obesity had been shown to be linked more to the abdominal distribution of fat, as measured by the Waist Hip ratio (WHR). Research studies on cardiovascular diseases, diabetes and obesity in Asian Indians often

reported that WHR was a risk factor. All the adult men with the waist-hip ratio of ≥0.90 and women with ≥0.80 were identified as obese (ICMR, 2010). For all the selected 513 hypertensives WHR were computed by dividing subject's waist circumference in cms by hip circumference in cms and compared with the standard values.

❖ **Waist Circumference**

Waist circumference was measured for all the five hundred and thirteen selected hypertensives. The subjects were asked to stand erect with weight evenly balanced on both feet, which were placed about 25 to 30 cm apart. A mark was made at the level of the lowest rib margin. The iliac crest in the mid axillary line was felt and a mark was made. The measuring tape was passed around the waist horizontally midway between the lowest rib margin and iliac crest and the circumference in centimeter was measured up to the nearest millimeter. The observer sat on a stool in front of the subjects while taking the measurement.

❖ **Hip Circumference**

Hip circumference was measured for all 513 hypertensives. Hip circumference should be measured around the widest portion of the buttocks, with the tape parallel to the floor. The circumference was measured at the point yielding the maximum circumference in centimeter up to the nearest millimeter. The subjects were asked to stand with feet close together, arms at the side and body weight evenly distributed with little clothing. The subject were relaxed, and the measurements were taken at the end of a normal expiration (WHO, 2008).

v. Waist Height Ratio (WHtR)

The WHtR is a simple and practical measure, and researchers have suggested that WHtR might be a better predictor of the risk of cardiovascular disease than BMI or waist-circumference (WC). WHtR was more strongly correlated with visceral fat-mass and clustering of cardiovascular risk factors in

adults. It incorporated WC as a measure of abdominal adiposity and adjusts for an individual's size by dividing by their height.

WHtR was computed for 513 selected hypertensives and compared with the standard values. According to Xin *et al* (2012), the reference values of WHtR for men and women was 0.536 and 0.492.

2. Food and nutrient intake

Most commonly, individual dietary surveys were undertaken for the purpose of establishing the existence, strength, direction and level of associations between dietary exposure and health outcome in the individual. The 24 -hour recall method was widely used to provide information on foods consumed by the individual over the previous 24 hours. The cooked amount of each food item consumed by the subjects was measured and then converted back into raw foodstuff using the formula given below. These raw equivalent was calculated for entire foods consumed from morning to bed time in a day and summed up to obtain the total consumption of a food items during the past 24 hour period.

$$R_i = \frac{T_R \times C_1}{T_c}$$

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

T_R = Total raw quantity of the food stuff used in that preparation

C_1 = Intake of the cooked amount of that preparation

T_c =Total cooked quantity of the food prepared

The nutrient intake of the subjects was computed for the raw equivalent for all the hypertensives using the food composition table as per nutritive value of Indian foods given by Gopalan *et al*., (2012) and compared with their recommended dietary allowances.

3. Blood Pressure

The selected 513 selected hypertensives were approached personally by the investigator and their Systolic Blood Pressure (SBP) and Diastolic Blood

Pressure (DBP) were measured using standard, validated digital sphgmomanometer (Omron). Three reading were recorded with the gap of 5 minutes and average of the three readings was recorded as the individual blood pressure.

PLATE - 2 INVESTIGATOR RECORDING THE BLOOD PRESSURE

The selected 513 hypertensives were classified with respect to their blood pressure based on JNC-VII classification as given below.

CLASSIFICATION OF HYPERTENSION

Category*	SBP mmHg		DBP mmHg
Normal	**<120**	**and**	**< 80**
Prehypertension	**120 – 139**	**or**	**80-89**
Hypertension Stage -I	**140 – 159**	**or**	**90-99**
Hypertension Stage -II	**≥160**	**or**	**≥100**

***JNC-VII (2008)**

Table –IV illustrates the classification of rural and urban hypertensives based on their blood pressure level.

TABLE – IV CLASSIFICATION OF RURAL AND URBAN HYPERTENSIVES BASED ON THEIR BLOOD PRESSURE LEVEL

Classification of hypertension*	Rural				Total	Urban				Total
	Men		Women			Men		Women		
Blood pressure (mm Hg)	No.	%	No.	%	Number (%)	No.	%	No.	%	Number (%)
Prehypertension SBP(120-129) / DBP (80-89)	77	61	63	49	140 (55)	85	66	76	59	161 (63)
Hypertension stage –I SBP (130-159) / DBP (90-99)	41	32	43	33	83 (32)	27	21	26	20	52 (20)
Hypertension Stage -2 SBP (>160) / DBP (>100)	9	7	23	18	32 (13)	16	13	27	21	43 (17)
Total	127	100	129	100	256	128	100	129	100	257

*JNC-VII (2008).

It was adverse to note that more than half of rural men (61 per cent), urban men (66 per cent), urban women (59 per cent) and nearly one part (49 per cent) rural women among the selected hypertensives were prehypertensives. It was more prevalent among men when compared to women.

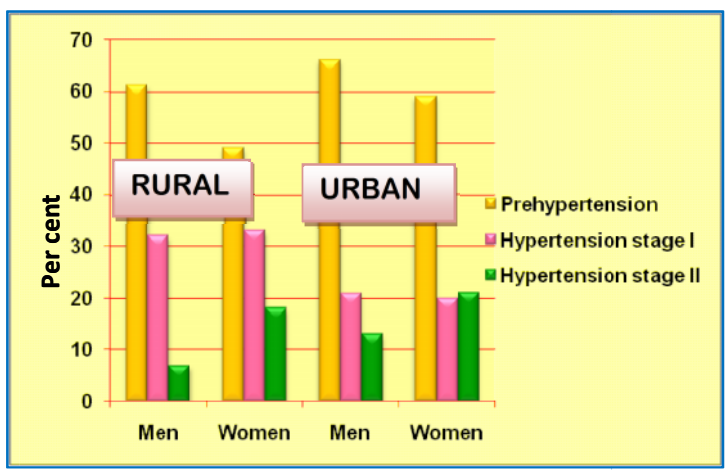


FIGURE -3 CLASSIFICATION OF HYPERTENSIVES

Hypertension stage -I was observed in 32-33 per cent of rural and 20-21 per cent of urban hypertensives and hypertension stage -II was found to be greater among women in both rural (18 per cent) and urban (21 per cent) against 7 per cent in rural men and 13 per cent in urban men.

e. Analysis of Biochemical parameters

Biochemical estimation was the most sensitive indicator of the health condition of an individual. For the sub samples of 180 hypertensives including 90 women and 90 men from urban areas, the blood parameters namely fasting glucose, postprandial glucose, glycosylated haemoglobin, lipid profile, serum sodium and potassium level were analysed using standard procedure in the laboratory before and after the interventions.

The subjects were asked to come in the early morning around 7 o' clock after 12 hours of fasting. Five ml of blood sample was collected from sub samples with the help of a experienced technician before and after the intervention. From each subject (90 men and 90 women) blood sample was collected from the antecubital vein. Care was taken to avoid hemolysis during the collection of blood for serum separation. Blood was allowed to clot for nearly 3 hours at room temperature. Then the clot was centrifuged and supernatant serum was removed and stored in well stopper bottles in a freezer and used for analysis of blood parameters.

1. Blood glucose Level

❖ Fasting and postprandial blood glucose level

Diabetes and hypertension frequently coexisted, leading to additive increased in the risk of life-threatening cardiovascular events (Bernard and Cheung, 2013). It was well documented that there was an association between the level of plasma glucose concentration and blood pressure in nondiabetic individuals. Moreover, it was reported that the prevalence of hypertension was increased in diabetic patients compared with nondiabetic individuals.

The blood sample collected from 180 subsamples (90 men and 90 women) after 12hrs of fasting was used to analyse the fasting blood glucose level and glycosylated haemoglobin. After that the selected hypertensives were asked to consume breakfast and two hours after the breakfast consumption, another sample of blood was collected and analysed for the postprandial blood glucose level. Blood glucose was estimated by GOD-PAP method.

❖ **Glycosylated Haemoglobin**

Glycosylated hemoglobin was a form of hemoglobin that was measured primarily to identify the average plasma glucose concentration over prolonged periods. It was formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose. Normal levels of glucose produce a normal amount of glycosylated hemoglobin. As the average amount of plasma glucose increased, the fraction of glycosylated hemoglobin increased in a predictable way. This serves as a marker for average blood glucose levels over the previous 3 months prior to the measurement as this was the lifespan of red blood cells. It was estimated by chromatographic spectropotometric ion exchange method for 180 sub samples.

2. Serum lipid profile

Abnormalities in serum lipid and lipoprotein levels (dyslipidemia) were recognized as major modifiable cardiovascular disease risk factors and had been identified as independent risk factors for essential hypertension. Many studies had shown that Total Cholesterol (TC), Triglycerides (TG), and virtually all fractions of lipoproteins tend to be more frequently abnormal among hypertensive patients than in the general population (Osuji *et al*., 2012)

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

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

- Very Low Density Lipoprotein (VLDL) cholesterol level was calculated from the estimated triglyceride level using the following formula.

$$\text{VLDL cholesterol} = \frac{\text{Triglyceride}}{5}$$ Where 5 was a constant factor

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

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

3. Serum sodium

High sodium and low minerals intakes especially potassium and calcium were the major factor of hypertension. In both normotensive and hypertensive humans, a large and sudden increase in dietary sodium usually causes a 2 to 4 mmol/L rise in plasma sodium. There were also significant correlations between the increase in plasma sodium and the reduction in plasma renin activity and aldosterone, and the rise in blood pressure (He *et al*., 2012). Hence serum sodium level was estimated for the sub samples (90 men and 90 women) using enzymatic method.

4. Serum potassium

Serum potassium had a fundamental role in blood pressure (BP) regulation, and there was evidence highlighting the importance of potassium homeostasis in hypertension. Studies observed the reverse relation between serum potassium and BP supported a close pathophysiological connection between serum potassium and essential hypertension.The Framingham Heart Study suggested that serum potassium level ≥ 5.2 mEq/L or ≤ 4.0 mEq/L may increase the risk of hypertension, (Lu *et al.,*2015). Serum potassium level was estimated for the sub samples of 180 hypertensives (90 men and 90 women) using enzymatic method.

B. Phase II – Formulation and evaluation of supplements

a. Selection of functional foods

Hypertension was not only among the common and leading causes of morbidity and mortality throughout the globe but also was the most important modifiable risk factor for life-threatening diseases. Surgical knife plus pills cannot fundamentally solve hypertension problems. There were lots of crops available against hypertension. The strategies of hypertension prevention in most of the countries were the functional food with whole grains and some vegetables as well as fruits for dietary. Mostly high potassium/low sodium diet was recommended by various studies (Zeng *et al*., 2011)

The functional foods which contained hypotensive components and also having other associated risk factor controlling properties were selected for the supplement based on various available data.

Numerous studies had proved the linear association between hypertension and potassium level in human. According to the studies, people who regularly consumed high-potassium foods had lower blood pressure, lower rate of heart disease and stroke than those who did not. A recent review of thirty-three studies examined the effect of potassium on blood pressure, and researchers concluded that participants who added 2,340 mg of potassium daily (from foods, supplements, or both) were able to lower risk of developing high blood pressure by 25 per cent (Adams, 2009).

Evidence showed that reducing sodium intake significantly reduced blood pressure in adults. WHO recommended a reduction in sodium intake to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart disease in adults (WHO, 2012).

Peptides from various sources had been shown to have clearly positive effects on health by functioning as antihypertensives, antioxidants, anticarcinogens, antimicrobials and anticariogenics among others. These properties had led to their labeling as functional or biologically active (i.e.bioactive)

peptides. Pulses and legumes were major dietary protein source in human beings (Campos *et al*., 2013).

A new animal study by Aley (2014) revealed that consuming pulses like beans, peas, lentils and chickpeas can reverse the changes that happen in blood vessels due to high BP. The notable finding of the study was the fact that lentils could alter the physical properties of blood vessels so that they resembled the vessels found in healthy animals.

In India, red gram dhal *(Cajanus cajan*) was very popular and preferred dhal among both rural and urban areas. Apart from protein, fiber, folic acid, it was low in calories. So it was good for people who were on weight loss diets. Weight reduction in turn had favourable association with hypertension.

Green gram *(Phaseolus aureus Roxb.)* was eaten on a regular basis in every Indian household in combination with rice and vegetables. Moong bean was rich in iron and potassium. Iron helped to maintain hemoglobin levels and potassium helped to lower blood pressure, a boon for hypertensive people. Vicilin, the major storage protein of mung bean, bioactive peptides reduced the activity of angiotensin-converting enzyme (ACE) that constricted blood vessels and raised blood pressure (Aleyne *et al*., 2014).

Cow pea *(Vigna catjang)* was widely used in India and in addition to peptides, it contained alkaloids which had antihypertensive properties. Cow pea peptides acted as antioxidants and inhibited the enzyme needed for biosynthesis of cholesterol and highly effective at inhibiting LDL oxidation (Campos *et al*., 2013).

Moth Bean *(Phaseolus aconitifolius)* seeds were good and potential reservoir of proteins and other essential minerals and vitamins. Moth bean was one of the major protein food source. It was rich in protein (23.6 g), calcium (202 mg) and it was an excellent supplement to cereal diet. Moth Bean was a cheap source of nutrients and forms a specific and perfect diet.

High food grade protein and good amount of potassium was found in coconut deoiled meal *(Cocos nuciferaL)*. Coconut meal flour was also being

marketed as functional foods. It had high dietary fiber content that aids in lowering cholesterol and provides other health benefits to the human body (Sivakami and Sarojini, 2013). High cholesterol level was found to be positively associated with hypertension and its other complications. Hence, including hypocholesterolimic foods in diet in turn prevent further complications in hypertensives.

Banana (*Musa paradisicum*) was a nutritious fruit and a good source of potassium. Banana was also a good source of vitamin B6 (22 per cent), potassium (12 per cent), vitamin C (17 per cent), magnesium (8 per cent), etc (Huang, 2013). Bananas also contained flavonoids which show anti-inflammatory, hypolipidemic (ability to lower LDL (bad) cholesterol), hypoglycemic and antioxidant activities. Because of their hypolipidemic properties, flavonoids in bananas can reduce the risk of heart disease, inflammation and aging. The Indian scientists reported that natural compounds in bananas act in a manner similar to anti-hypertensive drugs (Vijiyakumar *et al.,* 2015).

Thus, the foods rich in hypotensive components namely high potassium, low sodium and good quality of peptides were chosen to formulate the supplement. The selected ingredients were red gram dhal, green gram dhal, coconut deoiled, cow pea, moth beans and banana powder. Banana powder was a concentrated form and provided adequate amount of potassium. These foods were locally available throughout the year, low cost, familiar to the subjects and easy to process and store.

b. Formulation and organoleptic evaluation of supplement

The selected pulses and coconut deoiled meal were cleaned and powdered. To prepare banana powder, nendaram variety raw banana was chosen and it was peeled, sliced, shade dried and powdered. This variety was taken because it was used commonly by the south Indian people for making banana powder. The prepared banana powder was mixed with the pulse mix.

The selected food items coconut deoiled meal, banana powder; pulses were mixed in three different combinations in the ratio of 1:2:3, 3:2:1, 2:1:3 and

subjected to sensory evaluation. With the mix small quantity of jaggery was powdered and added to these mixes to increase the palatability of the mixes. The most acceptable food mix was selected for the formulation of supplement. The most acceptable combination of supplement selected for the clinical trial/intervention is given in the Table -V

TABLE – V ACCEPTABLE COMBINATION FOR THE SUPPLEMENT

Food items	Quantity (g)
Red gram dhal *(Cajanus cajan* (L.) Millsp)	10
Green gram *(Vigna radiate)*	10
Cow pea *(Vigna sinensis Savi)*	10
Moth bean *(Vigna aconitifolia)*	15
Banana powder *(Musa spp. - Rasthali – AAB)*	30
Coconut deoiled *(Cocos nuciferaL)*	15
Jaggery *(Caryota urens)*	10
Total	**100**

To facilitate regular consumption of the formulated supplement, it was incorporated into traditional recipes namely dosai, chappathi, adai, pongal, sweet bar (baked), uppuma, rotti, porridge and laddu and standardized. The prepared recipes were subjected to sensory evaluation using 9 point hedonic scale. Sensory evaluation was a scientific discipline used to evoke, measure, analyze and interpret those responses to products that were perceived by the senses of sight, smell, touch, taste and hearing. The most widely used scale for measuring food acceptability was the 9-point hedonic scale.

PLATE - 3 RECIPES PREPARED WITH THE SUPPLEMENT

The 9 point hedonic scale had nine points, and these points were given word description range from "dislike extremely" to "like extremely". The verbal anchors of the scale were selected so that the psychological distance between successive scale points was approximately equal. This equal-interval property helped to justify the practice of analyzing the responses by assigning successive integer values (1, 2, 3, … up to 9) to the scale points and testing differences in average acceptability using parametric statistics (Stone and Shedel, 1993). Hence the recipes were evaluated by panel of 21 experts using 9 point hedonic scale with numerical scores. The scores obtained for the recipes were given in the Table -VI.

TABLE - VI SCORE GIVEN BY THE EXPERTS FOR THE RECIPES

Recipes	Parameters					Total Score (Max-45)
	Appearance	Texture	Flavour	Colour	Taste	
1. Dosai	7.3	7.4	7.2	7.2	7.5	36.6
2. Chappthi	6.1	6.9	6.8	6.0	6.2	32.0
3. Adai	6.6	6.2	6.7	6.3	6.6	32.4
4. Pongal	5.8	5.9	6.1	6.0	5.8	29.6
5. Sweet bar (baked)	7.8	7.5	8.1	8.0	8.3	39.7
6. Uppuma	5.2	5.2	5.7	5.1	5.7	26.9
7. Rotti	6.1	6.0	6.3	6.2	6.2	30.8
8. Porridge	6.3	6.4	6.2	6.0	6.3	31.2
9. Laddu	6.4	6.3	6.2	6.1	6.3	31.3

The recipe namely sweet bar (baked), dosai, adai were found to be in the first three ranks. Among them the sweet bar (baked) recipe which had got high score and easy to prepare and distribute to the selected hypertensive was selected for the intervention trial. From 100g of the selected supplement, 6 medium size sweet bars were obtained. The preparation cost of the supplement comes around Rs.11. for a person per day. It was observed to be less when compared to the cost of pharmacological treatment for hypertension.

c. Nutrient content of the supplement

Nutrients namely energy, protein, fat, potassium and sodium present in the supplement were calculated using nutritive value of Indian foods by Gopalan *et al.,* (2012). The nutrient content of 100 g of supplement is given in the Table –VII.

TABLE - VII NUTRIENT CONTENT OF THE SUPPLEMENT

Nutrients	Quantity
Energy (Kcal)	323
Protein (g)	10.9
Fat (g)	0.5
Potassium (mg)	1196
Sodium (mg)	22.6
Fibre (g)	1.4

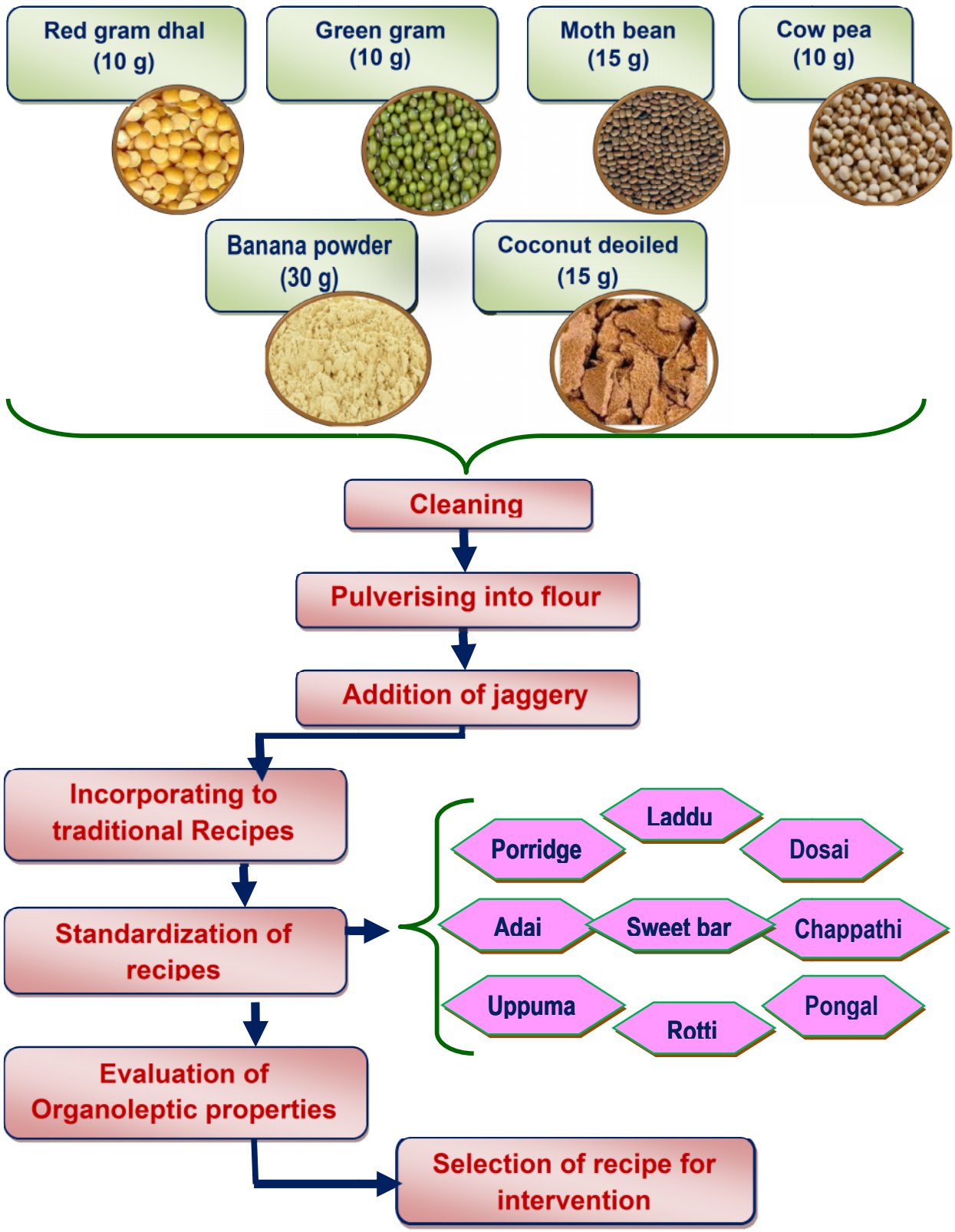
Hundred grams of selected mixture provided 323 kcal of energy, 10.9 g of protein, 0.5 g of fat, 1.4 g of fibre, 22.6 mg of sodium and nearly 26 percent of the daily requirement of the potassium.

C. Phase III - Dietary intervention

Intervention to provide a minimum level of nutrition or to stabilize or improve the nutritional status of individuals had become a high priority in public health during the past two decades. Two of the more popular interventions had been the use of direct food supplementation with or without nutrition education and fortification of staple foods. These interventions were usually established as “nutrition components” of comprehensive health service programmes.

Dietary modifications were mainstay for prevention and initial treatment of hypertension. Non-pharmacological therapy had an important role in both non-hypertensive and hypertensive individuals. In non-hypertensive individuals, including those with pre-hypertension, dietary intervention had the potential to prevent hypertension and more importantly to reduce BP and lower the risk of BP-related clinical complications (Gupta, 2013).

Figure - 4 FORMULATION AND EVALUATION OF SUPPLEMENT



Hence dietary intervention was planned for 180 days and executed in a systematic way. About 60 men and 60 women hypertensive subjects from urban areas were included for the dietary intervention based on the inclusive and exclusive criteria. The hypertensives were selected from urban areas to reduce the travel time for the investigator to distribute the supplement and observe the consumption regularly.

Inclusion Criteria:

- Blood pressure: SBP 120-129 mmHg/DBP80-89 mmHg (Prehypertensives)
- Age: 25 - 45 yrs
- Sex: Male and Female
- Duration of the disease : < 5 yrs
- Not taking antihypertensive medication
- Free of complications
- Willingness to participate in feeding trials

Exclusion Criteria:

- Blood pressure: SBP >129 mmHg / DBP >89 mmHg
- Age: <25 &>45 yrs
- Duration of the disease :>5 yrs
- Taking antihypertensive medication
- With complications : CVD, Diabetes, Kidney disorders

The included 120 prehypertensive subjects were grouped for dietary intervention as experimental and control group and given in the Table –VIII.

TABLE - VIII GROUPING FOR DIETARY INTERVENTION

S. No.	Groups	Number of subjects
1	**Experimental group**	
	Group (DIM)	30 men
	Group (DIW)	30 women
2	**Control group**	
	Group (CTM)	30 men
	Group (CTW)	30 women

DIM- Dietary Intervention Men **DIW -**Dietary Intervention Women
CTW-Control Men **CTW-**Control Women

Before starting the intervention, the selected hypertensives were well informed on the clinical trial and its subsequent analysis parameters. The written consents were obtained from the study subjects. The six pieces of sweet bar prepared from 100 gms of the selected food mix was given to the selected prehypertensives and instructed to consume three pieces in the midmorning and three pieces in the evening as a nutritious snack regularly. Supplementation was carried out for the period of six months (180 days). The consumption of the supplement was thoroughly monitored by the investigator.

Nutrition education was given to the experimental subjects. Nutrition education is an essential component in improving dietary habits and food choices, in order to reverse the under nutrition and improve the nutritional diagnosis. The positive impact of nutrition education on the nutritional status of adults had been confirmed by many studies.

PLATE- 4 NUTRITION EDUCATION MATERIALS PREPARED

Nutrition education was given to the experimental group through one to one counseling and group discussions. Pamphlets, booklet, posters and presentation slides were developed for the nutrition education and distributed to the participants. They were informed about causes of hypertension, its complications and role of dietary and lifestyle modifications in controlling blood pressure. The investigator visited the study subjects regularly and supervised their food and activity pattern along with the consumption of supplement. The group CTM and group CTW grouped under control groups did not receive any food supplementation or nutrition education.

The Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) levels of the experimental and control groups were recorded once in a week by the investigator regularly by using digital sphygmomanometer (Omron).

D. Phase IV - Lifestyle Intervention

Lifestyle changes had been shown to effect significant blood pressure (BP) reductions (Frisoli *et al.,* 2011). The American Heart Association (AHA) recommended exercise, stress management and weight management to prevent high blood pressure, thereby lowering the risk of heart disease. Practicing yoga helped to decrease the negative impacts of stress, including tension, shallow breathing and elevated heart rate. It also improved physical strength and flexibility (Sengupta, 2012).

Yoga was an ancient traditional Indian psychological, physical and spiritual exercise practice that had been studied for several decades for its role in the management of numerous chronic diseases. It was a form of physical activity consisting of various postures (Asana) and breathing techniques (Pranayama). A study on subjects with mild to moderate hypertension and reported that yoga can play an important role in reduce blood pressure, the risk for cardiovascular diseases (Mondal *et al*., 2014).

Using humor to decrease stress, diminish pain and improve quality of life had recently become a popular topic. The results showed that laughter and sense of humor was effective in short and long term (more than 2 months) effect on decreasing the blood pressure and can be used as a concomitant treatment of essential hypertension (Jalali *et al.,* 2008)

Hence, the lifestyle intervention was planned by including yoga asanas, meditation, pranayama (breathing exercise) and laughing therapy. For lifestyle intervention 60 hypertensives (30 women and 30 men) were selected for the experimental groups and the same control groups of dietary intervention group CTM and group CTW was treated as control groups for lifestyle intervention also. The hypertensives included for the experimental groups were selected based on the inclusion and exclusion criteria given below.

Inclusion Criteria:

- Blood pressure: SBP 120-129 mmHg/DBP80-89 mmHg (Prehypertensives)
- Age: 25 - 45 yrs
- Sex: Male and Female
- Duration of the disease : < 5 yrs
- Not taking antihypertensive medication
- Free of complications
- Willingness to participate in feeding trials

Exclusion Criteria:

- Blood pressure: SBP >129 mmHg / DBP >89 mmHg
- Age: <25 &>45 yrs
- Duration of the disease :>5 yrs
- Taking antihypertensive medication
- With complications : CVD, Diabetes, Kidney disorders

The grouping of hypertensives for lifestyle intervention is presented in Table -IX.

TABLE -IX GROUPING FOR LIFESTYLE INTERVENTION

S.No.	Group name	Number of subjects
1	**Experimental group**	
	Group (LIM)	**30 men**
	Group (LIW)	**30 women**
2	**Control group**	
	Group (CTM)	**30 men**
	Group (CTW)	**30 women**

LIM- Lifestyle Intervention Men **LIW -**Lifestyle Intervention Women
CTW-Control Men **CTW-**Control Women

Before beginning the intervention, the selected hypertensives were oriented on the importance of lifestyle modification strategies, its health benefits and the consequent analysis parameters. Written consent was obtained from the experimental and control groups. Training on lifestyle intervention strategies were given to the experimental groups by a professional trainer from reputed institute.

The training was given on simple yoga asanas in sitting, standing, kneeling, prone and supine positions, breathing exercise (pranayama) and meditation. They also instructed to simulate laughter for 45 seconds to one minute, beyond the typical burst of laughter, which was followed by deep breathing and gentle stretching. The training was given for a period of 4 days, 2hrs/day. After the training period, they were instructed to practice the same for 45 minutes/day for at least 6 days in a week, for a period of 120 days. This was thoroughly monitored by the investigator and trainer regularly.

PLATE – 5 TRAINING ON YOGA AND LAUGHING THERAPY

Nutrition education was given to the experimental groups by using specially prepared booklet, pamphlet, presentation of slides, group discussions and personal counseling on the causes, complication of hypertension, importance of healthy eating habit, significance of physical exercise and other associated factors which favoured blood pressure reduction.

The experimental groups were instructed to record their daily food intake, day and duration of yoga practiced and it was well monitored by the investigator along with the yoga expert.

The control groups namely (CTM and CTW) did not receive any training or nutrition education. Regular blood pressure measured was recorded for the experimental groups and control groups weekly once by the investigator.

The research design was registered in the CTRI trial registry (CTRI/2015/05/005815) and approved by the Institution Human Ethical Committee (AUW/IHEC-13-14/FHP-07).

E. Phase V - Evaluation and analysis of data

a. Evaluation of the impact of intervention among the selected hypertensives

To find out the impact of intervention, blood pressure was recorded weekly once for both experimental and control groups and anthropometry measurements, and blood parameters (blood glucose, serum lipid, serum sodium and serum potassium) of the experimental and control groups were recorded before and after dietary intervention period (180 days) and lifestyle intervention period (120 days). These results were evaluated using different parameters.

b. Consolidation and analysis of data

The data were systematically consolidated, tabulated and analysed using statistical package for the social sciences, version 17. All data were expressed as mean$\pm$SD Student's t-test was performed for experimental and control groups. Correlation analysis was done for the different parameters.