III METHODOLOGY

The research design pertaining to the study "MANAGEMENT OF DIABETES MELLITUS (TYPE II) WITH HERBAL SUPPLEMENTS" is presented under the following heads.

PHASE I
A. Selection of the locale
B. Selection of diabetic subjects
C. Formulation of tool for the conduct of the study

PHASE II
A. Development of an educational package for diabetics
B. Conduct of diabetic education campaign using the developed package
C. Collection of background information, dietary pattern, lifestyle pattern, personal and family history of diabetics
D. Assessment of nutritional status of the selected subjects
   a. Recording height, weight, waist and hip measurements and calculating Body Mass Index and Waist Hip Ratio
   b. Recording individual food and nutrient intake
   c. Analysis of biochemical parameters - blood glucose levels and serum lipid profile.

PHASE III
A. Selection, formulation and sensory evaluation of selected supplements
B. Parameters used to analyse the herbal supplements
   a. Determination of Glycemic Index
   b. Analysis of dietary fiber
   c. Analysis of Active Constituents and toxic elements

PHASE IV
A. Supplementation of different herbal powders for the selected diabetics

PHASE V
A. Evaluation of the effect of supplementation among the selected diabetics
B. Consolidation and analysis of data
PHASE I
A. SELECTION OF THE LOCALE

K.G. Hospital, Sakthi Herbal Center, Nature Cure Hospital, S.P.R. Diabetic Center, Balaji Diabetic Clinic and Jeeva Jothi Herbal Clinic in Coimbatore, S.K.M. Siddha Hospital in Erode and M.G. Diabetes Speciality and Research Center in Salem were selected as venue for the study. These hospitals were selected because of the easy accessibility and availability of adequate number of diabetic subjects who attended these hospitals periodically and were willing to cooperate. The authorities and diabetologists were very much interested in the research work related to the dietary management of diabetics. They had clinical laboratories of their own, which were well equipped with modern and sophisticated instruments for analysis of blood glucose and lipid profile. Doctors, nursing staff, dietitians and other coworkers assured their cooperation and were willing to help the investigator.

B. SELECTION OF DIABETIC SUBJECTS

Subjects for the study were selected using purposive sampling method. In purposive sampling method, a desired number of sample units are selected deliberately 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, 2005)

For the study 1000 Non Insulin Dependent Diabetes Mellitus (Type II) subjects in the age group of 45-60 years were selected from both sexes based on their willingness to participate in the study. The selected subjects were given an orientation regarding the conduct of the study and also they were briefed on the modalities and purpose of the study. They were also informed about the follow-up to be carried out for three consecutive months and the subsequent anthropometric and biochemical assessment to be carried out initially and finally (before and after the supplementation of 90 days).

Among the selected 1000 Type II diabetic subjects, 360 were chosen for the supplementation study, out of whom 330 were included in the eleven experimental groups (each group has 30 subjects) and the remaining 30 were in control group. The control group did not receive any supplementation during the study period of 90 days.
RESEARCH DESIGN

PHASE I
Total subjects N=1000
(Age 45-60 years)

COLLECTION OF BACKGROUND
INFORMATION
- Anthropometry
- Clinical Symptoms
- Dietary survey
- Biochemical Analysis
  - Blood Glucose – Fasting and Postprandial
  - Lipid Profile – Total cholesterol, LDL-C, HDL-C, VLDL-C and Triglycerides

PHASE II
Diabetic Education with the developed package (N=1000)

PHASE III
FORMULATION OF THE SUPPLEMENT AND SENSORY EVALUATION
ANALYSIS OF SELECTED SUPPLEMENTS
- Glycemic Index
- Dietary fiber
- Active constituents and Toxic elements

PHASE IV
SUPPLEMENTATION STUDY (N=360)

Experimental Group I (N=180)
TG +- Thenai - 50g+Green Gram-15g=(TG)
TG-AF (TG)+ Aavaram Flower Powder (2g)
TG-KL (TG)+ Kadazhahnjil Bark Powder (2g)
TG-KKZ (TG)+ Koral Kizhangu Powder (2g)
TG-KK (TG)+ Kotta Karanthai Plant Powder (2g)
TG-VL (TG)+ Vilvai Leaves Powder (2g)
*TG- Thenai Green Gram Group

Experimental Group II (N=150)
HC**-AF Aavaram Flower Powder Capsule (2g)
HC-KL Kadalazhinjil Bark Powder Capsule (2g)
HC-KKZ Korai Kizhangu Powder Capsule (2g)
HC-KK Kotta Karanthai Plant Powder Capsule (2g)
HC-VL Vilvai Leaves Powder Capsule (2g)
**HC – Herbal Capsule Group

Control Group (N=30)
No Supplement

PHASE V
EVALUATION OF SUPPLEMENTATION (N=360)
- Anthropometry - Height, Weight, Waist and Hip circumferences
- Clinical Symptoms
- Diet Recall and individual nutrient consumption
- Biochemical Analysis
  - Blood Glucose – Fasting, Postprandial and Glycosylated Haemoglobin
  - Lipid Profile – Total cholesterol, LDL-C, HDL-C, VLDL-C and Triglycerides
The criteria used for the selection of the diabetics for the supplementation study were as follows:

- age group between 45-60 years
- Fasting Blood Glucose (FBG) level >120 mg / dl.
- Post Prandial Blood Glucose (PPBG) level > 200 mg/dl.
- serum total cholesterol level >200 mg/dl.
- free from other complications and
- willing to take the supplements regularly for a period of three months.

C. FORMULATION OF TOOL FOR THE CONDUCT OF THE STUDY


Personal interview is a survey method of data collection which employs a questionnaire. The components of the personal interview are the researcher, the interviewer, interviewee and the interview environment (Pannerselvam, 2006).

A well structured interview schedule given in Appendix I was formulated to elicit background information about the selected subjects.

PHASE II

A. DEVELOPMENT OF AN EDUCATIONAL PACKAGE FOR DIABETICS

a. Collection of resource materials to be included in the software package

The first step in the development of software package consisted of drafting an effective education programme for diabetic subjects. This was carried out by reviewing and synthesizing numerous diabetic concepts and integrating them towards developing an interactive package.

Reliable information was collected from various literatures and modified to suit the Indian conditions. Before framing the educational package, nutritional professionals, diabetologists and computer professionals were consulted and the necessary guidelines
were obtained. The package was written to transfer academic knowledge into practical instructions and information for diabetic subjects to follow.

b. Collection of Visual aids to be used in the software package

Numerous pictures relating to diabetic concepts were collected from books, journals, pamphlets and materials available with diabetologists. Slides were also collected from diabetologists, which were developed as photos, scanned and used in the software package.

c. Developing screens using the resource materials and visual aids collected

After collecting the resource materials and visual aids, a script was prepared in a notebook indicating each slide in one page with necessary diagrammatic representation wherever required. On the whole 191 screens were developed.

The package thus framed consisted of the following heads; Introduction to diabetes, Prevalence, Symptoms, Classification, Causes, Diagnosis, Risk factor, Complications, Importance of herbs, Significance of dietary fiber, Management of diabetes by means of diet, drug, exercise, education and monitoring.

d. Collection of data relating to food exchange list and formulation of diets suitable for diabetic subjects

An exchange list is a grouping of foods in which specified amounts of all the foods listed, contained approximately equal amount of carbohydrates, protein and fat (Raghuram, 1998). All the formulated diets were given individual preferences by using food exchange list. Thus any food within a given list can be substituted or exchanged for any other food within the list – cereal, legume, vegetable, fruit, milk, fat and flesh food exchanges were computerized in the package. In the chapter “Diet”, model menus suitable for early morning, breakfast/dinner, in between snacks and lunch were given with suitable pictures and also whole day’s diet for 1200 k.calories, 1500 k.calories 1800 k.calories and 2100 k.calories were planned and their nutrient contents were calculated and presented for the benefit of the diabetic subjects.
e. Incorporation of audio effects to the developed software package

The investigator also introduced a human touch to the software by incorporating voice to each screen using "Microsoft agent" in addition to pictorial representation.

f. Internal evaluation of the software package and incorporation of corrections

Two senior members and five subject specialists who were well versed in the field were requested by the investigator to evaluate the developed package. Corrections indicated by the team of experts were carefully incorporated before the package was finalized. The education package was prepared with the contents covering all information about Diabetes and its management, which is recorded and given in Appendix II.

B. CONDUCT OF DIABETICS EDUCATION CAMPAIGN USING THE DEVELOPED PACKAGE

To conduct diabetic awareness campaigns the authorities of the hospitals/clinics in Coimbatore, Erode and Salem helped in providing place and other facilities to the investigator. As a first step an invitation for the conduct of diabetic educational campaign was sent to all the diabetic subjects who had already registered in the hospitals. This was done with the help of the doctors and their assistants. The subjects usually visited the hospitals in the specified dates by 7-8 AM to have their fasting blood sugar checked. And similarly after their breakfast they stayed back to have their post prandial checkup done. The investigator utilized that time effectively to conduct the educational campaigns (Plate I).

The awareness campaigns were well organized with the developed package. These campaigns gave the investigator a chance to explain the importance of good dietary habits, drug, exercise and monitoring in the management of diabetes. In addition they were educated on the nature of the disease and the possibility of development of acute and long term complications, if blood sugar is not kept under control. The general information on how to select the proper and adequate amount of food to take care of their energy needs, the importance of fiber rich diet and regular exercise were explained.

After showing the diabetic educational package to the subjects, the investigator simultaneously explained the nutritional significance and health benefits of herbs used in
Conduct of Educational Campaign

PLATE 1
the supplementation study with herbs. The subjects who expressed their willingness to join the supplementation programme were given a consent form (developed by the investigator) both in local language and in English. This consent form was signed by the subjects and their attendees. This was considered as a precautionary measure to make sure that the subjects fully understood the nature of the study and their role in it.

Diabetic education campaigns were conducted at the selected locale (Coimbatore, Salem and Erode) which enabled the investigator to select 1000 subjects and also proceed with the supplementation study. Awareness campaigns really helped the investigator to map out the subjects and conduct the in easy and quick manner. This method of conducting the campaigns proved very effective and fast when compared to meeting the subjects individually and explaining to them about the present study.

The subjects who were willing to join the supplementation programme were briefed about the modalities of the study and provided with supplements for a period of one month. They were instructed that they have to come for a check up to the hospital after one month. After the check up they were given the supplement for the subsequent month. During their visit at the end of first month, a quiz competition was conducted by the investigator to gage the awareness/understanding of the subjects on diabetes and its management. The top three scorers received small gifts just to encourage them and create interest in diet counseling. During the next visit (after 30 days) the subjects were asked to cook and bring any recipes suitable for diabetics. The quiz program as well as this diabetic cookery contest helped them to have a better understanding about nutritional and health concepts. The investigator also provided the participants with a few model menus which they could effectively use for better management of diabetes.

C. COLLECTION OF BACKGROUND INFORMATION, DIETARY PATTERN, LIFESTYLE PATTERN, PERSONAL AND FAMILY HISTORY OF DIABETICS

The background information, lifestyle pattern and dietary pattern were recorded for all the 1000 selected diabetics using the interview schedule given in Appendix I.

The background information such as name of the subject, age, sex, educational qualification, occupation, activity pattern, monthly income/total family income, type and composition of the family were collected. The information on food habits and dietary practices were recorded. A daily meal pattern for three consecutive days, cooking
methods used, food consumption pattern, types of oil used for cooking and knowledge about dietary fiber were also collected.

The lifestyle pattern such as exercise pattern, smoking and alcoholic habits, consumption of tea, coffee, health drinks, tobacco and pan masala were collected using the interview schedule. The details of the type and duration of diabetes mellitus, familial disposition of diabetes, frequency of monitoring, symptoms and complications they had experienced were also recorded.

**D. ASSESSMENT OF NUTRITIONAL STATUS OF THE SELECTED SUBJECTS**

**a. Recording height, weight, waist, hip measurement and calculating Body Mass Index and Waist Hip Ratio**

Anthropometry is the universally applicable, inexpensive and non-invasive method available to assess the composition and fat distribution of the human body. It reflects both health and nutritional status and also predicts performance, health and survival. Hence, it is used in various intervention programmes to monitor health and nutritional status of the selected population. Among the various anthropometric measurements, height and weight were adopted to obtain reliable data. Anthropometric measurements namely height, weight, waist and hip circumferences were recorded for all the 1000 subjects.

**i. Measurement of height**

Jelliffe and Patrice (19919) explain that the height of an individual is principally a measure of skeletal bony tissue. It is made up of the sum of the components; legs, pelvis, spine and skull.

Subjects were asked to stand erect on a flat surface with heels together and upper limbs hanging closely to the sides of the body. The investigator stood on the left side of the subject. The anthropometer rod (after assembling the four pieces and the sliding head piece properly), held in the right hand, should be placed at the back of the subject, touching heels, buttocks and back of the head. The chin of the subject should be held by left hand and the occipital protuberance is supported by the little finger of the right hand, while holding the rod with thumb and index finger. The head should be positioned such that the imaginary line drawn from tragus of the ear to the infra-orbital

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margin i.e. lower border of the socket of the eye (Frankfurt horizontal plane) is parallel to ground.

By holding the head in this position, a gentle upward pull is applied (taking care that the subject does not lift his/her heels) to straighten any curvature in the spinal cord. Then the sliding headpiece of the rod is brought down so as to touch the crown firmly pressing the hair, taking care that the blade is in the sagittal plane (mid-line of the body. At this juncture, the height is read from the window of the headpiece. This process is repeated thrice and the consistent reading is obtained using the procedure (Bramhan et al 2005). Height were recorded in cms, up to the nearest mm. (Plate II)

**ii. Measurement of weight**

Body weight is the most widely used simplest reproducible anthropometric measurement for the evaluation of nutritional status of the population. It is a more sensitive measure of nutritional adequacy than that of height and reflects recent nutritional status. Weight also provides a crude evaluation of overall fat and muscle stores (Whitney and Rolfes 2002)

To record the actual body weight, the subjects were made to stand on the platform of the digital electronic balance with out footwear with minimal clothing, ensure that he/she does not hold any other person for support (Bramhan et al 2005). Weight were recorded to the nearest 100 g. for the selected 1000 diabetic subjects as mentioned above. (Plate II)

**iii. Body Mass Index (BMI)**

According to World Health Organization (WHO, 2000) BMI is a simple index of weight for height that is commonly used to classify underweight, over weight and obesity in adults. The body mass index (Quetelet index) was calculated by dividing the individual’s weight in kilogram by the square of his or her height in meters. This was calculated to find out the grade of obesity of the selected subjects (Park and Park 2002).

After the computation of BMI, subjects were classified according to the norms given by the International Obesity Task Force (IOTF) and the values for different grades of obesity are given in table 1.
Anthropometric Measurements

Height

Weight

Waist Circumference

Hip Circumference

Biochemical Estimation

PLATE 2
### TABLE 3.1
**CUT OFF VALUES FOR DIFFERENT GRADES OF OBESITY**

<table>
<thead>
<tr>
<th>BMI (Kg / m²)</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 18.5</td>
<td>Underweight</td>
</tr>
<tr>
<td>18.5 – 22.9</td>
<td>Normal</td>
</tr>
<tr>
<td>23.0 – 24.9</td>
<td>At risk of obesity</td>
</tr>
<tr>
<td>25.0 – 29.9</td>
<td>Obese I</td>
</tr>
<tr>
<td>≥ 30.0</td>
<td>Obese II</td>
</tr>
</tbody>
</table>

www.IOTF.org

iv. **Measurement of waist and hip circumference (WHR)**

- **Waist circumference**

  In the recent past, particularly with increasing incidence of obesity, considering the significance of abdominal adiposity in diet related chronic diseases, waist and hip circumferences are used to evaluate the abdominal adiposity in subjects. The subjects were asked to stand erect with weight evenly balanced on both feet, which are placed about 25-30 cms apart. Mark 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 cms was measured up to the nearest mm. The observer was made to sit on a stool in front of the subject while taking the measurement. All the adult males with waist circumference of ≥ 102cm and women with ≥ 80cm were identified as having abdominal obesity. (Brahmam *et al.*, 2005). (Plate II)

- **Hip circumference**

  For measuring the hip circumference, a tape was placed horizontally over the buttocks and the circumference was measured at the point yielding the maximum circumference in cms up to the nearest mm (Brahmam *et al* 2005). According to Boyle *et al.*, (1993) the waist circumference should be taken at the narrowest circumference between ribs and hips. (Plate II)
All the adult men with the waist-hip ratio of ≥0.95 and women with ≥0.8 will be identified as obese (Brahmam et al., 2005). For all the selected 1000 subjects WHR were computed by dividing subject’s waist circumference in cms by hip circumference in cms and compared with the standard values.

b. Recording individual food and nutrient intake

Dietary survey constitutes an essential part of any complete study of nutritional status providing essential information on nutrient intake, sources of nutrients, food habits and attitudes (Swaminathan, 2001).

Dietary survey using 24 hours diet recall method was used to find out the quantity of foods consumed by the selected subjects. The raw equivalents were calculated and the nutrient intakes of the subjects were computed for the Experimental I (6 groups) & II (5 groups) totaling up to 330 diabetics and Control group (N-30) using the Food composition table (ICMR, 2004).

c. Analysis of biochemical parameters - blood glucose levels and serum lipid profile

Davidson (1990) has reported that Biochemical estimation is the most sensitive indicator of the health condition of an individual.

• Blood glucose

Selected subjects (control and experimental, N-360) were asked to come to the bio chemical laboratory early morning around 7 o’ clock after 12 hours of fasting. A sample of five milliliter of blood was collected with the help of the technicians and analyzed for fasting blood glucose and glycosylated hemoglobin, then the subjects were asked to consume the advised diet suggested by the dietitian in the hospital. After 2 hours of breakfast, another sample of blood was collected and analyzed for the post prandial blood glucose level. Blood glucose was estimated by GOD-PAP method

Enzymatic calorimetric determination of glucose (GOD PAP) was done according to the reaction

\[
\text{Glucose} + \text{O}_2 \rightarrow \text{Glucose Oxide} \rightarrow \text{Gluconic acid} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + \text{Phenol} + 4\text{-Aminoantipyrine} \rightarrow \text{Red quinine} + 4\text{H}_2\text{O}
\]
• **Glycosylated haemoglobin (HbA1C)**

Sugar in the blood stream can become attached to the haemoglobin in red blood cells. This process is called glycosylation. Once the sugar is attached, it stays there for the life of the red blood cell, which is about 120 days. The higher the level of blood sugar, the more sugar attaches to red blood cells. The haemoglobin A1c test measures the amount of sugar sticking to the haemoglobin in the red blood cells. Thus the HbA1c test can measure the amount of glycosylation that has occurred revealing the average blood glucose levels during the preceding three to four months before the test. The important advantage of HbA1c testing is that the sample can be drawn at any time, because it is not affected by short term changes like food intake, exercise, stress and hypoglycemic agents. HbA1c can delay or prevent the development of serious eye, kidney and nerve disease in people with diabetes.

Glycosylated haemoglobin was estimated by chromatographic--spectrometric ion exchange method for the experimental and control groups.

• **Serum lipid profile**

From each subject, five ml of blood sample was collected from the antecubital vein, and were taken preferably before breakfast to avoid the influence of food digestion on blood composition. Care was taken to avoid hemolysis, during the collection of blood for serum separation. Blood was allowed to clot for nearly 3 hours, at room temperature. Then the clot was centrifuged and supernatant serum was removed and stored in well stoppered bottles, in a freezer, and used for the analysis of lipids.

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

Triglycerides were estimated using the enzymatic method, suggested by Friedwald, (1972). The intensity of the colour developed is directly proportional to the triglyceride concentration and was estimated photometrically at 540nm.
Very Low Density Lipoprotein (VLDL) cholesterol level was calculated from the estimated triglyceride level using the following formula.

\[ \text{VLDL cholesterol} = \frac{\text{Triglyceride}}{5} \text{ Where 5 is a constant factor} \]

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

\[ \text{LDL cholesterol} = \text{Total cholesterol} - (\text{HDL cholesterol} + \text{VLDL cholesterol}) \]

For all the selected 1000 diabetics the above mentioned blood profile were noted from the hospital record at the start of the study period to select the subjects for the nutrition intervention.

The biochemical indices of metabolic control (Blood sugar- Fasting, Postprandial, HbA1C and Total cholesterol, LDL Cholesterol, HDL Cholesterol, VLDL Cholesterol and Triglycerides of the selected 360 Non Insulin Dependent Diabetic subjects (Experimental and control groups) were analyzed before and after the supplementation period to find out the effect of supplementation (Plate II).

**PHASE III**

**A. SELECTION, FORMULATION AND SENSORY EVALUATION OF SELECTED SUPPLEMENTS**

**a. Selection of the Supplement**

The nutritive value of Italian Millet (*Setaria Italica*) and Green Gram (*Vigna Radiata*), its validity as an antidote for several diseases and the hypoglycemic effect of herbs are well documented in literature. Hence the Italian millet (thenai), green gram and the following herbs were selected for supplementation. These mixes were selected because they are low cost, locally available and familiar foods, common in diet habits of South Indian's.
Traditional herbs known to us for centuries (which have a hypoglycemic and hypolipidemic effect) has not been exploited properly and also not documented scientifically. Therefore, the investigator tried this supplementation study to further validate the greatness of the traditional medicine. Facts about the selected millet, legume and herbs are given below:


• Italian / Foxtail Millet – Thinai / Setaria italica

Active principles - Coumarin - Setarin; Flavonoides - βsitosterol and kaempferol.

Action - Emollient, diuretic, appetizer; astringent, digestive, refrigerant and stomachic.

Diseases - Haemorrhage, rheumatism, cholera, fever, dyspepsia, poor digestion and food stagnancy in the abdomen.

• Green Gram - Vigna radiata

Active principles - Enzymes - Phosphoglucomutase, arabinolunase, galactokinase; Saponins - saponin I, II and III.

Action - Tonic, diuretic and galactagogue.

Diseases - Polyuria, fever and inflammations.

Millet proteins are deficient in lysine and tryptophan whereas legume proteins are very good source of lysine. Hence, blending millet with legumes in suitable proportions compliments the essential amino acids and forms proteins of high biological value. Both millet and legumes also contain a few non-nutritive phyto-chemicals and micronutrients. (Malleshi, 2001) So it was thought that it would be worth while considering a supplementation study with selected millet and legume in combination with herbs.
Herbs selected for the supplementation study

A) Aavaram Flower -(AF)  
(Cassia Auriculata)

B) Kadazhalinjl Bark-(KL)  
(Salacia Reticulata)

C) Kotta Karanthai Plant-(KK)  
(Sphaeranthus Indicus)

D) Korai Kizhangu-(KKZ)  
(Cyprus Rotundus)

E) Vilvai Leaves-(VL)  
(Aegle Marmelos)

PLATE 3
Herbs selected for the supplementation (Plate III)

- **Avarampoo - Cassia auriculata**

  **Active principles** - Alkaloids – Pyrrolizidine; Flavonoides - βsitosterol, kaempferol.

  **Action** - Astringent, tonic, refrigerant, alterative, hypolipidemic, antiperoxidative, hypoglycemic, cooling, purgative, febrifuge, emollient, expectorant, purgative, anodyne and ophthalmic.

  **Parts used** - Root, leaves, flowers, bark, seeds.

  **Diseases** - skin troubles, diabetes mellitus, urinary disorders, chylous urine, giddiness due to heart disease, excessive menstrual flow, nocturnal emissions and eye troubles.

- **Kadazhalinjil - Salacia reticulata**

  **Active principles** - Alkaloids - Mangiferin, Phlobatannin, leucopelargonidin; Ketones - 1, 3-diketones.

  **Action** - Astringent, abortifacient, bitter, thermogenic and stomachic.

  **Diseases** - Rheumatism, skin diseases, amenorrhea, anodyne, anti-inflammatory, depurative, emmenagogue, liver tonic, diabetes, haemorrhoids and inflammations.

- **Korai kilangu - Cyprus rotundus**

  **Active principles** - Flavonoid glucoside - Cyperene-1,2, cyperotundone, cyperolenone, patchoulenone. Essential oil - pinene, copadiene, rotundone, epoxyguaieine and cyperolone.

  **Parts used** - Tuber or bulbous root.

  **Action** - Diuretic, diaphoretic, astringent, stimulant, tonic, anti-inflammatory, hepato - protective, diaphoretic, demulcent, stimulant and repulsive.

  **Diseases** - bowel complaints, fever, diabetes, worm infestation, diarrhea, vomiting, cholera, uterine complaints, cervical cancer, antispasmodic, analgesic and menstrual pain.
**Kottai Karanthai - Sphaeranthus indicus**

**Active principles** - Alkaloid - Sphaerantine; Essential oil - Methyl chavicol, \( \alpha \)-ionone, \( \delta \)-cadiene; Acid - sesquiterpeneacid-2-hydroxycostic acid, \( \beta \)-eudesmol and ilicic acid.

**Action** - Aphrodisiac, rejuvenator, alterative, laxative, tonic, antihelminthic, bitter, acrid, sweet, thermogenic, diuretic, expectorant, febrifuge, stomachic, alterant, depurative, refrigerant and tonic.

**Diseases** - Skin diseases, diseases of vatham, jaundice, blood disorders, jaundice, disease of the spleen, enriches the blood, sore eyes, cures asthma, epilepsy, diabetes, leprosy, fever, tuberculosis, pectoralgia, cough and hernia.

**Vilvai leaves - Aegle marmelos**

**Active principles** - Essential oil - \( \alpha \)and \( \beta \)-phellandrene; Coumarins - marmesin, imperatorin, alloimperatorin, xanthotoxol; Alkaloid - Aegelenine, marmelosin, aegeline; Tannin - Phlobotanin, \( \beta \)sitosterol, marmesinin, leucoanthocyanins and anthocyanins.

**Parts used** - Fruit, root-bark, leaves.

**Action** - Astringent, stomachic, alterative, digestive, antiparasitical, antipyretic, aphrodisiac, aromatic, astringent, stimulant, febrifuge, haemostatic, nutritive, tonic and laxative.

**Diseases** - Habitual constipation, dyspepsia, diabetes, venereal diseases, flatulent colic, chronic diarrhoea, dysentery and heart diseases.

All the above listed herbs were shade dried and pulverized, powdered and used for supplementation (Plate IV). The herbal powder alone was not acceptable due to its poor taste. Hence herbal powder was mixed with the Italian millet which is a low cost, locally available, traditional and fiber rich (Plate V-Experimental I and II)
PROCESSING OF HERBS FOR SUPPLEMENTATION

A) Aavaram Flower -(AF)  B) Kadazhainil Bark -(KL)
C) Kotta Karanthai Plant -(KK)
D) Korai Kizhangu -(KKZ)
E) Vilvai Leaves -(VL)

Herbs (Dried Form)

Pulverising  Mixing  Sieving  Processing of Herbs

Herbal Powders

PLATE 4
FORMULATION OF HERBAL SUPPLEMENT

Herbal Powders

Mixed with

Thenai (Italian Millet) (Setaria Italica)

Green Gram (Vigna Radiata)

Experimental Group I

Thenai (50g) + Green Gram (15g) + Herbal Powder (2g)

Packed as

Herbal Capsules

Experimental Group II

Herbal Powder (2g)

PLATE 5
b. Formulation of the supplements (Hypoglycemic food mix)

Supplements were tried out using cleaned millet and coarsely powdered green gram in different forms like porridge, pongal and pan cake. As a first step porridge was eliminated because it is easily digestible. The second recipe selected was dosa (pan cake) had a very good acceptability but it was also eliminated because it required more oil for preparing when compared to pongal ( millet, dhal mixed recipe). So finally the investigator concluded that the supplement can be cooked in the form of pongal. For standardization of pongal, the investigator tried with different combinations like 30, 40, 50 and 60 g of millet with 10, 15 and 20 g of legume added with 2 and 3 g of each of the herbal powder. The above mentioned combinations were prepared in the foods laboratory of Avinashilingam University for Women and evaluated to choose the suitable supplement.

c. Sensory evaluation of the herbs incorporated recipes

Sensory evaluation is a multidisciplinary science that uses human panelists and their senses of sight, smell, taste, touch and hearing to measure the sensory characteristics and acceptability of food products. Thus, quality of food is judged in terms of appearance, color, taste, texture and flavour. (Chandrasekhar, 2002)

Sensory evaluation was done by the post graduate students of Avinashilingam University, using score card with five point hedonic scale with ranking, 1-unacceptable, 2-slightly acceptable, 3-moderately acceptable, 4-acceptable, and 5-highly acceptable. The scores obtained in the acceptability trials were statistically analyzed to obtain significant and trustworthy results for the best acceptable product. From the sensory analysis the highly acceptable pongal combination with herbal powder was selected based on the maximum score, which included pongal prepared using millet 50 g, green gram 15 g, along with 2 g of herbal powder.

B. PARAMETERS USED TO ANALYSE THE HERBAL SUPPLEMENTS

For the selected herbal supplements glycemic index, dietary fiber, active constituents and toxic elements were analyzed for its suitability for human feeding trials.
a. Determination of Glycemic Index (GI)

Diets with low GI helps to control weight, increase the body’s sensitivity to insulin, improve diabetes control, reduce the risk of heart disease, reduce blood cholesterol levels, reduce hunger, prolong physical endurance and help re-fuel carbohydrate stores after exercises (Matsuda et al., 2002).

To determine a food’s Glycemic Index (GI) rating, measured portions of the food containing 10-50 grams of carbohydrate were fed to the selected subjects after an overnight fast. Finger-prick blood samples were taken at 30 min intervals over the next 2 hours. These blood samples were used to construct a blood sugar response curve for the two hour period. The Area Under the Curve (AUC) is calculated to reflect the total rise in blood glucose levels after eating the test food. The GI rating (%) is calculated by dividing the AUC for the test food by the AUC for the reference food and multiplying by 100. The use of the standard food is essential for reducing the confounding influence of differences in the physical characteristics of the subjects.

Five subjects from each group (experimental and control) were randomly selected, the GI was calculated for the selected supplements using the above said procedure. The results were highly satisfactory for all the ingredients chosen, so it was given as a supplement for the selected experimental groups.

b. Analysis of Dietary fiber

Food habits with high fibre intakes are associated with low serum cholesterol concentration, low risk of coronary heart disease, reduced blood pressure, reduced blood sugar, enhanced weight control, reduced risk of certain forms of cancer and improved gastrointestinal function (Barnett, 2003).

The selected supplements were sent to Central Food Technological Institute (CFTRI), Mysore for analysis of dietary fiber. The analyzed results showed that the fiber content were (Millet-thenai (50g) containing 12.35 g of dietary fiber, Pulse - green gram (15g) containing 2.5 g of fiber and herbs (2g) containing 0.15-0.33 g). In total each supplement given per day contains about 15 g of dietary fiber. This supplemented quantity of fiber satisfies approximately 50 per cent of the recommended dietary allowances (ICMR 2004).
c. Analysis of active constituents and toxic elements

Active constituents and the toxic elements present in the supplement were also considered for the formulation of supplements and were analyzed in the Mettex Laboratory, Chennai using the following procedure.

- **Inorganic analysis** was done using an Atomic Absorption Spectrometer (AAS) with air acetylene.

  A known amount of sample was taken in a 500 ml beaker. 10 ml of HNO3 was added to 10 ml of 1+1 HCL and heated on a hot plate until the sample got dissolved. Cooling and filtering was done to remove insoluble material. The solution was transferred to 100 ml volumetric flask, the volume adjusted to 100 ml and mixed. A reagent blank prepared containing same amount of acids used in the preparation of sample. The standards and sample were aspirated into the AAS instrument.

  Calculation: \[ \text{per cent of the element} = \frac{A \times 100}{B}; \]
  
  \[ A: \text{concentration of the sample in ppm, } B: \text{Dilution factor} \]

- **Organic analysis**

  Steroids - Liebermann - Burchard test: Five mg of the substance in chloroform is treated with a few drops of acetic acid and acetic anhydride and two drops of conc. \( \text{H}_2\text{SO}_4 \), heated gently. Green to bluish green colour shows the presence of steroid.

  Triterperoids - Noller’s test: Five mg of the substance in a dry test tube is treated with a bit of Tin foil and 0.5 ml of \( \text{SOCl}_2 \) heated in water bath, pink colour shows the presence of triterperoid.

  Quinone; Perkins test: Five mg of the substance in alcohol is treated with alcoholic sodium hydroxide or potassium hydroxide. Deep colouration as red, purple, pink colour shows the presence of quinine.

  Flavonoids - Shimada test: Five mg of the substance in alcohol is treated with magnesium turnings and a few drops of conc. \( \text{HCL} \), red or pink colour indicates the presence of flavonoids.

  Alcohol -Jones reagent test: Five mg of the substance is treated with acetone and add one drop of Jones reagent is added and shaked the tube to mix the contents. Alcohol reacts in two seconds as indicated by the disappearance of the orange colour of the reagent and formation of a green or blue green precipitate or emulsion.
The content of the active constituents and toxic elements present in the selected herbal powders were under the permissible limits. So it was considered suitable for human feeding trials.

**PHASE IV**

A. **SUPPLEMENTATION OF DIFFERENT HERBAL POWDERS FOR THE SELECTED DIABETICS**

After analyzing the selected supplements using various parameters (dietary fiber, glycemic index and active constituents), different compositions were evolved to study the effect of supplementation of five different herbs on diabetes. For each herbal group 30 diabetics were selected for the supplementation for the period of 90 days. The following Table gives the composition of the supplements according to the groups selected.

**TABLE 3.2**

**COMPOSITION OF THE HERBAL SUPPLEMENTS**

<table>
<thead>
<tr>
<th>Experimental Group I</th>
<th>N</th>
<th>Millet</th>
<th>Legume</th>
<th>Herbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG* - I</td>
<td>30</td>
<td>Thenai</td>
<td>Green Gram</td>
<td>-</td>
</tr>
<tr>
<td>TG - AF</td>
<td>30</td>
<td>Thenai</td>
<td>Green Gram</td>
<td>Aavaram Flower (Cassia Auriculata),</td>
</tr>
<tr>
<td>TG - KL</td>
<td>30</td>
<td>Thenai</td>
<td>Green Gram</td>
<td>Kadazhalinjil bark (Salacia Reticulata),</td>
</tr>
<tr>
<td>TG - KKZ</td>
<td>30</td>
<td>Thenai</td>
<td>Green Gram</td>
<td>Korai Kizhangu (Cyprus Rotundus)</td>
</tr>
<tr>
<td>TG - KK</td>
<td>30</td>
<td>Thenai</td>
<td>Green Gram</td>
<td>Kotta Karanthai plant (Sphaeranthus Indicus)</td>
</tr>
<tr>
<td>TG - VL</td>
<td>30</td>
<td>Thenai</td>
<td>Green Gram</td>
<td>Vilvai Leaves (Aegle Marmelos)</td>
</tr>
</tbody>
</table>

**Experimental Group II**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HC **- AF</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>Aavaram Flower (Cassia Auriculata),</td>
</tr>
<tr>
<td>HC - KL</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>Kadazhalinjil bark (Salacia Reticulata),</td>
</tr>
<tr>
<td>HC - KKZ</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>Korai Kizhangu (Cyprus Rotundus)</td>
</tr>
<tr>
<td>HC - KK</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>Kotta Karanthai plant (Sphaeranthus Indicus)</td>
</tr>
<tr>
<td>HC - VL</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>Vilvai Leaves (Aegle Marmelos)</td>
</tr>
</tbody>
</table>

**Control Group**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TG Group*</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HC Group**</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

TG Group* - Thenai- 50 g, Green Gram-15 g, herbal powder -2 g
HC Group** - Herbal powder -2 g
a. Experimental Group I (TG Group – Thenai, Green Gram Group N = 180)

Italian millet and green gram were thoroughly cleaned, dried in a shade and used for supplementation for the subjects in Experimental Groups I. Green gram was coarsely powdered, mixed with whole millet, prepared as pongal and given for TG I group. For all the other groups in experimental group I, thenai and green gram was mixed with the specified herbal powder (as per the above table) and given as supplements for the five different groups. These ingredients were packed in a cover and distributed to the subjects with the request that the contents of each packet to be cooked daily and consumed in the form of pongal along with their regular meals.

b. Experimental Group II (Herbal Capsule Group - HC Group N =160)

For the Experimental Group II, 2 g of the above mentioned herbal powders were given in the form of capsule (with breakfast 2 capsules and with dinner 2 capsules each containing 0.5 mg of herbal powder). This was done since a few subjects said they could not cook and eat the supplements given for the experimental group I on a regular basis. Also the investigator desired to find out the effectiveness of herbal powder alone in lowering biochemical profile in terms of blood glucose and lipid profile and also to compare with the results of experimental group I (Italian Millet + Green gram +herbal powder). These capsules were also distributed for the five capsule groups each group consisted of 30 subjects like the Experimental group I and the supplementation was done for a period of 90 days for all the 11 groups.

c. Control Group ( N = 30)

Using the same criteria like experimental group thirty type II diabetic subjects were selected as control group for comparison. The control group did not receive any supplement during the study period.
PHASE V
A. EVALUATION OF THE EFFECT OF SUPPLEMENTATION AMONG THE SELECTED DIABETICS

To know the effect of supplementation, the anthropometry measurements (Appendix III), clinical symptoms, diet recall and biochemical profile (Blood Glucose, HbA1C and serum Lipid profile) of the experimental groups (11 groups) were recorded before and after supplementation period of 90 days (Appendix IV). Same procedure was followed for the control group also. So that comparison was done effectively.

B. CONSOLIDATION AND ANALYSIS OF DATA

The data collected were systematically consolidated and statistically analyzed for arriving at the results of the effect of supplementation on blood glucose and serum lipid levels among the selected type II diabetics and the findings are discussed and concluded in Chapter IV Results and Discussion.