



**Methodology**

### **III. METHODOLOGY**

The methodology pertaining to the study on “EFFECT OF SUPPLEMENTATION OF SELECTED NEUTRACEUTICALS ON TYPE II DIABETICS” involved the following steps:

- A. SELECTION OF THE VENUE**
- B. SELECTION OF THE SUBJECTS**
- C. FORMULATION OF TOOLS FOR THE CONDUCT OF THE STUDY**
- D. ASSESSMENT OF NUTRITIONAL STATUS**
  - 1. Anthropometric measurements**
    - a) Measurement of height
    - b) Measurement of weight
    - c) Calculation of Body Mass Index (BMI)
    - d) Measurement of Waist and hip circumference
  - 2. Recording individual food and nutrient intake**
  - 3. Estimation of biochemical parameters**
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    - b) Serum lipid level
- E. SUPPLEMENTATION OF SELECTED NEUTRACEUTICAL COMPONENTS**
  - 1. Supplementation with onion (*Allium cepa*)
  - 2. Supplementation with cinnamon (*Cinnamomum zeylanicum*)
  - 3. Supplementation with cumin seed (*Cuminum cyminum*)
  - 4. Supplementation with artichoke (*Cynara scolymus*)
  - 5. Supplementation with amla (*Embilica officianalis*)
  - 6. Supplementation with soyabeans (*Glycin max Merr*)
  - 7. Supplementation with bay leaves (*Laurus nobilis*)

8. Supplementation with flaxseed (*Linum usitatissimum*)
9. Supplementation with stevia leaves (*Stevia rebaudina*)
10. Supplementation with jambolin seed (*Syzygium cumini*)

## F. CONSOLIDATION AND ANALYSIS OF THE DATA

### A. SELECTION OF THE VENUE

The area selected for the conduct of the study was four private hospitals namely S.R.C. Diabetic Research Center, Baby Hospital, Kovai Medical Center and Hospital and Maruthi Medical Centre and Hospital, at Erode city of Periyar District in Tamil Nadu.

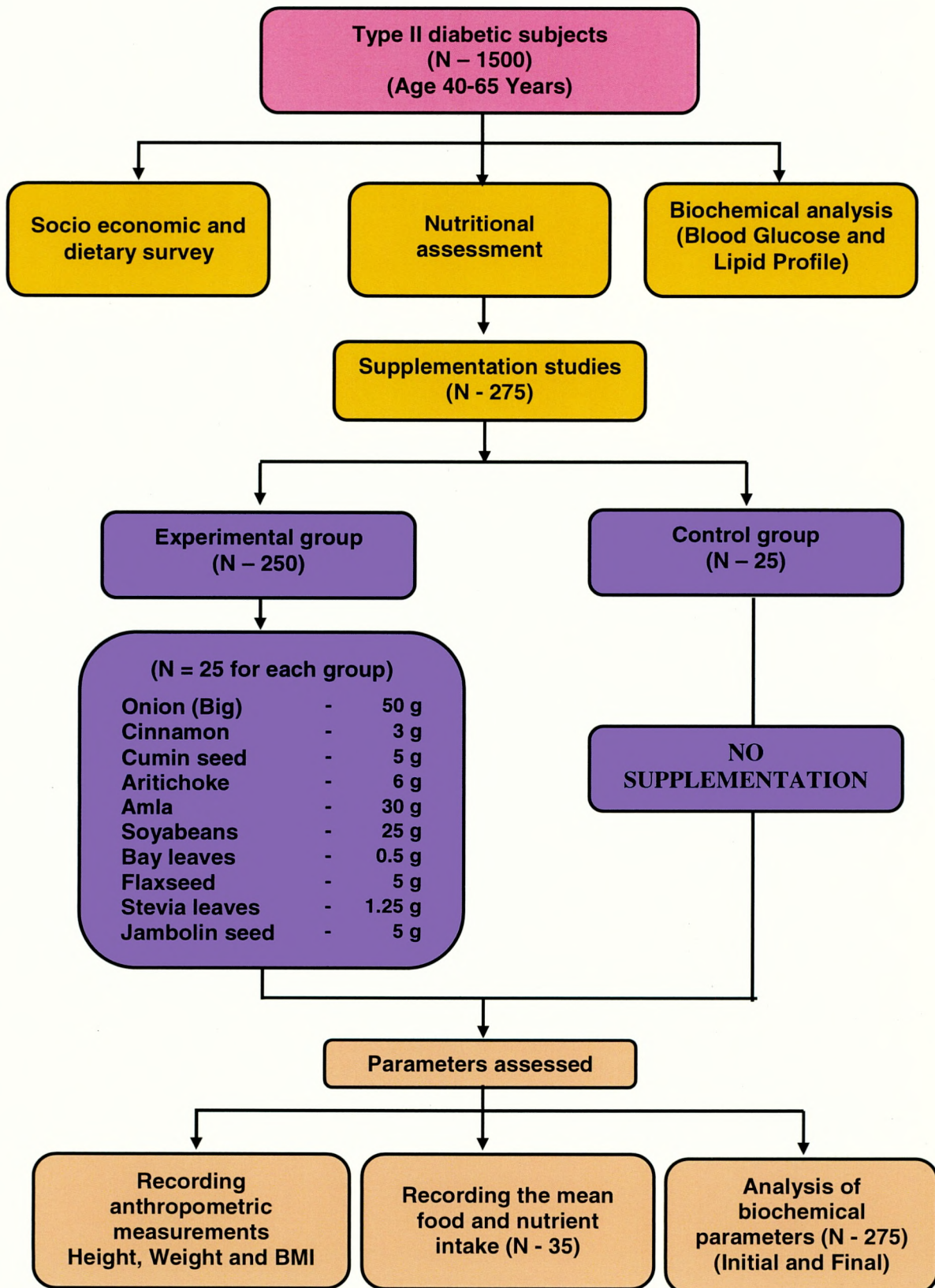
These hospitals were selected because of accessibility of the subjects and convenience of the investigator and also the diabetologists and authorities of the hospitals were very cooperative and willing to render their full support and help for the proposed research work. They also motivated the subjects to extend their cooperation for the collection of blood sample for biochemical analysis.

### B. SELECTION OF THE SUBJECTS

Non-Insulin Dependent Diabetes Mellitus (NIDDM) is more frequently encountered and accounts for over 90 per cent of all cases of diabetes in US. (www.LamMD.com). in the world.

Investigator visited the hospitals regularly and the required details were recorded for the available NIDDM subjects over a period of one year, who belonged to the age group of 40-65 years. Totally investigator contacted 1500 diabetic subjects (male-780 and female -720) for the collection of data to identify subsamples for further indepth study. Identification of the subjects were done for a period of eight months.

## RESEARCH DESIGN



In purposive sampling method, items for the sample are selected deliberately by the researcher; his choice concerning the items remains supreme (Kothari, 2005).

For supplementation study, 250 diabetic subjects as experimental group were selected by using purposive sampling method and they were divided into 10 groups, each group had 25 diabetic subjects. Each of these 10 groups received a specific supplement to find its hypoglycemic and hypolipidemic effect. For comparison, 25 diabetic subjects were selected as control.

### **C. FORMULATION OF TOOLS FOR THE CONDUCT OF THE STUDY**

According to Kothari (2005) interview method of collecting data involves presentation of oral-verbal stimuli and reply in turns of oral-verbal responses.

With the help of the specially designed interview schedule information regarding age, sex, educational status, nature of work, type of family, total monthly income of the family, food consumption pattern, life style history, family history of diabetes, type of treatment, exercise pattern, complications experienced and clinical method adopted to monitor their blood glucose level were collected from all the diabetic subjects (N=1500).

### **D. ASSESSMENT OF NUTRITIONAL STATUS**

Growth assessment is an essential component of health surveillance and the gold standard for growth assessment is anthropometric measurements using standardized equipments and procedures (Elizabeth, 2002). Nutritional status of the selected diabetic subjects (N=1500) was assessed using the following methods.

1. Anthropometric measurements like height, weight, body mass index and waist and hip ratio.

2. Recording individual food and nutrient intake.
3. Estimation of bio-chemical parameters.

### **1. Anthropometric measurements**

Anthropometry is the measuring of body size, weight and height. Measures obtained from anthropometry are sensitive indicators of health, growth and development.

#### ***a. Measurement of height***

Height is one of the excellent firstline detection of attack for various deficiency disorders. They are easy to obtain and generally reliable (Whitney, 1999). Height was measured using anthropometric rod for all the 1500 diabetic subjects. 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 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. Record the height in cm up to the nearest mm. Height was recorded for all the selected subjects (N=1500)

### ***b. Measurement of weight***

Weight is the important anthropometric measurement most common in use. Use of lever-actuated balance with 100gm of accuracy is recommended. The weighing scale should be placed on a firm and flat ground and zero-error has to be adjusted.

The subject was made to stand on the platform of the balance without footwear and with minimal clothing. The weight was recorded to the nearest 100g, for all the selected diabetic subjects.

### ***c. Body Mass Index (BMI)***

The weight for height ratio is a simple and widely accepted method which estimates total body mass rather than fat mass. It correlates very well with the amount of body fat. The most commonly used ratio is the Quetlet's index of Body Mass Index. It is the most widely used critical tool for measurement of obesity. It was calculated as weight in kilograms divided by the square of height in meters as shown below.

$$\text{BMI} = \frac{\text{Weight in Kgs}}{\text{Height in m}^2}$$

Since obesity is the leading cause of diabetes, the risk of diabetes is increased over 53 times the normal rate with severe obesity. So estimation of BMI was considered as essential. After computing the BMI value for each diabetic subject they were grouped according to the classification given by International Obesity Task Force (IOTF – 2002) for Asian ([www.iotf.org](http://www.iotf.org)).

#### ***d. Measurement of Waist and hip circumference***

##### **i) 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 subject should stand erect with weight evenly balanced on both feet, which are placed about 25-30 cm apart. Mark the level of the lowest rib margin. Feel the iliac crest in the mid-auxiliary line and make a mark. Pass the measure tape around the waist horizontally midway between the lowest rib margin and iliac crest and measure the circumference in cms up to the nearest one mm. It is advised that the observer should sit on a stool in front of the subject while recording the circumference of waist.

All the adult males with waist circumference of  $\geq 102\text{cm}$  and women with  $\geq 80\text{cm}$  will be identified as having abdominal obesity (Brahmam *et al.*, 2005).

##### **ii) Hip circumference**

Place the tape horizontally over the buttocks and measure the circumference at the point yielding the maximum circumference in cm up to the nearest mm. Waist hip ratio (WHR) was calculated using the formula given below and compared with standard values.

$$\text{WHR} = \frac{\text{Waist measurement (cm)}}{\text{Hip measurement (cm)}}$$

All the adult males with the waist-hip ratio of  $\geq 0.95$  and women with  $\geq 0.8$  will be identified as obesity (Brahmam *et al.*, 2005). The individual's height, weight and BMI of males and females are given in Appendix I and II respectively.

## 2. Recording individual food and nutrients intake

To obtain a general picture of each subjects typical dietary intake, the evaluative tool namely the food weightment survey was employed. From the 275 sub samples selected, 35 subjects were chosen includes experimental (25 subjects) and control (10 subjects) to conduct food weightment survey for a period of three consecutive days (Brahmam *et al.*, 2005).

Record the names of all the ingredients and the raw used for each of the preparation. As far as possible, obtain the equivalent quantities of raw foods (such as cereals/pulses etc.) available in the house and weigh them in the grocer's balance (supplied with the kit) and record the weights to nearest grams. This should be done after entering the menu of food preparations for whole day.

The total cooked volume of each of the preparations was recorded in terms of standard cups provided. For this purpose the housewives are asked to fill the vessel in which the preparation was made with water to the level up to which food was prepared. The volume of the cooked food was assessed by measuring this water with the standardized cups provided.

The quantity of each preparation consumed by each individual was assessed in terms of cups and was recorded accordingly. Intake of preparations, such as roti/chapatti, eggs etc. were assessed by noting the total number of pieces cooked and number consumed by each individual. Standard cups/spoons are used to assess the quantities of cooking oil, spices, sugar etc. It may be noted that the cups can be standardized in the respective institutions.

Record the quantity of "left over" foods, if any, under "left over" column, with remarks such as, "consumed later in lunch/dinner," or "next day" or "given to others" as the case may be. If the left over food was consumed later in the day, the

same has to be entered under “Menu” column and particulars of individual quantities consumed should be recorded.

After the completion of food weighment survey, the raw equivalents were calculated to find out the total consumption of different foods on a given day by each individual with the help of the Food Consumption Tables (Gopalan *et al.*, 2004), each individual’s nutrient intake was calculated for all the three days and average intake arrived at. The mean food and nutrient intake were compared with the Recommended Dietary Allowances (RDA) suggested by ICMR (2003) to assess the adequacy of the diet.

### **3. Estimation of biochemical parameters**

Biochemical tests are the most objective and sensitive measures of nutritional status.

#### ***a. Blood glucose level***

As the concentration of glucose in blood rises, more of it gets attached to hemoglobin and the combined molecule is chemically estimated as glycosylated hemoglobin. It reflects the general trend of glucose level in the blood during the previous 2-three months. In a normal population, the glycosylated hemoglobin concentrations vary from 4-7 per cent while in diabetes it is in the range of 8-18 per cent of the total hemoglobin depending on blood sugar level (Raghuram, 2000).

Regarding blood glucose both fasting, post prandial and glycosylated hemoglobin levels, data was recorded from the sources for all the diabetic subjects. Among the 1500 subjects, subsamples were selected for the supplementation studies using the following criteria.

1. Age group between 41-50 years

2. Fasting Blood Glucose (FBG) between 150-175 mg / dl.
3. Post Prandial Blood Glucose (PPBG) between 250-275 mg/dl.
4. Serum Total Cholesterol Level ranged between 225-275 mg/dl.
5. Selected subjects were free from other complications such as hypertension, cardiovascular diseases, kidney diseases and other related disorders.
6. Subjects using oral hypoglycemic agent alone were selected and they were asked to continue the same drugs during the study period.

Selected subjects (control and experimental, N-275) were asked to come 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 laboratory technicians and analyzed for fasting glucose and glycosylated hemoglobin and 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. Same biochemical parameters were also used to find the effect of supplementation on the selected subjects in the study group.

#### ***b. Serum lipid level***

Diabetes mellitus is an independent risk factor for cardiovascular disease. The subjects are at high risk when other additional risk factors like hypertension, hyperlipidemia and smoking especially a pattern with low HDL and LDL with an increased fraction of small dense atherogenic particles are associated. It is essential to record their serum lipid levels among the selected diabetic subjects.

Serum lipid profile is useful in determining the amount of different lipids in the blood. Hyperlipidemia is characterized by an elevated level of serum cholesterol or triglycerides or both cholesterol and triglycerides which are carried in the blood, bound to specific proteins called lipoprotein. These lipoproteins vary

as to the amount of protein and fat, they contain are identified according to their densities (Aronson, 1990).

The major cholesterol fraction, Low Density Lipoprotein Cholesterol (LDL), is the main causative factor for the occurrence of cardiovascular disease. High Density Lipoprotein cholesterol (HDL) scavenges cholesterol from the blood and tissues and delivers to the liver, where it is processed for excretion. HDL cholesterol is therefore called good cholesterol. The triglycerides are also important since they influence lipid deposition and clotting mechanisms.

Blood sample was collected from the experimental and control group, for the estimation of blood glucose and lipid profile.

Estimation of total cholesterol and high density lipoprotein cholesterol was done using the enzymatic method suggested by Buccolo *et al.* (1980) and Werner *et al.* (1981). In this procedure, serum low density lipoprotein and very low density lipoprotein are selectively precipitated by  $\mu\text{g}^{++}$  ions and phosphotungstate and removed by centrifugation. Cholesterol associated with HDL fractions remaining in the solution is carefully estimated by enzymatic method.

Triglycerides were estimated using the enzymatic method, suggested by Allain *et al.*, (1974). The intensity of the colour developed is directly proportional to the triglyceride concentration and is estimated photometrically at 540nm (530–570nm) or with a green filter.

Very Low Density Lipoprotein (VLDC) 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 and total cholesterol and VLDL cholesterol values using the following formula.

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

## **E. SUPPLEMENTATION OF SELECTED NEUTRACEUTICAL COMPONENTS**

### **1. Supplementation with onion (*Allium cepa*)**

Onions occupy a prominent place in human diet. It is popularly used both green and mature in vegetable curry salads, sambars, pickles, sauces etc. It enhances the flavour, taste and texture and increases variety in the diet.

Hundred grams of onion (big) contributes energy 50kcal, protein-1.2g, fat-0.1g, carbohydrate-11.1g, fiber-0.6g, and minerals-0.4g, calcium-46.9mg, phosphorus-50mg, iron-0.6mg, thiamine-0.08mg, riboflavin-0.01mg, niacin-0.4mg and vitamin C-11mg (Gopalan *et al.*, 2004).

Apart from the nutritional contribution of onion it also possess various medicinal properties. The principle active ingredients in onions are believed to be Allyl Propyl Disulphide (APDS) although other constituents such as flavonoids also play a role in reducing blood glucose level gradually. Onion extract was found to reduce blood glucose level during oral and intravenous glucose tolerance. Fresh onion extract possesses hypoglycemic property. However, beneficial effects were observed even for low levels (25g to 200 g) used in the diet (Agusti, 1975).

Regular uses of 50 grams of onion per day reduce the insulin requirement of a diabetic subject from 40 to 20 units, a day. Onion also produces a sustained hypocholesteraemic effect on long term basis. It also prevents the rise in serum cholesterol (Sharma *et al.*, 1975).

Having these points in mind, the investigator planned for the supplementation of onion extract to the selected 25 diabetic subjects (13 men and 12 women). About 50 grams of big onion use to extract fresh juice and it was mixed with 200ml of diluted butter milk which was found to be acceptable by organoleptic evaluation. The prepared juice was fed to the selected subjects daily for a period of 90 days (Plate I).

## **2. Supplementation with cinnamon (*Cinnamomum zeylanicum*)**

Cinnamon (*Cinnamomum Zeylanicum*) is a small evergreen tree 10-15 meters tall belonging to the family Lauracea and a spice obtained from the inner bark of this species.

Cinnamon is principally employed as a condiment and flavoring agent in various food preparations. The aroma of cinnamon has been described as sweet and pungent. It causes a mouth warming sensation. It contains 1.5 – 2.0 percent of volatile oil and the flavor intensity is due to the high amount of cinnamic aldehyde content (Gupta, 2003)

The nutritive value of 2.3 g of cinnamon powder: Energy 5 k.calories, Carbohydrates 2g, and does not contain other nutrients including protein, cholesterol and fat (Braudel, 1984).

The most active compound of the cinnamon is Methyl Hydroxyl Chalcone Polymer (MHCP). This compound increased the conversion of glucose to energy by twenty times. It also inhibits the formation of dangerous free radicals during the metabolic activities.

Subjects with diabetes get benefit by the regular inclusion of cinnamon in their daily diet, which control the elevated blood glucose and lipid levels (Silagy, 1994).

Anuradha (2004), also stated that oral supplementation of 4 grams of cinnamon powder for 90 days brought a significant reduction in blood glucose and lipid profiles among the selected diabetic subjects.

The amount of cinnamon powder given was 3g per day per individual and it was incorporated in the preparation of cinnamon bread. In bread preparation, 25g of wheat flour and 3 gm of cinnamon powder were used and its sensory attributes were analyzed and it was found to be acceptable (Plate I). The investigator prepared small pieces of bread and distributed daily as an evening snack, for a period of three months for the selected diabetic subjects in the experimental group (10 men and 15 women).

### **3. Supplementation with cumin seed (*Cuminum cyminum*)**

Cumin seed plants are small annual herb native to Mediterranean region. It is a most popular spice all over the world, especially in Latin America and all over Asia. The main constituents of the cumin seeds are 2.5-4 per cent essential oil and its main constituent is cumin aldehyde which is about 25-35 percent (Takayanagi *et al.*, 2003). The biologically active constituent of cumin seeds was characterized as cumin aldehyde. This cumin aldehyde is used as a lead compound and it was proved to be a new agent for anti-diabetic therapeutics (Lee, 2005).

According to Anuradha (2004), supplement of 4 grams of cumin seed powder for a period of three months showed a significant reduction in blood glucose and lipid profile values among the selected diabetic subjects.

Based on the above studies, the present work was carried out by the investigator to find out the effect of cumin seed supplementation on the blood glucose and serum lipid levels among the selected 25 diabetic subjects (11 men and 14 women) in the experimental group for the period of 90 days.

Five grams of roasted cumin seed powder was added into 45g of wheat flour and masala chapattis was prepared and advised the selected subjects to consume in dinner. Supplementation was carried out for the period of three months (Plate I).

#### **4. Supplementation with artichoke (*Cynara scolymus*)**

The globe artichoke, (*Cynara Scolymus*) is actually a member of the thistle family, although its leaves are larger, broader and soft rather than prickly. The flower petals and fleshy flower buttons are eaten as a vegetable throughout the world which has led to its commercial cultivation in many parts of South and North America as well as in Europe. The artichoke was used as a food and medicine by the ancient Egyptians (Puigmacia, 1986).

Artichoke is nutrient dense and medium artichoke will provide 25k calories of energy and trace amount of minerals like magnesium, chromium, manganese, potassium, phosphorus, iron and calcium. In addition to these important components, artichoke is a good source of fibre (12 per cent), vitamin C (10 per cent) and folate (10 per cent) and have no fat and cholesterol (Hindu, 1989).

The artichoke is popular for its pleasant flavour and unique taste, which are mainly due to a plant chemical component called as Cynarin. It is found in green parts of the plant. Other documented “active” chemicals include flavonoids, sesquiterpene lactones, polyphenols and caffeoyl quinic acids (Nichiforesco, 1965).

Artichoke has been used in traditional medicine for centuries to treat a specific liver and gall bladder diseases or disorders and also in treating various other ailments (Sayed (1980) and Ruppelt (1991). Artichoke also has the capacity to reduce the demonstrated lipid and has anti-cholesterolemic action by decreasing the rate of cholesterol synthesis in the liver and by mobilizing fat stores from the



**BIG ONIONS**



**ONION JUICE IN BUTTER MILK**



**CINNAMON**



**CINNAMON BREAD**



**CUMIN SEEDS**



**CUMIN SEED CHAPPATHI**

**PLATE I  
NEUTRACEUTICAL (ONION, CINNAMON, CUMIN  
SEED) SUPPLEMENTED PRODUCTS**

liver and other tissues. In addition, it also reduces triglycerides level of an individual (Daniel, 1993).

According to Bianchini (1975), the phytochemicals found in green parts of the artichoke like cynaropicrin and cynarin have shown to possess a hypoglycemic effect.

Hence to prove the efficacy of artichoke action in hyperglycemia and hyperlipidemia a suitable product was formulated after processing the artichoke and used for supplementation of the selected 25 diabetic subjects (12 men and 13 women).

The amount of artichoke powder given was six grams per day for an individual. It was incorporated into 20g of wheat flour and prepared as sugar free biscuits (Plate II). The investigator prepared four biscuits and distributed daily as mid morning and evening snack two at a time for a period of three months.

##### **5. Supplementation with amla (*Embilica officinalis*)**

Amla (Gooseberry) scientifically known as *Embilica officinalis* is one of the precious gift of nature. It is the richest source of vitamin C which is a water soluble vitamin needed for the cell integration and repair of tissues. It is an antioxidant that blocks some of the damage caused by free radicals. Amla fruit pulp contains 600 mg of vitamin C per 100 grams, which is 20 times more than the vitamin C present in orange juice.

Vitamin C is helpful for people with diabetes in a number of ways. People with diabetes have increased levels of free radicals which cause development of atherosclerosis and decreased level of antioxidant including vitamin C.



1. Globe artichoke



2. Pull of lower petals which are small and discolored



3. Cut stems close to base



4. Cutoff top quarter and tips of petal which is thorny in nature



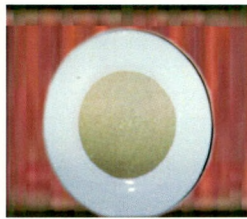
5. Plunge into acidified water(lemon juice)



6. Keep inside the pressure cooker for 5 minutes



7. Pullout petals and leaves, spread in plate



8. Dry and make it in to powder and store it



9. Incorporate dry powder and prepared as Biscuits

## PLATE II PROCESSING AND PREPARATION OF ARTICHOKE BISCUITS

Insulin helps in the body to take up the vitamin C that they need to function properly. Thus extra vitamin C in the form of supplements may be helpful in diabetes mellitus (Laight, 2000).

According to Manjunatha *et al.*, (2001) daily intake of amla reduces post prandial glucose in the oral glucose tolerance test and also reduces blood lipid level to a significantly greater extent than vitamin C from other sources.

For the supplementation study, twenty five diabetic subjects (12 men and 13 women) were selected. For each diabetic subject 30g of amla (Plate III) containing 180mg of vitamin C as supplement was provided for a period of three months and they were requested to take the amla as a whole along with the meals as suggested by Cunningham (1998). Investigator distributed the amla personally to each subject and explained the importance of amla in lowering the blood glucose level and the supplementation was carried out for three months.

#### **6. Supplementation with soyabeans (*Glycin max Merr*)**

Soyabeans (*Glycin max Merr*) are the legume belongs to Fabaceae family were grown as food for its high protein content. Soyabean is considered as a source of complete protein which contains significant amounts of all the essential aminoacids for the effective muscular formation of the human body.

Many traditional dairy products have been imitated using soyabean among which soyamilk plays a vital role. After making soyamilk, the solid residue that is left is called as Okara. It contains appreciable amount of protein and fiber and it has no flavour of its own and tastes like coconut (Shurtleff, 2002)

Okara is a yellowish pulp consisting of the insoluble parts of soyabean, which remains when pureed soyabeans are filtered in the product of soya milk. Okara is low in fat and rich in protein, calcium, iron, and riboflavin. It is used as a ingredient in various food products to improve the nutrient quality and quantity.



**AMLA**

**PLATE III**  
**NEUTRACEUTICAL (AMLA) SUPPLEMENTED PRODUCT**

Each 100 g of the okara contribute four grams of high quality protein, two grams of fat, 15 gm of carbohydrate, 100 mg of calcium and 1.5 mg of iron (Weingartner *et al.*, 1987).

Okara has an interesting role to play in diabetes in two different ways. In one way, inclusion of okara in daily diet slows the absorption of glucose in blood stream and in the other way, it has a low glycemic index (Zhan *et al.*, 1993).

According to Tusha (2003), 25 grams of okara included in the daily diet helped to control blood glucose; it reduces triglyceride and increases the HDL levels effectively.

Having all these points in mind, the investigator planned for the supplementation of okara for the twenty five diabetic subjects (15 men and 10 women). The amount of okara given was 25 grams per day per individual as and incorporated with rice flour and prepared as sevai (rice noodles) which is a common South Indian recipe. The investigator prepared one serving of lemon sevai cooked with 45 g of rice flour and 25g of okara flour and the same was distributed for each subject daily for a period of three months (Plate IV).

#### **7. Supplementation with bay leaves (*Laurus nobilis*)**

Bay leaf (leaf of the sweet bay tree) is an evergreen plant, indigenous to Asia minor bordering the Mediterranean. Bay leaves are large, glossy dark green, elliptical and pointed (about 8cm long and 3-4 cm wide).

Bay leaves are used as a whole and in the form of powder. The powder is a useful ingredient in some seasoning mixture and used in the place of leaves where there is preferable. Bay is used in curries, biriyanis, soups, sauces, fish and shellfish dishes, pickling and tomato juice.



Soy beans



Soaked for 5-8 hours

Pressure cook it for 10 minutes, drain the excess water. Grind the cooked beans.



Removal of milk



Remaining residue is dried



Okara powder (25 gram)



Rice flour (45 gram) + Okara powder (25 gram). Mix both and made into thick dough



Extrude it as sevai



Okara Sevai

**PLATE IV**  
**PROCESSING OF SOYA BEAN AND PREPARATION OF OKARA SEVAI**

Bay leaves contain approximately 1.5-2.5 per cent of essential oil, the principal compound is Cineole. Bay leaves also contains about 4-8 per cent volatile oil. A bay leaf has legendary medicinal properties. Bay leaves help to regulate the body's level of insulin, the hormone that is the carrier of blood sugar into the cells and help the body to use insulin more efficiently.

Anderson (2004) demonstrated that bay leaves help the body to use insulin more effectively at levels as low as 500 milligrams (about a half-teaspoon).

Five hundred milligram of bay leaves powder was given daily for an individual and in the experimental group consisting of twenty five diabetic subjects (10 men and 15 women). It was incorporated with 30g of wheat flour and prepared as chapattis, and this was distributed for all the subjects in the experimental group daily for a period of three months to study the effect of bay leaves powder on the blood glucose and lipid level of the selected diabetic subjects (Plate V).

## **8. Supplementation with flaxseed (*Linum usatissimum*)**

Flaxseed is an ancient blue flowering crop. It is also called as Linseed (*Linum usatissimum*). They are a hard and tiny seed which is widely used for thousands of years as a source of food.

Flaxseed is rich in protein. Various researchers highlight that its health benefits and are due to its fatty acid and fibre content. It contains nearly 21 per cent of protein, fat 42 per cent; PUFA 30 per cent of this 24 per cent is omega 3 fatty acid and 6 per cent of omega 6 fatty acids, 28 per cent of fibre, 6 per cent of carbohydrates and 3 per cent of other nutrients (Cunnane *et al.*, 1995).

Flaxseed is praised as the nutraceutical food of 21<sup>st</sup> century. It is the richest plant source of lignan precursors. Flaxseed contains Secoisolariciresinol Diglucoside (SDG) a potent antioxidant. This compound is effective in reducing

the blood sugar level and prevents the occurrence of both Type I and Type II diabetes and development of hypercholesterolemic atherosclerosis (Thompson, 1996). According to Tarpila *et al.*, (2002) people can receive 6 to 24 grams of flaxseed per day for the period of six months without any toxic effects.

The clean, sun dried flax seeds were carefully roasted, powdered and stored in air tight containers for the supplementation study.

For the 25 diabetic subjects (14 men and 11 women), the amount of flaxseed powder given was 5 grams incorporated into the wheat flour and prepared as wheat bread and distributed for each individual daily as a morning snack for a period of three months (Plate V).

#### **9. Supplementation with stevia leaves (*Stevia rebaudina*)**

Stevia is one of the most health restoring plants. It is a natural sweetener isolated from the herb stevia rebaudina, native to Paraguay, it is a small green plant bearing leaves which have a delicious and refreshing taste and 300 times sweeter than sugar (Columbus, 1997).

The natural sweet compounds in the stevia leaves are called Diterpene glycosides or steviol glycosides. The intensity of sweetness and taste is directly proportional to the presence of four major glycosides of the plant leaves, veins and stems. The normal proportions of these glycosides are stevioside (10 per cent), Rebaudioside A (14 per cent), Rebaudioside C (2 per cent) and dulcoside A (1 per cent).

Among the four glycosides, stevioside is used as an anti hyperglycemic agent. It has potential action on skeletal muscle, the major site of glucose disposal, by which it reduces the high blood glucose level. Stevia also regulates blood sugar through its high natural chromium content. It helps in increasing the number of islet cells on the pancreas for better insulin / blood sugar co-ordination.



**BAY LEAVES**



**BAY LEAVES POWDER**



**CHAPPATHI**



**FLAXSEED**



**FLAXSEED BREAD**

**PLATE V  
NEUTRACEUTICAL (BAY LEAVES, FLAXSEED)  
SUPPLEMENTED PRODUCTS**

Both Yamada *et al.* (1985) and Xili (1992) have calculated the “Likely maximum intake” of stevioside for human consumption. They confirmed that daily intake upto 125mg of stevioside is safe for human consumption.

Based on this recommendation of stevioside safe level intake of stevia leaf for diabetics can be calculated for supplementation.

#### **Calculation of recommended stevia leaves**

Amount of stevioside = 10 g/100g of stevia leaves

Stevioside requirement of a normal man = 125 mg/day

10grams of stevioside is present in 100g of stevia leaves

∴ 125mg is present in 1.25 grams of stevia leaves.

Therefore a therapeutic dose of 1.25 grams of stevia leaves which contains 125 mg of the active principle, the stevioside is recommended for the hypoglycemic effect.

Hence, to prove the authenticity of stevia action in hyperglycemia and hyperlipidemia, a suitable product is formulated and supplemented to the selected twenty five diabetic subjects (16 men and 9 women) for three months.

The amount of stevia leaf powder given was 1.25 g per day per individual and it was made into tea. The investigator prepared 100 ml of tea with 1.25g of stevia leaves powder and distributed for each individual daily for a period of three months (Plate VI).

#### **10. Supplementation with jambolin seed (*Syzygium cumini*)**

Jambu or Jaman fruit is found throughout India. This member of the myrtaceae is of wider interest for its medicinal applications than for its edible fruit (Morton, 1987). The fruit is cluster of just a few or 10 to 40, is round or oblong,

often curved. The skin is thin, smooth, glossy and adherent. The fruit have green or brown seeds upto 4 cm in length, though some fruit have 2-5 seeds tightly compressed within a leathery coat.

Jambolan seeds are found to contain an alkaloid, Jambosine and a glycoside, Jambolin or antimellin, which halts the diastatic conversion of starch into sugar.

Extracts of the seeds in liquid or powdered form are freely given orally 2-3 times a day, lowered blood sugar level without any ill effects among the subjects with diabetes mellitus (Morton, 1987). Supriya (1998) found that the jamun seed powder about 15g is administered orally thrice a day for 3-4 months in diabetes which showed a good level of hypoglycemic effect due to the presence of Ellagic acid an active principle in the jamun seed extract (Chatterjee, 2000).

Having these points in mind, the investigator planned for the supplementation of jambolan seed powder for the 25 diabetic subjects (13 men and 12 women).

Clean jambolan seeds were selected and powdered with skin. The investigator prepared one serving of two dosas (pan cakes) using 50 grams of rice batter with five grams of jambolin seed powdered and supplemented for each individual daily for a period of three months (Plate VI).

In all the formulation of ten supplements, sensory evaluation was systematically conducted by qualified panel members and three point sensory evaluations is carried out. The product which secured maximum score was considered as the highly acceptable product and selected as the supplement for the supplementation studies. During supplementation period, extra care is taken to ensure that the selected subjects in experimental group of study group were consuming the entire portion of supplementation without having any wastage.



**STEVIA LEAVES**



**STEVIA LEAVES TEA**



**JAMBOLIN SEED**



**DOSA**

**PLATE VI  
NEUTRACEUTICAL (STEVIA LEAVES, JAMBOLIN  
SEED) SUPPLEMENTED PRODUCTS**

Blood glucose and lipid profile values of control and experimental group before and after supplementation of nutraceuticals are given in Appendix III and IV respectively.

#### **F. CONSOLIDATION AND ANALYSIS OF THE DATA**

The data thus collected were consolidated analyzed and the statistical appraisal were done to find out the effect of selected nutraceutical components on blood glucose and lipid profile of the diabetic subjects. The results thus obtained are presented in Chapter 4 - Results and Discussion.