Methodology

III. METHODOLOGY

Methodology involved in the conduct of the present study **"Impact of supplementation of wheat germ, bran and grass on diabetic, hyperlipidemic and tuberculosis subjects"** is dealt under the following headings:

A. Selection of locale and subjects

B. Eliciting background information of the subjects

C. Assessment of anthropometric measurements and conduct of diet survey

D. Supplementation of wheat germ, bran and grass to diabetic, hyperlipidemic and tuberculosis subjects and

E. Evaluation of the impact of supplementation

A. Selection of locale and subjects

The locale selected for the study were the Districts of Chennai and Villupuram from the State of Tamil Nadu. The investigator was familiar with these places and hence could establish a good rapport with the populace in the selected districts, which provided the subjects for the study. The three main health issues considered for the study were diabetes mellitus, hyperlipidemia and tuberculosis. The diabetic subjects from two private diabetic clinics of Villupuram town, tuberculosis subjects from, the District Tuberculosis Centre of the Villupuram town and the hyperlipidemic subjects from the Chepauk Government dispensary, Ezhilagam, Chennai were selected for the study.

After an initial enquiry by the investigator about the feasibility of conducting the experimental study in the private and Government hospitals, the investigator approached the authorities of the respective hospitals in Villupuram and Chennai who whole- heartedly permitted to conduct the study. The concerned subjects in these hospitals were given a briefing about their ailment and explained the importance of wheat products and their potential to decrease the clinical and biochemical symptoms of their respective ailment. Later, they were briefed about the details of the study followed by deliberations. All the

volunteering subjects were considered for further selection procedure. The criteria for the selection of the subjects included that they should be non-smokers, non- alcoholics and within the age range of 40-55years.

The diabetic and hyperlipidemic subjects should have been under continuous treatment (minimum of three months) in the selected clinics / dispensary. In the diabetic group, subjects were included if their fasting blood glucose levels were ranging from 120-160mg/dl as specified by Ajgoankar (1962) and excluded if they were taking thyroid or steroid hormones, beta blockers, prednisone or diuretics that might affect serum lipids. In the hyperlipidemic group subjects were included only if their blood cholesterol levels were greater than150mg/dl and excluded if they had uncontrolled hypertension (systolic B.P >140mm Hg or diastolic B.P>90 mm Hg), symptomatic coronary or vascular disease, thyroid disease, diabetes, hepatic abnormality or renal disease. The tuberculosis subjects were excluded if they had tuberculosis as a disease or as a secondary infection . Data on the fasting and post prandial glucose for the diabetic group, total cholesterol , triglycerides, HDL,LDL and VLDL cholesterol for the hyperlipidemic group and Acid Free Bacilli (AFB) and tuberculin skin test for tuberculosis group were collected from the concerned hospitals . CD_4 was analyzed only for the volunteering TB subjects.

Based on the physicians' opinion on the clinical and biochemical picture obtained from the hospital records and the criteria framed by the investigator,105 diabetics from the private diabetic clinics at Villupuram town,60 tuberculosis subjects from the District Tuberculosis Centre, Villupuram and 105 hyperlipidemic subjects from Chepauk Government dispensary, Ezhilagam, Chennai were selected for the study. The ethical guidelines were followed and the study was approved by the Committee on Health Research Ethics, Avinashilingam University for Women, Coimbatore (H.E.C.2006.04).

B. Eliciting background information of the subjects

As a first step in the study an interview schedule was formulated to elicit information from all the 105 diabetic, 60 tuberculosis and 105 hyperlipidemic subjects on their socioeconomic details including age, sex, education, family type, monthly income, food habits and dietary pattern through interview cum observation method. Details on type and duration of disease and familial disposition of disease were also collected. A pilot study was performed on five per cent of the selected sample as suggested by Kothari (2005) before the conduct of the survey. Based on the results, relevant modifications were made and the proforma was finalized (Appendix I). The investigator administered the interview schedule to all the subjects and required information were collected.

C. Assessment of anthropometric measurements and diet survey of the subjects

1. Anthropometric measurements

Anthropometrics is the gold standard for assessment of nutritional status (Elizabeth, 2000). To add, anthropometry is the single point portable invasive method of assessing body composition reflecting health and nutrition and predicting performance, health and survival (Ramalingaswami, 1993). While height is used to assess the past nutritional status, weight helps to assess the present. Body Mass Index (BMI) is frequently used as a popular and rapid clinical measure of relative obesity and malnutrition (Priyatomako *et al.,* 2001).

Accordingly, the anthropometric indicators namely weight (kg), height (cm), BMI (kg/m^2), waist and hip circumferences were measured for all the 105 subjects in the diabetic and hyperlipidemic groups respectively before and after the supplementation period. Genton *et al,.* (2005) suggest that in conditions like tuberculosis where underweight is predominant the only useful parameter is anthropometrics and that would be weight loss, perhaps Mid Upper Arm Circumference (MUAC) and skinfold thickness. Therefore MUAC and skinfold

thickness were assessed along with height, weight and BMI for all the 60 tuberculosis subjects

a. Weight

Measurement of weight serves as the indicator to profess the presence and progress of ailment. The weight of all the selected subjects in the three groups were determined by making them stand barefooted and erect on a portable weighing scale to the accuracy of 0.1kg before supplementation (Brahmam *et al.*, 2005).

b. Height

Height is a constituent factor in the calculation of BMI and hence the height of all subjects was measured using a vertical measuring rod (anthropometer). The subjects were made to stand erect, barefooted on a levelled surface, with heels together and toes apart. The moving head piece of the anthropometer was placed in the sagital plane over the head of the subjects applying a slight pressure to reduce the thickness of hair. The reading was taken when the anthropometer was still in position to the accuracy of 0.1cm (Brahmam *et al.*, 2005).

c. Body Mass Index (BMI)

BMI determines if weight is appropriate for the height and thus has good correlation with fitness (Bamji *et al.,* 2004). WHO, (2000) has explained BMI as a simple index of weight for height that is commonly used to classify adults as underweight or overweight. BMI was calculated for all the selected subjects in the three groups using the following formula:

$$BMI = \frac{\text{Weight (kg)}}{(\text{Height})^2 \text{ (m)}}$$

d. Waist circumference

Waist circumference was measured for all the selected subjects. 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 upto the nearest millimeter. The observer sat on a stool in front of the subjects while taking the measurement (Brahmam *et al.*, 2005).

e. Hip circumference

Hip circumference was measured for all the subjects. For measuring the hip circumference, the measuring tape was placed horizontally over the buttocks and the circumference was measured at the point yielding the maximum circumference in centimeter upto the nearest millimeter (Brahmam *et al.*, 2005). According to Boyle *et al.*, (2003) the waist circumference should be taken at the narrowest circumference between ribs and hips. For all the selected subjects in the diabetic and hyperlipidemic groups Waist Hip Ratio (WHR) was computed by dividing subject's waist circumference in centimeter by hip circumference in centimeters .

f. Mid Upper Arm Circumference (MUAC)

MUAC was measured for all the 60 tuberculosis subjects. Assessment of protein compartments (muscle) can aid in the diagnosis of clients with wasting. MUAC provides an estimate of lean tissue and muscle mass of the patients and hence is a good indicator of wasting and weight loss. The MUAC was taken on the left forehand at the mid point between the tip of the acromion of scapula and the tip of the olecranon of the forearm bone of the patients. The arm was left freely hanging and flexible tape was used to measure the MUAC to the nearest millimetre with the tape still in position.

g. Skin fold thickness

Skin fold thickness is a measure of subcutaneous fat reserves which was evaluated for all the 60 tuberculosis subjects. The skin fold at triceps is more reliable than that of sub scapular in the assessment of underweight and is more sensitive to the socio economic environment (Knechtle and Kohler ,2007).

Triceps skin fold thickness was mid point where MUAC was measured. The skin fold was picked up between the thumb and the forefinger about one centimeter above the midpoint, taking care not to include the underlying muscle. The calipers were squeezed until they were equilibrated at the point for approximately three seconds. The measurements were read to the nearest millimetre and calibrated using previously published empirical equation (Felblinger, 2003).

2. Diet survey

According to Bamji *et al.,* (2004), diet is a vital determinant of health and nutritional status of people. Precise information of food consumption pattern of people through application of appropriate methodology is often needed not only for assessing the nutritional status of people, but also for elucidating the relationship of nutrient intake with deficiency as well as degenerative diseases. Precise information on food consumption pattern was collected through 24 hour recall method for one tenth of the selected subjects in all the three groups studied. The raw food equivalent of the cooked food was determined and the intake of macro and micro nutrients were computed using the values given in the 'Nutritive Value of the Indian Foods (ICMR, 2004).

D. Supplementation of wheat germ, bran and grass to diabetic, hyperlipidemic and tuberculosis subjects

1. Grouping of subjects

Kothari (2005) defines sampling as the selection of some part of an aggregate or totality on the basis of which a judgment or inference about the aggregate or totality is made. For the present study purposive sampling method was adopted, as the method has definite view point in selection, considering the nature, scope and criteria fixed for the study (Burney, 2003). The selection of subjects for the study, mainly depended on the discretion of the investigator, keeping in view the criteria fixed for the study.

During the initial rapport with the selected subjects, willingness to participate and cooperate till the completion of study was sought from the interview and obtained in the form of a consent letter. The 105 diabetics and 105 hyperlipidemics were divided randomly into seven groups of 15 each respectively. Group A received wheat germ, group B received wheat bran, group C received wheat grass, group D received wheat germ and bran, group E received wheat germ and grass and group F received wheat bran and grass as supplements. Group G served as the control group and did not receive any supplements other than the usual medications.

Wheat bran's nutraceutical potential in alleviating symptoms of certain diseases were correlated only with the high insoluble fibre content (Borel *et al.*,2005). Further the experienced physicians suggested that wheat bran supplementation would irritate the bowel movements of tuberculosis subjects and also inhibit the absorption of the medication. Thereby the supplementation of wheat bran to the tuberculosis subjects was forgone on ethical reasons and only 60 tuberculosis subjects were selected and divided into four groups of 15 each. Group A received wheat germ, group B received wheat grass and group C received wheat germ and grass as supplement. Group D served as the control group and did not receive any supplements. Figure I gives the grouping of subjects and the supplements tested.

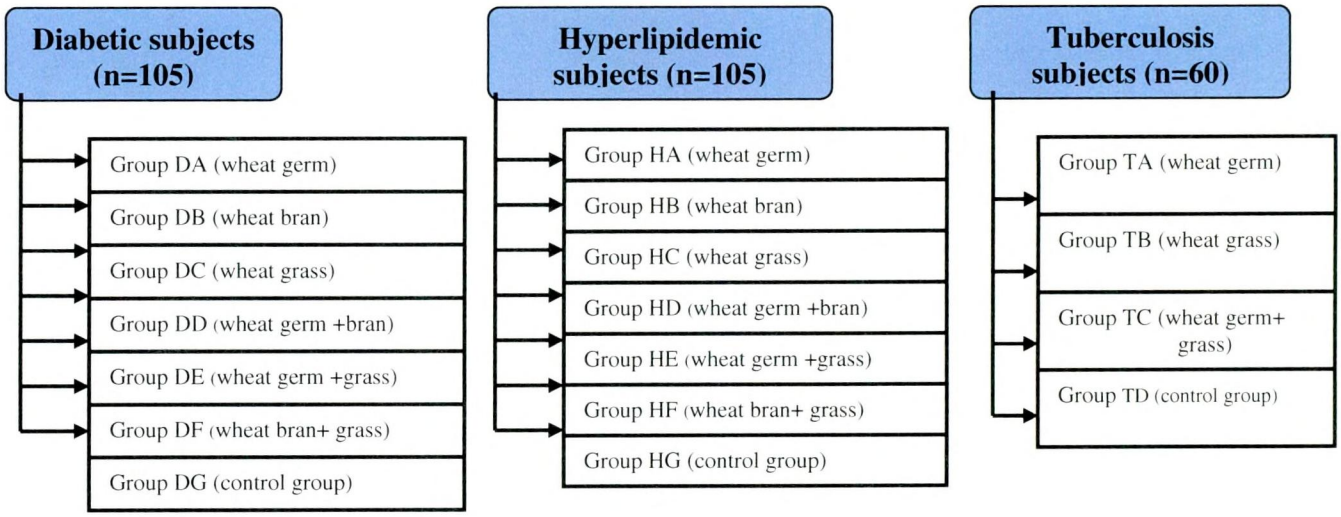


Figure I

Grouping of subjects

2. Procurement of wheat germ and bran

Wheat germ and wheat bran were sponsored by Yamuna flour mill, Thrisur, Kerala State for the study purpose. On a monthly basis 216 kg of wheat germ and 36 kg of wheat bran were procured from the sponsor for a period of six months. Plates 1 and 2 present the procurement and packaging of wheat germ and bran.

3. Germination and preparation of wheat grass juice

Among all types of grasses, wheat grass is the best (Fahey *et al.*,2005). Wheat grass is considered to be one of the best foods for all people in different age groups because of its nutrient content. The process of wheat grass cultivation consists of two steps namely germination of wheat grains and cultivation of wheat grass. Plate 3 gives the process of germination and preparation of wheat grass juice.

a. Germination of wheat grains

Superior good quality whole wheat was procured, and cleaned properly. The wheat grains were soaked in cold water for 12 hours. The process of soaking helps the wheat grains to become tender. It also reduces the phytin content of

Procurement of wheat germ

120g sachet of wheat germ
weighed for the experimental group

Packing of the
weighed wheat germ
for the experimental group

60 g wheat germ
weighed for supplementation

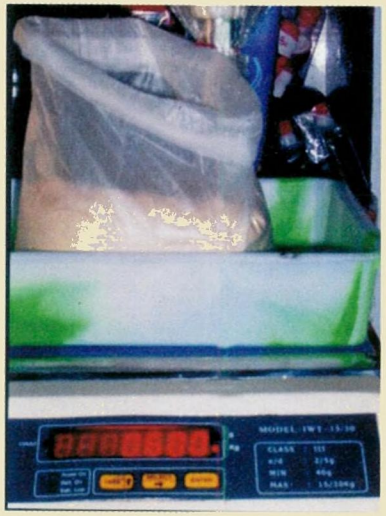
20g of wheat germ mixed in 100ml of toned Milk

Subject consuming 20 g of wheat germ in toned milk

PLATE 1

PROCUREMENT OF WHEAT GERM AND PREPARING FOR DISTRIBUTION

Wheat Bran Purchased
in Wholesale



Measuring 600 gram of Wheat Bran

Wheat Bran Packets Ready for Distribution
(20gram per day for 30 days)

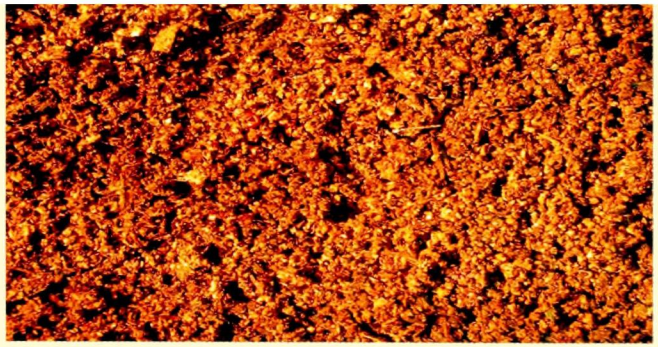
50 g wheat flour mixed with 20g of Wheat Bran

Chappathies prepared from wheat flour and wheat Bran
mixture

PLATE 2
PREPARATION OF CHAPPATHI FROM WHEAT BRAN

Land before Cultivation of wheat grass

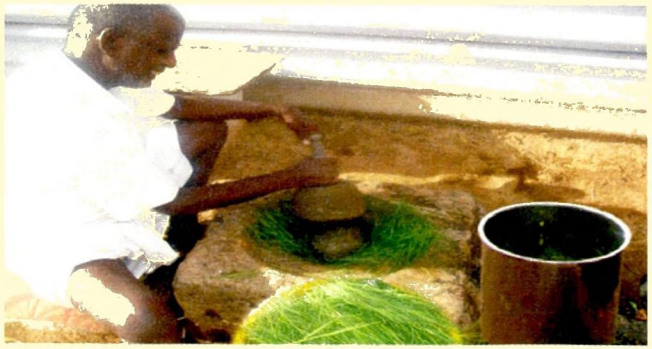
Germinated wheat



Germinated wheat seeded

Cultivated Land

Wheat grass ready for harvest



Grinding of wheat grass

Transport of wheat grass juice

PLATE 3

GERMINATION AND PREPARATION OF WHEAT GRASS JUICE

wheat. After 12 hours of soaking the water was strained and the soaked grains were tied in wet woven cotton cloth and hung for a period of 12 hours. Water was sprinkled over the cotton cloth at least thrice during germination period. Moisture and warm temperature is needed during the germination period. During this process, enzymes get activated, thus increasing the availability of nutrients and digestibility. It also increases ratio of non essential amino acids and content of vitamins like riboflavin, niacin and biotin. It also increases the action of cytases and pectinases. It releases minerals like calcium, zinc and iron from their bound form. This process also reduces trypsin inhibitor factors (Jensen *et al.*,2005). Wheat sprouts contain four times more folic acid and six times more vitamin C than unsprouted wheat or ordinary grass (Davis *et al*, 1999).

b. Cultivation of wheat grass

After 12 hours of germination, the germinated wheat were sowed in a shady place .Since wheat can grow in all temperatures, shady place is preferred to avoid excess nutrient loss due to exposure to direct sunlight. The sowed seeds started to grow and on the seventh day, the grass reached the length of 15 to 18cm which was then harvested (Ben and Goldin , 2002).150g of wheat was required to cultivate 100g of wheat grass.

4. Analysis of wheat germ, bran and grass

a. Nutrient content

The proximate principles, amino acid composition, vitamin and mineral content of wheat germ, bran and grass were estimated. The proximate principles namely moisture, total ash, total fibre, carbohydrate, total fat and protein were analyzed by the standard procedures of National Institute of Nutrition (NIN, 1999). The amino acids for wheat germ, bran and grass were analyzed using High Performance Liquid Chromatography (HPLC) (Heinrikson and Meridith,1984). The minerals namely calcium, phosphorus, iron, sodium, zinc, copper and selenium and vitamins namely vitamin A, vitamin C, vitamin D,

vitamin E, thiamine, riboflavin, niacin, folate and vitmainB_{12} were analyzed using standard procedures of NIN (1999).

b. Phytosterol content of wheat germ

The phytosterol content of wheat germ was estimated by mass spectrophotometry (Ostlund *et al.*,1996) Table I gives the phytosterol content of wheat germ.

TABLE I

PHYTOSTEROL CONTENT OF WHEAT GERM

Phytosterols	Content (mg/g)
Beta sistosterol	3.04±0.15
Campesterol	1.02±0.04
Stigmasterol	0.04±0.00
Total phytosterol	4.10±0.19

$\bar{X}$ ±SEM for triplicate samples

5. Determination of dosage and supplementation of wheat germ, bran and grass

a. Determination of dosage of wheat germ, bran and grass

Wheat germ is a good source of phytosterol and one such phytosterol is beta sistosterol which is a plant sterol found in almost all plants. According to Cara *et al.*, (2001) beta sistosterol regulates blood sugar and insulin levels in Type II diabetics by stimulating the release of insulin in the presence of non-stimulatory glucose concentrations and inhibiting glucose -6- phosphatase. In the liver, the enzyme glucose -6- phosphatase is the primary pathway for conversion of dietary carbohydrates to blood sugar. Glucose -6- phosphatase dephosphorylates glucose -6- phosphate to yield free D- glucose. Free D-glucose passes into the blood, thus elevating blood sugar levels.

Another study by Bourdon *et al.*, (1999) reveals that beta sistosterol reduces cholesterol levels as it has a close chemical resemblance to cholesterol which enables it to block the absorption of cholesterol by competitive inhibition. The recommended requirement of beta sistosterol is 150-200 mg for exhibiting

its property in reducing blood sugar and total cholesterol (Heimbach *et al.*,2007). Kharb (2000) considers that beta sistosterol not only boosts immunity but also enhances lymphocyte proliferation and natural killer - cell activity. Based on this literature and on the estimation of the phytosterol content in wheat germ it was decided to supplement 60 g of wheat germ which would provide 182.4 mg of beta sistosterol to diabetic, hyperlipidemic and tuberculosis subjects.

The WHO recommends 20 to 40 g of dietary fiber a day. Higher intake of dietary fiber is associated with increased glycemic index of the diet thereby improving the blood glucose levels in diabetics (Sunkin *et al.*,2000). Moreover, plasma lipids and cholesterol assimilation decreased on intake of dietary fiber in hypercholesterolemic adults (Greenwald *et al.*, 2006). Nutrient analysis of wheat bran revealed that 100g of wheat bran provides 42.8g of dietary fiber. Cade (2006) reported the beneficial effect of 20g of wheat bran supplementation on colon cancer patients. As 20g of wheat bran provides 8.56g of dietary fiber and also has been well tolerated by the colon rectal cancer subjects, it was decided to supplement 20 g of wheat bran to the diabetic and the hyperlipidemic group subjects in the study group.

The study conducted by Meyerowitz *et al.*,(2003) where 100g of wheat grass was supplemented to anemic subjects an increase in the blood haemoglobin levels was observed after a period of five months. Thereby, 100g wheat grass was supplemented in the present study to the diabetic, hyperlipidemic and tuberculosis subjects.

Details of the daily dosage of wheat germ, bran and grass individually and in combination to the experimental groups is given in Table II.

TABLE II
DETAILS OF DOSAGE OF WHEAT GERM ,BRAN AND GRASS SUPPLEMENTED TO THE EXPERIMENTAL GROUPS

Wheat products	Dosage of supplementation		
	Diabetic	Hyperlipidemic	Tuberculosis
Wheat germ	60g	60g	60g
Wheat bran	20g	20g	-
Wheat grass	100g	100g	100g
Wheat germ +grass	60g+100g	60g+100g	60g+100g
Wheat germ+ bran	60g+20g	60g+20g	-
Wheat bran +wheat grass	20g+100g	20g+100g	-

b. Supplementation of wheat germ, bran and grass

Before starting the feeding trials, all the 225 subjects in the experimental groups were educated about the beneficial effect of the supplements in alleviating the disease conditions. Sixty grams of wheat germ and 20g of bran were supplied in sachets to the diabetic and hyperlipidemic group every fortnight at the clinic premises. Wheat grass juice was supplemented for the 45 subjects each in the diabetic and the hyperlipidemic group and 30 subjects in the tuberculosis group. 3000ml of fresh juice was prepared daily and distributed to the selected subjects.

The following procedure was adopted for feeding the supplements:

- 30g of wheat germ in 100ml of toned milk was consumed twice a day (at midmorning and at bed time)
- 10 g of wheat bran mixed in 50g of wheat flour was prepared as chappathi and taken twice a day (breakfast and dinner)
- Three kilogram of seventh day wheat grass was crushed using a stone grinder so as to avoid the mechanical disintegration of the chlorophyll molecule in wheat grass and 100 ml of the same was distributed to the each subjects which was consumed at mid morning.

Thus on a daily basis 60g of wheat germ, 20g of wheat bran and 100g of wheat grass were consumed by each subject in the respective experimental groups for a period of six months. Figure 2 gives the research design of the study.

E. Evaluation of the impact of supplementation

Impact of supplementation of wheat germ, bran and grass on selected subjects was evaluated by the following methods

1. Clinical examination and
2. Biochemical assessment

1.Physiological symptoms clinical exa.

The physiological symptoms of diabetes and tuberculosis were evaluated before and after the supplementation period using a check list. As no symptomatic subjects were selected from the hyperlipidemic group the physiological symptoms screened for diabetes and tuberculosis include:

a. Diabetes

Polydypsia, polyphagia, nocturia, constipation, insomnia, shivering, giddiness, excessive sweating , burning sensation in extremities, impaired vision, burning sensation during micturation, hesitancy during micturation and frequency of micturation.

b.Tuberculosis

Nausea, weakness, rapid weight loss, fever, night sweats, cough, chest pain and haemoptysis.

2.Biochemical analysis

Biochemical changes can be expected to occur prior to clinical manifestation. Therefore biochemical tests which can be conducted on easily accessible body fluids such as blood and urine can help to diagnose disease at the sub clinical stage (Davidson, 1990).All the biochemical parameters were evaluated initially and after six months of supplementation for all the subjects. Three months after the withdrawal of supplementation five subjects from each

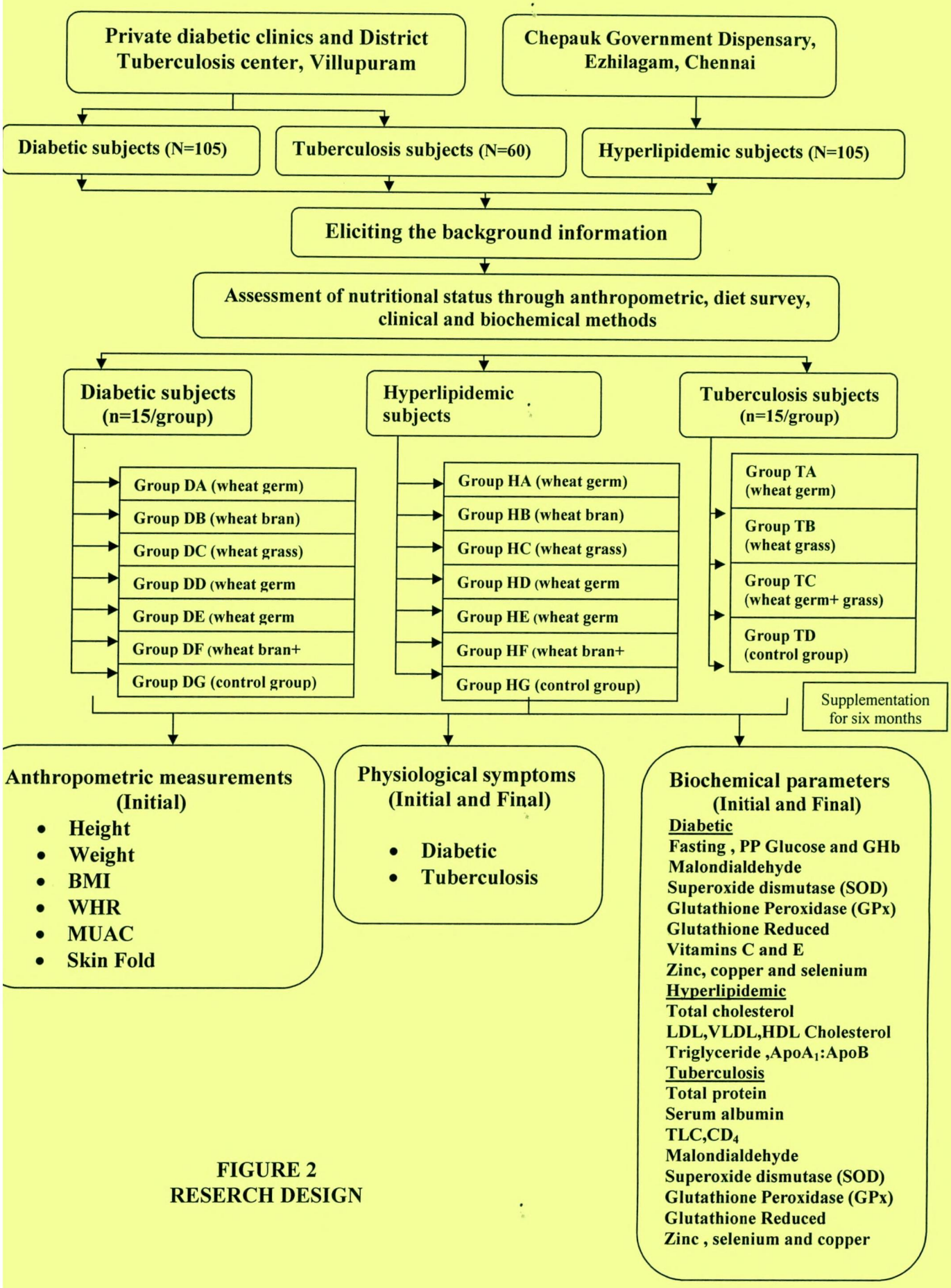


FIGURE 2
RESERCH DESIGN

group were randomly assessed for the same parameters to establish the sustainability of the supplements. Lipid peroxidation and antioxidant activity were evaluated for the diabetic and the tuberculosis group subjects initially and after six months of supplementation.

The procedure for collection of blood and the method followed in the estimations is elaborated below:

10ml of venous blood was collected after overnight fasting in different containers-

Sodium fluoride bulb

1.0ml of blood for glucose estimation.

EDTA bulb

5.0ml of blood was added. 0.2ml of blood was used for reduced glutathione estimation. Remaining blood was centrifuged. Separated plasma was used for the estimation of vitamin C. Red blood cells were washed three times with ice cold normal saline and used for the estimation of glutathione peroxidase, superoxide dismutase and glycosylated hemoglobin.

Plain bulb

Remaining blood was used for the estimation of lipid profile, malondialdehyde and vitamin E, zinc, selenium and copper.

The various parameters evaluated for the three ailments selected are given in Table III.

a. Diabetes mellitus

(i)Fasting and postprandial blood glucose

Fasting and Post prandial glucose were measured using commercial kits from Accurex , India , on automated analyzer.

(ii)Glycosylated hemoglobin (GHb)

Glycosylated hemoglobin was determined by the method of Fluckiger *et al* .,(1976).

(iii) Superoxide Dismutase (SOD)

Superoxide dismutase was determined by method of Winterbourn *et al.,* (1975). Hemoglobin was measured by Drabkin's method.

(iv) Malondialdehyde

MDA levels were estimated by thiobarbituric acid (TBA) reaction (Gavino *et al.*,1981).

(v) Glutathione Reduced

The level of glutathione reductase in erthyrocyte was determined by a method of Beutler *et al.*,(1963).

(vi)Glutathione Peroxidase (GPx)

The glutathione peroxidase activity was determined by the procedure of Paglia *et al* ., (1967).

(v) Vitamin C

The vitamin C was determined by titration method as given in Varely *et al.*,(1988).

(vi) Vitamin E

The vitamin E was determined by method of Baker and Frank (1988)

(vii) Serum selenium

The serum was diluted with double-distilled de-ionized water in the proportion of 1:5. Selenium and iron were assayed by flame atomic absorption spectrophotometry with Zeeman background correction (Hitachi, Tokyo, Japan).

(viii) Serum zinc and copper

The serum was diluted with de-ionized water in the proportion of 1:10. Zinc and copper were assayed by flame atomic absorption spectrophotometry (Model PU 9100 X, Philips Analytical, Cambridge, Great Britain).

b.Hyperlipidemia

Lipid profile

Total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol and triglyceride were measured by enzymatic calorimetric method using commercially available kits (manufactured by diagnostic division of Reddy's Laboratory, Hyderabad, India). Serum Apo A_1 and B levels were estimated by immunoturbidimetric method (Rifai.,1988).

c.Tuberculosis

(i)Serum albumin and total protein:

Albumin determination was made on serum sample by the method of Bartholomew and Delaney (1964) and total protein was determined by the Biuret method of Reinhold (1953).

(ii) Total Leucocyte Count (TLC)

The total leukocyte count was determined in a Neubauer chamber using the method of Wintrobe *et al.*,(1961).

(iii) CD_4 count

CD_4 count was estimated by flowcytometry using the method of Giorgi *et al.*,(1981).

(iv) Superoxide Dismutase (SOD)

Superoxide dismutase was determined by method of Winterbourn *et al.*, (1975). Hemoglobin was measured by Drabkin's method.

(v) Malondialdehyde

MDA levels were estimated by thiobarbituric acid (TBA) reaction (Gavino *et al.*,1981).

(vi) Glutathione Reduced

The level of glutathione reductase in erthyrocyte was determined by a method of Beutler *et al.*,(1963).

(vii)Glutathione Peroxidase (GPx)

The glutathione peroxidase activity was determined by the procedure of Paglia and Valentine,(1967).

(viii) Serum selenium

The serum was diluted with double-distilled de-ionized water in the proportion of 1:5. Selenium and iron were assayed by flame atomic absorption spectrophotometry with Zeeman background correction (Hitachi, Tokyo, Japan).

(ix) Serum zinc and copper

The serum was diluted with de-ionized water in the proportion of 1:10. Zinc and copper were assayed by flame atomic absorption spectrophotometry (Model PU 9100 X, Philips Analytical, Cambridge, Great Britain).

TABLE III

PARAMETERS FOR BIOCHEMICAL ASSESSMENT

Ailment	Biochemical parameters
Diabetes mellitus	Fasting glucose*
	Post Prandial (PP) Glucose*
	Glycosylated haemoglobin (GHb)*
	Antioxidants: Superoxide dismutase (SOD)
	Malondialdehyde (MDA)
	Glutathione Peroxidase (GPx)
	Glutathione Reduced(GHx)
	Vitamin C
	Vitamin E
	Serum zinc
	Serum copper
	Serum selenium
Hyperlipidemia	Total cholesterol*
	Triglycerides*
	HDL cholesterol*
	LDL cholesterol*
	VLDL cholesterol
	$ApoA_1$:ApoB
Tuberculosis	Total protein*
	Serum albumin
	Total Leucocyte Count (TLC)
	Cell Differential count (CD_4)*
	Antioxidants: Malondialdehyde
	Superoxide dismutase (SOD)
	Glutathione Peroxidase (GPx)
	Glutathione Reduced
	Total Antioxidant
	Serum zinc
	Serum copper
	Serum selenium

* Parameters performed after three months of withdrawal of treatment