

METHODOLOGY

The methodology involved in the conduct of the present study titled, “**Effect of Interventions on Vitamin A and Iron Nutritional Status among Primitive Tribal Children in Nilgiris District**” is presented under the following headings:

Phase I

Screening of Primitive Tribal Children for VAD and IDA

- A. Selection of Area and Children
- B. Screening for Clinical Symptoms of VAD and IDA

Phase II

Assessment of Socioeconomic and Nutritional Status

- A. Formulation of Tools and Selection of Children
- B. Conduct of Socioeconomic Survey
- C. Assessment of Nutritional Status
 1. Age of Children
 2. Nutritional Anthropometry
 3. Serum Retinol and Blood Hemoglobin
 4. Dietary Survey

Phase III

Formulation, Acceptability, Nutrient Analysis and Storage Stability of Spirulina Supplement

- A. Selection of Spirulina as Supplement
- B. Formulation of Spirulina Incorporated Food Supplement (SIFS)
- C. Assessment of Acceptability
- D. Nutrient Analysis of Supplements
- E. Storage Stability of Spirulina Incorporated Food Supplement

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Nutrition Interventions to the Target Groups

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- B. Conduct of Supplementation
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- C. Imparting Nutrition Education
 - 1. Audio- Visual Aids
 - 2. Nutrition Exhibition
 - 3. Positive Deviance Hearth
- D. Raising Kitchen Garden
 - 1. Procuring Seeds
 - 2. Developing a Model Kitchen Garden
 - 3. Laying Kitchen Gardens

Phase V

Impact Evaluation

- A. Nutritional Supplementation on
 - 1. Nutritional Anthropometry
 - 2. Biochemical Analysis
 - 3. Clinical Examination
 - 4. Dietary Pattern
- B. Nutrition Education and Kitchen Garden on
 - 1. Knowledge, Attitude and Practice

Ethical Approval for this study was obtained from Institutional Human Ethics Committee with the approval no. AUW/IHEC-13-14/FHP-20

Phase I

Screening of Primitive Tribal Children for VAD and IDA

A. Selection of Area and Children

The study was conducted in Nilgiris District, since the district abounds in tribal population. According to 2011 census, the total population of the district is 7.35 lakhs, out of which the total population of Scheduled Tribes is 32,813, constituting 4.5 percent of the general population. Of these, 29,702 belonged to Primitive Tribal Groups (Tribal Research Centre, 2007), with a total of 8347 households. Hence this district was selected for the study based on purposive sampling technique.

Nilgiris District is divided into six taluks, viz., Udthagamandalam, Gudalur, Pandalur, Coonoor, Kotagiri and Kundah. The Primitive Tribal Population is not evenly distributed in the six taluks - 32.08 percent live in Pandalur taluk, 24.10 percent of them live in Kotagiri, 14.33 percent are in Gudalur, 13.16 percent live in Kundah and 6.96 percent live in Coonoor Taluk. The Scheduled Tribes found in Nilgiri District are Thodas, Kotas, Kurumbas, Irulas, Paniyas, and Kattunayakans (Plate 1).

The total population of primitive tribal children in the Nilgiris is 7810 (TRC, 2007), of whom, nearly 60 percent, i. e., 4376 tribal children in the age group of 1 –14 years were screened in the present study, for clinical symptoms of VAD and IDA, by stratified systematic sampling method in order to ensure that the selection is proportional and representative of the whole population. Random sampling technique was used for the selection of 317 PTG children for assessment of nutritional status, in order to ensure that every member in the population has an equal chance of being included in the study without any bias.

The map showing the settlements of PTGs in the Nilgiris District is given in Figure 4. Details of Primitive Tribal Children screened from each village are shown in Table I.

Table I

Screening of Primitive Tribal Children (N=4376)

Tribes	Taluks	No. of settlements	No. of households	No. of Children
Thodas	Udhagamandalam Coonoor Kotagiri	36 Munds	209	241
Kotas	Udhagamandalam Coonoor Kotagiri Gudalur	6 Kokkals	420	452
Kurumbas	Udhagamandalam Coonoor Kotagiri Gudalur Pandalur	51 Padis	1038	1070
Irulas	Udhagamandalam Coonoor Kotagiri Gudalur	42 Padis	1199	1231
Paniyas	Udhagamandalam Coonoor Kotagiri Gudalur Pandalur	62 Padis	1124	1156
Kattunayakans	Gudalur	18 Oorus	189	226
Total		215	4179	4376

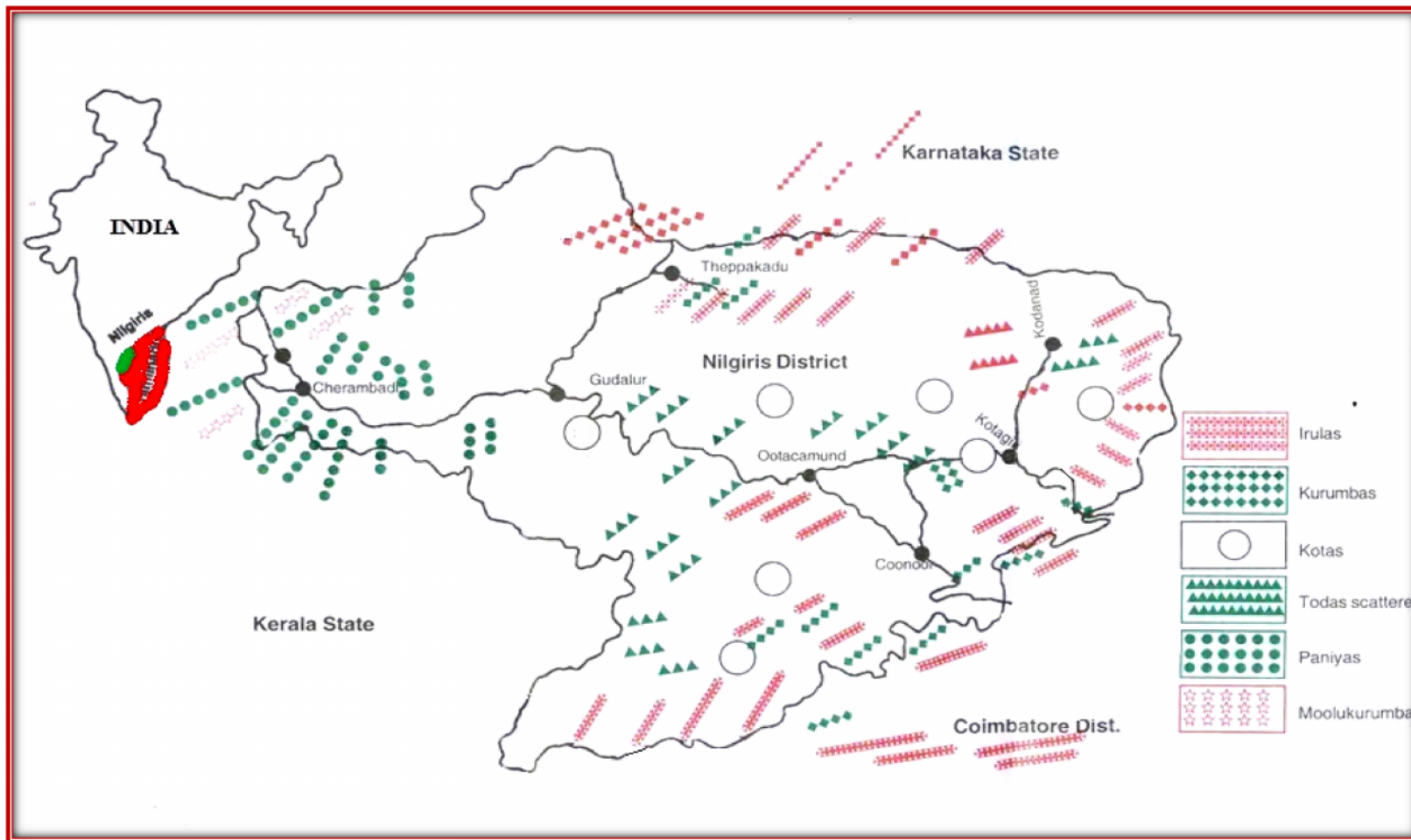


Figure 4
Map showing Primitive Tribal Settlements in Nilgiris District

B. Screening for Clinical Symptoms of VAD and IDA

Clinical examination has always been and remains a widely used tool which is most practical as a direct method for assessing the nutritional status of an individual (Jelliffe and Jelliffe, 1990). All the children were screened clinically by the investigator (with the help of a medical practitioner) for symptoms of VAD such as Bitot's Spot, Night blindness, corneal xerosis, keratomalacia, Xerophthalmia, conjunctival xerosis and IDA such as pale pallor, pale conjunctiva, fatigue and Koilonychia (Plate 2). The clinical examination proforma of Jelliffe (1966) was used for the purpose (Appendix I).



Plate 2. Examination for Clinical Symptoms

The stages of Xerophthalmia are regarded both as disorders and clinical indicators of VAD, and thus can be used to estimate an important aspect of morbidity and blinding disability as well as the prevalence of deficiency.

Classification of Xerophthalmia (WHO, 1982)

XN	Night Blindness
X1A	Conjunctival Xerosis
X1B	Bitot's Spots
X2	Corneal Xerosis
X3A	Corneal Ulceration/Keratomalacia (< 1/3 corneal surface)
X3B	Corneal Ulceration/Keratomalacia (≥ 1/3 corneal surface)
XS	Corneal Scars
XF	Xerophthalmic Fundus

Phase II

Assessment of Socioeconomic and Nutritional Status

A. Formulation of Tools and Selection of children

To elicit information on socio-economic status, lifestyle, dietary pattern, Food Frequency and health status of the selected population, a specially designed interview schedule was developed (Appendix II), based on NNMB-NIN Schedule. Food and nutrient intakes were computed from 24 hour Food recall schedule (ICMR, 2010).

Of the 4376 children, 317 children showing moderate to severe clinical symptoms of VAD and IDA were selected for the socioeconomic, dietary and anthropometry assessments.

B. Conduct of Socioeconomic Survey

Socio – economic factors profoundly affect nutritional status. Ethnic background and educational level of members of the household influence overall food availability and food choices. Levels of income also influence the diet (Rolfes *et al.*, 1998).

The investigator visited the tribal areas which consisted of remote tribal hamlets (Plate 3), with highly vulnerable sources of drinking water (Plate 4). The families were met individually and in groups through frequent visits and a good rapport was established with them. This facilitated easy elicitation of information and execution of the study. All the respondents were interviewed personally by the investigator. Details were gathered by observing the children in their homes and schools (Plate 5).



Plate 3. Scenic view of the remote Tribal Hamlets and Houses



Plate 4. Open and Highly Vulnerable Sources and Storage of Drinking Water



Plate 5. Socio Economic Survey

With the help of the interview schedule, socio-economic details such as age, type of family, educational status, monthly income, and monthly expenditure pattern were collected. Information about life style pattern such as habit of chewing tobacco/betel leaves/betel nuts, habit of taking tea/coffee etc. was elicited. Dietary details regarding dietary habit, meals consumed, food preferences and frequency of food consumption were recorded.

C. Assessment of Nutritional Status

Anthropometry offers a reliable method to assess the nutritional status of children (Bhasin *et al.*, 1990). It is the single most universally applicable, inexpensive and non-invasive method available to assess the size, proportion and composition of human body. The WHO has recommended various indices based on anthropometry to evaluate the nutritional status of children (WHO, 1995).

Assessment of nutritional status was done by using a combination of methods (i.e.) through indicators like nutritional anthropometry, food and nutrient intake.

1. Age of Children

Accurate age assessment in tribal areas of India is often difficult because of illiteracy and parents do not keep any documentary evidence to the festivals and other records of birth as stated by Rao (2009). However, since rural folk are habituated to festivals and other local events related mainly to agriculture or other important activities, age assessment was done accurately using calendar based on local occurrences. The local events calendar contains the dates on which the various festivals and other celebrations occurred for the previous 4-5 years. The age of children was assessed by finding out the festival during which the child was born and the number of such festivals that occurred after the child's birth and referring to the calendar and immunization card/ANC card available with the mother.

2. Nutritional Anthropometry

a) Parameters

i) Standing height, ii) Body weight iii) Mid Upper Arm Circumference iv) Head Circumference and v) Chest Circumference (for children 1-5 years of age) were the parameters measured using standard procedures outlined by WHO, 2006 (Plate 6).

i) Standing height

All the 317 children were taken height using Vertical Measuring Bar at monthly intervals.



Plate 6. Nutritional Assessment of the Primitive Tribal Children

ii) Body weight

All the 317 children were weighed every month using digital weighing balance.

iii) Mid Upper Arm Circumference

Mid Upper Arm Circumference was taken on the left hand, the mid-point between the tip of the acromion of scapula and the tip of the olecranon process of the (fore-arm bone) ulna, with the arm flexed at the elbow using fiber glass tape for all the 317 children.

iv) Head Circumference

The head circumference was measured for 150 children in the age group of 1-5 years by passing the tape around the head over the supra-orbital ridges (just above the eyes) of the frontal bone in front, and the most protruding point of the occiput on the back of the head. Slight pressure was exerted to allow for the thickness of the hair.

v) Chest Circumference

The chest circumference was taken at the nipple level preferably in mid inspiration using fiber glass tape for 150 children in the age group of 1-5 years

The description of the procedures is given in Appendix III. From the parameters the following indices were computed:

b) Body Mass Index

BMI is considered to be the most suitable, objective anthropometric indication of nutritional status (Gibney *et. al.*, 2005). It has been proposed as a good index to assess the current form of malnutrition in a community (Kumari and Singh, 2003). Body Mass Index is computed by taking the body weight in kilograms divided by the square of the height in meters (Raghu, 2002). In the present study, it was calculated for all the children using the formula,

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

c) Height for age

The anthropometric data can be expressed in a number of ways in relation to reference data, a) by the use of mean and standard deviation values, b) by calculating percentage of the median value of reference population which is assigned as 100 per cent, and c) by comparing with percentiles of the reference data, where median value is the 50th centile.

The WHO (2007) recommends the use of SD classification to categorize the children into different grades of nutritional status. The pre-school children were classified according to stunting (height for age) computed using NCHS reference values, as provided below:

SD Classification	Height for Age
>Median -2SD	Normal
<Median -2SD to <Median -3SD	Moderate stunting
<Median -3SD	Severe stunting

d) Weight for Age

The most widely used weight for age classification is the Gomez classification (Gomez *et al.*, 1956), in which the children are classified as having first, second or third degree malnutrition if their weight for age is in the range of 75-90 per cent, 60-75 per cent or less than 60 per cent respectively of the reference median. According to this classification, all the children, whose weights are 90 per cent and above, are categorized as normal. The IAP (1972) recommends the following classification: 80 per cent, 70-80 per cent, 60-70 per cent, 50-60 per cent and <50 per cent as normal, first, second, third and fourth grade of malnutrition respectively, as most of the classification based on weight-for-age, use arbitrary cut-off points.

The World Health Organization recommends the use of SD classification (WHO, 2007) which is popularly used to categorize the children into different grades of nutritional status. Accordingly, pre-school children were classified according to weight for age using NCHS reference values, as provided below:

SD Classification	Weight for Age
>Median -2SD	Normal
<Median -2SD to <Median -3SD	Moderate underweight
<Median -3SD	Severe underweight

e) Mid Upper Arm Circumference and Head Circumference for age

Mid Upper Arm Circumference and Head Circumference for age (1-5years children) were calculated using Z Scores (WHO, 2007).

3. Serum Retinol and Blood Hemoglobin

Biochemical tests cannot be applied on a large scale in the nutritional assessment of a whole community. They are often carried out on a sub – sample of population (Park, 1998). Hence, three milliliters of blood were drawn from totally 60 (every 5th child). They were divided into four groups of 15 each to form the Experimental group I (EI), Experimental group II (EII) Placebo group I (PI) and Placebo group II (PII). Hematological tests help to determine the happenings inside the body and are useful in detecting the nutrient levels in blood and also give an estimation of the amount of nutrients stored in the body (Jelliffe and Jelliffe, 1991).

Estimation of blood Hemoglobin is the simplest biochemical test and was determined using Sahlis method (Mukherjee, 2004), Serum Retinol was estimated using HPLC method (Raghuramulu, 2003). The procedures for the biochemical analyses are enclosed in Appendix IV.

Hb concentration is the most reliable indicator of anaemia at the population level (WHO, 2008). Haemoglobin thresholds used to define anaemia (WHO, 2001).

Age or gender group	Haemoglobin threshold (g/l)
Children (5.00–11.99 years)	115
Children (12.00–14.99 years)	120

The prevalence of Hb values below the population-specific Hb threshold is used to classify countries by the level of the public health problem (WHO, 2001).

Table II

Classification of Anaemia as a Problem of Public Health Significance

Prevalence of anaemia (%)	Category of public health significance
≤4.9	No public health problem
5.0–19.9	Mild public health problem
20.0–39.9	Moderate public health problem
≥40.0	Severe public health problem

4. Dietary Survey

Data regarding the diet consumed by the different categories of the population, information on meal pattern, frequency of food consumption, eating behavior, food and nutrient intake and food preferences of the children were obtained through diet survey.

Details regarding food and dietary pattern of the children were assessed and intake of nutrients was computed from the Tables of Food composition (Gopalan *et al.*, 2009).

i) Food Frequency

Qualitative and quantitative assessment of food intake is a technique used for the assessment of nutritional status as it furnishes useful indications about dietary inadequacies. Food consumption survey helps to elicit the quality and quantity of food consumed by the individual (Sharma and Prakashan, 2006). The type of various foods and the frequency of food consumption over a period of time was assessed using an interview schedule (Appendix II).

ii) Assessment of 24 Hour Food Recall

Food consumption is one of the important determinants of nutritional status. Hence dietary assessment forms an integral part of nutrition survey (Deshpande *et al.*, 2001). Diet surveys constitute an essential part of any complete study of nutritional status of individuals or group providing essential information on nutrient intake levels, sources of nutrients, food habits and attitudes. In the present study, through the 24 hour food recall method, the amount of raw ingredients used for cooking, the total amount of food consumed by the individuals were measured using standard cups and utensils (Kapil *et al.*, 2004) and recorded for the sub sample of 167 children. The average food intake of the children was calculated and compared with the suggested allowances of ICMR (2010). The raw equivalents for these food items were calculated and the nutrient available from this food intake was computed using the Tables of Food Composition and compared with RDA, ICMR, (2010). The proforma is appended in Appendix V.

Phase III

Formulation, Acceptability, Nutrient Analysis and Storage Stability of Spirulina Supplement

A. Selection of Spirulina as Supplement

Spirulina has a relatively higher provitamin A concentration (Belay 1997). An excessive dose of β -carotene may be toxic, but when the β -carotene is ingested from *Spirulina* or another vegetable it is usually harmless since the human organism converts it into vitamin A, up to the quantity it needs (Henrikson 1994). *Spirulina* is a very rich source of vitamin B12, and that is why these cyanobacteria are of great value for people needing supplements in the treatment of pernicious anemia (Becker 1984; Richmond 1984; Belay 1997). *Spirulina* contains 4 per cent to 7 per cent lipids and essential fatty

acids such as LA and GLA (Othes and Pire 2001). The latter is claimed to have medicinal properties and prerequisite for arachidonic acid and prostaglandin synthesis (Dubacq and Pham-quoc 1993). GLA lowers low-density lipoprotein, and is 170-fold more effective than LA (Cohen 1997; Sanchez and others 2003). The fatty acid composition of *Spirulina* is generally characterized by high levels of the ω -6 series (De Caire *et al.*, 2000). Deficiency in linolenic acid is associated with vision (Neuringer and Connor 1986) and nervous system defects, regulation of blood pressure, cholesterol synthesis, inflammation, and cell proliferation (De Caire *et al.*, 2000; Gyenis *et al.*, 2005). Iron is not appropriately absorbed in some nutritional complements. Iron is 60 per cent better absorbed in *Spirulina* than ferrous sulfate and other components. Consequently, it could represent an adequate source of iron for anemic pregnant women (De Caire *et al.*, 2000). *S. platensis* contains about 13.6per cent carbohydrates; some of which are glucose, rhamnose, mannose, xylose, and galactose (Shekharam *et al.*, 1987). *Spirulina* does not have cellulose in its cell wall, a feature that makes it appropriate and important food stuff for patients who have poor intestinal absorption and for geriatric patients (Richmond, 1984). A new high-molecular-weight polysaccharide with immunostimulatory activity has been isolated from *Spirulina* and is called "Immulina." This highly water-soluble polysaccharide represents the dry matter between 0.5per cent and 2.0per cent (w/w) (Pugh *et al.*, 2001).

Spirulina is well known to have very high iron content. It was tested against a typical iron supplement, iron sulfate. *Spirulina*-fed rats absorbed 60 percent more iron than rats fed the iron supplement. This study suggested that there is a highly assimilable form of iron in *spirulina*. An earlier study also showed that it was effective in correcting anaemia in rats (Johnson and Shubert, 1986).

Studies on *Spirulina* supplementation have revealed health benefits such as prevention of skeletal muscle damage (Lu *et al.*, 2006). According to Volterille *et al.*, (2008), *spirulina* provided adequate protein quality to maintain body growth and muscle protein synthesis rates were increased by the ingestion of *spirulina* diet in young rats. Duan *et al.*, (2001) opine that *spirulina* has a certain factor which inhibits cancer, high blood pressure, diabetes and hastens the body to absorb Selenium and Molybdenum and is beneficial to cardiac muscles. The experiments indicated that *spirulina* is a good health care food with high nutritional and medicinal potentials. It exhibits therapeutic functions such as antioxidant, antibacterial, antiviral, anticancer, anti-inflammatory,

anti-allergic and anti-diabetic and a wide plethora of beneficial functions. Spirulina consumption appears to promote the growth of intestinal micro flora as well (Kulshrestha *et al.*, 2008). Premakumari *et al.*, (2011) carried out supplementation of spirulina in the form of candies for PTG children from the Nilgiris and revealed the efficacy of spirulina candies as a supplement to promote overall growth in under five tribal children. Supplementation for only six months could improve the nutritional status of children to a statistically significant extent ($p < 0.01$; $p < 0.05$) Therefore, Spirulina was selected as a food supplement in the present study.

Though spirulina is acclaimed to be an inexpensive source of complete proteins providing all essential amino acids and all micronutrients (except Vitamin C), yet, macro nutrients are not provided by the candies and it is well known that macronutrients are indispensable during childhood. Simpore (2006) has reported that spirulina plus misola (millet, soja and peanut) are good food supplements for undernourished children. Hence a food supplement was formulated using spirulina and inexpensive, locally available cereals and millets.

B. Formulation of Spirulina Incorporated Food Supplement (SIFS)

A Spirulina Incorporated Food Supplement (SIFS) was prepared using the same quantity of spirulina (1g) as in the candies used as a supplement for E I. Spirulina powder was purchased from Antenna Nutritech Foundation, Madurai. Wheat, Maize, jowar, Roasted Bengal gram, ragi and jaggery were included to constitute 30g of the health mix (based on the ICDS health mix formula). These ingredients were purchased from the local grocery shops. It was ensured that all ingredients were of good quality. They were cleaned well, dry roasted separately, one by one and powdered in the mill, sieved and stored in air-tight containers. The procedure for formulation and preparation of the SIFS is appended in Appendix VI (Plate 7). It was thought of interest to compare the efficacy of the SIFS with that of the spirulina candies among the PTG children.

Table III shows the composition of Spirulina Incorporated Food Supplement

To prepare 100 grams of Spirulina supplement 18 g of wheat flour, 16 g of Maize flour, 16 g of jowar flour, 12 g of Bengal gram flour, 5 g of ragi flour, 30 g of jaggery powder and 3.33 g of Spirulina powder were mixed. In one serving (30g based on acceptability trials) the composition of wheat flour, corn flour, jowar flour, Bengal gram flour, ragi flour, jaggery powder and Spirulina were 5.4g, 4.8 g, 4.8 g, 3.6g, 1.5g, 9 g and 1 g respectively.

Table III
Composition of Spirulina incorporated Food Supplement

Ingredients	100 g	Per Serving (30g)
Wheat flour	18	5.4
Maize flour	16	4.8
Jowar flour	16	4.8
Bengal gram flour	12	3.6
Ragi flour	5	1.5
Jaggery powder	30	9.0
Spirulina	3.33	1.0

One serving of SIFS provides 6.2, 9, 0.8, 18.7, and 25.6 per cent of suggested allowances of energy, protein, Vitamin A, vitamin C, iron and calcium.

Rajbir *et al.*, (2004) mixed one gram of spirulina powder with one serving(30) of panjiri (Indian and Pakistani sweet snack from Punjab region, made from whole-wheat flour fried in sugar and ghee, heavily laced with dried fruits) and fed for five days a week for a period of two months to 60 girls aged 7-9 years and their hematological profile and intellectual status were analyzed. An statistically significant improvement ($p < 0.01$) in the haematological as well as intellectual status among respondents of Spirulina supplemented group when compared to Control group showed positive effect of spirulina and proved to be an effective source of protein as well as iron and retinol.

C. Assessment of Acceptability

Spirulina candies being sweet candies were highly acceptable and popular with the children. However acceptability trials were conducted for the Spirulina Incorporated Food Supplement (SIFS). Three different formulations of SIFS were prepared using 30 g of Health mix powder each with 1.0, 1.5 and 2.0g of spirulina powder respectively. These were administered in the form of laddu of 60 g weight (one per child) and tested for acceptability. Acceptability trials were performed in the morning, preferably between 10.30 and 11.00 am, using a child friendly scale. A child friendly five point scale, modified from the Age appropriate Hedonic Scales to measure the Food Preferences of young children, 1996; Bredie, 2011) (Appendix VII) ranging from “super good” to “super bad” or a scale utilizing “smiley” faces was given to twenty children of 7-14 yrs age group for evaluating colour and appearance, texture flavor, taste and overall acceptability of the supplement.

Table IV
Nutrient Content of Spirulina Candies

Parameter*	100 g	Per Serving (6.4g)
Moisture%	1.1	0.0704
Total Ash %	1.0	0.064
Fat%	0.16	0.01024
Protein%	6.6	0.4224
Crude Fibre%	0.18	0.01152
Carbohydrates%	91.0	5.824
Sodium (mg/100g)	448	28.672
Potassium(mg/100g)	121	7.744
Calcium(mg/100g)	869	55.616
Iron(mg/100g)	4.8	0.3072
Magnesium (mg/100g)	292	18.688
Zinc(mg/100g)	1.2	0.0768
Manganese(mg/100g)	0.2	0.0128
Copper(mg/100g)	1.5	0.096
Beta Carotene(mg/100g)	39.2	2.51
Energy (kcal)	392	25.09

***(National Agro foundation, 2011)**

Moisture, total ash, fat, protein, crude fibre, carbohydrates, sodium, potassium, calcium, iron, magnesium, zinc, manganese, copper, beta-carotene and energy for 100 g were 1.1 per cent, 1.0 per cent, 0.16 per cent, 6.6 per cent, 0.18 per cent, 91.0 per cent, 448mg, 121 mg, 869 mg, 4.8mg, 292mg, 1.2mg, 0.2mg, 1.5mg, 39.2mg and 392kcal respectively and for per serving (6.4 g) it was 0.0704 per cent, 0.064 per cent, 0.01024 per cent, 0.4224 per cent, 0.01152 per cent 28.67mg, 7.74mg, 55.62mg, 0.31mg, 18.69mg, 0.08mg, 0.01mg, 0.1mg, 2.5mg and 25.09kcal respectively.

1) Nutrient content of ordinary candies

One gram of ordinary candy provides 4 kcal of energy, each ordinary candy weighed 3.2 g. Two candies (per serving) provided (6.4*4) 25.6 kcal of energy. Table V gives the percentage of total daily energy provided by the candies.

Table V
Percentage of Energy provided by the candy

Age in Years	Energy (K cal)*	% of Energy provided by the candy
7-9	1950	1.31
10-12 Boys	2190	1.16
10-12 Girls	1970	1.29
13-14 Boys	2450	1.04
13-14 Girls	2060	1.24

*RDA (ICMR, 2011)

Ordinary candies were selected as placebo since they provided only (negligible amounts) 1.04 to 1.31 per cent of the total daily energy expenditure of the children.

2) Nutrient content of Food Supplement

Table VI shows the nutrient content of Nutrient content of food supplement

Table VI
Nutrient Content of Food Supplement

Nutrients	100 g	Per Serving (30g)
Energy (kcal)	350	105
Protein (per cent)	8.5	2.55
Iron(mg)	30	4.098
Calcium(mg)	280	84
Vitamin A (mcg)	250	0.417
Vitamin C(mg)	25	7.5
Vitamin B1 (mcg)	325	97.5
Vitamin B2 (mcg)	625	187.5

One hundred grams of Food supplement provided 350 kcal of energy, 8.5 per cent of protein, 30 mg of iron, 280 mg of calcium, 250 mg of Vitamin A, 25 mcg of vitamin C, 325 mcg of Vitamin B1, and 625 mcg of Vitamin B2. Per serving (30g) showed that it was 105 kcal of energy, 2.55 per cent of protein, 4.098 mg of iron and 84 mg of calcium, 0.417 mcg of Vitamin A, 7.5mg of vitamin C, 97.5 mcg of vitamin B1 and 187.5 mcg of vitamin B2 respectively.

E. Storage stability of Spirulina Food Supplements

Storage stability of a product depends on the ability of the supplement to remain edible during the storage period without bacterial or fungal invasion and without change in colour, texture, flavour, odour, appearance and taste. Since the supplement has to be stored for future use it was tested for shelf life/microbial activity. It has been suggested by Gahalawat and Sehgal, (1993) that supplementary foods should have a shelf life of six months.

The candies being sugar concentrates with negligible amounts of free moisture packed in air tight pouches had a proven shelf life of six months at ambient temperature (National Agro foundation, 2011).

For testing shelf life 30 g of SIFS was packed in triplicates in poly propylene covers and was stored in air tight containers at room temperature. Each set of triplicate was opened at monthly intervals and analysed for total microbial count for a period of six months i.e. both the number of bacterial and fungal colonies was determined by Serial Dilution Technique (AOAC, 1990). Plate count method is based on the principle that when material containing bacteria or yeast and mould is cultured, every viable bacteria develops into a visible colony on a nutrient agar medium and every viable yeast and mould develops into a visible colony on a Malt Agar medium. The numbers of colonies are the same as the number of organisms contained in the sample. The detailed procedure for the estimation of total microbial count is given in Appendix VIII. The health mix powder containing 1.0g of spirulina powder was adjudged the best in terms of overall acceptability by the children and was analysed for its nutrient content and microbial storage safety. It did not develop microbial contamination even at six months and hence was named as Spirulina Incorporated Food Supplement (SIFS) and utilized in the supplement study.

Nutrition Interventions to the Target Groups

A. Selection of PTG Children

Since supplementation involved candies, it was felt that children (<6 years) would find it difficult to swallow them and would probably encounter choking hazard. Hence children in the age group of 7-14 years were included. The number of children selected should neither be excessively large, nor too small. It should be optimum since an optimum size is one which fulfills the requirements of efficiency, representativeness, reliability and flexibility (Kothari, 2006). Two hundred and forty PTG children in the age group of 7-14 years were included for supplementation [four groups (two experimental and two placebos) of 60 each] based on their acceptance, willingness and cooperation. Children were dewormed before starting supplementation. The children were enthusiastic and eager to participate in the supplementation study. At the end of the study excluding the dropouts there were 52 children in E I, 51 children in E II and 32 children each in P I and P II. Tendency to dropout was high among the children because of the nomadic existence of their families.

B. Conduct of Supplementation

The four groups of children, constituting two experimental groups were administered the food supplements (E I- Supplemented with SC and E II with SIFS) and two placebo groups (P I- supplemented with ordinary candies and P II - supplemented with Food Supplement without spirulina powder), for a period of 180 days (Plate 8).

Clinical examination, nutrition anthropometry and biochemical tests were performed on the 167 children before and after supplementation. The impact of supplementation was assessed using the same parameters.

1. Spirulina Candies

Spirulina candies were purchased from Antenna Nutritech, Madurai. Two candies (weighing 3.2g each) containing one gram of spirulina was supplemented per day per child to 52 children in the E I group. Weekly rations of 14 candies were packed and handed over to mothers/caregivers and to older children (beneficiaries) with strict instructions as to their consumption.

Ordinary cane sugar candies were given to the 32 children in the placebo group (P I) (2 per day, on a weekly basis), with strict instructions as to their consumption with stringent monitoring as for the experimental groups by a supervisor.

2. Spirulina Incorporated Food Supplement

Two hundred and ten grams of SIFS was doled out in seven air tight packets (Zip lock covers of 30 g each) for the seven days in a week and given to each of the 51 children in E II group. They were instructed to take 30g of Spirulina Incorporated Food Supplement mixed with 10 ml of water (in the form of ladoos). This was supplemented to 51 children in the E II.

Food Supplement was doled out in air tight packets (Zip lock covers of 30 g each) for the seven days in a week and given to each of the 32 children in P II group. They were instructed to take 30g of Food Supplement mixed with 10ml water (in the form of ladoos).

The Parents/ care givers/ teachers were given strict and clear instructions regarding the distribution of candies, preparation and administration of the SIFS only to the children in E I and E II respectively. Supervisors were allocated from each tribal village to monitor the intake of supplements. Appropriate attendance records were maintained for the purpose. Moreover, the daily consumption of supplements was ensured by strict and careful monitoring by the ANM, Anganwadi Worker and the investigator by regular, surprise and periodic visits.



Spirulina Incorporated Food Supplement



A Tribal boy enjoying SIFS



PTG Children on SIFS



Spirulina Candies



A tribal girl enjoying spirulina candy



PTG Children on spirulina candy

Plate 8. Conduct of supplementation

C. Imparting Nutrition Education

In the present study, nutrition education was given only to mothers, because educating a woman is educating a family. According to Amritha (2008), it offers a great opportunity to them to learn about the essentials for health and take steps to improve the quality of their diets, their well being and influence the attitude and consumption of healthy diet by the children and family as a whole.

According to Sasicoumar *et al.*, (2006), the health issues in tribal villages is due to poor personal hygiene and poor environment. The villagers do not have the right concept of health and its promotion. Nutritional and health education should be imparted in the tribal local dialect preferably using audio-visual materials. Hence, in the present study, nutrition education was given in the local dialect,(Paniya language and Tamil) to 100 mothers drawn from ten different tribal hamlets, over and above the hamlets selected for supplementation with a view to provide intervention with greater coverage. Various educational methods like audio- visual aids, exhibition, Positive Deviance Hearth (PD Hearth) were used to teach the group.

1. Audio- Visual Aids

Charts and posters with pictures denoting do's and don'ts in food preparation, charts regarding personal and environmental hygiene, family health, communicable disease prevention and control were prepared using colourful pictorial depictions to enhance the attractiveness and facilitate easy understanding by viewers.

'Nutrition and Health' (NIN, 2009) is a comprehensive, multilingual and multimedia presentation that attempts to address the basic concerns of a common man on nutrition for a healthy living. It is designed to answer the crucial questions - what to eat, when to eat, how to eat and how much to eat. The presentation used in the study was in the local dialect, namely Tamil. The content in the CD was well structured to enable easy understanding. The information was aligned under four key heads:

- Know your food
- Nutrient requirement and their sources
- Food and Diseases
- Food Safety

2. Nutrition Exhibition

Inexpensive and locally available nutritious food items and recipes rich in iron, beta- carotene, calories, protein and fibre were prepared separately and displayed. Their

nutritional benefits were explained to the tribals who visited the exhibition. Exhibits were put up on nutritional deficiency diseases such as VAD and IDA. Their causes, symptoms, methods of prevention and treatment were explained to them (Plate 9).

A total of 25 sessions were conducted for groups of primitive tribal women, for a period of six months with an interval of seven days.

3. Positive Deviance Hearth

Positive Deviance is a development approach that helps a community and its members find existing, sustainable solutions to a community problem by understanding the behaviors of positive deviant individuals within the community (Child Fund India-NAWA, 2009-10)

The duration of the programme in one tribal hamlet was two hours per day for 12 days, involving children, mothers and care givers. Sessions were scheduled at a time that was convenient to the participants (working mothers/caregivers/pregnant women/adolescent girls).



Plate 9. Nutrition Exhibition



Plate 10. Positive Deviance Hearth

The programme involved de-worming, growth recording and monitoring at the start and end of the session. Training of mothers/care givers to hand-wash their children, nutritious food preparation from locally available affordable items and feeding, integration of health and nutrition education messages and practices were taught to the participants. Sessions were repeated for children who did not rehabilitate in the stipulated time. Referral service was given to sick children who did not gain weight (Plate 10).

D. Raising Kitchen Garden

The main purpose of the kitchen garden as a vegetable production system in the present study was to enhance the availability and consumption of micronutrients, as well as to increase income in areas where there is an insufficient supply of vegetables and fruits.

Some of the tribal women were agricultural labourers who were employed on the fields of other land lords as daily wage earners. The concept of kitchen garden was new to them and was adopted by 100 willing families with small land holdings around their houses from 10 different tribal hamlets.



Seeds Distributed



Imparting know-how on Kitchen Garden



Model Kitchen Garden in Progress



Preparation of soil



Observing the plants grow



Fencing for Kitchen Garden



Interaction with Tribal Women



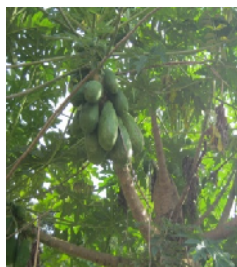
Analyzing the growth of plants



Fruit Harvest



Beetroot



Papaya



Tomato



Bitter gourd



Cabbage

Bumper Harvest

Plate 11. Kitchen Garden

1. Procuring Seeds

Kitchen garden seeds such as carrot, beetroot, yellow pumpkin, beans, tomato, papaya, bitter gourd, bottle gourd, lady's finger, cabbage, fenugreek and spinach were procured from Tamilnadu Agricultural University, Coimbatore and Department of Horticulture, Ootacamund.

2. Developing a Model Kitchen Garden

A kitchen garden model was developed by the investigator with inputs from the Department of Horticulture, Ootacamund with basic vegetation.

The kitchen garden model is appended (Appendix VIII).

3. Laying kitchen Gardens

One hundred kitchen gardens were laid in 10 tribal hamlets. One kitchen garden was laid by the investigator in one area as a model from each tribal hamlet involving all the participants. Since, houses in a tribal hamlet are far flung, ten tribal women from each hamlet were assembled in a local balwadi/ community centre. The women were oriented about the importance of kitchen garden and all the aspects of raising kitchen garden.

The model of the garden was sketched on the black board and explained to all the ten women. They were then given hands on training by involving them in the preparation of land, fencing, sowing of seeds and watering. The seeds were packed separately and distributed to the women for laying 100 kitchen gardens (Plate 11).

Phase V

Impact Evaluation

A. Nutritional Supplementation on

1. Nutritional Anthropometry

Changes in the nutritional anthropometry for all children were assessed every month for six months and recorded.

2. Biochemical Analysis

Changes in the blood hemoglobin level for 45 children were assessed twice once before the start and the other after 180 days of supplementation and prevalence of anemia was assessed.

3. Clinical Examination

The signs and symptoms of nutritional deficiencies were assessed again after six months of supplementation and changes if any were recorded.

4. Dietary Pattern

Frequency of food consumption and details of food and nutrient intake (24 hour food recall method) were collected from the workers after the nutrition education to assess the impact of nutrition education.

B. Nutrition Education and Kitchen Garden on

1. Knowledge Attitude and Practice Scores

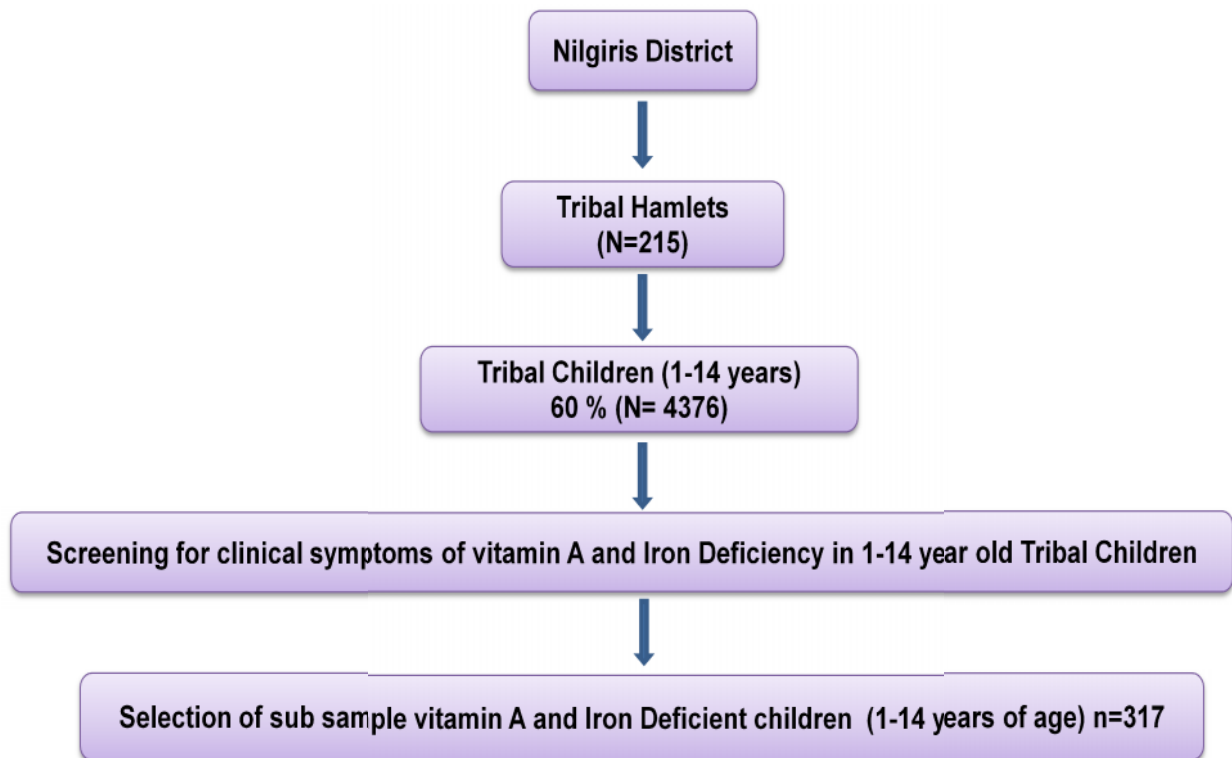
A detailed questionnaire of 60 questions was formulated (Appendix IX) with 20 questions in each aspect related to nutritional knowledge, attitude and dietary practices and data were collected from the mothers and care takers before and after nutrition education. Knowledge aspects comprised of questions regarding knowledge of food and nutrients and their functions. Attitude questions covered the importance of nutrition and need for nutrition education. Questions related to practices mainly comprised of daily dietary intake and practices relating to hygiene and sanitation.

A tentative score was allotted for each question, giving equal weightage. In the case of yes or no questions, the correct alternative was given full scores and the other was given zero. The only exceptions were questions pertaining to practices where a gradation was given with the most desirable practice given full score and the least desirable, graded zero. These final scores were further analyzed statistically to evaluate the KAP level of all the selected mothers and care takers.

The experimental design of the study is given in figure 5.

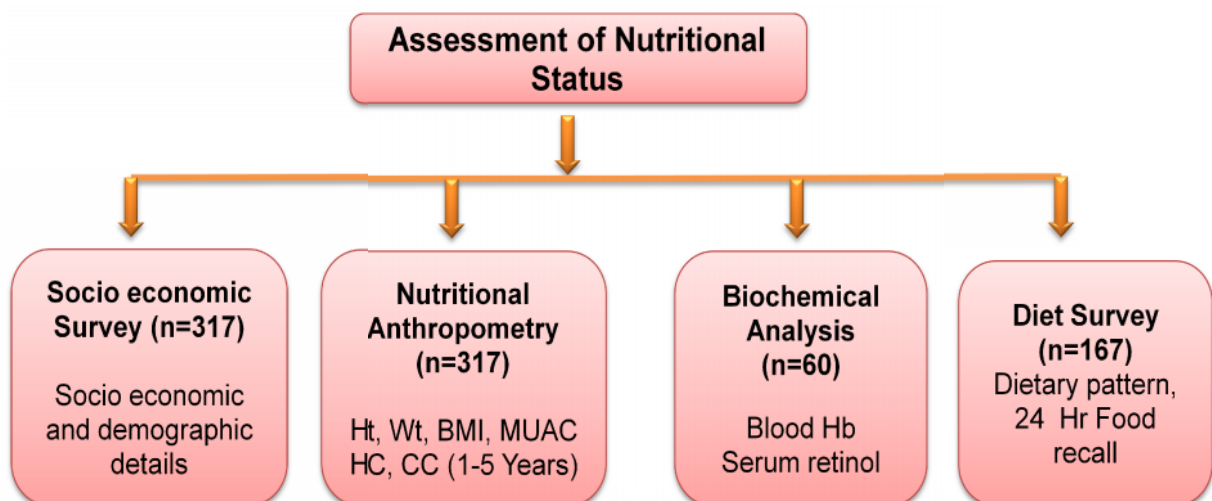
Phase I

Screening of Primitive Tribal Children for VAD and IDA



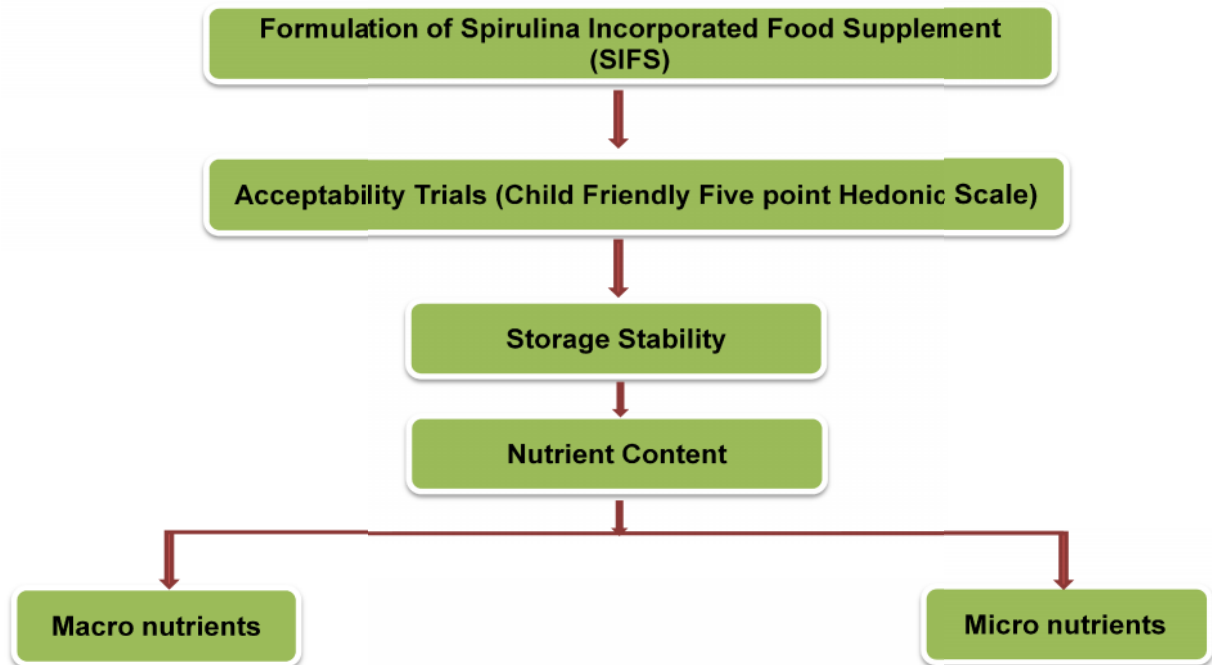
Phase II

Assessment of Socioeconomic and Nutritional Status



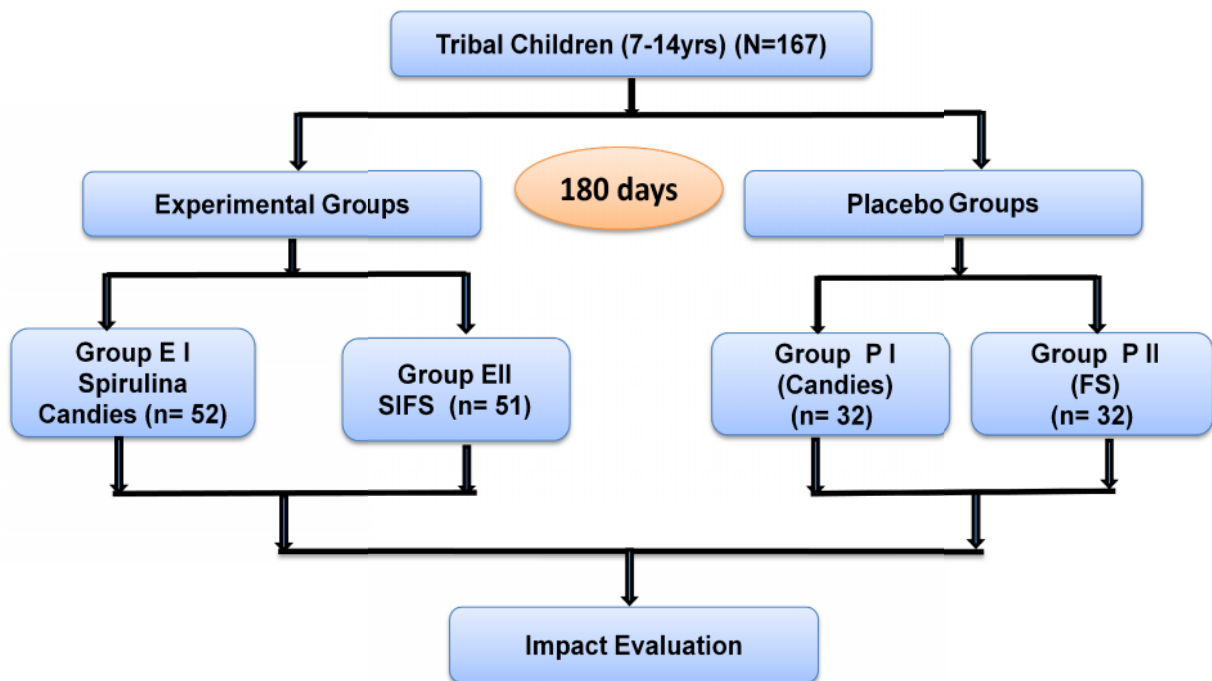
Phase III

Formulation, Acceptability, Nutrient Analysis and Storage Stability of Spirulina Supplements



Phase IV

Supplementation and Impact Evaluation



Phase V

Nutrition Education and Kitchen Garden

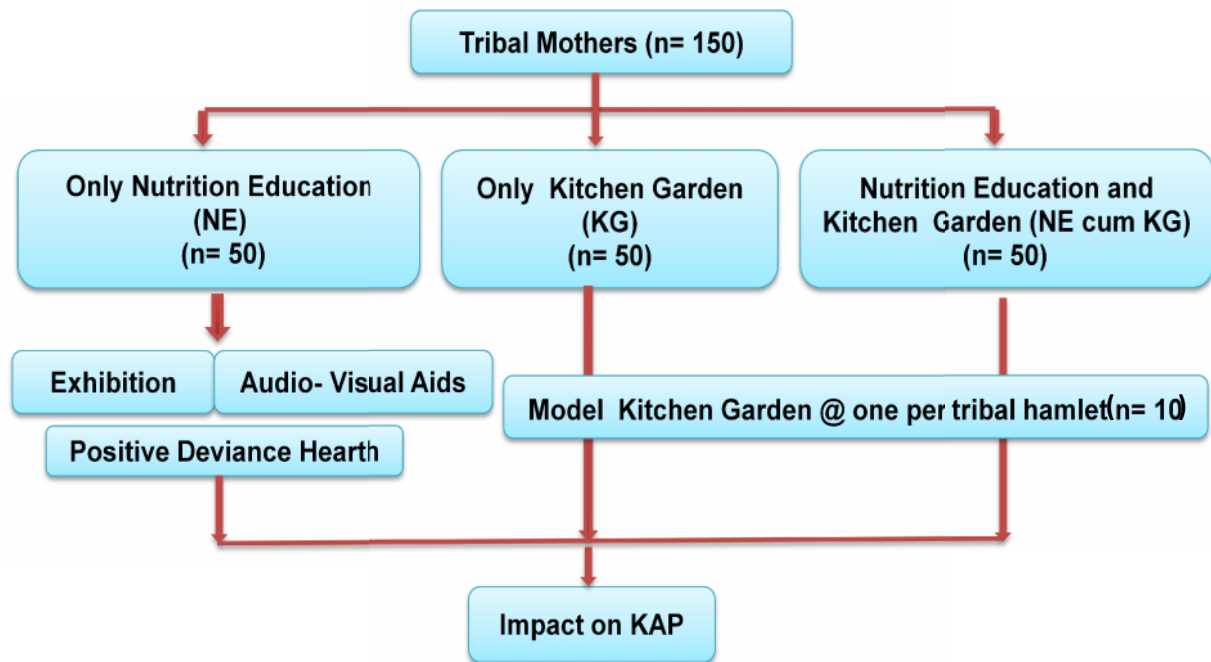


Figure 5

Experimental design of the study