METHODOLOGY

III.METHODOLOGY

The experimental procedure for the present study entitled **"Impact of Nutritious Supplement on Selected Anaemic Adolescents in Coimbatore City, Tamil Nadu"** was carried out in the following phases:

PHASE I: Assessment of Nutritional Status of Adolescent Boys and Girls

A. Selection of Area and Adolescents

B. Collection of Background Information of the Adolescents

C. Assessment of Nutritional Status of the Selected Adolescents

PHASE II: Formulation and Supplementation of Nutritious Supplement

A. Formulation of the supplement

B. Supplementation to the selected underweight anaemic adolescents

C. Impact of Supplementation

PHASE III: Imparting and Evaluating Health Oriented Education Programme (HOEP)

A. Development of Health Oriented Education Programme (HOEP)

B. Administration of HOEP

C. Assessing the impact of HOEP

D. Analysis and interpretation of data

PHASE I: Assessment of Nutritional Status of Adolescent Boys and Girls

A. Selection of Area and Adolescents

The study was conducted in schools located in semi urban areas of Coimbatore, Tamil Nadu. Three government and seven private schools in three semi urban areas namely Kavudampalayam, Ashokapuram and Vellakinaru were selected. These schools were selected because most of the school going adolescents comes from different income groups. The authorities extended their support and were accessible. No such research was conducted previously in these schools.

Among four government and ten private schools, three government and seven private schools were selected based on permission granted to the investigator from the concerned authorities. These schools were selected by convenience sampling technique. Convenient sampling is a procedure comprising the sample, which are simply available in a convenient way to the researcher (Kothari, 2001). Figure 1 presents the selection of schools for the present study.

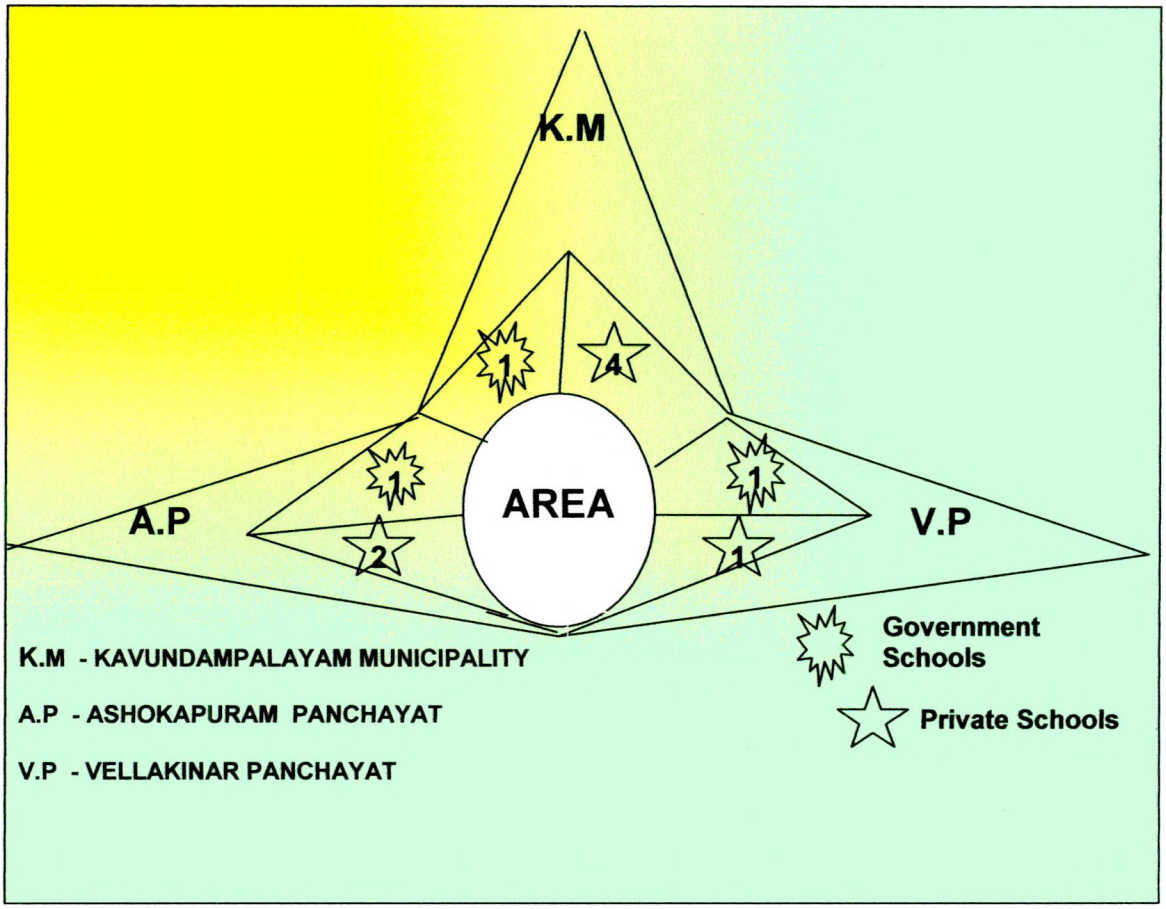

Figure 1

SELECTION OF AREA

A total of 3166 adolescents were selected for the study using purposive sampling method. Krishnaswami (1999) states that this method involves purposive or deliberate selection of particular unit of the universe. These selected adolescents were drawn as a cluster in the age group of 10 to18 years, from the class of 6^{th} to 12^{th} standards, 1823 (57.5%) were boys and 1343 (42.5%) were girls (Figure 2).

Table I shows the list of schools and adolescents selected for the study

TABLE I

List of Schools and the Adolescents Selected for the Study

SCHOOL	NO. OF ADOLESCENTS (N=3166)			
	Boys (N=1823)		Girls (N=1343)	
Private School (PS)	**N**	%	**N**	%
PS **I**	94	**3**	71	**2**
PS **II**	109	**3**	114	**4**
PS **III**	109	**3**	87	**3**
PS **IV**	218	**7**	135	**4**
PS **V**	73	**2**	96	**3**
PS **VI**	144	**5**	176	**6**
PS **VII**	102	**3**	91	**3**
Government School (GS)				
GS **I**	284	**7**	203	**6**
GS **II**	386	**12**	164	**5**
GS **III**	354	**11**	206	**7**

B. Collection of Back ground Information

To collect details on the socio economic background of the selected adolescents an interview schedule was framed. Patnaick (2000) refer an interview schedule as a set of question in a questionnaire. Background information such as age, sex, type of family, occupation and education of the parents and lifestyle pattern such as physical activity, leisure time activity, eating while watching T.V. Dietary pattern such as type of food consumed, meal pattern, skipping of meals, habit of dinning outside, snacking pattern, consumption of hot beverages, frequency of consuming fleshy foods of the selected adolescents was elicited using an interview schedule these information was personally collected by the investigator (Appendix I).

C. Assessment of Nutritional Status of the Selected Adolescents

The principal aim of assessing the nutritional status of a community is to map out the magnitude and geographical distribution of malnutrition as a public health problem (Jelliffe and Jelliffe, 1989).

Nutritional status usually cannot be determined from a single measurement alone (Gopalan, 1998). Three pronged approach comprising of dietary, biochemical and clinical components including the anthropometry component is suggested by Sachdev and Chaudhari (1994) for the effective assessment of nutritional status.

The assessment of nutritional status was carried out using the following parameters

1. Measurement of Body Mass Index
2. Clinical examination
3. Estimation of blood haemoglobin and
4. 24 hour dietary recall method

1. Measurement of Body Mass Index

Growth assessment is the best single measure to define the health and nutritional status of school children. There is a definite relationship between children's age, weight and height deviation from the established relationship which reflects negligence in the up bringing of the adolescent (Anwer and Awan, 2003). To arrive at the nutritional assessment indices body adiposity such as height and weight was taken for the selected adolescents (3166) using standardized procedures (Brahamam et al., 2005).

a. Height

Height is the best stand by measurement of skeletal growth (Saureborn et al., 1993) The linear measurement of the selected adolescents was measured using a stadiometer. The adolescents were asked to remove the shoes and stand against the stadiometer touching the heels, buttocks, shoulders and back of the head. The head was held comfortably erect, upper limbs hanging closely to the sides of the body. The investigator stood on the left side of the selected adolescent and height was recorded to the nearest 0.1cm using a scale which was used to pin point the point reached by the head.

b. Weight

Weight was measured to nearest of 0.5kg in the standard portable bathroom scale on which the selected adolescent was asked to stand barefoot, erect without touching anything nearby.

Body mass index (BMI) for age correlates with other measures of health risk. Body mass index for the present study was also carried out in the same manner using the formula suggested by WHO (1995).

$$\text{Body Mass Index (BMI)} = \frac{\text{Weight (Kg)}}{\text{Height (M}^2\text{)}}$$

The selected 3166 adolescents were categorized according to nutritional status based on BMI as suggested by NHANES I* (Figure2). BMI for age /sex centile is given below

BMI FOR AGE /SEX CENTILE	NUTRITIONAL STATUS
<5th centile	Underweight
5 to15th centile	Risk of underweight
15th to85th centile	Normal weight
85th to 95th centile	Overweight
>95th centile	Obese

*National Health and Nutrition Examination Survey (NHANES 1, 2004).

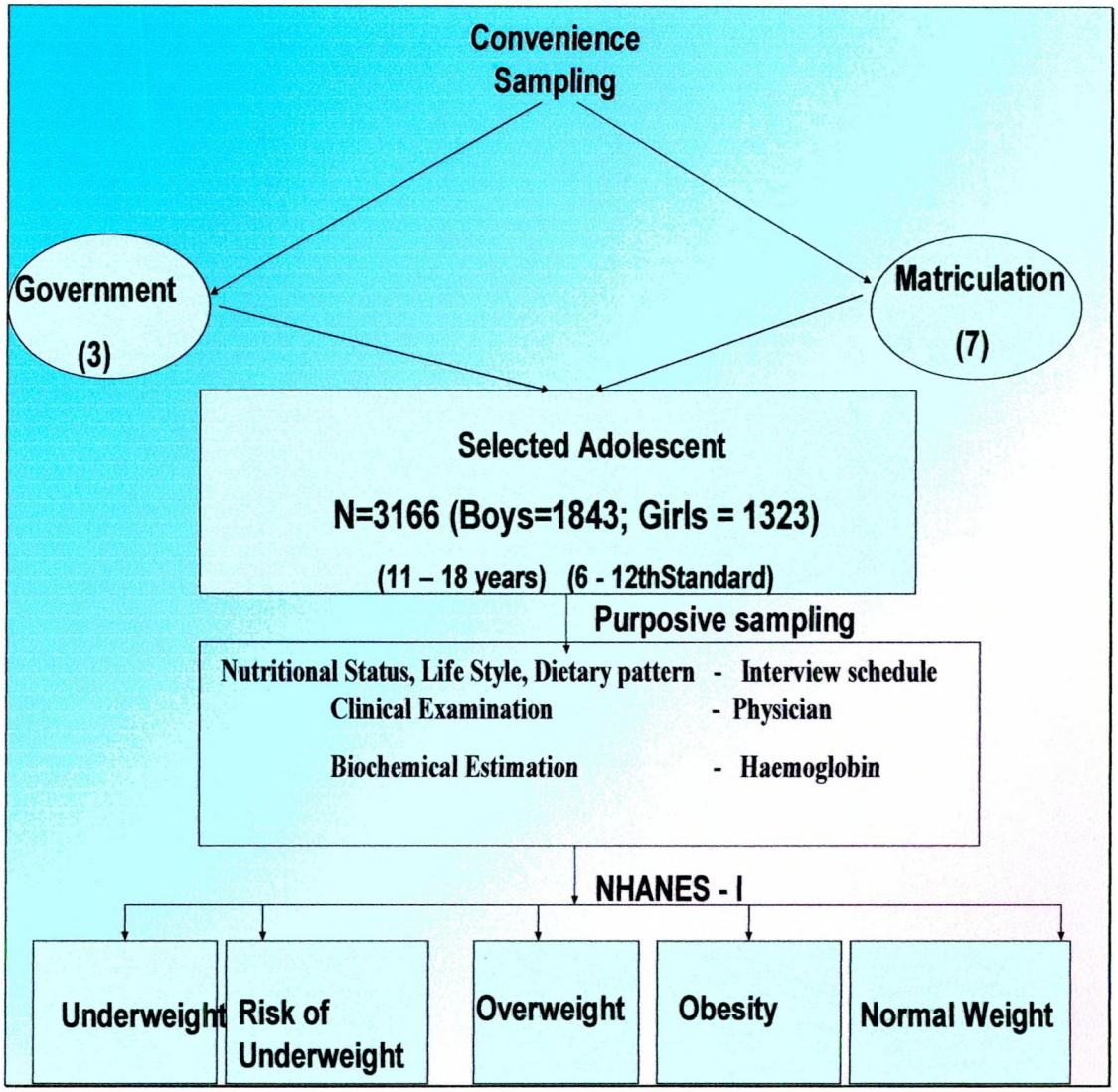

Figure 2

SELECTION OF ADOLESCENTS

2. Clinical Examination

Clinical examination has always been and remains a widely used practical, direct method to assess the nutritional status of an individual (Jelliffe and Jelliffe, 1989). It involves looking for changes in the body which are indicative of a particular deficiency. Using the ICMR clinical assessment proforma (Appendix II), health check up was carried out for all the selected adolescents with the help of local physician to screen for the presence of infection or disease if any. The investigator identified the obvious signs and symptoms of anaemia.

3. Estimation of Blood Haemoglobin

Biochemical assessment is considered as the most effective and objective for assessing the sub clinical state of deficiency due to lowered intake or absorption or impaired transport or abnormal utilization of nutrients (Bert, 1998). They also provide an accurate indication of the nature of short term nutritional problems (Foster, 1992).

Haemoglobin concentration (Hb) is widely used to measure for assessing iron deficiency anaemia (UNICEF and WHO, 1999). International Committee for Standardization Haematology (ICSH) recommends the cyamethaemoglobin (CMH) method, a standard for estimation of haemoglobin in different types of anaemia.

The blood haemoglobin was estimated for the selected adolescents who belonged to the underweight category which was assessed using the BMI. So the investigator analyzed blood haemoglobin levels for all the selected underweight adolescents (Boys= 819, Girls= 512) using the cyamethaemoglobin method. On the basis of blood haemoglobin values, the adolescents were classified as moderate anaemic, mild anaemic and non anaemic (WHO, 1989).

4. Eliciting Details on Dietary Pattern and Assessing Mean Food and Nutrient Intake of the Selected Adolescents

The details of food consumption by the selected adolescents are very important for diagnosing the nutritional status. Dietary survey provides essential information on nutrient intake levels, source of nutrients, food habits, attributes on special condition and diseases. The mean nutrient intake of these subjects was assessed by 24 hour recall method for three consecutive days. This formed the basis for formulating the supplement for the intervention programme.

Dietary adequacy of the selected sub sample in the supplemented group (underweight moderate and mild anaemic adolescents N=295) and non supplemented group (192 underweight anaemic adolescents, 179 underweight adolescents, 285 risk of underweight adolescents N=656). was determined quantitatively by 24 hour recall diet survey for three consecutive days. All the food items consumed by the selected adolescents during the previous day (including those cooked in the house or as well as from out side) was recorded in the diet survey form as shown in Appendix III. Ravikumar et al., (1999) reported that 24 hour recall method has been the procedure used for the several studies.

The success of 24 hour recall method depends on the memory, co- operation and communication ability of the selected subject and skill of the investigator (Walter, 2004). Various types of standardized utensils were used to assist in recalling and assessing the quantity of food consumed. From the weight of raw equivalent of the cooked food consumed, the nutrient intake was determined as per standard criteria using the ICMR food composition tables (Gopalan et al., 2002).

PHASE II: Formulation and supplementation of nutritious supplement

A. Formulation of the Supplement

An ideal food for prevention and control of under nutrition should be of high nutritive value, acceptable, readily available at economical price, familiar to the community and have good tolerance both in illness and good health (Swaminathan, 1999). Totally ten variations were formulated using different ingredients like deoiled coconut meal flour, rice flakes flour, defatted soya flour, ragi flour, samai flour, garden cress seeds and whole wheat flour. The proportions of the ingredients were chosen such that iron and protein were high. Deoiled coconut meal (cocous nucifera) was used as the major ingredient for the formulation of the supplement, because deoiled coconut meal has high energy density, high in iron (69.4mg), protein (23.8g) and fibre (9.8g) besides being rich in carbohydrates (47.9g) and phosphorus (649mg) (Gopalan et al., 2002). Rice flakes a traditional rice based product of India is rich in iron (20mg) and calories (346 kcals) and defatted soya flour which has high quality protein (40 g) and it is an excellent source of iron, calcium and vitamin B (Mariani, 1986). With its easily digestible (Ihekoringe and Ngoddy, 1985), it is also rich in lysine and low in sulphur amino acids were the other ingredients used for the supplement. Refined wheat flour, powdered sugar and fat were used along with other ingredients for preparing the supplement.

Deoiled coconut meal which was hexane free was procured from oil mills in Coimbatore and Kangayam, Tamilnadu. The meal available as thin flakes was shade dried for 5 to 7 days and finally powdered using micropulverizer. Rice flakes were powdered separately to fine texture. By changing the proportion of the ingredients, ten variations were finally selected for acceptability trials.

The composition of the ten variations is presented in Table II.

Table II

Composition of the Ten Selected Variations

INGREDIENTS	VARIATIONS IN AMOUNT(g)									
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
Deoiled coconut meal flour	25.0	22.5	20.0	20.0	**20.0**	17.5	17.5	17.5	15.0	14.0
Rice flakes flour	15.0	15.0	20.0	15.0	**15.0**	17.5	17.5	17.5	25.0	6.0
Defatted Soya flour	2.5	5.0	2.5	2.5	**7.5**	7.5	5.0	7.5	2.5	22.5
Refined wheat flour	7.5	7.5	7.5	7.5	**7.5**	7.5	7.5	-	7.5	7.5
Powdered sugar	25.0	25.0	25.0	25.0	**25.0**	25.0	25.0	25.0	25.0	25.0
Fat	25.0	25.0	25.0	25.0	**25.0**	25.0	25.0	25.0	25.0	25.0
Garden cress seeds powder	-	-	-	-	-	-	2.5	-	-	-
Whole wheat flour	-	-	-	-	-	-	-	7.5	-	-
Ragi flour	-	-	-	5.0	-	-	-	-	-	-
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

All the ingredients were evenly mixed with powdered sugar, fat and biscuits were prepared. They were subjected to sensory evaluation.

1. Need for Selecting Biscuit as Nutritious Supplement

Adolescents tend to eat differently than they did as children. With school activities and active social events, teens tend to lead to meal skipping, snacking throughout the day and eating more away from the home (Duryff and Larson, 2002).

Biscuits are capable of being enriched with additional proteins, vitamins and minerals to enhance their nutritive value. They are suited for promotion of protein and iron rich foods for middle and low income group (Fox and Cameron, 1999).

Bakery products are gaining extreme popularity as processed foods which offer ready to eat convenience as well as have comparatively long shelf life. Biscuits are more convenient and are ideally suited for storage and distribution to a large number of people. For the present study biscuits were prepared by traditional "creamery method" described by Whitley (1970).

2. Nutritive Value of the Supplement

All the raw ingredients used in ten variations were weighed accurately and the energy, protein, fat, carbohydrates, iron and fibre contents were calculated using the nutritive value of Indian foods (Gopalan et al., 2002). Nutrient content is shown in Table III.

Table III

Nutrient Content of the Supplement

VARIATIONS	AMT (g)	ENERGY (Kcals)	PROTEIN (g)	FAT (g)	CARBO-HYDRATES (g)	IRON (mg)	FIBRE (g)
I	100	489	9	26	55	21	3
II	100	489	10	26	55	19	3
III	100	490	8	26	56	18	2
IV	100	489	8	26	56	18	2
V	**100**	**490**	**10**	**26**	**54**	**18**	**2**
VI	100	490	10	26	55	17	2
VII	100	492	9	26	55	19	2
VIII	100	490	10	26	55	17	2
IX	100	492	7	26	58	16	2
X	100	490	15	26	42	13	2

3. Sensory Evaluation and Chemical Analysis of the Supplement

A quality of a food product is assessed by means of human sensory organs, the evaluation is said to be sensory or subjective or organoleptic (www.food.sci.uoguelph)

Organoleptic evaluations of the ten variations of biscuits were carried out by a panel of 10 judges. The judges included the staff members and research scholars of Avinashilingam University for Women, Coimbatore.

All the ten variations of biscuits were given different code numbers. Each panel judges were given three biscuits per sample to assess. The quality factors such as colour, flavour, texture, taste and overall acceptability using 5 point hedonic structure scale followed by Ihekoringe and Ngoddy (1985) was adopted for scoring the biscuits as shown in Appendix IV. The scoring scales were 5=Very Good, 4=Good, 3=Fair, 2=Poor and 1=Very Poor (Amerine et al., 1973).

The panel judges were provided with clean tap water to rinse their mouth after tasting each type of variations. Room temperature was maintained throughout the testing session.

The mean scores obtained by the panel judges for the ten variations is presented in Table IV.

Table IV

Comparison of Mean Scores Obtained for Different Variations (Mean ±SD)

Variations	APPEARANCE	FLAVOUR	TASTE	TEXTURE	OVERALL ACCEPTABILITY
I	2.80 ± 0.42	2.50±0.53	2.90± 0.57	2.30± 0.82	2.62 ± 0.58
II	2.70±0.48	2.70 ±0.67	2.80 ± 0.63	3.00 ± 0.47	2.80 ± 0.56
III	2.90 ±0.74	2.90± 0.74	2.40 ± 0.70	2.80 ± 0.42	2.75 ± 0.65
IV	3.00±0.47	2.50±0.85	2.20± 0.79	2.90 ± 0.32	2.65±0.60
V	**4.10± 0.74**	**3.90± 0.74**	**4.10± 0.74**	**4.10 ± 0.74**	**4.05 ± 0.74**
VI	3.40±0.70	2.80±0.90	2.60 ±0.90	3.20 ± 0.42	3.00±0.73
VII	3.80±0.63	2.40±0.97	2.70±0.67	3.00 ± 0.47	2.97 ±0.68
VIII	2.80 ±0.42	3.30 ± 0.67	2.90±0.57	2.60 ± 0.70	2.90 ±0.59
IX	2.50± 0.53	2.70 ±0.67	2.80 ±0.42	2.80 ± 0.63	2.70 ± 0.56
X	2.90 ±0.74	3.00 ±.47	3.00 ±0.47	3.00 ± 0.47	2.97± 0.53

Out of the ten variations tested, **variation V** obtained high mean scores and was found to be the most acceptable variation. The selected variation was analysed in the laboratory for protein (Macrokjeldhal method), fat (Volumetric Gerber's method), carbohydrates (Anthrone method), iron (NIN method), crude fibre (AOAC method,1995) and calorie (Atwater method) contents.The total procedure adopted for chemical analysis is given in Appendix V.

B. Supplementation to the Selected Underweight Anaemic Adolescents

1. Selection of Sub Sample

Supplementation of the selected **variation V** was administered to 114 underweight moderate anaemic and 181 underweight mild anaemic adolescents. Hundred grams (8 No) of biscuits were provided to the selected underweight anaemic adolescents daily for a period of three months. Four biscuits were given during mid morning and four in the mid afternoon during the school interval time. Raivard et al., (2006) reported that daily iron supplementation is able to correct iron deficiency much more than twice / weekly in adolescents.

Due to the full co-operation rendered by the school authorities and teachers, the packed biscuits were distributed to the class teacher concerned. The selected sub sample was requested to collect the biscuits from the teachers during the interval period.

Kumari et al., (2004) reported that foods rich in vitamin C such as oranges, tomatoes, amla and guava would help in the absorption of iron from plant foods. Jialal (2003) study suggested that iron absorption increases with increase in vitamin C intake especially 25 to 50 mg / day. Brock (2002) proved that ascorbic acid increases iron absorption 2 to 3 folds. Hence the investigator also provided amla (20g to 25g) which provides 100 to 120 mg of ascorbic acid to the sub sample daily to increase the bio availability of iron.

Since worm infection is very common among Indian school children and is known to affect haemoglobin levels, the selected sub sample were given recommended doses of albendozle tablet as suggested by Kulkarni and Sathey (1981) prior to the feeding trail with due consultation of the physician .

In order to avoid monotony in the supplement, different flavours like pineapple, coconut, vanilla, badam were used in biscuit preparation. The entire selected sub sample participated enthusiastically in the supplementation programme.

Orientation programme on importance of nutrition and sound health was given to the selected sub sample to prevent dropouts from the study. Before supplementation due permission was obtained from the parents of the experimental group and attendance was maintained and the investigator was vigilant for ensuring regular consumption of the biscuits by the sample throughout the study period.

Those who could participate regularly alone served as a sub sample for the final evaluation. There were no dropouts in the final study because it was carried out from the month of September to November and January to March. During holidays the selected adolescents were advised to take biscuits at home during mid morning and evening. These biscuits were packet in separate packets for a day and these adolescents were advised to eat them regularly. During the supplementation period normal home diet was followed by the selected sub sample.

2. Impact of Supplementation

The impact of supplementation for the target group namely underweight mild anaemic (N=114) and underweight moderate anaemic (N=181) was studied using the following parameters before and after supplementation.

Anthropometric measurements like height and weight, clinical examination and blood haemoglobin was carried for selected 295 underweight anaemic adolescents. Biochemical parameters like serum iron, serum ferritin, total iron binding capacity, transferrin saturation, serum total protein, serum albumin and serum globulin was carried out only for 103 (35 %) of the selected adolescents because only this group was co-operative for offering their serum samples for biochemical estimation.

Table VII shows the biochemical estimation adopted to find the impact of supplementation

Table VII

Biochemical Estimation Adopted For the Impact of the Supplementation

BIOCHEMICAL ESTIMATION	METHODS ADOPATED	NORMAL VALUES (Deb, 2000).
Serum Ferritin	RA Auto Analyzer-50	>12 µg/dl
Serum Iron	RA Auto Analyzer-50	50-175 µg/dl
Total Iron Binding Capacity (TIBC)	RA Auto Analyzer-50	200-450 µg/dl.
Transferrin Saturation	$T.S = \frac{\text{SerumIron}}{\text{TIBC}} \times 100\mu g/dl$	35 percent and above
Serum Total Protein	RA Auto Analyzer-50	6.8-8.3g/dl
Serum Albumin	RA Auto Analyzer-50	3.7-5.8g/dl
Serum Globulin	RA Auto Analyzer-50	2.2-3.0g/dl

PHASE III: Imparting and Evaluating Health Oriented Education Programme (HOEP)

A. Development of Health Oriented Education Programme (HOEP)

According to WHO, today's emphasis is on health expectancy rather than life expectancy. If today's children fall a victim to food and life style diseases, it is a national loss and it has to be checked as early as possible (Mishra, 2006).

School children in India are most often fast generation learners and can be trained to be "change agents" of the community to increase their nutritional status and combat nutritional deficiency diseases in the community. School programmes will have greater impact and be sustained longer if they are tied to community activities, programmes and other private and non-governmental organizations.

An educational tool namely (HOEP) was developed by the investigator. HOEP materials such as posters, charts, rotating charts, hand outs and power point presentations were developed and were used to create awareness regarding good health, ways and means of combating nutritional deficiencies and sound eating habits. These included posters, food pyramid for healthy adolescents, leaflets providing messages of anaemia and underweight, charts highlighting consequences of anaemia, underweight, protein energy malnutrition, niacin deficiency, thiamine deficiency, vitamin A deficiency, calcium deficiency and so on. Software showing importance and consequences of underweight, anaemia and healthy life style (Appendix VI) was also created.

The HOEP also emphasized on basic nutrition concepts and demonstrated low cost nutritious recipes. Lectures and discussions were held for the sub sample. Interactive session was held during the Parents – Teachers Meeting. In the case of government schools the parents were requested to come for the programme. Special care was taken to pass on the message to the concerned parents through the prepared visual aids.

Teachers were also trained for this interpersonal communication. Life style education was imparted to them and they acted as "Agent Communicators" during the intervention period.

A medical, a dental and an eye camp was organized in three schools as an incentive to the school children and the school authorities for extending their co-operation. Individual attention and care was taken to check the selected underweight anaemic adolescents and medical advice was given by the doctors.

B. Administration of HOEP

HOEP was imparted to the supplemented group (underweight moderate and mild anaemic adolescents N=295) and non supplemented group (192 underweight anaemic adolescents, 179 underweight adolescents, 285 risk of underweight adolescents N=656). Using the audio visual aids prepared HOEP was imparted in the groups in their respective class room in every 15 days for a period of two months.

Lecturing was minimized and other methods that bring out creative interaction were adopted to make learning continuous. Participatory approach, instructional approach and child to child approach were followed while teaching.

1. Participatory Approach (PA)

An informal atmosphere was created which involved learning by question and answers, talking and writing on the class room board. Priority was given to the message on improving the dietary practices, intake of food rich in nutrients like protein, iron, calories, vitamin C and fiber.

2 Instructional Approach (IA)

The Instructional approach was made functional to sustain interest in selected adolescents through charts, posters, leaflets, pictures, rotating charts and CDs involving information on nutrition deficiency diseases, importance of nutrients rich food in diet and maintaining good nutritional status.

3. Child to Child Approach (CCA)

Here the selected adolescents learned simple preventive measures related to nutrition and health problems appropriate to the community. They pass on what they learn, to their siblings, parents and friends as well as to their neighbours.

C. Assessing the Impact of HOEP

1. Pre and Post Test

Multiple choice questions (Appendix VII) were prepared to find the pre and post knowledge, attitude and practices of the selected adolescents. Scores were allotted for each question and the impact was assessed.

Scoring was done on the following basis. Scores of one, half and zero were awarded respectively to each correct, partially correct and wrong answer. Gain in scores and percent improvement was calculated using the following equation.

$$\text{Gain in scores} = \text{Scores of post test - Scores of pre test}$$

$$\text{Percentage of improvement} = \frac{\text{Gain in scores}}{\text{Pretest scores}} \times 100$$

Food and nutrient intake was calculated using 24 hour food recall method for three consecutive days both before and after counselling to evaluate the impact.

D. Analysis and Interpretation of Data

The collected data were consolidated and statistically analyzed by SPSS 11.5 Version. Paired't'test was used to find the impact of supplementation and HOEP.