The methodology involved in this study titled “Haemopoietic Micro-nutrient Fortified Rice on the Nutritional Status and Cognitive Performance of Anaemic Adolescents” consist the following steps:

A. SELECTION OF LOCALE

B. SELECTION OF THE PARTICIPANTS (ADOLESCENTS)

C. COLLECTION OF SOCIO-DEMOGRAPHIC PROFILE OF THE ADOLESCENTS

D. ASSESSMENT OF FOOD AND NUTRIENT INTAKE BY THE ADOLESCENTS

E. EVOLVING THE INTERVENTION (FORTIFIED RICE)
   E.1. Rationale for the composition of rice premix
   E.2. Procurement of rice premix and preparation of fortified rice
       E.2.a. Nutrient availability of the rice premix
   E.3. Ethical clearance
   E.4. Details of rice premix and fortified rice
       E.4.a. Acceptability trial
       E.4.b. Cooking quality
       E.4.c. Shelf life
       E.4.d. Enhancers and inhibitors of iron absorption
       E.4.e. Bioaccessibility of iron
   E.5. Feeding of fortified rice
       E.5.a. Random assignment of anaemic adolescents
       E.5.b. Deworming of all the anaemic adolescents
       E.5.c. Preparation and serving of meals

F. IMPACT OF INTERVENTION
   F.1. Anthropometric measurements
       F.1.a. Recording body Weight
Methodology

F.1.b. Recording Height
F.1.c. Computation of Body Mass Index (BMI)

F.2. Determination of Biochemical Parameters
F.2.a. Estimation of Haemoglobin
F.2.b. Estimation of Serum ferritin
F.2.c. Estimation of serum iron
F.2.d. Estimation of Total Iron Binding Capacity (TIBC)
F.2.e. Computation of Transferrin saturation
F.2.f. Estimation of serum folate
F.2.g. Estimation of C - Reactive Protein (CRP)

F.3. Clinical Examination of the anaemic adolescents

F.4. Cognitive performance of the anaemic adolescents
F.4.a. Assessing the intelligence
F.4.b. Assessing the memory status
F.4.c. Assessing the attention and concentration
F.4.d. Assessing the academic performance

F.5. Recording the Prevalence of Acute Morbidity

G. CONSOLIDATION AND STATISTICAL ANALYSIS OF THE DATA

A. SELECTION OF LOCALE

Micro-nutrient malnutrition is a major public health problem. The extent and magnitude of this problem varies considerably depending on the socioeconomic status of the affected population groups and geographical location.

The study was carried out in a rural area in Uthiramerur taluk, Kancheepuram district of Tamilnadu (Figure 5) for the following reasons;

1. Permission and co-operation of the Correspondent, Head Master and Hostel Warden of the School and

2. Consent of the parents of adolescents in the school to participate in the study and the assent of the participants.
B. SELECTION OF THE PARTICIPANTS (ADOLESCENTS)

A total of 250 adolescents in the age group of 10-18 years residing in the hostel and studying in standard VI to XII were screened for blood haemoglobin levels determined by gold standard cyanmethaemoglobin method and results were compared with WHO (2011) cut off values for classifying anaemia. The haemoglobin status showed that 88 per cent (220) of the adolescents were anemic. Out of 220, ten were from class XII who will not be present for a period.
of one year. Seven of them showed poor attendance and two of them dropped from the school making finally the total number of participants as 201.

C. COLLECTION OF SOCIO-DEMOGRAPHIC PROFILE OF THE ADOLESCENTS

A proforma on general information (Appendix-I) consisting of name, sex, date of birth, number of siblings, size of the family, parents' educational and occupational details, family income, birth order of the adolescents was administered to the parents of all the selected adolescents and the information was collected by interview schedule.

D. ASSESSMENT OF FOOD AND NUTRIENT INTAKE BY THE ADOLESCENTS

Haemopoietic micro-nutrients are iron, zinc, folate, pyridoxin, cyanocobalamin, ascorbic acid, riboflavin and vitamin A. Out of these iron, zinc, folate and vitamin B₁₂ were considered for the fortification.

Dietary survey of food weighment method was employed in order to obtain data on the food and nutrient intake of the adolescents for seven consecutive days as the menu in the hostel was seven days cycle menu. It was carried out on a sub sample of 24 (12%) of selected adolescents belonging to different age groups and both gender (10 to12 years Boys-4, Girls-4, 13 to15 years Boys-4, Girls-4 and 16-18 years Boys-4, Girls -4).

The raw weight of all the ingredients used for the preparation, cooked weight of the food, the amount served to the individuals and leftovers on the individual’s plate were weighed and recorded to obtain data on individual food intake. From the food intake, the raw ingredients consumed by the individuals was calculated using the formula,

\[
\frac{\text{Cooked food consumed by the individual} \times \text{Total raw ingredients used for cooking}}{\text{Total cooked food weight}}
\]

From the raw ingredient, the nutrient intake was calculated using the Table (Gopalan et al., 2007). The mean nutrient intake was compared with the RDA (NIHFW, 2012) to assess the deficit in the diet. The details are presented in Table IV.
### TABLE IV

**MEAN MICRO-NUTRIENT INTAKE BY THE ANAEMIC ADOLESCENTS BEFORE THE INTERVENTION**

(N=24)

<table>
<thead>
<tr>
<th>Age in Years</th>
<th>Gender</th>
<th>Iron (mg)</th>
<th></th>
<th>Zinc (mg)</th>
<th></th>
<th>Folate (mcg)</th>
<th></th>
<th>Vitamin B₁₂ (mcg)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RDA*</td>
<td>Al</td>
<td>Deficit</td>
<td>RDA*</td>
<td>Al</td>
<td>Deficit</td>
<td>RDA*</td>
<td>Al</td>
</tr>
<tr>
<td>10-12</td>
<td>Boys (4)</td>
<td>21</td>
<td>7.8</td>
<td>13.2</td>
<td>9</td>
<td>5.9</td>
<td>3.1</td>
<td>140</td>
<td>138</td>
</tr>
<tr>
<td></td>
<td>Girls (4)</td>
<td>27</td>
<td>6.5</td>
<td>20.5</td>
<td>9</td>
<td>4.9</td>
<td>4.1</td>
<td>140</td>
<td>126</td>
</tr>
<tr>
<td>13-15</td>
<td>Boys (4)</td>
<td>32</td>
<td>8.5</td>
<td>23.5</td>
<td>11</td>
<td>6.4</td>
<td>4.6</td>
<td>150</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Girls (4)</td>
<td>27</td>
<td>7.1</td>
<td>19.9</td>
<td>11</td>
<td>5.3</td>
<td>5.7</td>
<td>150</td>
<td>128</td>
</tr>
<tr>
<td>16-18</td>
<td>Boys (4)</td>
<td>28</td>
<td>9.0</td>
<td>19.0</td>
<td>12</td>
<td>7.0</td>
<td>5.0</td>
<td>200</td>
<td>146</td>
</tr>
<tr>
<td></td>
<td>Girls (4)</td>
<td>26</td>
<td>8.5</td>
<td>17.5</td>
<td>12</td>
<td>6.5</td>
<td>5.5</td>
<td>200</td>
<td>135</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>26.8</td>
<td>7.90 (30%)</td>
<td>18.9 (70%)</td>
<td>10.7</td>
<td>6.0 (56%)</td>
<td>4.7 (44%)</td>
<td>163</td>
<td>135 (83%)</td>
</tr>
</tbody>
</table>

*Recommended Dietary Allowance (ICMR, 2010), Al - Actual intake
†Upper limit of RDA was considered as cut off for calculation of vitamin B₁₂ deficit
The mean intake of iron, zinc, folate, vitamin $B_{12}$ by the anaemic adolescents was found to be 7.9 mg, 6.0 mg, 135 mcg and 0.98 mcg respectively.

E. EVOLVING THE INTERVENTION (FORTIFIED RICE)

E.1 Rationale for the composition of Rice premix

The mean RDA of iron, zinc, folate and vitamin $B_{12}$ for Indian adolescents in the age range of 10 to 18 years is 26.8 mg, 10.7 mg, 163 mcg and 0.2-1 mcg respectively and the nutrient intake by the food weighment survey was estimated to be 7.9 mg, 6.0 mg, 135 mcg, and 0.98 mcg respectively. A gap of 18.9 mg, 4.7 mg, 28 mcg and 0.02 mcg of iron, zinc, folate and vitamin $B_{12}$ was planned to be filled through the supplementing fortified rice. As the day scholars would also be participating in the noon meal, the breakfast was selected for feeding with fortified rice.

Sample of the regular rice which was used in the school was sent to the manufacturer of rice premix to avoid differences in appearance of the rice after cooking.

E.2. Procurement of Rice premix and Preparation of fortified rice

Iron deficiency anaemia is the most serious public health problem among micro-nutrient deficiencies. Rice is the staple food for more than half of the population in India. Therefore fortification of rice with micro-nutrients could make some sense to address the problem (Bamji, 2011). Fortification of wheat and rice is commonly practiced in many countries as a cost effective strategy to control and prevent the problem. One of the available technologies for fortifying rice is using Rice premix\ultra Rice. Using these technologies an international nonprofit organization Promoting Appropriate Technology in Health (PATH) has developed many different varieties of ultra Rice\Rice premix.

The investigator approached PATH (-a catalyst for global health) and got approval for the sponsor of Rice premix for the current investigation. The Rice premix was produced by a cold extrusion process by a PATH approved manufacturer in Kolkata (plate1). The fortificants used for rice premix preparation were micronized ferric pyrophosphate, Zinc as zinc oxide IP, Folic acid IP, Vit $B_{12}$.
as 0.1 per cent mannitol base. One gram of the produced Rice premix provides 19.6 mg of iron, 4.1 mg of zinc, 60.2 mcg of folate, 0.1 mcg of Vitamin B₁₂.

**RICE PREMIX**
**PLATE 1**

**Preparation of Fortified Rice**

Information on food consumption quantity revealed that the mean intake of rice (breakfast) was 120.8 g. Hence in order to meet the existing haemopoietic micro-nutrients deficit, the rice premix was mixed with the regular rice in the ratio of 0.8: 99.2 so that 100g of fortified rice provide an extra amount of 15.68 mg of iron, 3.28 mg of zinc, 34.6 mcg of folic acid and 0.038 mcg of vitamin B₁₂ (apart from the nutrients present in regular rice).

**E.2.a. Nutrient availability of the Rice premix**

Details regarding the nutrient availability of the Rice premix based on loss of nutrients during processing, storage and soaking together with bioavailability of iron are presented in the following Table V.
### TABLE V

NET NUTRIENT AVAILABILITY OF THE RICE PREMIX

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Rice premix Quantity (per gm)</th>
<th>Processing</th>
<th>Storage / Transport</th>
<th>Soaking / Cooking</th>
<th>Percentage Remaining</th>
<th>Net availability of Rice Premix (Per gm)</th>
<th>(per 0.8 gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (mg)</td>
<td>19.6</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>100</td>
<td>19.6</td>
<td>15.68</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>4.1</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>100</td>
<td>4.1</td>
<td>3.28</td>
</tr>
<tr>
<td>Folic Acid (mcg)</td>
<td>60.2</td>
<td>20.0%</td>
<td>10.0%</td>
<td>8.0%</td>
<td>72%</td>
<td>43.3</td>
<td>34.60</td>
</tr>
<tr>
<td>VitaminB₁₂ (mcg)</td>
<td>0.1</td>
<td>10.0%</td>
<td>10.0%</td>
<td>28%</td>
<td>48%</td>
<td>0.048</td>
<td>0.038</td>
</tr>
</tbody>
</table>

### E.3. Ethical clearance

The methodology, proforma for data collection and consent form were placed before the Ethical Committee of the University and school authority. After thorough scrutiny the committee approved the study and gave clearance to the proposed feeding trial (Appendix II). The approved number of the study is HEC 2011. 24, HEC 1.2011

### E.4. DETAILS OF RICE PREMIX AND FORTIFIED RICE

#### E.4.a. Acceptability trial

Sensory evaluation is a science which uses human senses for measuring the appearance, colour, texture, flavour and taste. Any formulated or prepared food/recipe failed to satisfy the human being, it is said to be an incomplete food. Therefore, overall acceptability was determined in cooked rice using 9-point hedonic scale (Appendix-III) on three consecutive days.

The total panel consisted of 24 healthy adolescents of both sexes (boys12, girls12) from the school. The investigator explained how to evaluate food samples and record results on a sensory evaluation sheet prepared in the local language. The plain cooked regular rice and plain cooked form of fortified rice were served to assess the acceptability.

#### E.4.b. Cooking quality

The cooking quality of the fortified rice was assessed by water uptake ratio and cooking time.
1. **Water uptake ratio**: This was determined by cooking five samples each of two gm of rice premix in 20ml distilled water for a minimum cooking time of 15 minutes in a boiling water bath and the superficial water was then drained. The cooked sample weight was accurately recorded. The water uptake ratio was calculated as the ratio of cooked weight to uncooked weight of the fortified rice. As the food was steam cooked in the school, 2 kg of rice premix and regular rice was cooked in the same kitchen using the steamers separately. The duration of time taken for cooking, cooked weight of both the samples was recorded. This was appraised at the beginning, at 6th and 12th month of the study period.

2. **cooking time**: This was determined by boiling two gm of rice premix kernels in 100 ml distilled water, removing a few kernels at different time intervals during cooking and pressing them between two glass plates until no white core was left. Optimum cooking time was taken as the established cooking time, plus two (2) additional minutes (Oko, et al., 2012).

**E.4.c. Shelf life**

In order to study the shelf life in terms of TPC, moisture and microbiological growth, samples of ordinary rice and rice premix were taken and analyzed in the laboratory by Spread plate method (Jidenai and jidenai,2006), Vacuum oven method,(AOAC 925.09), Ecoli and coliform by MFHPB-34, Salmonella EFLA MFHPB 24 respectively.

**E.4.d. Enhancers and inhibitors of iron absorption**

The primary regulatory mechanism of iron balance is from absorption through gastro-intestinal tract. Since humans have no physiological path way for the excretion of iron, the regulation of the intestinal absorption of iron is crucial. In humans, the dietary, luminal and systemic factors affect the bioavailability of iron (Nair and lyengar, 2009). Hence the iron absorption enhancers and inhibitors present in the breakfast with regular rice (as the fortification was done only in the breakfast) was analyzed.

**Enhancers of iron absorption**

The best known enhancer of iron absorption is vitamin C which was estimated by Vyadac instrument: application notes-HPLC-UV.
Inhibitors of iron absorption

The inhibitors of iron absorption including calcium, phytate, oxalate, tannin, crude fibre and polyphenols in the ordinary rice were analyzed by chemical method, titration method, Pearson's composition analysis of food 9th edition 1991. AOAC chapter 32, 18th edn, 920.86 respectively.

E.4.e. Bioaccessibility of iron

Bioaccessibility of iron is the proportion of iron that is actually available for the absorption by the body. In the present study the bioaccessibility of iron was estimated by Luten et al., 1996 (Appendix-IV)

E.5. Feeding of fortified rice

E.5.a. Random assignment of anaemic adolescents

The study was a randomized control feeding trial among adolescents in the age group of 10 to 18 years. The selected 220 anaemic adolescents were randomly assigned into control and experimental group by using computer generated random numbers. Out of 220 selected adolescents, 201 completed the study with 101 in the experimental group and 100 in the control group.

E.5.b. Deworming of all the anaemic adolescents

Deworming was carried out by administering albendazole (400mg) tablets for all the selected participants at baseline and at the interval of six months i.e the selected adolescents had three doses of deworming drugs.

E.5.c. Preparation and serving of meals

The selected school hostel had steamer for the cooking rice. Out of the two steamers, one was used for cooking the breakfast with regular rice and the other was used for the fortified rice.

The fortified rice and regular rice were used for preparation of various recipes such as Pongal (rice steamed with green gram dhal, pepper, ginger and cumin seeds and served with Sambar which was prepared with red gram dhal, onion, tomato and spices), Tamarind rice (rice steamed and mixed with thick gravy prepared with tamarind juice and seasoned with red chillies and spices and served with Beetroot curry which was prepared by boiling the beetroot and seasoned with
spices), Lemon rice (rice steamed and mixed with lime juice and seasonings and served with Cabbage curry which was prepared by boiling cabbage seasoned with spices), Tomato rice (rice steamed and mixed with gravy prepared with tomato, onion and spices), and Rice and dhal porridge (rice and dhal steamed with excess water and served with chutney which was prepared with Roasted Bengal gram and spices). All the adolescents were served with required quantity of meals on all day. Information on food consumption quantity of breakfast of 25 per cent of each age group was collected to assess their quantity of food intake which ranged from 90 to 143 g of raw rice and the mean intake was observed to be 120.8 g/head.

The side dishes such as chutney, sambar, cabbage and beetroot curry were cooked commonly for both groups. The meal was served ad libitum to children with potable water. Care was taken to avoid sharing or exchange of their meals. The study period was from November to October. The food was served for 250 days, six days per week except on Sundays. The number of days stayed in the hostel by the adolescents was also recorded. The entire process of cooking, serving and consumption was monitored by the investigator/warden/a teacher residing in the school on every day (Plate 2).

SERVING OF BREAKFAST MEAL FOR THE ADOLESCENTS
PLATE 2
F. IMPACT OF INTERVENTION

F.1. Anthropometric Measurements

Anthropometry involves obtaining physical measurements of an individual and relating them to standards that reflect the growth and development of the individual. These physical measurements of another component of the nutritional assessment are useful for evaluating over nutrition and under nutrition. They can be used to monitor the effects of nutritional interventions (Kathalene, 2008).

Anthropometry is a universally applicable inexpensive and non invasive method to assess body weight, height and other components. These measurements in turn can be sensitive reflectors of overall health and wellbeing of people. There are two situations which arise when assessment of nutritional status is based on anthropometry. First, detection of loss/gain of body components relate to previous measurements. Second, relating to the values of any one person to normal value in order to identify over nourishment or undernourishment. A data are more valuable when they reflect accurate measurements and are recorded over a period of time (Kathalene, 2008). Therefore height and weight of the selected adolescents were recorded at an interval of three months for a study period of one year.

F.1.a. Recording Body Weight

This is the most widely used simple and reproducible anthropometric measurement for evaluation of nutritional status in relation to age, body weight indicates current nutritional status.

The body weight of the adolescents was taken (Plate 3) with a portable digital weighing balance. The weight was recorded under basal condition. The adolescents were asked to remove foot wear and stand on the weighing balance with one foot on either side of the scale, without holding anything with hands. The weight was read on the scale to the nearest 0.1 kg. It was recorded twice to get an accurate value.
Methodology

RECORDING BODY WEIGHT
PLATE 3

RECORDING BODY HEIGHT
PLATE 4
F.1.b. Recording Body Height

This linear measurement reflects skeletal growth. Inadequate dietary intake can slowdown linear growth and height deficit may be a measure of duration of malnutrition.

Stature meter (Plate 4) was used to record the body height of all the selected anaemic adolescents, the accuracy of the stature meter was ensured before starting the procedure. The stature meter was fixed on the wall by using nail and lines were drawn on either side of the tape to ensure that the tape is winding down vertically without any obliquity while measuring.

The bare footed adolescents were made to stand straight against the wall with heels together, knees, buttocks, shoulder and back of the head touching the wall and they were made to look straight ahead with head held in Frankfurt plane, Then the horizontal limb of the stature meter was firmly placed on top of the head and the height was measured to the nearest 0.1cm. It was recorded twice to get an accurate value.

F.1.c. Computation of Body Mass Index (BMI)

BMI measurement requires weight and height measurements. It can indicate over nutrition or under nutrition. BMI accounts for differences in body composition by defining the level of adiposity according to the relationship of weight to height thus eliminating dependence on frame size (Singh, 2012).

The BMI of the adolescents were calculated from the recorded weight and height using the following formula,

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

Categorization of nutritional status of adolescents was done according to (WHO, 2007) classification of BMI for age z score and height for age z score.
F.2. Determination of Biochemical Parameters

Biochemical test is the most objective and sensitive measure of nutritional status (Agarwal, 2006). Biochemical investigations conducted on easily accessible body fluids such as blood can help to detect nutritional deficiencies at a subclinical stage and also confirm clinical diagnosis at a diseased state. Biochemical estimation helps to confirm clinical and dietary data so that diagnosis can be made and nutritional and medical care can be planned and implemented effectively. Modern analytical instruments (e.g. High Performance Liquid Chromatography), techniques (e.g. Radio or Enzyme Immunoassay) and computerization have greatly increased the capability of nutritional bio-chemical testing

Biochemical parameters namely blood haeomoglobin was estimated for all the selected adolescent whereas serum ferritin, serum folate, serum iron, Total Iron Binding Capacity (TIBC) and CRP were estimated and computation of transferrin saturation from TIBC and serum iron for a sub sample of 20 per cent (N=41) of the selected adolescents at the beginning and at end of the study.

COLLECTION OF BLOOD SAMPLE
PLATE 5
F.2.a. Estimation of Haemoglobin

The blood haemoglobin level is considered as a simplest and easiest way to determine anaemia. Hence blood haemoglobin was estimated (Raghuramulu et al., 2003), for all the selected adolescents by cyanmethaemoglobin method. This was carried out at baseline, midterm and at the end of the intervention.

Four millilitres of blood was collected (plate 5) from 20 per cent (N=41) of sub sample from the total 201 adolescents for the estimation of haemoglobin, serum iron, TIBC, serum ferritin, serum folate and CRP level. All these estimations were done at the beginning and at the end of the study.

F.2.b. Estimation of Serum Ferritin

Serum Ferritin reflects the status of Iron Storage in the body. It is generally considered as the test of choice for estimating iron storage. However, its level increases in infections, inflammation and liver disease.

A two step immunoassay to determine the presence of Ferritin in human serum using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocol referred to as Chemiflex was employed for the estimation of Serum ferritin.

F.2.c. Estimation Serum Iron

Serum iron shows a consistent and progressive fall when negative iron balance occurs (Agarwal, 2006). Serum iron was estimated by the standard Dipyridyl method (Varley, 1998).

F.2.d. Estimation of Total Iron Binding Capacity (TIBC)

Total Iron Binding Capacity and transferrin saturation indicate iron supply to tissues. TIBC is lowered in chronic disease and raised in iron deficiency (Agarwal, 2006). TIBC is generally measured to assess the body’s ability to transport iron in the blood. TIBC measures all the proteins in the blood that are available to bind with iron, including transferrin. Since transferrin is the primary iron-binding protein, the TIBC test is a good indirect measurement of transferrin (www.labtestsonline.org). The body produces transferrin in relation to the need for
iron. When iron stores are low, transferrin levels increase and vice versa. In healthy people, about one-third of the binding sites on transferrin are used to transport iron (http://www.healthcare.siemens.com/clinical-specialities/anemia) TIBC was estimated by saturation method (Haematol, 1978).

F.2.e. Computation of Transferrin saturation

Transferrin saturation value is most consistently helpful than either serum iron or TIBC. However it does not reflect iron stores but relative to efficiency of moving iron out of iron processing cells (reticuloendothelial macrophages, hepatocytes are absorptive erythrocytes) and erythron was calculated (Agarwal, 2006) using the following formula;

\[
\text{Serum iron} \times 100
\]

TIBC

F.2.f. Estimation of Serum Folate

Serum folate is an indicator of recent folate intake, repeated low values of serum folate within an individual over the course of a month is indicative of low folate status or folate depletion (WHO vims 2012).

The ‘ARCHITECTFolate’ is a two step assay for the quantitative determination of folate in human red blood cells using Chemiluminescent Microparticles Immunoassay was used for the estimation serum folate (Appendix V)

F.2.g. Estimation of C – Reactive Protein (CRP)

C-reactive protein is the best known among the acute-phase proteins, a group of proteins whose concentration increases in blood as a response to inflammatory disorders (acute-phase response). CRP is normally present in low concentration in blood of healthy individuals (< 5 mg/L). It is elevated upto 500 mg/L in acute inflammatory processes associated with bacterial infections, post-operative conditions. The measurement of CRP represents a useful laboratory test for detection of acute-infection as well as for monitoring
inflammatory processes and also in acute-rheumatic and gastrointestinal diseases.

Endpoint determination of the concentration of CRP by photometric measurement of the antigen-antibody reactions of antibodies to human CRP with CRP present in the sample was used for the estimation (Appendix - VI)

F.3. Clinical examination of the anaemic adolescents

Clinical examination is a very important aspect in assessing the health and nutritional status of the individual. The various clinical symptoms associated with specific nutrient deficiencies can be used as a valuable tool to assess the nutritional status (Shronts, 2003).

As it is used as an exposure measure of individual risk and an outcome to determine individual responses to dietary interventions, it was carried out on all selected adolescents by a medical practitioner at baseline and end line of the study. The clinical assessment schedule used is given in Appendix - VII.

F.4. Cognitive performance of the anaemic adolescents

Cognition is the scientific term which primarily refers to abilities like new learning ability, memory, speech and reading comprehension. Usage of this term varies in different disciplines. Humans are generally equipped with a capacity for cognitive function at birth, however these cognitive functions are influenced by optimal nutrition.

Many studies that have assessed the effect of multiple micro-nutrient fortified foods on cognitive performance in school age children in developing countries have shown that the beneficial effects on at least one of the cognitive performance indicators measured (Moretti et al., 2006).

In the present study, cognitive performance, such as general intelligence, memory, attention and concentration, academic / scholastic performance of all the selected adolescents were assessed at the beginning and at the end of the study by using the appropriate psychometric test. A special care was taken to ensure
that all adolescents had their breakfast before the test began in the morning, because missing breakfast can impair cognitive performance (Plate 6).

CONDUCT OF TEST FOR COGNITIVE PERFORMANCE

PLATE 6

F.4.a. Assessing the intelligence

Health can affect the intelligence in various ways. Conversely, the intelligence can affect health. Health effects on intelligence have been described as being among the most important factors in the origins of human group differences in Intelligent Quotient (IQ) test scores and other measures of cognitive ability (Deary, 2008). The present study efforts to ascertain the effects of supplementation of haemopoietic micro-nutrients on the IQ of adolescents Intelligence level of all the 201 anaemic adolescents was assessed using Colored Progressive Matrices (CPM) and Standard Progressive Matrices (SPM) depending upon the age of the participants.

Coloured Progressive Matrices (CPM): CPM is the test used to assess the Intelligence Quotient (IQ) level of the participants between the age range of 5-11 years of age. The test consists of a total of 36 items in 3 sets (A, Ab, B) with 12 items per set. The total raw score gives us the corresponding IQ.
Standard Progressive Matrices (SPM): 

SPM was developed by J.C. Ravens in 1938. This test was used to assess the abstract intelligence of the participants between the age range of 12+ years. This test comprises of 60 items of progressive difficulty which is divided into 5 subsets (A, B, C, D, E) with 12 items each. The total raw score gives us the corresponding IQ level as per the given norm table.

F.4.b. Assessing the memory status

The WHO/UCLA version of (Maj et al., 1993) Rey's Auditory Verbal Learning Test (RAVLT) was used to measure the verbal learning and memory of all the 201 anaemic adolescents in the present study. RAVLT measures of immediate memory, delayed verbal recall and verbal memory loss are sensitive parameters of the cognitive deficits in children.

RAVLT was individually administered to all the selected adolescents. It consists of 2 lists of 15 nouns each. ‘List A’ consists of 15 nouns read aloud for five trails. Each trail is followed by an immediate recall. The order of presentation of words remains fixed across the trails. The five trails are learning trails and provide a learning curve indicating the rate of learning. An interference list of 15 words ‘List B’, is presented after completing 5 trails of ‘List A’. This is followed by an immediate recall of List B. This is immediately followed by a recall of ‘List A’. Immediate recall of List B and then of List A is to assess the presence/absence of provocative/retroactive interference. A delayed recall of ‘List A’ is taken after a 30-minute delay period. Scoring for each trail is the number of words correctly recalled. The different scores calculated for this test are as follows (Spreen and Risser, 1995).

1. Number of words recalled on each of the five trails.
2. Sum of words recalled across the five trails: Total learning score.
3. Number of words recalled following ‘List B’: Proactive interference.
   Proactive interference is said to occur when learning of ‘List A’ interferes with the learning of ‘List B’.
4. Number of words recalled on delayed recall.
5. Number of repetitions and intrusions on each of the five trails.

6. Amount of loss from trial five to the recall of ‘List A’ following ‘List B’ recall:
   Retroactive interference. Retroactive interference occurs when learning of
   ‘List B’ interferes with the learning of ‘List A’.

**F.4.c. Assessing the attention and concentration**

Colour trails test is a measure of focused attention. It is also a measure of
mental or conceptual tracking and cognitive flexibility.

Colour trails test (D’Elia et al., 1996) was included in the present study for
all the selected adolescents because of its wider applicability. This test is designed
to minimize the influence of language and covers a wide age range from childhood
to adulthood. It has two parts, ‘Part A’ and ‘Part B’. On ‘Trail A’ circles numbered
1 to 25 are in two colors yellow and pink. All odd numbered circles are in pink and
even numbered circles in yellow. The subject is required to serially connect the
number 1 to 25, irrespective of the colors. ‘Trail B’ shows all numbers from 1 to 25
twice in pink and in yellow. The subject is required to connect the numbers serially
from 1 to 25 alternating between pink and yellow circles disregarding the numbers
in the circles of the alternate colour. Time taken for both trails A and B were noted
separately. Errors are also recorded.

Time taken in seconds and errors of omissions and commissions for ‘Trail
A’ and ‘Trail B’ separately comprise the score for this test.

Attention and concentration of both control and experimental groups were
assessed by using a Colour trail test. The investigator explained the procedure in
the local language to subject and scores were recorded.

**F.4.d. Assessing the academic performance**

Academic Performance of the selected 201 adolescents was collected/calculated from their marks in science and maths subjects they study. All baseline
parameters were repeated at the end of one year of supplementation.
F.5. Recording the Prevalence of Acute Morbidity

The self-reported acute onset of common ailments and attendance, reasons for absenteeism among the adolescents was recorded throughout the study period. The proforma used to record the acute morbidity pattern is annexed in Appendix-VI.

G. CONSOLIDATION AND STATISTICAL ANALYSIS OF DATA

The data thus collected were consolidated, analysed and the statistical analysis were done to see the effect of supplementing haemopoietic micro-nutrients on the nutritional status and the cognitive performance of the anaemic adolescents. Owing to variations in the data was very small parametric tests was carried out for analysis. The statistical methods that were used were percentage, mean, standard deviation, paired “t” test, independent “t” test and cross tabulation, chi-square. The above statistical analyses were done using SPSS package version 16.
**RESEARCH DESIGN**

- **Selection of Area**
  (Uthirameuru Taluk, Kancheepuram District, Tamil Nadu)

- **Screening of Adolescents for Anaemia (N=250)**

- **Deworming of adolescents (Albendazole 400 mg)**

- **Selection of Adolescents for the study (n=220) on the basis of prevalence of anemia**

- **Food Weight Survey on 10% of the total participants**

- **Evolving the composition of Micro-nutrient Rice Premix**

- **Preparation of Fortified Rice (By mixing Rice Premix with Regular Rice)**

- **Intervention (250 days)**

  - **Experiment Group**
    (Fortified Rice)
  - **Control Group**
    (Regular Rice)

- **Impact of Interventions**

  - **Assessment of Nutritional Status**
  - **& Cognitive Performance**

**Socio demographic profile**

**Anthropometric Measurement**: Height, Weight, BMI (n=220)

**Clinical and Morbidity Pattern**: (n=220)

**Biochemical parameters**: Haemoglobin (n=220)
  - Serum ferritin, Serum iron, TIBC (n=41)
  - Transferrin saturation, Serum folate, C-Reactive Protein

**Cognitive Performance**: Intelligence (CPM, SPM)
  - Memory status (RAVLT)
  - Attention & Concentration- Colour trails