

THE EFFECT OF SUPPLEMENTATION OF DRUMSTICK LEAVES  
ON PRESCHOOL CHILDREN

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## INTRODUCTION

Malnutrition has today emerged as the major health problem of the world. According to conservative estimates, atleast 1,90,00,000 infants and children of the world are on the verge of death from gross malnutrition (Gupte, 1979). In India alone under nutrition is said to claim around 500,000 deaths among infants and children every year (Loccit, 1977). Of these 35 to 60 per cent of all deaths occurring in these age group are preventable. Malnutrition in its serious forms is a very depressing health problem of today, affecting over half of the Indian population. Infants, preschool children and school children are the most critically affected. In India, 50 per cent of the preschool children have moderate to severe malnutrition (Devadas et al., 1976).

Malnutrition contributes directly or indirectly to high mortality and morbidity. Malnutrition interferes with a child's motivation, power of concentration and learning capacity. It results in poor school performance of the child and low aspiration to higher education. Thus malnutrition becomes a huge educational waste (Cravioto et al., 1978). It leads to retarded physical growth and may affect the mental development. Nearly 300 million children in the world today are retarded physically and perhaps mentally due to malnutrition which is a proven killer of young children (Boerma, 1981).

The complexity of the problem of malnutrition becomes evident when socio-economic and environmental factors are taken into consideration. The problem of malnutrition is assuming its seriousness not completely because of poverty but also because of ignorance and illiteracy of the people (Gopala Rao et al., 1980). The poverty Index when expressed in terms of deficient caloric intake, shows that 70 per cent of Indian population is below poverty line (Devadas et al., 1978). There is a very high illiteracy rate of 73 per cent which places a serious handicap in extending information and new ideas of nutritional improvements (WHO, 1979). But inadequate food intake, mainly due to poverty is the major cause of under nutrition (UNICEF, 1981).

Fulfilment of the nutritional needs of children in zero to six years of age is utmost important in fostering national health. However, the preschoolers are comparatively more neglected than the other groups of children (Devadas, 1971). There is a great need to mobilize the nation's efforts to improve the nutritional status of children (Boerma, 1981). Taskar (1973) has shown that in families of poor socio-economic status, the man in the family often gets a larger share of the available food than rest of the family members. The children of poor socio-economic group were shown to receive nutrients, significantly below the recommended allowances. The dietary surveys done in 1970 and 1972 by ICMR shows the very low intake

of milk and leafy vegetables in most of the states of India. The low intake of milk and green leafy vegetables is characteristics of so many group groups of people of India (Mc Davitt et al., 1976). In tropical regions in the urban or the rural environment, food intake is much more limited and monotonous. Besides the staple food, the emphasis is often on dry legumes and taste comportsing spices. The use of leafy vegetables is usually limited to some tens of grams (Grubben, 1978). The balanced diet recommended by ICMR include 100g of leafy vegetables daily. In the recent national survey it was found that on an average of only 16g of green leafy vegetables are consumed per day by an adult (Krishna kumari Menon, 1980). The use of green leafy vegetables as a source of vitamins on young children has been widely endorsed (PAG, 1973). Leafy vegetables play a predominant role in nutrition as the cheapest source of minerals and vitamins (Sankaran, 1973).

Leafy vegetables are particularly rich in the mineral nutrient iron, which on inclusion solve the problem of anemia in both children and women. The inadequacy of milk intake, leading to calcium deficiency, can be met with the regular intake of greens, in rural children (Gopalan, 1975). Vitamin A can be obtained in the 'readymade' state from food sources such as butter, egg, liver, etc. which are all expensive. Alternately, many green leafy vegetables contain carotene which can be converted to vitamin A in the body. An average daily intake

of about 50g of green leafy vegetables which may not cost more than a few paise provides the required amount of vitamin A to the child (Gopalan et al., 1975).

Many types of green leaves such as palak, amaranth, fenugreek leaves, drumstick leaves, mint etc are consumed all over the country as vegetables and most of them are rich sources of calcium, iron, carotene, vitamin C, riboflavin and folic acid. These vegetables are therefore inexpensive sources of many nutrients which are essential for growth and maintenance of normal health. Deficiency of these nutrients is commonly seen in our country and steps should, therefore be taken to encourage cultivation of green leafy vegetables in kitchen garden and school gardens. Consumption of such vegetables in adequate amounts especially by pregnant and nursing women and by children should also be encouraged (Ganapathy et al., 1980).

There are number of perennial trees which are often used as leafy vegetables. Of this leaves, chekkurmanis, Agathi, Tamarind, Drumstick and Curry leaves are very high in nutritive value (Choudhury, 1975). Among the green leafy vegetables available in India, drumstick leaves are rich in carotene, inexpensive and available throughout the year (Gopalan et al., 1971). The dark green leafy vegetables such as drumstick leaves, amaranth, agathi etc have much higher carotene content than pale green vegetables such as cabbage, lettuce and brussels (WHO, 1976).

The free school meal programme in several parts of India is one of the important measures undertaken to overcome malnutrition among primary school children (Gopala Rao et al., 1980). UNICEF and CARE are augmenting the school lunch programme through the contributions of protective foods such as butter oil. Corn, Soya Milk (CSM) and bulgar wheat. However food aid from foreign sources can not be eternal. Therefore local substitutes for food aid must be found and should be enriched with locally available low cost but rich sources of supplements. This study was an attempt in that direction. It was undertaken to improve a preschool lunch programme by supplementing it with drumstick leaves, which is available in the villages all the year round, at practically no cost.

40 children from a Balwadi (preschool) of Pannimadai village were selected for the study and divided into two groups of equal strength. One formed the control and the other experimental <sup>supplemented</sup> with drumstick leaves, while the control received the lunch alone without any supplement. The effect of supplementation was studied in terms of changes in serum vitamin A, vitamin C, Calcium and haemoglobin.

## II REVIEW OF LITERATURE

The literature pertaining to the study, "Effect of supplementation of drumstick leaves on preschool children" are presented under the following headings.

- A. Malnutrition among Indian children
- B. Prevalence of vitamin and mineral deficiencies
- C. Etiology of the deficiency of vitamins and minerals
- D. Preventive measures
- E. Green leaves-rich sources of vitamins and minerals
- F. Factors influencing the bio-availability of nutrient from drumstick leaves.

### A. Malnutrition among Indian children

Malnutrition in its serious forms is a very depressing health problem of today affecting over half of the Indian's population (Devadas et al., 1978). With a new child being born every one and a half second and millions of them to poverty and misery, it is time that we should get concerned (Agarwal et al., 1978). In India, there are about 115 millions of children below the age of 6 year (Datta, 1978). In India alone gross undernutrition is said to claim around 500,000 deaths among infants and children every year (Loccit, 1977). Of these 35-60 per cent of all deaths occurring in these age group are preventable. 18-20 per cent of maternal death are attributed to anaemia (Gopalan, 1978). There are about 22 million live births per

year, but as infant mortality is 113 per thousand, the net addition to the population of preschool children is of the order of 20 million per year. 40 per cent of all deaths in the country still take place among children below the age of five and the death rate among preschool children is 21 to 35 per thousand (ICMR, 1979). Gopala Rao (1980) have reported that 75 per cent of the child population can be classified as not healthy due to major and minor illnesses. The tragedy of malnutrition is not so much that it is responsible for high mortality but it cripples and permanently damages the growing generation (Gopalan et al., 1971). Today's malnourished children if they survive to an adulthood will constitute a population segment having clinically illhealth which inturn will seriously handicap the social and economic progress of developing countries. Instead of being asset, they will be a liability (Devadas et al., 1978). Fulfilment of nutritional needs of children in the zero to six years age range is of utmost importance in fostering national health. However preschoolers are comparatively more neglected than other groups of children (Devadas, 1971). Preschool children (1-5 years) are nutritionally vulnerable segments of population (NIN, 1979).

#### B. Prevalence of vitamin and mineral deficiencies

Vitamin A deficiency is one of the most devastating public health problem in India. 5 to 11 per cent of children in Southern and Eastern India are found to have vitamin A

deficiency (Elnagar et al., 1981). Vitamin A deficiency leading to blindness affects children below six years of age. It is estimated that around 25,000 children become blind every year (UNICEF, 1981).

Xerophthalmia is a major cause of childhood blindness in many developing countries, particularly in rice eating regions of South Asia. Estimates suggest 20,000-100,000 survivors are permanently blinded each year, while an equal number may die (Sommer, 1980). 25 per cent of the survivors of severe xerophthalmia remain totally blind, about 50-60 per cent are partially blind and only 15-25 per cent escape with unimpaired sight. There are as many as 40 million blind people in the world. Of this blindness 80 per cent is in developing countries. Two thirds of this blindness is preventable or curable (Xerophthalmia club, 1978).

Most of the deaths associated with xerophthalmia occur in infants, and children in the second year of life. In Denmark during the First World War the fatality rate was 24 per cent; in Indonesia it was 35 per cent and in Jordan it was 60 per cent (WHO, 1976). Vitamin A deficiency was found to be a serious public health problem in Indonesia, India, Ceylon, East Pakistan, North and South Vietnam, The Philippines, Jordan, Syria, Iraq, Haiti and Brazil. In many of these countries, the consequences of vitamin A deficiency are an untold misery for parents, who see their children having life long disability with partial or complete blindness (Roels, 1978).

In a clinical survey carried out among 714 preschool children of 12-36 months of age in the rural areas of Coimbatore in the Tamil Nadu Nutrition Study (1973), it was found that vitamin A deficiency syndrome seen as Bitot's spot occurred in 71 per cent of the children, while the severe nutritional eye deficiency syndrome were extremely rare.

Blindness due to xerophthalmia almost invariably occurs in early childhood, resulting in the disability of individuals and placing a heavy burden on society. Vitamin A deficiency is associated with high mortality; although it is rarely the sole cause of death in man, it is an important contributory cause when present. Vitamin A deficiency is widely prevalent in the densely populated countries of Southern and Eastern Asia and also in parts of Africa, the Middle East and Latin America (WHO, 1976).

Nutritional anemia mainly due to iron deficiency and to a lesser extent folate deficiency is prevalent in many areas in the world (Bober, 1979). Iron deficiency is seen in both developing and industrialised countries and is particularly prevalent among young children (Dallman et al., 1980). Iron deficiency anaemia is a wide spread nutritional problem in our country. It is most common among pregnant women and preschool children, as their requirement of iron are commonly high. Almost 50 per cent of the pregnant women in our country are anaemic (ICMR, 1977). The incidence of iron deficiency is estimated to be as high as 85 per cent among preschool children of India (UNICEF, 1981+).

The survey carried out by Vermury (1980) in three areas of Colombia reported that 65 per cent of the preschool children are anemic and iron consumed by them were less than recommended dietary allowances. 75 per cent of them consumed less than the recommended allowances for vitamin A. In tropics, anemia is a common clinical problem which often leads to infant death. The etiology is frequently multifactorial involving nutritional deficiencies, malnutrition of hematinics, abnormalities of hemoglobin (Wright, 1979). Children below 14 years constitute approximately 40 per cent of the total population in India. Of this, 20 per cent of them are found to have iron deficiency anemia (Devadas et al., 1973).

The growth rate of Indian children is relatively slow at all ages, the somatometric measurements are inferior to those of contemporary Western standards. Since calcium intake of Indian children is far below the recommended levels, it is possible that calcium deficiency is one of the factors responsible for poor growth of children (Pushpa Bansal et al., 1969). Dietary surveys done in eight states of India indicated very low intake of milk and leafy vegetables and also our intake of dietary calcium is below the recommended allowance (Chaney and Rose, 1978).

22 per cent of children in 6-12 years age group in India show one or more signs of deficiency disease, the common being vitamin B-complex inadequacy, ocular lesions of vitamin A deficiency and follicular hyperkerotosis (Gopalan, 1978).

Acute deficiency of ascorbic acid intake leads to the classic disease of scurvy. Most cases of scurvy encountered in the United States, occur in individuals in the age range of 7 months to 2 years. Deficiency in the vitamin has also been implicated in certain anemias, often in association with either iron or folate deficiency.

Studies on preschool children by the ICMR indicated the wide spread deficiencies of vitamin A, D B-complex and ascorbic acid and also dental caries, anemia and rickets.

#### C. Etiology of the deficiency of vitamins and minerals

The main cause of development of deficiency diseases of vitamins and minerals is due to low dietary intake of these nutrients. Inadequate intake of vitamin A and iron in the diet is the major etiological factor which leads to blindness and anemia respectively. The pregnant women and children under five years of age are particularly vulnerable to these diseases (Kardjati et al., 1979). The low intake of milk and green leafy vegetables is characteristic of so many groups of people of India (Mc Divitt et al., 1976). Milk and green leafy vegetables are inadequate in the diet of Indians (UNICEF, 1976). The dietary surveys carried by Devadas et al., (1978) indicated that the home diet of children contain only 137 $\mu$ g of  $\beta$ -carotene as against the ICMR allowances of 1200 $\mu$ g. The balwady supplement also provided only 16 $\mu$ g of  $\beta$ -carotene, resulting in a deficient intake of vitamin A.

Also all children received a minor quantity of green leafy vegetables resulting in a deficient intake of iron. Vermury et al., (1980) found that among the preschool children not receiving supplementary iron or vitamin A, 65 percent consumed less than the RDA for vitamin A. National average revealed that the dietary intake of both iron and vitamin A for children consuming nonsupplementary food are very low. There is abundant provitamin A in green leaves, but they are not incorporated into the diet of weanlings (WHO, 1976). In India both dietary protein and iron are generally inadequate. Diets were found to be grossly deficient in pulses, milk and dairy products. The result was deficiency of niacin, riboflavin, vitamin A, iron and calcium (Ganapathy, et al., 1980). Diet surveys carried out in India (ICMR, 1974) have shown that the average calcium intake is not more than 500mg per day. Of these, more than 60 per cent of this calcium is derived from cereals and other vegetable foods. Further, the availability of calcium in such foods may be limited by associated factors like phytate, oxalic acid and high protein content. Average intake of calcium of the predominantly rice eating population may be lower than recommended allowances (ICMR, 1974).

The interplay of several factors such as unsatisfactory nutrition and recurrent infection, results in poorer rates of growth of preschool children of lower socio-economic groups compared with those of children from better socio-economic backgrounds (Pereira, 1975).

Though India is considered to be the home of vegetarianism, vegetables and fruits occupy relatively less important positions in the Indian diet for various reasons. Of these, poverty and ignorance attributed a greater part. The poverty Index when expressed in terms of deficient calorie intake, shows that 70 per cent of Indian population is below poverty line. There is also a very high illiteracy rate of 73 per cent which places a serious handicap in extending information and new nutritional revolutionary ideas (Devadas et al., 1978).

#### D. Preventive Measures

Good nutrition is one of the most important environmental factors affecting the health of man kind. Various agencies have undertaken for the improvement of the nutritional status of the preschool group through supplementary feeding programmes at balwadis and the extended school lunch programme (Pereira et al., 1975). Supplementary feeding programme are aimed to prevent mainly protein calorie malnutrition, night blindness and anemia (Elnaggar et al., 1981).

The most rational and feasible method of control and prevention of vitamin A deficiency in children would be to improve their dietaries and ensure adequate intake of the vitamin (Devadas and Murthy, 1975). The average daily intake of about 50g of green leafy vegetables which may not cost more than a few paise provides the required amount of vitamin A to the child. A regular intake of green leafy vegetables in such amounts will

also help to build up a store of vitamin in the body (Gopalan et al., 1975). The dietary improvement cannot be done so easily due to huge obstacles like ignorance poverty and illiteracy. The prevention of xerophthalmia is mainly based on an increased production of foods rich in vitamin A and on the nutritional education of public, but recently more immediate measures such as oral administration of large doses of vitamin A to preschool children and of the possible fortification of food with vitamin A have been introduced to overcome the problem. Strongly recommended preventive measure is the fortification of skimmilk used in developing countries with vitamin A (WHO, 1973).

Among the nutritional disorders affecting children and pregnant women, anemia is one of the most important one. The cause for this in most cases is iron deficiency. Anemia can be prevented by consumption of food stuffs like green leafy vegetables, and also administration of iron salts is an effective, easy and inexpensive method of overcoming this condition (Gopalan et al., 1971).

Methods of preventing iron deficiency among infants and children included four possible approaches such as encouragement of prolonged breast feeding, avoidance of excessive cow's milk during the first six months of life, inclusion of iron enhancers such as meat and ascorbic acid rich foods and avoidance of excessive weight gain (Dallman et al., 1980). Suggested preventive interventions are modifications of school lunch programmes and food fortification. Oral administration of ferrous sulphate is recommended as the treatment of choice, with specific guidelines outlined concerning dosage levels.

Therapeutic supplementation with iron or folate is often necessary, when the incidence of nutritional anemia is high and the situation needs to be improved quickly as in the case of pregnant women and children. Large amount of additional iron or folate are supplied in the form of tablets or for children in a liquid preparation (Bober, 1979).

The calcium deficiency can be prevented by increasing the dietary calcium or by oral administration of calcium tablets. Rice is very deficient in calcium and therefore insufficiency of calcium is one of the most important defects of the rice based diet (Wilson et al., 1971). The encouragement of increased amount of milk and green leafy vegetables obviously meet the calcium requirement and prevent the retarded growth, weak bone and tooth. The rich sources of calcium are milk, cheese, green leafy vegetables, cereals and millet ragi. Of these, millet ragi and green leafy vegetables are rich sources of calcium, as well as they are very inexpensive (Gopalan et al., 1971).

Acute deficiency of ascorbic acid leads to the disease scurvy. The recommended daily allowance of vitamin C for adults is 70mg per day, although many nutritionist recommended 100 to 200mg per day. Recently several scientists have recommended the use of massive dose of 1000 to 3000mg or more per day to increase the resistance against colds (Tietz, 1970). The recommended daily intake of vitamin C for 0-9 years of age are 20mg. Pauling (1970) has claimed that substantial daily doses of vitamin C have proven to be beneficial in the prevention of the common cold. The

ascorbic acid needs of the body can be easily met by the increased intake of vegetables and fruits mainly the citrus fruits (Birch et al., 1974).

E. Green leaves - rich sources of vitamin and mineral

Plant proteins have been down graded in human nutrition studies (Mushtag, 1974). There is a common notion among people that a nutritious diet necessarily involve the use of costly food articles. It is true that foods of animal origin are highly nutritious, but they are expensive and in short supply. Nature provides us food articles, which are nutritious and can be obtained at practically no cost. Judicious use of such food items, not only brings down the cost of a meal but also makes it rich in terms of nutrition. Unfortunately, there exist some hurdles in the way of use of such foods. Often people are influenced by traditional food taboos and beliefs which have no scientific basis. This is further complicated by the fact that people are carried away by the status value attached to a food unmindful of its nutritive value. This situation is largely due to lack of correct knowledge regarding the nutritional importance of various available foods (Krishnakumari Menon, 1980).

Green leafy vegetables are rich sources of calcium, iron, carotene, vitamin C, riboflavin and folic acid. These vegetables are therefore, inexpensive sources of many nutrients which are essential for growth and maintenance of normal health (Ganapathy et al., 1980). Palak, amaranth, goger, methi leaves, drumstick

leaves, mint, fenugreek, agathi etc. are consumed most commonly all over the country as vegetables (Gopala Rao et al., 1980). Many of the dark green leafy vegetables such as amaranth, drumstick leaves, curry leaves, agathi keerai, chekkurmanis and manathakali leaves eaten traditionally have much higher carotene content than the pale green vegetables such as leaves of cabbage, lettuce and brussels (WHO, 1976). Lucerne, dubgrass and drumstick leaves have high supplementary values to a poor rice diet (Patwardhan et al., 1966). Consumption of certain foods for special purposes like citrus fruit for vitamin C content, dark green leafy vegetables for pro vitamin A, vitamin C and some minerals is an usual thing (Nutrition reviews, 1973).

Moringa is one of the perennial tree of which, both the leaves and fruits are edible. In Muringa leaves B<sub>1</sub>, B<sub>6</sub> and niacin and protein nitrogen are very rich. Moringa leaves contain 6780ug of B carotene, 0.44g of calcium and 7.0mg of iron per 100g of leaves. Moringa leaves are characterized by higher <sup>l</sup>leaves of protein and protein nitrogen and can be recommended as a regular dietary source. Drumstick fruit is a regular and common vegetable used but its leaf is not commonly used. Its medicinal use is more popular (Gopala Rao et al., 1980). Moringa oleifera, traditionally called drumstick leaves are rich sources of vitamin A and C and also have high content of iron and calcium (Krishnakumari Menon, 1980). Amaranthus plays a predominant role in nutrition as the cheapest sources of minerals

and vitamins. Among the leafy vegetables this species is found to be cultivated widely, in South India, (Sankaran, 1973).

Vitamin A can be obtained in the ready-made state from such foods as butter, egg, liver etc, which are all expensive. Alternatively many green leafy vegetables and some fruits contain carotene which can be converted to vitamin A in the body. An average daily intake of about 10g of green vegetables which may not cost more than a few paise provides the required amount of vitamin A to the child. Regular intake of green leafy vegetables in such amounts will also help to build up a store of vitamin in the body to provide for the lean seasons (Gopalan et al., 1975). Leaf protein is an extremely rich source of  $\beta$ -carotene. A few grams of leaf protein will provide the entire daily dietary vitamin A activity of fresh green vegetables (Bray, 1975). About 15 to 20g-a handful of carotene rich leaves in a child's daily diet can maintain adequate food levels and prevent xerophthalmia (Underwood, 1980).

Average Indian diet, and in particular, rice diet when it included little or no milk, is deficient in calcium (Aykroyd et al., 1980). Milk which is a good source of calcium is usually not within the reach of the poor classes. Green leaves have a high supplementary value and are also cheap and rich in calcium content (ICMR, 1979). On a comparable dry weight basis some greens are four times as rich in calcium as milk (ICMR, 1969).

Green leaves containing large amount of oxalates particularly soluble oxaltes should not be consumed as sources of calcium. But Moringa oleifera has the advantage as it is having only insoluble oxalate.

In India, dietaries which are mostly vegetarian, liberal amounts of green leafy vegetables have been advocated as a source of iron in nutrition programmes to combat anemia, because of their easy availability and low cost (Apte et al., 1969).

The food values of leafy vegetables rests mainly on their vitamin and mineral contents (Smith, 1975). Deficiency of these nutrients is commonly seen in our country and steps should therefore be taken to encourage cultivation of green leafy vegetables and consumption of such vegetables in adequate amounts, especially by pregnant and nursing women and by children should be encouraged (Ganapathy et al., 1980). If people are conscious of their health it is not difficult to select the leafy vegetables which are cheapest on cost and richest in nutrients (Gopala Rao et al., 1980).

F. Factors influencing the bio-availability of Nutrients from drumstick leaves

Geervani and Arundhathi Devi (1981) studied the influence of fat on the utilisation of carotene from drumstick leaves. It showed that 5 per cent of dietary fat is essential for carotene absorbtion and even when the level is small, it still has a beneficial effect on carotene absorption. It was also

observed that serum and liver vitamin A level of rats fed with drumstick leaves was significantly less than rats fed with pure  $\beta$ -carotene.

Jayarajan et al., (1980) have shown that the diets of preschool children are not only deficient in vitamin A but their fat content is also low. The data obtained suggest that consumption of green leafy vegetables will produce an increase in vitamin A nutritional status of children but a small amount of additional fat in the diet will have greater beneficial effect.

Small supplement of fat given to young boys without any other change in the diet increased absorption of  $\beta$ -carotene from 5 per cent to 50 per cent, and at the same time raised their serum levels (Lala et al., 1970). Experiments reveal that as little as 5g of fat added to the diets of children is enough to enhance the carotene absorption (ICMR, 1974).

The reports of FAO (1967) showed considerable differences in liver storage of vitamin A in animals fed on low and high protein diets. Both the sources of carotene and the quality of protein are important in determining the physiological potency of carotenoid pigments (Geervani et al., 1981). The incidence of vitamin A deficiency is particularly high among children suffering from protein calorie malnutrition and it has been suggested that a close interrelationship exists between the metabolism of vitamin A and protein (Gopalan, 1973). In animals maintained on

diets providing 4 per cent protein, the absorption and conversion of  $\beta$ -carotene has been found to be impaired (Mathews and Beaton, 1963).

Vitamin 'E' is a factor which affects the biological utilisation of vitamin A (Baurenfeind et al., 1974). Animals deficient in vitamin E, absorb vitamin A poorly. This poor absorption can be corrected by dosing with vitamin E (WHO, 1976). Oral supplementation with d-alpha-tocopherols increased the utilisation of vitamin A about six folds.

Iron in the Indian diet is mainly derived from cereals, some come from pulses and some from green leafy vegetables. The high phytate, low calcium and low ascorbic acid content which are characteristic of high cereal diet tend to make much of the iron which is present in the diet unavailable to the body (Chaney and Rose, 1978).

Iron absorption is not altered in vitamin A deficiency. No change in the absorption of iron in vitamin A deficient children was noted, when  $\text{FeSO}_4$  is orally administered (Mohanram, 1977).

Certain nutrients in the diet are found to improve the absorption of calcium, important among them are vitamin D, protein and ascorbic acid. Poor absorption occurs when diets are deficient in protein; increase in the protein content of a low protein diet, improves calcium absorption (Hegsted et al., 1972).

Numerous studies have been done to explore the influence of ascorbic acid on absorption of calcium. In an experiment with college women, absorption of calcium was improved by the addition of ascorbic acid to the diet. Supplementation of diet with an additional 25 mg of ascorbic acid significantly increased the absorption of calcium (Wilson et al., 1971).

Oxalic acid and phytic acid will interfere with calcium absorption. Oxalic acid combines with calcium to form insoluble calcium oxalate which makes the calcium unavailable to the body. So the availability of calcium depends on the oxalate content (Singh et al., 1969).

### III EXPERIMENTAL PROCEDURE

This investigation was designed to determine the effect of supplementation of locally available drumstick leaves (Moringa oleifera) on preschool children. This was done by estimating the serum vitamin A, vitamin C, calcium and haemoglobin before and after supplementation.

The procedure consisted of the followed steps:

- A. Selection of the target children
- B. Estimation of  $\beta$ -carotene, vitamin C, calcium and iron in fresh and cooked leaves of Moringa Oleifera.
- C. Conducting food weighment survey.
- D. Conducting the feeding trial - supplementation of the diet of the selected children with drumstick leaves.
- E. Evaluation of the feeding trial through,
  - i. Estimation of serum vitamin A.
  - ii. Estimation of serum ascorbic acid.
  - iii. Estimation of serum calcium.
  - iv. Estimation of haemoglobin.
  - v. Measurement of heights and weights.
  - andvi. Record of incidence of sickness.

#### A. Selection of the target children:

Malnutrition is a major nutritional problem affecting children, especially during the first 6 years of life. Inadequate food intake mainly due to poverty is the major cause of under

nutrition. Apart from general malnutrition, vitamin A deficiency leading to blindness is a major problem particularly in children below six years of age. It is estimated that around 25,000 children become blind every year. Iron deficiency anemia is another major problem and the incidence is estimated to be as high as 85 per cent among preschool children (UNICEF, 1981). Therefore, a preschool (Balwady) situated in Pannimadai village near Coimbatore was selected for conducting the study. 40 children from this balwady 3-5 years of age, participated in the feeding programme. Their age, initial heights and weights were recorded. All the children were physically fit and none suffered from any disorder which might therefore interfere with the study. The selected 40 children were divided into two groups, comparable in age, sex, heights and weights. One formed the control and the other, experimental group.

B. Estimation of  $\beta$ -carotene, vitamin-C, iron and calcium in fresh and cooked leaves of Moringa Oleifera:

Drumstick leaves is a common leafy vegetables source of Indian diet. It is rich in  $\beta$ -carotene, vitamin C and iron and calcium.

The  $\beta$ -carotene content of fresh samples and cooked samples of drumstick leaves were estimated using the procedure of Holden (1978) detailed in Appendix-I. The vitamin C content was determined by dye method detailed in Appendix-II. The iron content was estimated by potassium ferricyanide method as given in Appendix-III. Estimation of calcium in fresh and cooked drumstick leaves was done by Clark and Callip method described in Appendix-IV.

### C. Conducting food Weighment survey

Ramasastri et al (1973) opine that food weighment survey is a positive step towards building better eating habits by finding out the needs. A three day weighment survey would be as efficient as that of the seven day weighment, if the dietary variation in the day today life is not large. Since the dietary pattern of low socio-economic groups in India (Taskar et al., 1967).does not vary greatly day to day a three day weighment was done. Fiften children from both experimental and control groups were selected randomly and a three day food-weighment survey was carried out in their families. During the study period the children had their usual diet at home.

The raw ingredients used for each cooked meal and the total cooked wieght of the food were recorded. The amount of food consumed by the individual child was also weighed for a period of three days. The raw ingredients consumed were computed from the quantities of cooked food consumed. From the figures thus obtained the mean intake of all the nutrients such as  $\beta$ -carotene, vitamin-C, calcium and iron of every child per day from her/his diets was calculated using the value reported by the ICMR (1981).

### D. Conducting the feeding trial - Supplementation of the diet of the selected children with drumstick leaves

Based on the food weighment survey and the  $\beta$ -carotene analysis of the drumstick leaves, the supplementation amount

is formulated. The control group received the balwady diet alone without any green leafy vegetables and the experimental group received the drumstick leaves in addition to balwady diet.

The food trial was in operation for a period of 40 days as follows:

The balwady diet of the children of experimental group was supplemented with drumstick leaves as a source of B-carotene, ascorbic acid, iron and calcium. These leaves were obtained daily from a local vendor. The content of these vitamins and minerals in both fresh and cooked forms were estimated in five different days. The cooking losses were estimated and accounted for, in the finalisation of supplement. 248g of fresh drumstick leaves which provided 1200µg of β-carotene were added as a supplement in the form of porial. This level represents the daily requirement of β-carotene for preschool children as recommended by ICMR (1981) and also about 25.6mg of ascorbic acid, 103.1mg of calcium and 1.49mg of iron. Here also the distribution and consumption were supervised by the investigator daily. Care was taken so that the plate wastes were practically nil. Whenever there was some left over, they were recorded.

#### E. Evaluation of the feeding trial

##### i. Estimation of serum vitamin A

The vitamin A levels in the serum of the target children was determined before and after the supplementation. Nearly about 5.0ml of blood was drawn from the anticubital vein from

each of the children, with the help of a trained technician and the serum was separated. The serum vitamin A was estimated adopting the trifluoro acetic acid method of Neeld and Pearson as modified and suggested by Roels et al (1966), quoted by Roels and Mahadevan (1967) given in Appendix-V.

ii. Estimation of serum Ascorbic acid

Serum ascorbic acid was determined by the 2,4 dinitro phenyl hydrazine method of Roe and Kuether (Varley 1976).

The procedure is given in Appendix VI.

iii. Estimation of serum calcium

Serum calcium level was determined by doing EDTA titration. 0.25ml of serum is digested using triple acid and then titrated with EDTA using ammonium purpurate as indicator. The method makes use of the remarkable chelating properties of disodium ethylene diamine tetra acetate (Versenate (or) sodium versenate). This EDTA method of calcium estimation is done before and after supplementation, as given in Appendix VII.

iv. Estimation of Haemoglobin

Initial and final haemoglobin level of all the children were estimated by cyanmethaemoglobin method of NIN (1971), following the procedure given in Appendix VIII.

v. Records of height and weights and incidence of sickness

Initial and final record of heights and weights of the target children was maintained. The incidence of sickness of

these children was recorded throughout the study period to see whether or not supplement had any ill effects on the children.

The data obtained was statistically analysed. The mean, standard deviation and co-efficient of correlation were found out for the different groups and compared. The 't' test was also used to find out the level of significance of the differences and for the comparison of groups.

#### IV RESULTS AND DISCUSSION

This study was designed to assess, the effects of supplementation of the diets of a selected group of preschool children with drumstick leaves, for a period of 40 days. The results of the investigation are discussed under the following heads.

- A. Background information on the socio-economic status of the target children and their families.
- B. Findings of the food weightment survey.
- C. The body heights and weights of the target children.
- D.  $\beta$ -carotene, ascorbic acid, iron and calcium content of the drumstick leaves.
- E. Effects of supplementation in the target children.
  - i. Changes in serum vitamin A levels
  - ii. Changes in blood haemoglobin levels.
  - iii. Changes in serum ascorbic acid.
  - and iv. Changes in serum calcium.
- F. Cost analysis of the supplement.

A. Background information on the socio-economic status of the target children and their families

All the children belonged to families of low income group. For 96 per cent of the families the monthly income ranged from rupees 75 to 325 thus all the children belonged to families with low (less than Rs.100) per capita income 98 per cent of the heads of families were daily wage earners and mill labourers and the rest were school teachers, 85 per cent of the parents were

illiterate showing the very low educational status of the selected families. All the children were from nuclear families.

B. Findings of food weightment survey

Food weightment survey was carried out on three consecutive days in the families of 15 children, selected randomly from both experimental and control groups. Table-I presents the mean amounts of food consumed by the experimental and control children, compared with the daily allowances. recommended by ICMR (1981).

TABLE - I

MEAN DAILY FOOD CONSUMPTION OF THE TARGET CHILDREN

(3 day food weighment survey on 15 children)

Foods (g)	Recommended daily allowances (ICMR 1981)	Amount consumed by the children of	
		Control Group	Experimental Group
Cereals (rice, wheat and Maize)	222.5	185	189
Pulses	35	12	13.1
Green leafy vegetables	45	1	0.8
Other vegetables and roots and tubers	40	12	10.9
Fruits	50	2.3	2.6
Milk and milk products	275	5.0	3.7
Fat and oils	20	0.9	1.1
Sugar and Jaggery	35	15	12.8
Fleshy foods	30	1	0.8
Nuts and oil seeds	--	2	1.5

Consumption of all the food items was below the recommended daily allowances of ICMR(1981). The consumption of fleshy foods and green leafy vegetables were very low in amount. These findings are in confirmity with those of Devadas et al (1978). The daily dietary consumption of experimental and control group of children was found to be the same and had no significant difference.

The common breakfast items were iddli, dosai and left over cold rice. Bulgar wheat uppuma was served in the Balwady for lunch. In 85 per cent of the families, food was cooked only once in a day. Table-II shows the nutrient contribution of the foods consumed by the target children.

TABLE - II

MEAN NUTRIENT CONTRIBUTION OF THE HOME DIET AND BALWADY DIET  
CONSUMED BY THE TARGET CHILDREN

Nutrients	Recommended daily allowances(ICMR, 1981)	Amount consumed by the target children
Energy (K.cal)	1470	1021.94
Protein (g)	25.7	20.9
Calcium (mg)	400-500	78.29
Iron (mg)	15-20	5.74
$\beta$ -carotene (mcg)	1200	126.02
Thiamin (mg)	0.6	0.63
Riboflavin (mg)	0.7	0.38
Vitamin - C (mg)	30-50	2.07

The daily intake of all nutrients was found to be far below the recommended daily allowances of ICMR(1981). The energy intake was only 1021.94 kilocalories per child per day which is 69.5 per cent of the daily allowance for this age group. Similarly the intake of protein was also found to be 18.7 per cent short of the recommended daily allowance of ICMR (1981). Alos, only 17.4 per cent of the calcium, 32.8 per cent of iron, 10.1 per cent of  $\beta$ -carotene and 5.2 per cent of vitamin-C of the daily allowances were met. This information was utilised in planning the drumstick leaves as a supplement of minerals and vitamins. Table-III presents the mean intake of the nutrients by the children in the experimental group through home diet, balwady diet and supplement.

TABLE - III

MEAN DIETARY INTAKE OF NUTRIENTS BY THE EXPERIMENTAL CHILDREN  
THROUGH HOME DIET BALWADY DIET AND SUPPLEMENT

Source of food for the children	Energy (k.cal)	Protein g	Calcium mg	Iron mg	$\beta$ -caro- tene mcg	Thia- min mg	Ribo fla- vin mg	Vita min C mg
Home diet	678.85	13.50	44.79	3.45	116.06	0.57	0.34	1.80
Balwady diet	340	7.8	30	2.5	10	0.07	0.05	0.2
Supplement	18	1.25	103.1	1.49	1200	0.015	0.01	25.6

TABLE - IV

## MEAN DAILY NUTRIENT INTAKE BY THE CONTROL AND EXPERIMENTAL CHILDREN

Nutrients	Control children	Experimental children
Energy (K.cal)	1025.03	1026.85
Protein (g)	19.29	22.5
Calcium (mg)	81.35	177.9
Iron (mg)	5.53	7.44
$\beta$ -carotene (mcg)	126	1200
Thiamine (mg)	0.62	0.65
Riboflavin (mg)	0.37	0.404
Vitamin C (mg)	2.14	27.6

As indicated by Table-IV, no significant difference in the intake of energy and protein was found between the two groups. But a significant difference in the intake of calcium, iron,  $\beta$ -carotene, thiamine, riboflavin and vitamin-C was noted. Inclusion of 24.8g of drumstick leaves supplying 1200<sup>c</sup>mg of  $\beta$ -carotene, 25.6mg of vitamin-C, 103.1mg of calcium and 1.49mg of iron with the lunch made so much difference in the intake of these nutrients between the control and experimental children.

### C. Body heights and weights of the target children

Table-V shows the mean body heights (cm) and weights (kg) of the experimental and control children at the beginning and at the end of the study.

TABLE - V

MEAN BODY HEIGHTS AND WEIGHTS OF THE TARGET CHILDREN				
Group	Height(cm)		Weight (kg)	
	a	b	a	b
Control	93.8 ± 6.65	93.95 ± 6.75	12.5±4.36	12.7±1.9
Experimental	92.6 ± 6.17	93.08 ± 6.16	12.08±	12.64±1.71
			1.68	

a = beginning of the study

b = at the end of the study

The difference in mean increments in the body heights and weights attained by both the groups of children was not significant. The individual heights and weights of the children are presented in Appendix-IX.

D. β-carotene, ascorbic acid, iron and calcium content of the drumstick leaves:

Samples of drumstick leaves which were fed to the target children were analysed for above said nutrients in both fresh and cooked forms. The supplement was analysed on five different days, taking triplicate samples each time.

i) β-carotene content of fresh and cooked drumstick leaves:

Table-VI shows the mean β-carotene content of the fresh and cooked samples of the supplement and the cooking loss of β-carotene.

TABLE - VI

β-CAROTENE CONTENT OF RAW AND COOKED DRUMSTICK LEAVES AND  
THE COOKING LOSS

β-carotene content of drumstick leaves				Cooking loss of β-carotene mcg/100g	Percentage β-carotene lost on cooking
Raw mcg/100g		cooked mcg/100g			
Mean	Range	Mean	Range		
6802.53	6368.66	4843.73	4446.7 to		
±	to	±		1958.8	28.8
10.94	7233.33	12.14	5373.33		

The fresh samples of drumstick leaves contained a mean β-carotene content of 6802.53 ± 10.94mcg per 100g which ranged from 6368.66 to 7233.33mcg. The cooked samples contained a mean β-carotene content of 4843.74 ± 12.14mcg per 100g which ranged from 4446.66 to 5373.33mcg per 100g. The mean cooking loss of β-carotene per 100g was found to be 1958.8mcg constituting a per centage ~~of~~ loss of 28.8. The drumstick leaves were cooked for 10 minutes with a minimum amount of water and a pinch of salt and then seasoned using minimum amount of oil. Care was taken so that the leaves were just cooked and the cooking loss was minimal.

Devadas et al (1980) have reported a loss of 24.5 per cent β-carotene in amaranth. Murthyet al (1976) have reported a 25 per cent loss of β-carotene in amaranth as a result of cooking. According to Rajalakshmi et al (1974), the losses varied from 27 to 40 per cent when the greens were cooked.

ii) Ascorbic acid content of fresh and cooked drumstick leaves:

Table-VII shows the ascorbic acid content of raw and cooked drumstick leaves along with cooking loss.

TABLE - VII

ASCORBIC ACID CONTENT OF RAW AND COOKED DRUMSTICK LEAVES  
AND THE COOKING LOSS

<u>Ascorbic acid content of drumstick leaves</u>				<u>Cooking loss of ascorbic acid mg/100</u>	<u>Percentage ascorbic acid lost on cooking</u>
Raw mg/100g		cooked mg/100g			
Mean	Range	Mean	Range		
224.43 ± 14.1	207.0 to 242.5	103.27 ± 14.0	88.38 to 110.7	121.16	53.99

The mean ascorbic acid content of fresh drumstick leaves was 224.43 ± 14.1mg per 100g, which ranged from 207 to 242.5mg per 100g. The mean ascorbic acid content of cooked drumstick leaves was 103.27 ± 14.01mg per 100g, with a range of 88.38 to 110.7mg per 100g.

The cooking loss of ascorbic acid was 121.26mg per 100g per 100 of leaves indicating the per centage loss to be 53.99.

Devadas et al (1971) have reported 54.54 per cent of loss in ascorbic acid in amaranthus on cooking.

iii) Iron content of the raw and cooked supplement, cooked drumstick leaves and the cooking loss:

TABLE - VIII

IRON CONTENT OF RAW AND COOKED SUPPLEMENT AND THE COOKING LOSS

<u>Iron content of drumstick leaves:</u>				Cooking loss of iron mg/100g	Percentage of iron lost on cooking
Raw mg/100g		Cooked mg/100g			
Mean	Range	Mean	Range		
6.23 ± 0.4	5.66 to 6.74	6.01 ± 0.5	5.5 to 6.49	0.22	3.56

The fresh samples of drumstick leaves contained a mean iron content of  $6.23 \pm 0.4$ mg, with a range of 5.66 to 6.74mg per 100g. The cooked samples contained a mean iron content of  $6.01 \pm 0.5$ mg which ranged from 5.5 to 6.49mg per 100g. The mean cooking loss of iron per 100g was found to be 0.22mg that is 3.56 per cent. Devadas et al (1968) have registered a cooking loss of 4.7 per cent of iron in amaranth.

iv) Calcium content of fresh and cooked drumstick leaves:

The supplement, drumstick leaves was analysed for the calcium content in both fresh and cooked forms. Table-IX indicates the mean calcium content of the fresh and cooked supplement along with the percentage loss of calcium on cooking.

TABLE - IX

## CALCIUM CONTENT OF RAW AND COOKED SUPPLEMENT AND THE COOKING LOSS

Calcium content of drumstick leaves:

Raw mg/100g		Cooked mg/100g		Cooking loss of calcium mg/100g	Percentage of calcium lost on cooking
Mean	Range	Mean	Range		
445.93	405.3	416.4	360.4	29.55	2.7
$\pm$	to	$\pm$	to		
13.8	488.3	11.2	421.3		

The mean calcium content of fresh drumstick leaves was 445.93  $\pm$  13.8mg per 100g, and the values which ranged from 405.3 to 488.3mg per 100g. The mean calcium content of cooked drumstick leaves was 416.4  $\pm$  11.2mg, which ranged from 360.4 to 421.3mg per 100g. The loss on cooking was 29.55mg per 100g of leaves, thus indicating a cooking loss of 2.7 per cent.

E. Effects of supplementation of drumstick leaves in the target children

i) Changes in serum vitamin A:

The mean initial and final serum vitamin-A levels of the control and of the experimental groups of children who were fed with drumstick leaves along with the balwady diet are given in Table-X and in Figure-I. The individual values are given in Appendix-X.

TABLE- X

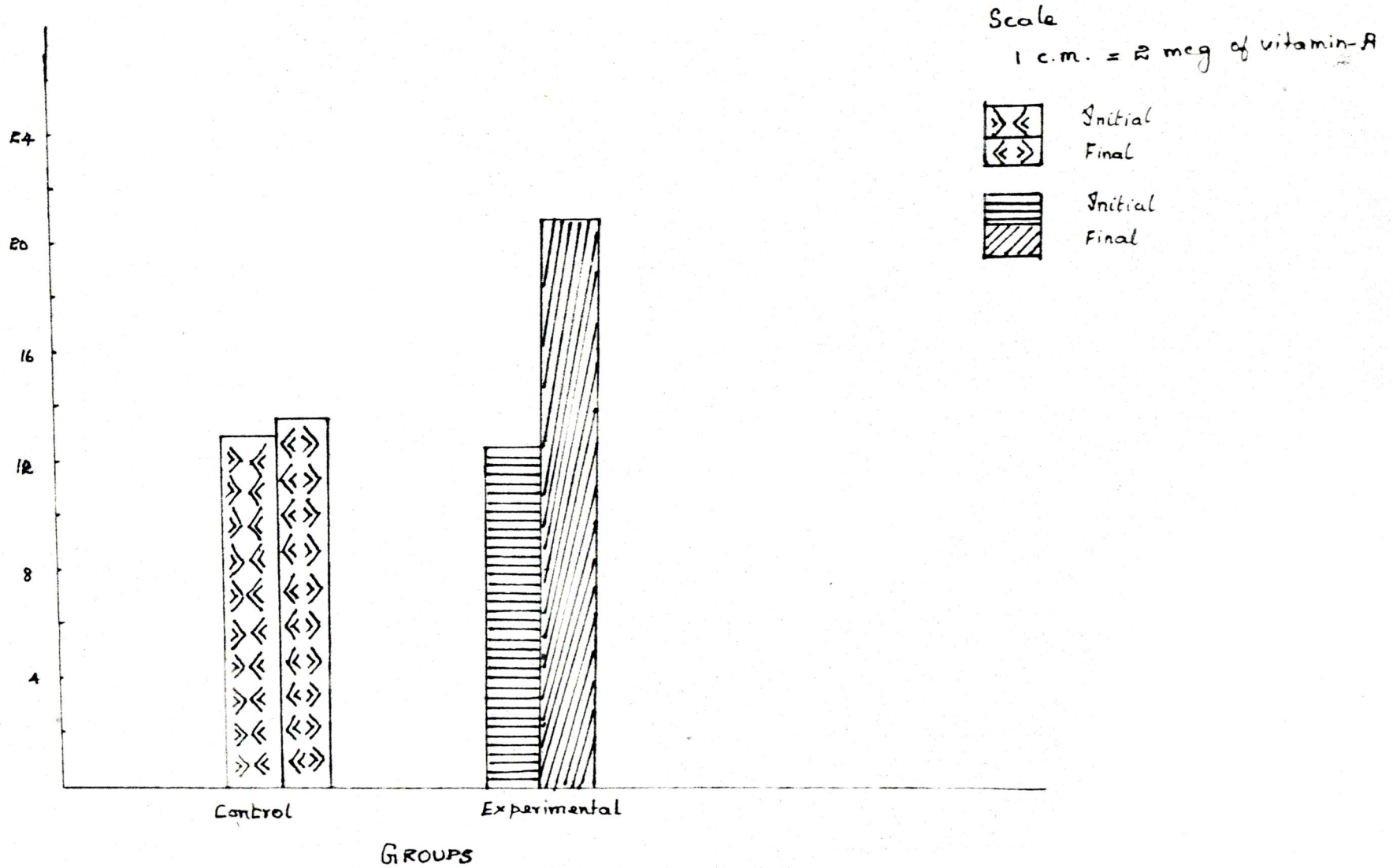
EFFECTS OF SUPPLEMENTATION OF DRUMSTICK LEAVES ON SERUM  
VITAMIN-A

Group	Serum vitamin-A level		Difference	't' value
	Before Supplemen- tation mcg/100ml	After Supplemen tation mcg/100ml		
Control	13.025 $\pm$ 2.51	13.31 $\pm$ 2.66	0.285 $\pm$ 0.3	21.018**
Experi- mental	12.295 $\pm$ 3.18	20.74 $\pm$ 3.17	8.445 $\pm$ 1.67	

\*\* indicates significant at one per cent level.

Levels of vitamin-A in the serum of children in the ~~content~~ of control group who did not received any supplement, showed ~~no~~ appreciable change during the study period of 40 days, where as the children in the experimental group showed a remarkable rise in their serum vitamin-A levels. The mean initial levels of serum vitamin-A in the selected children was below 13.03 micro gram per 100ml. Supplementation of 24.8g of drumstick leaves containing 1200 microgram of  $\beta$ -carotene per child per day for a period of 40 days had resulted in a mean increament of 8.45  $\pm$  1.6 microgram per 100ml of serum in the experimental children. Control group also has registered an increment of 0.285  $\pm$  0.3 microgram per 100ml which is insignificant when compared with the mean initial value for the group. The increment in serum vitamin-A registered by the experimental group is significant at one per cent level, when compared to the control.

# MEAN INITIAL AND FINAL SERUM VITAMIN-A LEVELS OF THE CONTROL AND THE EXPERIMENTAL GROUPS



Pereira and Begum (1968) have also reported an increase in the serum vitamin-A levels in children after feeding green leafy vegetables for 3 months. Lala and Reddy (1970) found increments in serum vitamin-A levels in as short a period as 15 days by feeding undernourished children with 40g of amaranth (Amaranthus tricolor). Devadas et al (1980) have also reported a significant increase in the serum vitamin-A level in preschool children by supplementing Amaranthus gangeticus for a period of two months.

ii) Changes in blood haemoglobin levels:

Table-XI and Figure-II give the mean initial and final blood haemoglobin levels of the target children along with 't' value. The individual haemoglobin values are given in Appendix-XI.

TABLE - XI

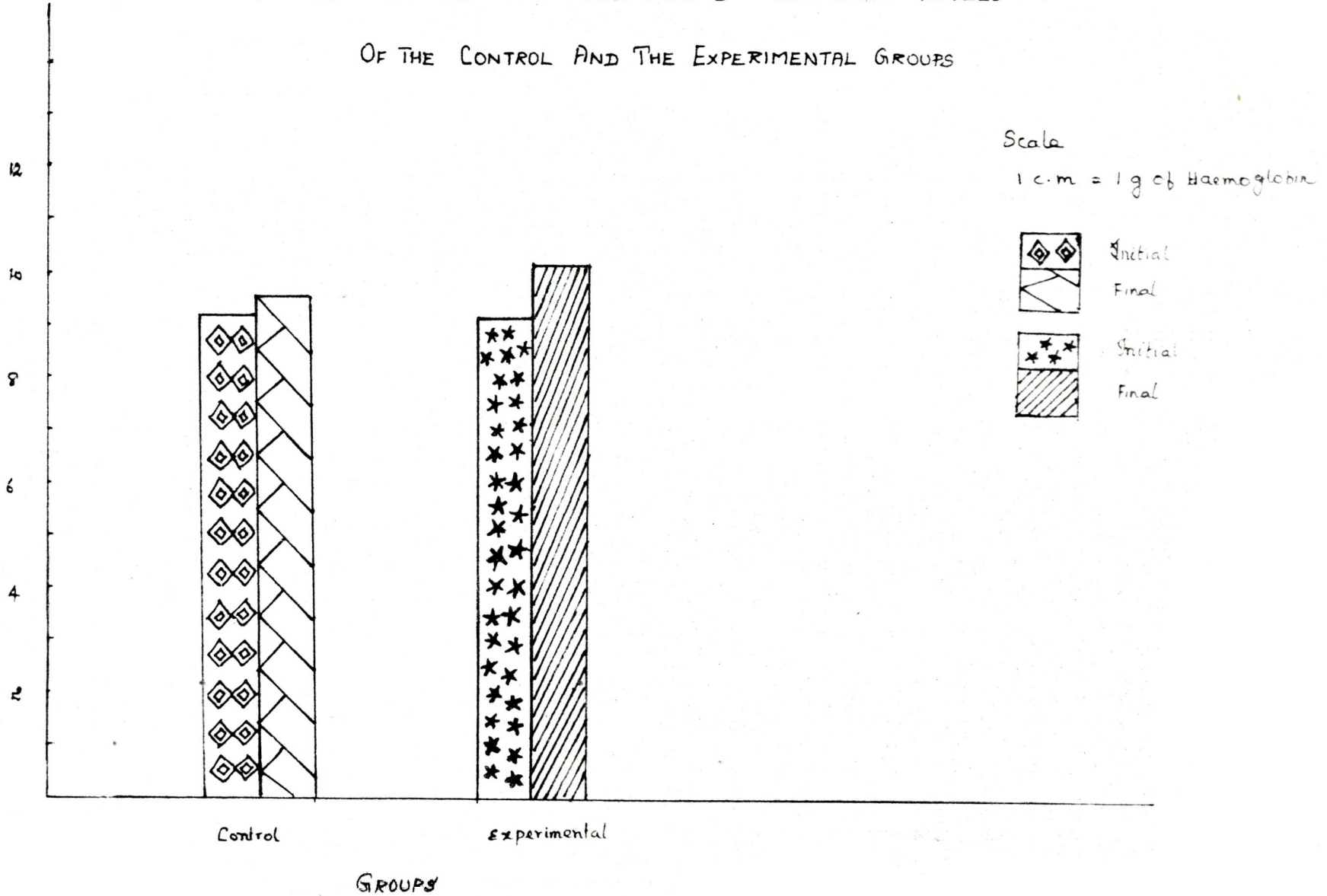
MEAN INITIAL AND FINAL BLOOD HAEMOGLOBIN LEVELS OF TARGET CHILDREN

Group	Initial level g/100ml	Final level g/100ml	Difference g/100ml	't' value
Control	9.23 $\pm$ 0.85	9.5 $\pm$ 0.84	0.27 $\pm$ 0.15	14.22**
Experi- mental	8.86 $\pm$ 0.78	9.88 $\pm$ 0.85	1.04 $\pm$ 0.18	

\*\* = indicates significant at one per cent level.

# MEAN INITIAL AND FINAL BLOOD HAEMOGLOBIN LEVELS

OF THE CONTROL AND THE EXPERIMENTAL GROUPS



According to Sauberlich (1979) haemoglobin values below 10g per 100ml of blood for preschool children is considered as deficient; 10-10.9g as low and 11.0g or above as acceptable. As per the above standards all the target children were found to have deficient levels of haemoglobin. At the end of the study an over all increase in the blood haemoglobin level had been registered by children of control as well as the experimental group. The mean increment in the blood haemoglobin level was  $1.04 \pm 0.18$ g per 100ml in the case of experimental group and it was only  $0.27 \pm 0.15$  per 100ml in the case of control group. The increment seen in the control group was not significant, when compared with the experimental group. The increment in blood haemoglobin level recorded by the experimental group was significant at one per cent level, when compared with that of control children. This increment is caused by supplementation of drumstick leaves containing 1.49mg of iron per child per day for a period of 40 days.

iii) Changes in serum ascorbic acid after supplementation

The mean initial and final serum ascorbic acid levels of the control as well as experimental children are given in Table-XII along with 't' values and in Figure-III. The individual values are given in Appendix-XII.

TABLE - XII

## INITIAL AND FINAL SERUM ASCORBIC ACID LEVELS OF TARGET CHILDREN

Group	Initial level mg/100ml	Final level mg/100ml	Difference mg/100ml	't' value
Control	0.735 $\pm$ 0.33	1.005 $\pm$ 0.45	0.27 $\pm$ 0.24	8.72**
Experi- mental	0.725 $\pm$ 0.40	1.72 $\pm$ 0.26	0.995 $\pm$ 0.26	

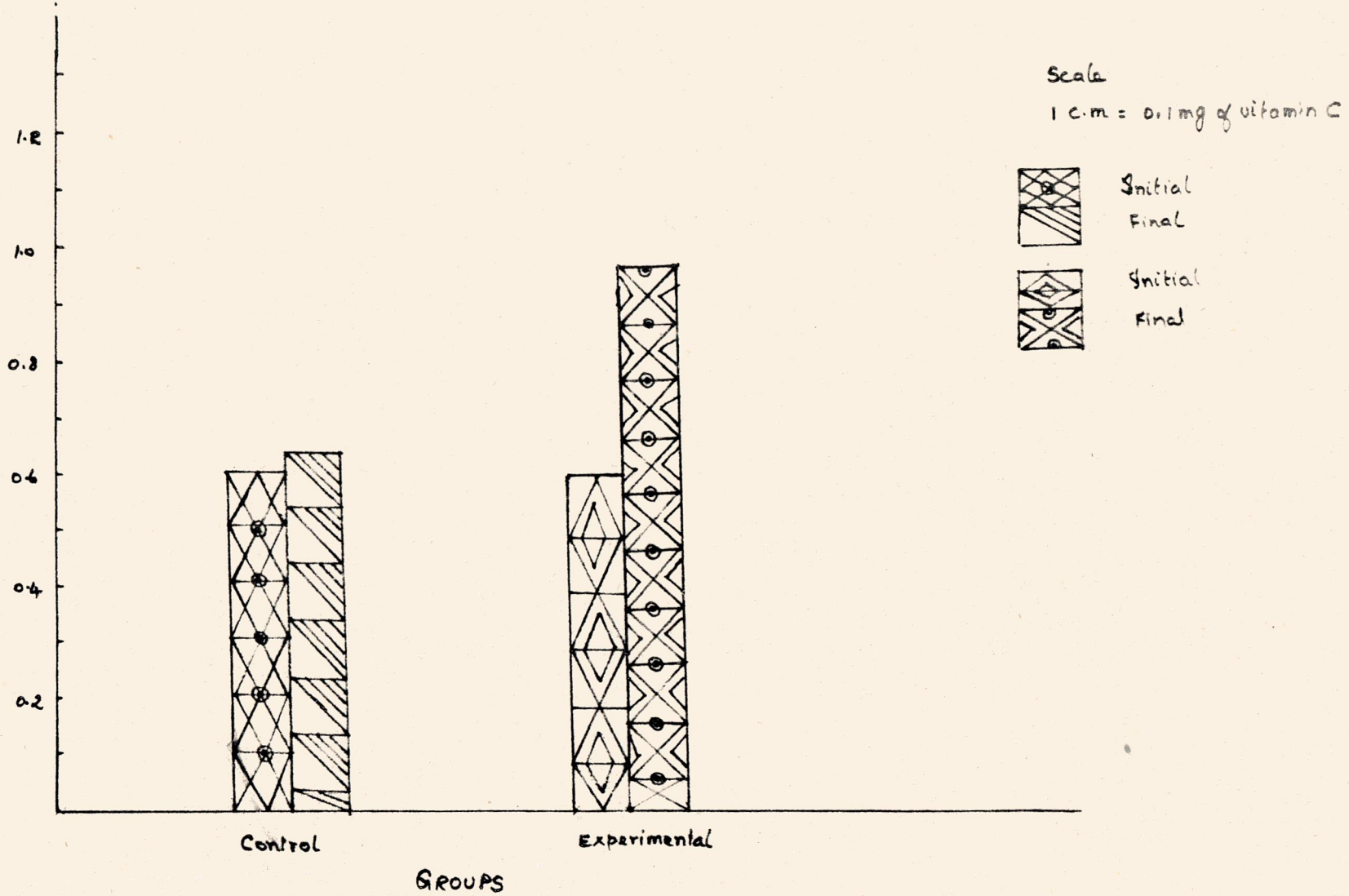
(\*\* = indicates significant at one per cent level)

Levels of ascorbic acid in the serum of children in the control group who did not receive the supplement showed no appreciable change during the study period of 40 days, where as the children in the experimental group, showed a significant ( $p < 0.001$ ) increase in their serum ascorbic acid levels. The mean increment registered by the experimental and control group of children were  $0.995 \pm 0.27$  and  $0.27 \pm 0.24$  respectively. The increment in the control group over the initial value is not statistically significant.

Supplementation of 24.8g of drumstick leaves containing 25.6mg of vitamin-C in the cooked form per child per day for a period of 40 days showed a mean increment of  $0.995 \pm 0.27$  in the serum of experimental children.

# MEAN INITIAL AND FINAL SERUM VITAMIN-C LEVELS

## OF THE CONTROL AND THE EXPERIMENTAL GROUPS



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iv) Changes in serum calcium levels:

Table-XIII and Figrue-VI <sup>shows</sup> the mean initial and final serum calcium levels of the target children of both the experimental and control groups. The individual values are given in Appendix-XIII.

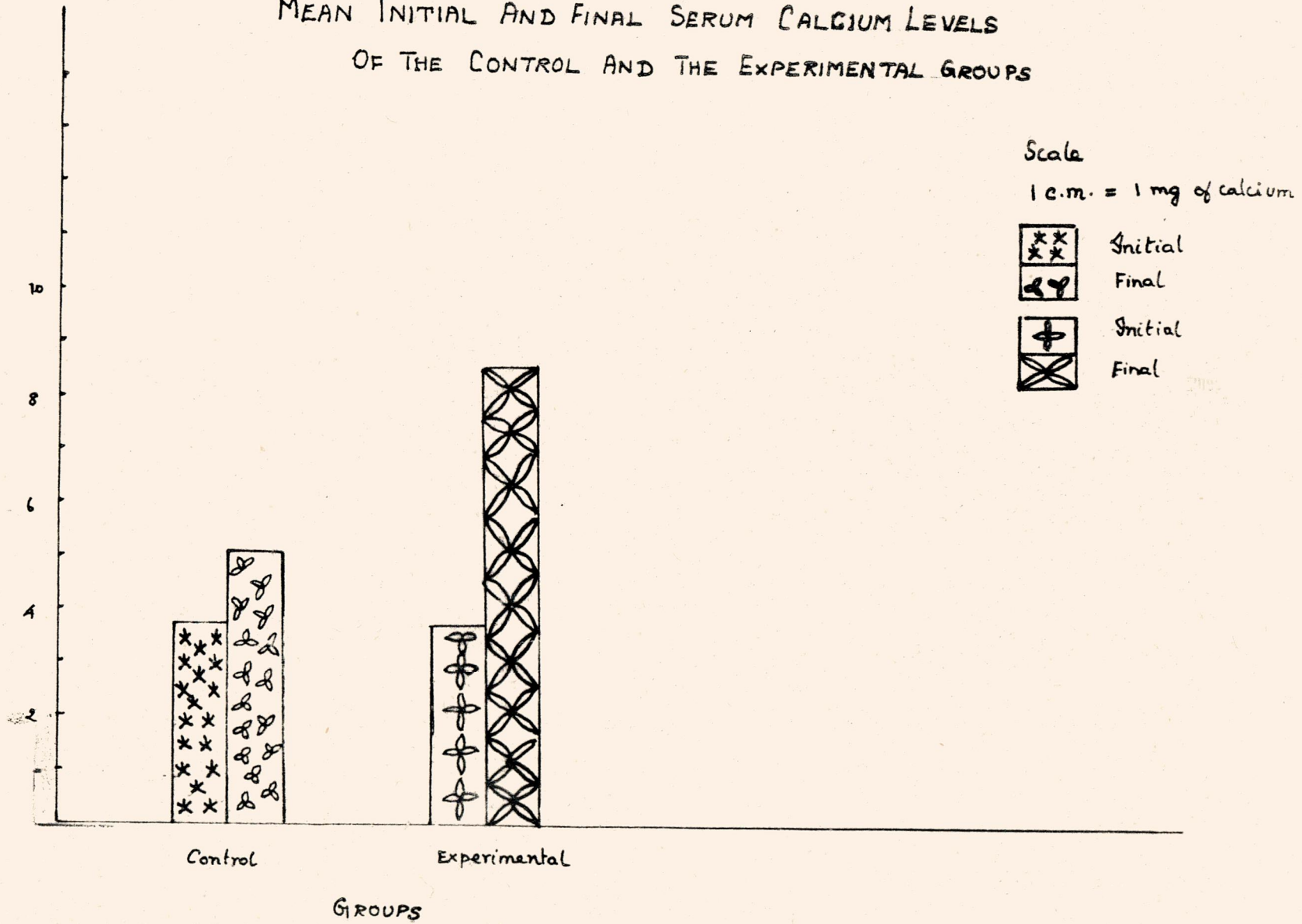
TABLE - XIII

SERUM CALCIUM LEVELS OF THE TARGET CHILDREN				
Group	Serum Calcium level		Difference mg/100ml	't' value
	Initial mg/100ml	Final mg/100ml		
Control	6.1 ± 0.41	6.29 ± 0.43	0.185 ± 0.13	24.94**
Experi- mental	5.9 ± 0.56	9.68 ± 0.58	3.78 ± 0.62	

(\*\* = (P < 0.0001))

The mean increment in the serum calcium level of the control group was 0.185 ± 0.13 while that of the experimental group was 3.78 ± 0.62mg per 100ml. This increment was found to be statistically significant (P < 0.0001) when compared with the mean <sup>of</sup> control group. This variation is caused by the supplementation of 24.8g of drumstick leaves containing 103.1mg of calcium per day for a period of 40 days to the experimental children. There was no significant increase in the serum as their diet contained less calcium than that of the experimental children.

# MEAN INITIAL AND FINAL SERUM CALCIUM LEVELS OF THE CONTROL AND THE EXPERIMENTAL GROUPS



F. Cost analysis of the supplement:

Table-XIV presents the comparative cost, nutritive value and amount of different foods that would supply 1200mcg of  $\beta$ -carotene, 103.1mg of calcium, 1.49mg of iron and 25.6mg of vitamin-C, which are contributed by 24.8.g of cooked drumstick leaves.

TABLE - XIV

COST AND NUTRITIVE VALUE OF DRUMSTICK LEAVES AND OTHER FOODS

Foods	Amount (g)	Cost (paise)	Calcium (mg)	Iron (mg)	$\beta$ -carotene (mcg)	Vitamin-C (mg)
Drumstick leaves	24.8	2.0	103.1	1.49	1200	25.6
Egg	30	40	18	0.63	1200	0
Milk	90	25	103.1	0.2	180	2
Jaggery cane	16.45	7.5	13.16	1.49	27.64	0
Lemon	50	45	3.5	1.16	0	25.6

Based on the cost of the food items as prevailed during the study period in Coimbatore town an analysis of cost and nutritive value of drumstick leaves were compared with a few other itmes selected for comparison. Analysis of cost and nutritive value of drumstick leaves, egg (a source of vitamin-A), milk (a source of calcium), jaggery (a source of iron) and lemon fruit (a source of vitamin-C) showed that drumstick leaves as a cheap source of the selected nutrients costing only 2 paise. But this amount (1200mcg) of  $\beta$ -carotene is supplied

by 30g of egg which costs 40 paise. 103.1mg of calcium present in 2 paise of drumstick leaves when given in the form of milk costs 25 paise, 1.49mg of iron (contributed by 24.8g of cooked drumstick leaves costing 2 paise) when supplied in the form of jaggery (cane) costs 8 paise, 25.6mg of ascorbic acid when given in the form of lemon costs 45 paise. This cost comparison obviously indicated that drumstick leaves as not only a low cost source, also as a rich source of all the four nutrients selected for the study. All other food stuffs are not only costly but supply only one of the selected nutrients, the rest being negligible. Thus the dark green leafy vegetable, drumstick leaves have far reaching implications towards the improvement of the nutritional status of children and other vulnerable groups. The Applied Nutrition Programmes, the school lunch and preschool feeding programmes need to emphasis the beneficial role of drumstick leaves.

## V. SUMMARY AND CONCLUSION

This investigation was undertaken on 40 per school children drawn from a low socio-economic community and participating in a balwady feeding programme, to determine the efficacy of drumstick leaves as a source of  $\beta$ -carotene, ascorbic acid, iron and calcium. The selected 40 children were divided into two groups of twenty each to form a control and an experimental group. The children of the experimental group were supplemented with drumstick leaves. The study was conducted for a period of 40 days.

$\beta$ -carotene, ascorbic acid, iron and calcium were estimated in fresh and cooked samples of drumstick leaves on five different days. The efficacy of the leafy vegetables as a rich source of vitamins and minerals was evaluated by collecting data on (i) changes in serum vitamin-A levels, (ii) changes in blood haemoglobin levels (iii) changes in serum ascorbic acid levels and (iv) changes in serum calcium levels as a result of supplementation. The daily dietary intake of  $\beta$ -carotene, ascorbic acid, iron and calcium was surveyed in the control and experimental children to know the amount of these nutrients affecting the serum levels through dietary intake.

The findings of the weighment survey showed that the intake of almost all the nutrients was much below the recommended

daily allowances of ICMR (1981) in the target children. The energy and protein intake was 30.5 and 18.7 per cent short of the recommended daily allowances of ICMR (1981).

Also, only 17.4 per cent of calcium, 32.8 per cent of iron 10.1 per cent of  $\beta$ -carotene and 5.2 per cent of vitamin-C of the daily allowances were met by the target children. On supplementation of 24.8g of drumstick leaves 69.85 per cent of energy, 87.54 per cent of protein, 44.48 per cent of calcium, 49.6 per cent of iron, 100 per cent of  $\beta$ -carotene and 92 per cent of ascorbic acid of the daily allowances were met by the experimental children, due to the supply of 32.82 kilocalories of energy, 1.66g of protein, 103.1mg of calcium, 1.49mg of iron, 1200mcg of  $\beta$ -carotene and 25.6mg of ascorbic acid present in 24.8g of drumstick leaves.

The values got by estimating fresh and cooked drumstick leaves showed that there was a significant loss of vitamins and minerals on cooking. The mean  $\beta$ -carotene content of fresh leaves was  $6802.53 \pm 10.94$ mcg per cent, and for cooked leaves it was  $4843.73 \pm 12.14$ mcg per cent. The cooking loss of  $\beta$ -carotene was 28.8mcg per cent. Ascorbic acid content of drumstick leaves had a mean of  $224.43 \pm 15.01$ mg per cent in fresh form and  $103.27 \pm 14.0$  per cent in cooked form. The percentage loss was 53.99. The mean iron content of fresh leaves was  $6.23 \pm 0.4$ mg per cent, of cooked leaves was  $6.009 \pm 0.5$ mg per cent, having a cooking loss

of 3.6 per cent. The mean calcium content of fresh leaves was  $445.93 \pm 13.8$ mg per cent, of cooked leaves was  $416.38 \pm 11.2$ mg per 100g with cooking loss 3.72mg per cent. The results showed that the percentage loss of vitamins was more than the minerals.

Changes in serum vitamin-A levels indicated that those children supplemented with drumstick leaves showed an appreciable mean increment  $8.445 \pm 1.67$ mcg per 100ml serum, where as the children of control group who did not receive any supplement showed an increment of  $10.285 \pm 0.298$ mcg per 100ml of serum which was not statistically significant. The increment in serum vitamin-A of the experimental group was significant at one per cent level, when compared with that of the control group.

The mean haemoglobin levels of the target children revealed that those children fed with drumstick leaves recorded a higher increment  $1.04 \pm 0.185$ g per cent, while the control group recorded an increment of  $0.265 \pm 0.14$ g per cent only. The mean increment of haemoglobin registered by the experimental group was significant at one per cent level, when compared with that of the control.

The experimental children showed a marked rise in serum ascorbic acid level  $0.995 \pm 0.272$ mg per 100ml, whereas in the control group it was  $0.27 \pm 0.24$ mg per 100ml. Statistical analysis showed that this increment in experimental children was significant at one per cent level, when compared with that of the control children.

The children fed with drumstick leaves recorded higher values ( $3.78 \pm 0.165$ mg per cent) of mean serum calcium, while the increment of control group was only  $0.185 \pm 0.13$ mg per cent. The increment note in experimental children was significant ( $P < 0.001$ ) when, compared with that of the control children.

Cost analysis revealed that 24.8g of the cooked drumstick leaves costing 2 paise contribute 1200mcg of  $\beta$ -carotene supplied by 30g of egg costing 40 paise, 103.1mg of calcium present in 90g of milk costing 25 paise, 1.49mg of iron in 8 paise of jaggery and 25.6mg of ascorbic acid in lemon costing 45 paise. Thus drumstick leaves <sup>was</sup> worked out to be the cheapest source and also a nutritious food stuff.

The study reveals that the inclusion of green leafy vegetables such as drumstick leaves which is a rich source of  $\beta$ -carotene, vitamin-C, iron and calcium in the food would increase the serum level of these nutrients appreciably and would alleviate the deficiency symptoms of these nutrients in children.

The following recommendations are presented based on the findings of this study.

1. The inclusion of drumstick leaves should be a regular feature in Balwady and school feeding programmes.
2. Cultivation of drumstick leaves in the Balwady and school gardens should be favoured.
3. The importance of drumstick leaves as a source of many nutrients should be made familiar among the villagers and illiterates so that its utilisation will be increased.

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A P P E N D I C E S

## APPENDIX - I

### Estimation of $\beta$ -carotene

About 1.0g of the fresh drumstick leaves sample is chopped quickly with sharp knife into a small mortar and then ground rapidly with sand and 5ml acetone. Petroleum ether (40-60°C) 5ml is added and the grinding continued briefly. Most of the pigments will now have been extracted. Particles of sand and the sample material will settle out and the extract is decanted into a 25ml volumetric flask. Three further extractions are made, each with about 4ml petroleum ether, the extracts are added to the flask and the volume is made up to the mark. The flask, with all the pigments now in solution of petroleum ether containing 20% acetone, is kept in the dark until the next stage. If any debris has been carried over into the flask it should settle to allow a sample to be removed with a pipette. Usually a 10ml sample is taken so that the determination can be repeated if necessary.

The carotene is separated from the other pigments of a column of alumina ( $Al_2O_3$  active; for chromatography absorption analysis, Brockman activity II). A glass tube with 2.5cm in diameter with the bottom end narrowed and plugged with glass wool is used. Alumina is mixed with an equal weight of anhydrous sodium sulphate and the mixture placed in the column to a depth of about 5cm. A batch of the absorbent mixture can be made up and stored tightly, stoppered until wanted.

The sample containing the pigments is added carefully to the top of the column and when all the fluid has passed into the absorbent, petroleum ether is added. The carotene travels down leaving the other pigments behind and starts to come off in the first runnings of the column. Solvent is added until the eluate comes off colourless. The eluate is made up to a known volume eg. 20ml and the absorbance is read on a spectrophotometer at 450nm.

## APPENDIX - II

### Estimation of Ascorbic acid

#### Principle:

Ascorbic acid is a strong reducing agent which reduces the dye 2,6 dichlorophenol indophenol which is blue in colour, to its colourless leucoform. This is made use of in the estimation.

#### Reagents:

##### a. Stock standard ascorbic acid solution

100mg of pure dry crystalline ascorbic acid was weighed accurately, dissolved in 4% oxalic acid and made upto 100ml with oxalic acid in a standard flask.

##### b. Working standard

10ml of the stock standard solution was diluted to 100ml in a standard flask with 4% oxalic acid.

##### c. Dye solution

42mg of sodium bicarbonate and 52mg of 2,6 dichlorophenol indophenol were taken in a 250ml standard flask, and dissolved in 50ml of distilled water and made upto the mark with water.

##### d. 4% Oxalic acid

#### Procedure

Weighed out 5g of drumstick leaves and soaked in 4% oxalic acid for 5 minutes and ground well and the resultant solution was centrifuged. The supernatant was transferred into 100ml standard flask. This was repeated for three or four

times and then made up the solution in the flask to 100ml with 4% oxalic acid.

#### Standardisation of the Dye

Pipetted out 5.0ml of the standard ascorbic acid solution into a conical flask and then added 10.0ml of 4% oxalic acid. The conical flask was placed in an ice container and contents were titrated against the dye contained in the burette. The end point was the appearance of a distinct pink colour which persisted for 30 seconds. The amount of the dye consumed is equivalent to the amount of the ascorbic acid present.

#### Estimation of Vitamin C

Pipetted out 5.0ml of the experimental solution into a clean dry conical flask and added 10.0ml of 4% oxalic acid. The contents were titrated against the standardised dye in the cold. The end point was noted then calculated the amount of the dye got from the above experiment.

## APPENDIX - III

### Estimation of calcium:

#### Preparation of mineral solution

About 2.0g of the drumstick leaves were accurately weighed in a chinadish and was heated in the oven at 100°C for 6 hours. The moisture free sample was completely transferred into a silica crucible and it was heated for some time by placing it on clay pipe triangle above the burner. The burner should be adjusted to a low flame till all the plant material in the crucible gets charred. Then it was transferred to a muffle furnace for about 3½ hours at about 6000°C. It was then cooled in a desiccator and weighed. To ensure completion of ashing the crucible was again heated in the muffle furnace for ½ an hour cooled and weighed. This was repeated till two consecutive weights are the same and the ash was almost white or greyish white in colour.

The ash obtained in the above process was moistened with a small amount of glass distilled water (0.5 to 1.0ml) and 5.0ml of distilled Hcl were added to it. The mixture was evaporated to dryness on a boiling water bath. Another 5.0ml of hydrochloric acid was added again and the solution was evaporated to dryness as before. A few ml of water was then added and warmed over a boiling water bath and filtered into

a 25ml standard flask using whatman No.40 filter paper. The volume was made up to 25ml with distilled water and suitable aliquots were used for the estimation of calcium and also iron.

2.0ml of the prepared mineral solution was taken in centrifuge tubes. Added 2.0ml of 4% ammonium oxalate. Mixed well and let stand overnight. In the next day it was centrifuged and the supernatant was discarded. The precipitate was washed strongly with 3.0ml of 2 per cent ammonia solution. Centrifuged and discarded and the supernatant. This was done to remove the excess of ammonium oxalate. The precipitate was then warmed with 2.0ml of 2N sulfuric acid so that the solution of oxalate was complete. Removed the tubes from the bath and the hot contents (70-75°C) were titrated against N/100 potassium permanganate to a faint pink color which persisted for about a minute. As a blank, titrated 2.0ml of hot 2N sulfuric acid to the same end point. The difference in the two titre values gives the volume of 0.01N potassium permanganate solution to titrate the precipitated calcium oxalate.

## APPENDIX - IV

### Estimation of Iron:

#### Reagent

1. 3N potassium thiocyanate:  
73g of potassium thiocyanate was dissolved in 250ml of distilled water.
2. Saturated potassium persulphate solution.
3. Concentrated sulphuric acid.
4. Standard Iron solution:

17.2mg of ferrous ammonium sulphate was dissolved in distilled water. Added 1.ml of concentrated sulphuric acid and made upto 100.ml with water in a standard flask.

#### Working standard

10.0ml of the stock standard is diluted to 100ml with water so that 1.0ml contains 10 $\mu$ g of iron.

#### Procedure

About 2.0g of fresh leaves was ashed and dissolved in concentrated hydrochloric acid and made upto 25.0ml with distilled water. In to a series of clean test tubes added 1, 2, 3, 4 and 5ml of the standard iron solution. The volumes of each were made upto 7.7ml with distilled water. 1.0ml of the ash solution was taken and made upto 7.7ml with water. Then added 0.4ml of saturated potassium persulphate solution, 0.3ml of concentrated sulfuric acid and 1.6ml of 3N potassium thiocyanate. The color developed was read in

a colorimeter against a reagent blank using a 540mp within 10 minutes. A standard graph was drawn by plotting concentration of iron on X axis and the klett reading on Y axis.

## APPENDIX - V

### Estimation of serum vitamin A (Retinol)

The serum vitamin A was estimated by the trifluoro acetic acid method of Neeld and Pearson as modified and suggested by Roel et al quoted by Gyergy and Pearson (1967).

### Procedure

#### Reagents

1. Absolute ethanol: purified for spectrophotometry.
2. n-Hexane: Fisher certified reagent special for spectrophotometry.
3. Chloroform: Merck reagent special for spectrophotometry.
4. Trifluoro acetic acid: reagent grade (Sigma).
5. 1N alcoholic KOH.
6. a) stock vitamin A solution: 344mg of vitamin A acetate (300mg of vitamin A) was dissolved in chloroform and made upto 100ml, 1ml of stock contains 3000mcg of retinol  
b) Intermediate standard:
  1. 0.1ml of stock diluted to 100ml with chloroform (3mcg/ml).
  2. 0.1ml of stock diluted to 50ml with chloroform (6mcg/ml).
  3. 0.15ml of stock diluted to 50ml with chloroform (9mcg/ml).
  4. 0.1ml of stock diluted to 25ml with chloroform (12mcg/ml).  
c) Working standard: Each intermediate standard was again diluted in the ratio 1:10 and from each standard finally 1.0ml was taken.

### Method:

The serum (0.5ml or less) was saponified with an equal volume of 1N ethanolic KOH in a water bath at 60°C for 20 minutes. The mixture was cooled and vigorously shaken in a glass-stoppered tube with an equal volume (1.0ml) of n-hexane for 10 minutes. The tube was centrifuged for 1 minute at 100g to separate the layers. An aliquot (0.8ml) of the n-hexane layer was pipetted off for determination of retinol. The n-hexane was evaporated from this aliquot in a water bath at 60°C in a stream of oxygen free nitrogen. The last traces of n-hexane are removed by nitrogen blowing at room temperature. The residue was taken up in (0.5ml) chloroform; 1 drop of acetic anhydride was added followed by (0.1ml) trifluoro acetic acid. The mixture was shaken vigorously and the optical density at 620m $\mu$  was determined exactly 30 seconds after the addition of the trifluoro acetic acid.

### Correction of vitamin A transmission reading at 620 m $\mu$ for carotene interference at that wavelength

Trifluoro acetic acid in chloroform solution reacts with carotene, and the absorption of the reaction product at 620m $\mu$  interfered with the vitamin A determination, done at the same wavelength.

A series of the solutions of the  $\beta$ -carotene standard in petroleum ether were made and aliquot portions of it were evaporated and the residue was taken up in chloroform and

optical density was read at 452m $\mu$ . A second series of solutions of the  $\beta$ -carotene standard in petroleum ether was made, aliquot portions of it were evaporated from centrifuge tubes and exactly the same procedure was applied to the residues remaining in the centrifuge tubes as was described for serum. The optical density at 620 m $\mu$  contributed by the  $\beta$ -carotene TFA complex and the true value of the optical density due to vitamin A was employed in the final calculation.

## APPENDIX - VI

### Estimation of Ascorbic acid

The serum ascorbic acid is estimated by Roe and Kuther method.

### Reagents

1. 2,4 Dinitro phenyl hydrazine reagent:  
Weighed 2g of 2,4 dinitrophenyl hydrazine, 250mg of thiourea and 30mg of copper sulphate and made upto 100ml with 9N sulphuric acid. Keep in refrigerator. Stable for one week.
2. 10% Trichloro acetic acid.
3. 65% sulphuric acid.

### Stock standard

100mg of ascorbic acid was weighed and made upto 100.0ml with 4% oxalic acid.

### Working standard

1.0 ml of the stock standard solution was diluted to 100ml with 4% oxalic acid.

### Procedure

To 0.8 ml of serum 3.2 ml of 10% trichloro acetic acid was added. Mixed well and allowed to stand for 5 minutes and centrifuged at 2000rpm.

Transferred 3.5ml aliquot to a small test tube and added 0.2ml of dinitrophenyl hydrazine reagent. Stopped

and incubated at 37°C for 3 hours. Chilled in ice bath, added 0.8ml of cold 65% sulphuric acid and mixed well. Allowed to stand for 30 minutes at room temperature. The yellow colour produced was read at 520m $\mu$  in a colorimeter.

Blank comprised 0.5ml trichloroacetic acid treated as for the serum. Standard comprised 0.5, 1.0, 1.5, 2.0, 2.5, 3.0ml of ascorbic acid solution corresponding to the  $\mu$ g values 5,10,15,20,25 and 30 respectively and the above procedure was repeated.

## APPENDIX - VII

### Estimation of serum calcium by EDTA method (Jackson, 1976)

#### Principle

The method makes use of the remarkable chelating properties of disodium ethylene diamine tetra acetate (versenate or Sodium versanate). It forms chelates which is soluble with many divalent cations.

#### Reagent

1. Ethylene diamine tetra acetic acid disodium salt (EDTA 0.02N):- Dissolved 19g of the reagent in 5 litres of distilled water, standardised against calcium carbonate solution and adjusted to exactly 0.02N solution.
2. Murexide: Mixed 40g of potassium sulphate with 10g of ammonium chloride and 0.02g of ammonium perpurate.
3. 10% Sodium hydroxide.

#### Procedure

About 1.0ml of the serum with 13ml of triple acid (Nitric acid, Sulphuric acid and Perchloric acid in the ratio of 9:2:1) for 1 hour in a microkjeldahl flask in order to get the element calcium in free form in the resulting solution.

5.0ml of the triple acid extract was taken in a china dish and added 10.0ml of 10% sodium hydroxide and added about 0.1g of murexide indicator. This solution was titrated against

0.02N versenate solution until the color changed from red to violet at the end point an additional drop of versenate solution should produce no further change.

1.0ml of the 0.02N EDTA = 0.0004 mg of calcium.

The amount of calcium present in 100ml of the sample is equal to  $0.0004 \times 1000 \times 100 \times$  titre value of calcium in mg/100 ml.

## APPENDIX - VIII

### Estimation of Haemoglobin by cyanmethaemoglobin method

#### Reagents

1. Drabkins diluent solution:

Sodium bicarbonate	- 1.0g
Potassium cyanide	- 0.05g
Potassium ferricyanide	- 0.2g
Distilled water	- 1.litre.

This solution is preserved in dark brown bottle and preferably under cold storage. Its preparation and handling should be done with great care. This solution should not be used after it forms a precipitate at the bottom of the storage.

#### Procedure

1. Exactly 5.0ml of Drabkins diluent solution is measured into a dry test tube from a burette or a pipette with suction bulb.
2. Exactly 0.02ml of blood is transferred from a standard hemoglobin pipette into a diluent solution. Usual care in filling and cleaning of loaded Hb pipette must be observed.
3. The pipette is rinsed three times with the diluent solution without allowing the formation of air bubbles in the solution.
4. The blood and the diluent are thoroughly mixed by rotating the tube.
5. Ten minutes time is allowed for the formation of the cyanmethaemoglobin.

6. 5.0ml of the diluent solution is used as blank.
7. The readings are taken in a photoelectric colorimeter at 540mp.

#### Calibration Procedure

1. Total blood iron is determined by Wong's method. This determination would give absolute amount of Haemoglobin.
2. Exactly 0.02ml of this known blood sample is measured into 5.0, 7.5, 10.0, 12.5 and 15.0ml of the diluent solution. These are now equivalent to blood samples containing respectively 100, 67, 50, 40 and 30% of the original hemoglobin concentraion.
3. The intensity of the color is read using a green filter (540mp) against a blank set at zero O.D.
4. A standard graph is drawn using these hemoglobin concentration and corresponding O.D. values.

APPENDIX - IX

BODY HEIGHTS AND WEIGHTS OF THE TARGET CHILDREN

Group	S.No.	Age (months)	Sex	Height (cm)		Weight (Kg)	
				a	b	a	b
	1	48	Boy	89	89.0	10.0	10.1
	2	45	Boy	85	85.1	13.0	13.0
	3	58	Boy	103	103.2	13.5	13.5
	4	59	Boy	101	101.2	15.5	15.7
	5	52	Boy	94	94.1	12.5	12.4
CONTROL	6	59	Boy	100	100.2	16.0	16.3
	7	48	Boy	94	94.0	14.0	14.1
	8	56	Boy	100	100.3	13.0	13.0
	9	58	Boy	102	102.4	13.0	13.0
	10	56	Boy	101	101.4	11.0	11.0
	11	42	Girl	82	82.0	13.5	13.6
	12	56	Girl	98	98.1	13.0	13.0
	13	52	Girl	94	94.0	12.0	12.1
	14	52	Girl	91	91.3	11.5	11.5
	15	48	Girl	95	95.2	14.0	14.2
	16	54	Girl	96	96.2	12.0	12.0
	17	37	Girl	86	86.1	10.0	10.0
	18	36	Girl	81	81.0	8.0	8.2
	19	42	Girl	90	90.1	12.0	12.0
	20	46	Girl	94	94.0	12.5	12.7

Group	S.No.	Age (months)	Sex	Height (cm)		Weight(Kg)	
				a	b	a	b
Experiment	1	56	Boy	90	90.8	11.5	12.0
	2	48	Boy	89	89.9	11.0	11.7
	3	59	Boy	102	103.0	15.0	15.8
	4	58	Boy	95	95.2	13.5	13.5
	5	50	Boy	93	93.0	11.0	11.2
	6	51	Boy	90	90.0	11.5	11.7
	7	58	Boy	99	99.3	13.0	14.0
	8	60	Boy	103	103.5	14.5	15.6
	9	49	Boy	89	89.5	12.0	12.3
	10	45	Boy	88	88.7	12.1	12.6
	11	57	Girl	101	101.9	14.0	14.5
	12	56	Girl	82	82.5	10.5	11.6
	13	56	Girl	98	98.5	14.0	14.8
	14	45	Girl	94	94.7	12.5	12.9
	15	49	Girl	91	91.0	8.0	9.1
	16	46	Girl	95	95.3	13.0	13.5
	17	50	Girl	96	96.1	12.0	12.6
	18	48	Girl	86	86.9	11.0	11.2
	19	39	Girl	81	81.8	10	10.5
	20	36	Girl	90	90.0	11.5	11.7

APPENDIX - X

INITIAL AND FINAL LEVELS OF SERUM VITAMIN-A OF THE TARGET CHILDREN

S.No.	Control			Experiment		
	Amount of vitamin-A $\mu\text{g}/100\text{ml}$			Amount of vitamin-A $\text{mcg}/100\text{ml}$		
	Initial	Final	Difference	Initial	Final	Difference
1	12.1	12.2	0.1	11.0	19.5	8.5
2	13.4	13.7	0.3	12.2	21.3	9.1
3	10.9	10.9	0	16.3	22.7	6.4
4	10.5	10.0	0.5	14.7	22.3	7.6
5	12.6	13.2	0.6	19.0	27.4	8.4
6	13.5	13.6	0.1	8.4	18.3	9.9
7	11.1	11.3	0.2	10.5	23.1	12.6
8	12.2	12.6	0.4	11.3	22.8	11.5
9	18.0	18.8	0.8	14.6	23.6	9.0
10	14.2	14.9	0.7	8.8	18.9	10.1
11	15.6	16.1	0.5	10.3	19.1	8.8
12	11.9	12.0	0.1	17.1	25	7.9
13	16.6	16.8	0.2	12.5	19.3	6.8
14	14.1	14.5	0.4	11.6	19.2	7.6
15	15.2	15.3	0.1	12.0	20.1	8.1
16	16.6	16.9	0.3	16.3	22.6	6.3
17	8.8	9.2	0.4	12.7	21.1	8.4
18	10.6	10.7	0.1	10.5	19.1	8.6
19	9.3	9.5	0.2	6.8	14.0	7.2
20	13.3	14.0	0.7	9.3	15.4	6.1

APPENDIX - XI

INITIAL AND FINAL LEVELS OF BLOOD HAEMOGLOBIN OF THE TARGET CHILDREN

S.No.	Experiment			Control		
	Amount of Haemoglobin g/100ml			Amount of Haemoglobin g/100ml		
	Initial	Final	Difference	Initial	Final	Difference
1	8.2	8.9	0.7	9.3	9.5	0.2
2	8.6	9.5	0.9	9.2	9.3	0.1
3	9.3	10.5	1.2	10.3	10.3	0
4	9.4	10.4	1.0	10.1	10.2	0.1
5	9.8	11.3	1.5	8.5	8.8	0.3
6	8.0	9.0	1.0	8.2	8.5	0.3
7	8.7	9.5	0.8	10.2	10.4	0.2
8	8.5	9.8	1.3	9.4	9.8	0.4
9	8.8	10.0	1.2	9.6	9.9	0.3
10	8.4	9.1	0.9	10.1	10.4	0.3
11	8.9	9.7	0.8	9.5	9.9	0.4
12	9.7	10.9	1.2	9.6	9.7	0.1
13	9.1	10.2	1.1	9.9	10.3	0.4
14	9.2	10.5	1.3	9.8	10.2	0.4
15	9.5	10.4	0.9	10.3	10.6	0.3
16	8.8	9.5	0.7	8.6	9.0	0.4
17	8.8	10.3	1.5	7.9	8.0	0.1
18	7.9	8.9	1.0	8.1	8.7	0.6
19	7.1	8.0	0.9	7.8	8.1	0.3
20	9.9	11.2	1.3	8.3	8.4	0.1

APPENDIX - XII

INITIAL AND FINAL LEVELS OF SERUM ASCORBIC ACID OF THE  
TARGET CHILDREN

S.No.	Experiment			Control		
	Amount of ascorbic acid mg/100ml			Amount of ascorbic acid mg/100ml		
	Initial	Final	Difference	Initial	Final	Difference
1	0.4	1.6	1.2	0.6	0.7	0.1
2	0.3	1.3	1.0	0.4	0.6	0.2
3	0.6	1.5	0.9	0.3	0.3	0
4	0.4	1.5	1.1	0.5	0.9	0.4
5	1.1	1.9	0.8	0.5	0.6	0.1
6	0.7	2.0	1.3	0.6	0.9	0.3
7	0.5	2.1	1.6	0.8	0.9	0.1
8	0.5	2.0	0.5	0.4	0.4	0.0
9	1.2	1.8	0.6	0.3	0.5	0.2
10	1.8	2.4	0.6	0.9	1.0	0.1
11	0.9	1.8	0.9	1.1	1.4	0.3
12	0.8	1.8	1.0	1.0	1.1	0.1
13	0.5	1.6	1.1	0.9	1.2	0.3
14	0.4	1.8	1.4	1.2	1.4	0.2
15	0.5	1.4	0.9	0.6	1.6	1.0
16	0.3	1.5	1.2	0.7	1.3	0.6
17	0.8	1.6	0.8	0.9	1.3	0.4
18	0.6	1.5	0.9	0.4	0.4	0.1
19	0.5	1.6	1.1	1.2	1.7	0.5
20	0.7	1.7	1.0	1.4	1.8	0.4

APPENDIX - XIII

INITIAL AND FINAL LEVELS OF SERUM CALCIUM OF THE TARGET CHILDREN

S.No.	Experimental group			Control group		
	Amount of calcium mg/100ml			Amount of calcium mg/100ml		
	Initial	Final	Difference	Initial	Final	Difference
1	6.0	9.5	3.5	6.3	6.4	0.1
2	7.2	10.4	3.2	5.8	6.0	0.2
3	6.2	8.9	2.7	5.9	6.0	0.1
4	5.9	9.5	3.6	6.0	6.5	0.5
5	5.1	9.6	4.5	6.5	6.6	0.1
6	6.3	10.8	4.5	6.3	6.5	0.2
7	5.9	10.0	4.1	6.6	6.9	0.3
8	5.2	8.9	3.7	6.5	6.6	0.1
9	5.2	9.4	4.2	5.6	5.8	0.2
10	6.1	9.6	3.5	6.3	6.5	0.2
11	6.3	9.4	3.1	5.5	5.6	0.1
12	5.3	9.9	4.6	6.6	6.7	0.1
13	6.5	11.2	4.7	6.1	6.5	0.4
14	6.0	10.0	4.0	6.0	6.3	0.3
15	5.3	9.2	3.9	6.4	6.4	0
16	5.9	9.5	3.6	6.0	6.3	0.3
17	6.0	9.7	3.7	5.5	5.7	0.2
18	5.4	9.6	4.2	5.9	6.0	0.1
19	5.5	9.4	3.9	6.5	6.6	0.1
20	6.7	9.1	2.4	6.8	7.0	0.2