EFFECT OF SUPPLEMENTATION OF RED PALM OIL, IRON AND VITAMIN C ON VITAMIN A AND IRON STATUS OF ADOLESCENT GIRLS

BY

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CERTIFICATE

Ms.KUNA APARNA has satisfactorily prosecuted the course of research and that the thesis entitled **EFFECT OF SUPPLEMENTATION OF RED PALM OIL, IRON AND VITAMIN C ON VITAMIN A AND IRON STATUS OF ADOLESCENT GIRLS** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any university.

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CERTIFICATE

This is to certify that the thesis entitled **EFFECT OF SUPPLEMENTATION OF RED PALM OIL, IRON AND VITAMIN C ON VITAMIN A AND IRON STATUS OF ADOLESCENT GIRLS** submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN HOME SCIENCE** for Acharya N.G.Ranga Agricultural University, Hyderabad is a record of the bonafide research work carried out by **Ms.KUNA APARNA** under my guidance and supervision. The subject of the thesis has been approved by the student's Advisory committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. The published part has been fully acknowledged. All the assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

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LIST OF ABBREVIATIONS

Abs	:	Absorbance
BMI	:	Body mass index
DDRPO	:	Deacidified Deodorised Red Palm Oil
FAO	:	Food and Agriculture Organization
g/dl	:	gram per deciliter
gm	:	gram
HPLC	:	High Performance Liquid Chromatography
IDA	:	Iron Deficiency Anaemia
IQ	:	Intelligence Quotient
IU	:	International units
kg	:	kilogram
mg	:	milligram
mt	:	meter
ml	:	milliliter
ml/min	:	milliliter per minute
g	:	microgram
g/dl	:	microgram per deciliter
NIN	:	National Institute of Nutrition
NNMB	:	National Nutrition Monitoring Bureau
PORIM	:	Palm Oil Research Institute of Malaysia
ppm	:	Parts per million
RDA	:	Recommended Daily Allowances
RPO	:	Red Palm Oil
UNICEF	:	United Nations Children's Educational Fund
WHO	:	World Health Organization

DECLARATION

I, **Ms.KUNA APARNA** hereby declare that the thesis entitled **EFFECT OF SUPPLEMENTATION OF RED PALM OIL, IRON AND VITAMIN C ON VITAMIN A AND IRON STATUS OF ADOLESCENT GIRLS** submitted to Acharya N.G.Ranga Agricultural University for the Degree of **MASTER OF SCIENCE IN HOME SCIENCE** is a result of original research work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

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ABSTRACT

The present study was conducted to assess the improvement in vitamin A and iron status of adolescent girls on supplementation with Red palm oil, iron and vitamin C. Deodorised Deacidified Red Palm Oil (DDRPO) is a partially refined palm oil with its carotene content retained upto 85 per cent and therefore an excellent source of natural vitamin A. 15 volunteers with depleted levels of vitamin A and iron were selected from a school and information regarding general dietary patterns and intake of protective foods was collected.

After collection of baseline data, all the subjects were supplemented for 30 days with placebo, 45 days with placebo + iron tablets, 45 days with DDRPO laddu + iron tablets, 45 days with DDRPO laddu + iron tablets + vitamin C tablets. At the end of each supplementation period serum retinol, -carotene and blood haemoglobin were estimated.

By the end of the study, blood haemoglobin, vitamin A and -carotene status were found to be improved significantly. Maximum haemoglobin was achieved with both Vitamin A and iron supplementation rather than with iron supplementation alone and further improved with addition of vitamin C. Vitamin A and -carotene status improved significantly with DDRPO supplementation.

Improvement in vitamin A status may contribute to the control of anaemia in adolescent girls.

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INTRODUCTION

CHAPTER I

INTRODUCTION

The extent and magnitude of micronutrient deficiencies in India are a matter of continuing concern. Iron deficiency anaemia and vitamin A deficiency are among the major public health nutritional problems in many developing countries including India.

Deficiencies in the intake or absorption of vitamin A and iron have serious consequences for health, mental and physical function. The clinical manifestations of these nutritional deficiencies such as xerophthalmia and anaemia have been identified as major nutritional problems with increasing public health significance. During rapid growth, micronutrient intake must increase or else growth failure or deficiency diseases develop. It is during these periods that deficiency symptoms are most prevalent. For this reason, adolescents and women of reproductive age are high priority large groups for micronutrient deficiencies.

Adolescence is a significant period of human growth and maturation; unique changes occur and many patterns are established during this period. In addition, the proximity of adolescence to biological maturity and adulthood may provide final opportunities for preventing adult health problems. Human growth and maturation are continuous processes and transitions from childhood into adulthood are not abrupt. In contrast with the previous age groups, which are defined by chronologic age, adolescence begins with pubescence, the earliest signs of development of secondary sexual characteristics, and continues until morphologic and physiologic changes approximate adult status, usually near the end of the second decade of life. It is due to all these reasons that micronutrients are very essential during this important period in human development.

Vitamin A deficiency is not a new disease. Thousands of years ago, the ancient Egyptians recognised reversible night blindness. Hippocrates in ancient Greece, knew that the eating of liver was a treatment for night blindness. Today Vitamin A deficiency is a systemic disease with most prominent manifestations occuring in the eye. It is the most widespread nutritional disorder causing blindness to 60 million children in developing countries particularly in tropical and subtropical countries. Severe forms of the deficiency result in corneal blindness and the incidence is particularly high in young children of poor communities. Majority of the children suffer from subclinical deficiency of Vitamin A without signs of xerophthalmia, with serum retinol levels below 20 μg/dl.

Sommer (1989) reported that 25-50 million children world wide may be suffering from the physiologic consequences of Vitamin A deficiency. 5 million of them are developing xerophthalmia and of these, annually 2,50,000 to 5,00,000 go blind. About half a million children in India become blind every year as a result of vitamin A deficiency (Reddy and Vijayaraghavan, 1991d). More than 124 million children in the world were estimated to be affected by Vitamin A deficiency and a predicted 1 to 2.5 million deaths are preventable by improvement of Vitamin A nutriture (Humphrey *et al.*, 1992). Vitamin A deficiency continues to be a major public health problem in many parts of the world including India, and the Nutrition Foundation of India suggests exploring the natural sources available in the country, to combat the disorder through dietary improvement (Gopalan *et al.*, 1992).

Vitamin A is unique structurally, essential nutritionally, elegant in its photo-receptor role, and yet is tantalizingly mysterious in its other biological involvements. Vitamin A is only present in animal foods. However, it can be made in the body from yellow pro-vitamin A i.e., carotenoids found largely in foods of plant origin. Among 500 known carotenoids, 50-60 possess pro-vitamin A activity. The most important pro-vitamin A is β-carotene and this is converted to vitamin A in the body.

Recently, newer carotene rich foods that offer considerable promise were discovered. Some of them are *Red palm oil, spirulina, buriti, bhakri, santi, pingu, some oil seeds, Dunaliella sp., marunggay leaves and blakestea sp.*

Among them Red palm oil (RPO) (*Elaeis quineensis*), the unrefined, unbleached version of the more conventional palm oil

(PO) is the only vegetable oil which contains a very high quantity of carotenoids in the unsaponifiable, non-glyceride fraction (Goh, 1985). It is as yet unfamiliar to the Indian consumer, Systematic studies have been carried out at National Institute of Nutrition, Hyderabad on the nutritional and safety aspects of RPO (Manorama, 1994). Thus RPO is reported to be completely safe and useful in controlling nutritional blindness among children. The total carotenoid content of RPO has been reported to range between 500 to 700 μg/g with 85-90 per cent constituting of α and β-carotenes, the most active precursors of vitamin A (Ng and Tan, 1988). Palm kernal oil contains 0.30 μg β-carotene/g of oil.

Long before biochemists realized that iron is a necessary trace element and essential for life, there was a natural tendency to associate iron with strength. The ancient Greeks concocted potions of iron fillings dissolved in vinegar, hoping that drinking this liquor would empower them with the properties of the element.

Today anaemia is a widely prevalent condition, common in humans of all ages living in all continents, the most common cause being iron deficiency. It is defined as a condition in which the haemoglobin content of blood is lower than the normal as a result of deficiency of one or more essential nutrients regardless of the cause of such deficiency (WHO, 1968). The population groups most likely to be at risk are young children and women of child bearing age, particularly the pregnant and lactating mothers.

According to the UNICEF and WHO (1995) an estimated 2000 million population both in developed and developing countries suffer from anaemia. In India, the prevalence of anaemia is greater in 1-5 year age group (27%) followed by pregnant women (75%), adult women (65%) and adult men (45%) (FAO and WHO, 1993).

Nutritional anaemia is the end result of severe deficiency of one or more nutrients such as iron, folic acid, vitamin B_{12}, pyridoxine, protein and vitamin C. Of these, iron deficiency is by far the most common cause of nutritional anaemia.

Lack of knowledge of appropriate dietary content, insufficient amounts of animal protein in the diet, poor absorption and bioavailability of iron from the foods, insufficient utilisation of iron, poor nourishment are implicated as the major causes for the higher prevalence rates of iron deficiency. Apart from these, infections like malaria, worm infestations and other diseases either enhance or aggravate the condition.

Because the condition is so widespread, interventions to improve iron status usually involve the provision of pharmaceutical supplements. However, a review by Northrop-Clewes *et al.* (1996) indicated that the vitamin A status of target groups should be an important consideration when carrying out iron supplementation programs, especially in places where infections are highly prevalent.

Moreover iron-deficiency anaemia and hypo-vitaminosis A are two of the most prevalent nutritional problems in many parts of India. In some cases the average dietary intake of iron may be adequate yet iron deficiency anaemia persists, indicating the importance of other nutritional and environmental factors in its etiology.

Vitamin A and its derivatives are important not only for the normal functioning of the eye but also for the normal differentiation of other cell systems in the body, including parts of the haematological system. Arrays of epidemiological studies have indicated that vitamin A deficiency and anaemia often co-exist, and that there are significant associations between serum retinol and bio-chemical indicators of iron status.

Several studies in humans and experimental animals have shown that there is an interaction between vitamin A nutriture and iron nutrition and metabolism. These studies indicate that the lack of vitamin A may lead to a widespread anaemia characterized by low serum iron and elevated levels of this mineral in storage depots, particularly the liver. Epidemiological studies in both children and adults support this concept and nutritional interventions with Vitamin A in humans revealed a positive effect on the iron nutrition. This information suggested that vitamin A deficiency impairs the utilization of iron and that improving vitamin A nutriture may benefit the hematological condition of populations.

Taking into consideration the above evidences, the study was planned with the following broad objectives.

General objective

To study the improvement in vitamin A and iron status of adolescent girls on supplementation with Red palm oil, iron and vitamin c.

Specific objectives

- To assess the effect of iron supplementation alone on iron and vitamin A status of adolescent girls.
- To assess the effect of iron and DDRPO supplementation on iron and vitamin A status of adolescent girls.
- To assess the effect of iron, DDRPO and vitamin C on iron and vitamin A status of adolescent girls.
- To assess the overall impact of integrated supplementation of the three nutrients on Vitamin A and iron status of adolescent girls.

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REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

This section reviews the scientific evidence available on micronutrient malnutrition, prevalence of vitamin A deficiency and anaemia, interrelationship between vitamin A and iron, interrelationship between iron and ascorbic acid and Red palm oil as a source of vitamin A. The literature available on the above aspects has been sub divided and presented under the following sections namely:

2.1 Micronutrient malnutrition

2.2 Prevalence of vitamin A deficiency

2.3 Prevalence of iron deficiency anaemia

2.4 Interrelationship between vitamin A and iron

2.5 Interrelationship between iron and ascorbic acid

2.6 Red palm oil as a source of vitamin A

2.7 Dietary intake and nutritional status of adolescent girls

2.1 MICRONUTRIENT MALNUTRITION

Vitamins and minerals cannot be synthesized by the human body, therefore they must be provided by the diet. They are needed in small amounts, that is micrograms or milligrams daily hence they are called "micro nutrients". They are necessary for the regulatory systems in the body, for efficiency of energy metabolism and for other functions like cognition, efficient functioning of immune system, and reproduction. Deficiencies of these nutrients cause illness, death, learning disabilities and impaired work capacity.

Deficiencies in the intake or absorption of vitamin A and iron have serious consequences for health, and mental and physical functions. The clinical manifestations of these nutritional deficiencies such as xerophthalmia and anaemia have been identified as major problems with increasing public health significance.

WHO reported that in 1991 nearly 14 million preschool children had eye damage due to vitamin A deficiency (WHO, 1992a). Around 10 million of these children are located in Asia. Each year it is estimated that between 250,000 to 500,000 preschool children go blind from vitamin A deficiency. About two-thirds of these children die within months of going blind.

Anaemia is assessed by low haemoglobin with cut offs determined by WHO. The overall anaemia prevalence of women in developing countries is estimated at 42 per cent, equivalent to just over 370 million women. In pregnant women (with a cut off 11 g/dl haemoglobin) the prevalence is estimated at 51 per cent, and in non pregnant women, 41 per cent (with a cut off of 12 g/dl). While there is considerable variation in prevalence regionally from around

64 per cent in South Asia to 23 per cent in South America, it is striking that anaemia is prevalent throughout the developing world.

Eliminating micronutrient deficiencies can have major yet subtle social and economic benefits. When nutritional deficiencies are eliminated, adverse consequences, such as reduced IQ, impaired growth, reduced work capacity, and death associated with pregnancy and child birth are reduced. Food fortification provides maximum benefit for minimum investment.

Hathcok recently published a recompilation of the safety range for vitamins and minerals, defining the NOAEL (Non-Observed Adverse Effect Level) and LOAEL (Lowest Observed Adverse Effect Level) for every micronutrient in some settings. Almost all country prevalence data relate to vitamin A deficiency in preschool children, which means that prevalence data from other age groups are frequently not available.

Table 1: Safety levels of (Vit.A and iron) micronutrients

Micronutrient	RDA	No. of times the RDA	
		NOAEL	LOAEL
Vitamin A	3.333 IU	3	6.5
Iron	10 mg	6.5	10

Source: Alberto Nilson *et al.* (1998)

It is estimated that some 5 million children, in Asian countries suffer from xerophthalmia of which almost a quarter go blind (Sommer, 1989). It has been reported that vitamin A deficiency is resulting in blindness in more than 60 million children in the world (vitamin A Report, 1990). Further, it is estimated that 2,00,000 cases of blindness among children were reported anually in less industrialized parts of the world (Sommer, 1981).

2.2 PREVALENCE OF VITAMIN A DEFICIENCY IN INDIA

Surveys in different parts of India have shown that about 5-10 per cent of children have clinical signs of vitamin A deficiency (Reddy, 1991a). India has an estimated population of 86 crores with an under five population of 21 per cent among whom approximately 80,000 corneal blindness and 40,000 blind cases are reported anually (Devadas, 1984).

Severe forms of the disease involving cornea are seen most frequently in children in the age group of 1-5 years (Reddy, 1991b). NFI (1991) reported about 30,000 cases of corneal xerophthalmia in preschool children per year, of which, nearly half would result in permanent blindness.

2.3 PREVALENCE OF IRON DEFICIENCY ANAEMIA

Iron deficiency anaemia (IDA) is the most prevalent nutritional deficiency worldwide. It is a major public health problem with adverse consequences especially for women of reproductive age and for young children. Over 90 per cent of affected individuals live in developing countries. The consequences of IDA are numerous as iron plays a central role in the mechanism for oxygen transport and is essential in many enzyme systems. Of greatest concern is that IDA in infants and children is associated with impaired physical and cognitive development. In adults, IDA is associated with weakness and fatigue which reduce capacity for physical work and productivity. In pregnant women, it contributes to maternal mortality and morbidity and increases the risk of foetal morbidity, mortality and low birth weight (ACC/SCN, 1992). Severe anaemia may be a contributory factor in upto 50 per cent of maternal deaths in developing countries.

2.3.1 Anaemia - A Global problem

Iron deficiency anaemia has attracted the attention of scientists from various disciplines for many years and its magnitude has enhanced tremendously during the last half of the century, throughout the world. In children between the age group of 1-3 years the incidence of anaemia is around 60 per cent or more, which is distinctly higher than in age group 3-5 years where incidence is around 30-40 per cent. Anaemia is equally common among adolescent girls (WHO, 1970).

About 80 per cent of tropical anaemia is caused by iron deficiency and those most "at risk" are mensturating women and pregnant women (Bour, 1977). Johnson *et al.* (1982) found that among all the age groups in both the sexes; adult women especially the pregnant and lactating women were most affected by nutritional anaemia. Bruce Tagoe *et al.* (1978) studied the prevalence of anaemia in residents of South Ghana. It was observed that moderate anaemia with Haemoglobin <10 g/dl was common particularly among women of 15-29 years age.

According to Demaeyer and Tegman (1985) in Africa 48 per cent of women of child bearing age, about 50 per cent of pregnant women and 39 per cent lactating women are affected by anaemia. In East Asia 14 per cent of women in reproductive age and 41 per cent pregnant women are anaemic, whereas in South Asia, about 57 per cent of women and 56 per cent of pregnant women are anaemic. About 14 per cent of European women are affected by Anaemia.

A review of world wide prevalence of anaemia indicates that about 25 per cent of world's population (13,000 million people) is anaemic, of these 90 per cent belong to the developing countries of the world (Carriers, 1986).

2.3.2 Anaemia-A national problem

Since 40 years, focus of nutritional research in India has been on the prevalence of anaemia and it is catching the attention of scientists even today. Rao and Rao (1958) conducted a study on the general health and nutritional status of rural population in South India, and reported that mean Haemoglobin values in adult women was 10.96 g/dl; which is below the WHO norms. Similar results were observed by Rao *et al.* (1960).

Swaminathan *et al.* (1960) reported that about 64 per cent of women were found anaemic with Haemoglobin values less than 12 per cent and about 22 per cent of women suffered from moderate to severe anaemia with Haemoglobin <10 g/dl).

Of all the countries, for which the information is available, India has probably the highest prevalence of nutritional anaemia among women (WHO, 1976).

About 70.5 per cent of women in rural areas around Hyderabad are found to be anaemic (NIN, 1980). Saralakumari (1982) reported the mean Haemoglobin levels of 9.85 g/dl in tribal women of Andhra Pradesh as against 10.05 per cent among non-tribals of the same region. Over 15 per cent of urban and 75 per cent of low income group of rural Indian women are anaemic particularly in IIIrd trimester of pregnancy (ICMR, 1983-84).

Agarwal *et al.* (1986) reported the prevalence of anaemia as 57 per cent and 55 per cent in urban slums and rural areas of Varanasi in India respectively. Around areas of Hyderabad 65 per cent of adult women and 75 per cent of pregnant women suffer from anaemia (Narasinga Rao, 1987).

In India, iron deficiency is common, despite the intake of dietary iron of 28.4 mg/day (NNMB, 1988-90), which almost satisfies the recommended dietary allowance of 28 and 30 mg/day for average Indian adult man and woman respectively (ICMR, 1989).

In a study by Vasanthi *et al.* (1990), it has been shown that 37 per cent of rural girls <12 years have Haemoglobin <12g/dl and prevalence of IDA is 25 per cent in adolescent girls irrespective of their rural or urban slum stress.

A recent study by Schultink *et al.* (1995) showed that in preschool children with low iron status, twice weekly iron supplementation has an effect on iron status similar to that of daily supplementation.

The above studies indicate that majority of women than men suffer from IDA in developing countries like India.

2.4 INTER-RELATIONSHIP BETWEEN VITAMIN A AND IRON

The aetiology of iron deficiency anaemia and vitamin A deficiency is complex. Inadequate diet, unsatisfactory methods of preparation of food, faulty food habits, poor environment, sanitation, associated infections and infestations are the main factors which continue to perpetuate anaemia and vitamin A deficiency.

The haematologic influence of vitamin A was reported as early by Sure *et al.* (1929). In a study of vitamin A deficiency in rats, anaemia was observed in early pregnancy, which proceeded to an increase in haemoglobin concentration and erythrocyte number, apparently when the animal manifested symptoms of xerophthalmia. Koessler *et al.* (1926) had also concluded that blood regeneration cannot take place in the absence of vitamin A.

Sebrell and Harris (1954) reported that resumption of vitamin A feeding in depleted rats, showed an outburst of haematopoiesis in bone marrow and spleen and greater deposits of hemosiderrin in liver and spleen disappeared. Many other workers have shown impairment of haematopoietic function and anaemia in the vitamin A depleted rats (Mejia *et al.*, 1976; and Mejia *et al.*, 1979ab). It can be seen from these studies that anaemia might be a component of vitamin A deficiency, but might be masked by the dehydration which goes with severe vitamin A deficiency. This dehydration leads to a misleading rise in haemo concentration.

Gardner *et al.* (1979) suggested that when anaemia is observed in Vitamin A deficiency, it is probably not a result of a haemolytic process. They found that vitamin A deficiency results in decreased incorporation of 59 iron into red blood corposcles, which was 30-40 per cent of control values. Mejia *et al.* (1979b) also observed a significantly low incorporation of 59 iron into red blood corposcles. They found a significant reduction in plasma and blood volumes of vitamin A depleted rats. Moreover, there was a greater isotope accumulation in the liver and spleen of depleted rats.

Hodges *et al.* (1975) and Hodges *et al.* (1978) observed that induced vitamin A deficiency in adult men lead to hypoferimea, suggesting that iron deficiency may have resulted from vitamin A deficiency. They found significant positive correlation between plasma vitamin A levels and blood values for haemoglobin and haematocrit values, but these blood values were not correlated to dietary and medicinal iron intake. Vitamin A deficiency can thus be said to have an adverse effect upon absorption or utilization of dietary or medicinal iron. This concept is strengthened by the observation that haemoglobin and haemotocrit levels rose following restoration of vitamin A in the diet of these men. This effect was seen also by Hodges *et al.* (1978).

The role of vitamin A in haematopoiesis has been demonstrated in children also (Mc.Laren *et al.* (1968). Positive correlation between plasma retinol and serum iron was found by Mejia *et al.* (1977). They observed this correlation in children with adequate intake of iron. There was no correlation when dietary iron was low. Low haemoglobin and haemocrit levels in children with low levels of vitamin A were also noticed by other workers (NIN, 1977; NIN, 1980a; and Devadas and Suraj, 1980).

Mohanram *et al.* (1977) not only found low levels of haemoglobin and haematocrit in children with low plasma retinol levels, but also observed a significant increase in the haemoglobin, haematocrit and plasma iron levels on supplementation of vitamin A.

Hodges (1978) found that vitamin A supplementation has a positive effect on the iron utilisation in the body. Similar results were obtained by Meijia *et al.* (1979); Mejia and Arroyave (1982); Rekha (1983); Vijayalakshmi and Devadas R P (1987).

Ezzat *et al.* (1970) reported that iron supplementation produced a direct positive response in growth and red blood cell counts whereas the limited vitamin A supplement produced delayed decreases in growth and red cell number. Mejia *et al.* (1979) reported that anaemia may be a component of vitamin A deficiency, but might be masked by the dehydration that accompanies severe depletion of vitamin A. These data suggest that vitamin A and iron are interrelated factors in haemotologic responses of the rat.

David Staab *et al.* (1984) conducted a study to understand the relationship between vitamin A and iron in liver and their data supported the hypothesis that vitamin A is involved in the regulation of iron release from the liver. Mejia and Chew (1988) reported that simultaneous adminstration of vitamin A and iron resulted in a better response of serum iron and per cent transferin saturation than when the supplement consisted only of vitamin A or iron alone. Vitamin A benefits hematological condition and iron metabolism.

Bloem *et al.* (1989) and Bloem *et al.* (1990) not only provided further evidence of a causal association between vitamin A and iron metabolism but also observed that in areas where vitamin A deficiency is endemic, periodic massive vitamin A dose programs can also improve iron status of the population.

Richard *et al.* (1992) reported that vitamin A supplementation helps mobilize hepatic stores of iron and may enhance hematopoiesis in children with vitamin A deficiency. Annet *et al.* (1994) observed in rats with iron deficiency that low tissue iron levels coincided with high iron absorption and high total iron-binding capacity, and reported that changes in iron metabolism with vitamin A deficiency differed from those with iron deficiency alone.

In a study conducted by Meena Panth *et al.* (1990) at NIN it was reported that there was an increase in maternal and cord vitamin A levels with vitamin A and iron supplementation to 450 pregnant women. Also it was reported that there was no significant decline in haemoglobin occuring at 26-28 weeks of gestation. The birth weights were also not altered by vitamin A supplementation.

A study of pregnant Indonesian women showed that 100 per cent of the anaemic women were cured by combination therapy of vitamin A with iron, wehreas only 40 per cent were cured by vitamin

A alone and only 60 per cent by iron alone (Dojko Suharno *et al.*, 1993). This clearly has important programmatic and policy implciations. Recent work from Nepal by Stoltzful *et al.* (1997) showed that vitamin A supplementation improved only mild anaemia, and only in those women who were not infected by worms.

A review by Northrop-Clewes *et al.* (1996) indicated that vitamin A status of target groups should be an important consideration when carrying out iron supplementation programs, especially in places where infections are highly prevalent. Furthermore the study showed that vitamin A may have a protective effect during iron supplementation.

Recent expreiments by Layrisse *et al.* (1998) demonstrated that vitamin A prevented the inhibiting effect of polyphenols and phytates on iron absorption. It was also demonstrated that vitamin A had the same effect on iron absorption as phytase.

2.5 INTER-RELATIONSHIP BETWEEN IRON AND ASCORBIC ACID

Several studies have demonstrated the enhancing effect of ascorbic acid on the abosorption of the non-haem iron (Sayers *et al.*, 1973; Bjourn-Rasmussen and Hallberg, 1974; Hallberg *et al.*, 1986; 1989). Dramatic response occurs with ascorbic acid, which facilitates absorption both by reducing ferric and ferrous and by forming a chelate with ferric iron at acid pH that remains soluble in the alkaline milieu of the small intestine (Conrad and Schade, 1968). It was demonstrated that ascorbic acid given at different doses from 25 to 200 mg with 50 g maize enriched with 5 mg of iron as ferrous sulphate, progressively enhance iron absorption indicating a dose dependent enhancing effect of ascorbic acid on iron (Bjorn-Rasmussen and Hallberg, 1974). A much lower dose of 15mg ascorbic acid was also found to enhance iron absorption by more than 25 fold from rice meal (Gillooly *et al.*, 1983).

Ascorbate and iron are metabolically inter-related. High dietary intake of either nutrient was found to modify tissue iron distribution (Smith and Bidlack, 1979). Derman *et al.* (1980) and Rathee and Pradhan (1980) showed that ascorbic acid increases iron availability.

Inhibitory effects of various dietary factors on iron absorption were found to be counteracted significantly by ascorbic acid, for example, the addition of 100mg ascorbic acid to a meal containing isolated soy protein had almost six fold increase in iron absorption (Mork *et al.*, 1982). The dose dependent inhibitory effect of sodium phytate on iron absorption was also found to be counteracted by the addition of ascorbic acid (Hallberg *et al.*, 1989). Crystalline ascorbic acid and native ascorbic acid in foods appeared to have the same effect in promoting absorption of iron and about 50 mg of vitamin in each main meal is desirable for optimum effect on iron absorption (Hallberg *et al.*, 1986).

It has been reported that citric acid when added to a rice meal, in an amount equimolar to ascorbic acid did not increase iron absorption significantly. However, when amount of citric acid was increased to 1 gm there was a significant improvement in the iron absorption from the same meal (Gillooly *et al.*, 1983). The study showed that citric acid is less effective in improving iron absorption while compared to ascorbic acid. On the other hand, Hazell and Johnson (1982) found that enhancement of iron availability measured from diffusable iron was far more effective with citric acid compared to ascorbic acid. The same authors also reported an increased iron availability with an amount of citric acid present in many foods eaten in the average U.K. diets whereas ascorbic acid required was much larger in amounts than that normally found in foods, to increase iron availability (Hazell and Johnson, 1987).

Hunt *et al.* (1990) reported that in iron -depleted women consuming a diet with predicted poor iron availability ascorbic acid supplementation enhanced body iron retention for 5.5 weeks.

2.6 RED PALM OIL AS A SOURCE OF VITAMIN A

Red palm oil is an unconventional oil. It is an unrefined, unbleached version of the more conventional palm oil universally used. Red palm oil is bright red in colour. It is obtained from mesocarp of the fruit of oil palm (*Elaeis guineensis*) which originated in west coast of Africa. After the 15th Century, it was introduced to other parts of Africa, South East Asia, Malaysia and Latin America.

Cultivation of oil palm was started in India by Oil palm India Limited under the Technology Mission on Oilseeds (TMO) which was set up in the year 1986 to meet the vegetable oil shortages in India (Abraham, 1988). The committee identified areas in India for commercial plantation. About 2.5 lakh hectares in Andhra Pradesh (Krishna, East and West Godavari districts), Tamilnadu (Nagarcoli), Karnataka (Cauvery, Tungabhadra, Ghattaprabha, Mallaprabha areas), 1.0 lakh hectares in Orissa (Berhampore, Gangam) and 0.5 lakh hectares each in Assam, Tripura and Eastern states were earmarked for cultivation of oil palm (Narsinga Rao, 1992).

2.6.1 Chemical Composition of Red palm oil

Information on the chemical composition and physical behaviour of palm oil is essential for judging how best it can be used in its end products. A complete analysis of RPO by the Palm Oil Research Institute of Malaysia (PORIM) gave the following composition.

Table 2: Chemical composition of Red palm oil

Free fatty acid	3.2%
Monoglycerides	0.3%
Diglycerides	7.7%
Triglycerides	88.4%
Carotenes*	800 pm
Tocopherols and Tocotrienols	850 ppm
Sterols	300 ppm
Terpenes	800 ppm
Phospholipids	1150 ppm

Source: Berger (1980)

2.6.2 Carotenoids in Red palm oil

Carotenoids are sometimes present in traces in many vegetable oils but are unusually high in RPO. The carotenoid content of RPO from Malaysia and Zaire was found to vary between 500 and 700 ppm (Clegg, 1973). Larger amounts (800-1600 ppm) have been reported from Nigeria, Togo, Ivory coast and Dahomey.

A typical analysis of the composition of caretenoids shows that α and β-carotenes are the major components and the rest are β-carotene, lycopene and xanthophylls.

RPO has the highest vitamin A derived activity (500 IU vitamin A/g) primarily due to its β-carotene content. β-carotene may also contribute to the vitamin A activity to some extent. The bio-availability of β-carotene from RPO was found to be on par with synthetic vitamin A as per the studies of Manorama and Rukmini (1992) on school children. Apart from their vitamin A activity, palm oil carotenoids were found to be protective against membrane peroxidation and free radical formation thus inhibiting tumor progression (Sundaram, 1989).

2.6.3 Acceptability studies and supplementation trials with Red palm oil

RPO has widespread acceptability as a cooking oil in many parts of Africa. It is a major source of dietary fat in Nigeria being used extensively in the preparation of African salad. The widespread acceptability of RPO is due to its high solid glyceride content which gives it the desirable consistency without hydrogenation. Moreover it is readily digested, absorbed and utilized because of the occurrence of oleic acid in the second position of triglyceride structure.

The acceptability of RPO in certain Indian foods was tested by three investigators so far. Parvathi *et al.* (1988) reported better acceptability of RPO for shallow fat frying and seasoning than for deep fat frying and the cooked foods were reported to have better storage stability.

Manorama and Rukmini (1992b) conducted acceptability studies with recipes made using RPO. RPO was found to be well accepted in preparations where its yellow/orange colour blended well with the natural colour of certain India food items like 'upma', 'tamarind rice', deep fried products and cake.

In a study conducted by Saritha Mahapatra (1995) on red palm oil supplementation and massive Vitamin A supplementation programme in 36 school children, it was reported that 8g RPO (equivalent to 50,000 IU of vitamin A in Besan laddu) was as efficient as massive vitamin A dose in providing protection from vitamin A deficiency for three months after cessation of supplementation.

A study conducted by Srilatha (1994) showed that efficacy of RPO supplementation was comparable with massive dose of Vitamin A. RPO was found to be equally efficient in maintaining normal serum vitamin A levels. Even morbidity was comparatively less than the control group.

When the efficacy of periodic (monthly) dose of vitamin A and RPO was compared, RPO was found to be more efficient than vitamin A. The final serum vitamin A levels had increased and morbidity was also found to be lower in the group that received RPO in comparision with those who received periodic vitamin A supplements.

Manorama and Rukmini (1992b) proved that Red palm oil was nutritionally rich and unique in comparision with other edible oils as it has high content of β-carotene (400 ppm) which can be made an ideal choice for combating vitamin A deficiency in developing countries. Toxicological studies also indicate that RPO is safe for human consumption (Manorama *et al.*, 1989).

Indian School children fed on supplementary food prepared with RPO for 60 days had significant increases in serum retinol levels as well as increased liver retinol store suggesting the ready bio-availability of β-carotene (Manorama *et al.*, 1996).

2.6.4 Deodorised Deacidified Red Palm Oil (DDRPO)

Even though the benefits of edible grade RPO cannot be questioned based on the above available data, the problem of its viscosity and odour still remain. Studies have indicated its acceptability in food preparations, but reservations do exist due to the strong fruity odour, which the community at large is likely to reject.

A feasible alternative to this is deacidified deodorised RPO which is partially refined to get a clear light, transparent dark coloured oil with its carotene content intact. PORIM, Malaysia has developed such a product (Kalyan Sundaram and Yusuf Basiron, 1997) which has been patented and is now marketed by Global Palm products, Malaysia and distributed by Global Nutrition Products Private Limited, Ahmedabad, India.

A comparision of DDRPO with unrefined and unprocessed RPO indicated that DDRPO was totally devoid of Free Fatty Acids (FFA) due to partial refining, and around 80-85 per cent of carotenes and vitamin E was retained. Peroxide value was 'nil' indicating good shelf life and keeping quality.

Table 3: Comparision of DDRPO with RPO

	FFA	Carotene (ppm)	Vitamin E (ppm)	PV
Crude palm oil	4.52	623	1066	2.2
DDRPO	0.08	434	959	Nil
Molecular dist. procedure	0.04	513	1000	Nil

* Peroxide value
Source: Kalyan Sundaram and Yusuf Basiron (1997)

This oil has been found to be more readily acceptable than RPO in the diets. The thick viscous texture and odour was taken care of. However the product which is now available is of considerably high cost but the extensive use of oil in future can bring down the cost.

2.7 DIETARY INTAKE AND NUTRITIONAL STATUS OF ADOLESCENT GIRLS

Dietary intake and nutritional status during adolescence are affected not only by socio-economic and cultural factors but also by certain food fads and fallacies.

A study conducted by the National Institute of Nutrition (1994) in Hyderabad showed that adolescent girls from high income families also suffered from chronic energy deficiency. Iron intake of the subjects was only 23.3 per cent of the RDA. The researchers found that adolescent girls perceived expensive foods to be better than green leafy vegetables.

Vijayalakshmi (1997) found the intake of pulses, milk and milk products, fleshy foods and green leafy vegetables to be low among adolescent girls in rural Ranga Reddy district of Andhra Pradesh. As a result only 57.8 per cent of the protein requirement and 47.6 per cent of iron requirement were met by their diets. The mean BMI of these subjects was 17.1, showing chronic energy deficiency.

Chaturvedi *et al.* (1996) studied Rajasthani adolescent girls from low socio-economic status. The diets of these subjects were deficient in calories by 26-36% and in protein by 23-32%. They had chronic energy deficiency with BMI ranging from 14 to 16.3.

In a study on Tamil adolescent girls in Hyderabad, Mythili (1980) observed that the consumption of cereals, pulses, green leafy vegetables and fruits by them was inadequate inspite of their availability.

Pushpamma *et al.* (1982) noted that the intake of rural adolescent population in Andhra Pradesh was low in all foods except cereals. The intake of vitamin A and vitamin C were the most inadequate.

Narsinga Rao (1989) found 13-15 year old adolescent girls in Andhra Pradesh to have a daily iron intake of only 19.7 mg. In addition their poor food intake could be the reason for their low body weight of 34.7 kg as compared to a reference weight of 47.8kg. Gopalan (1989) had also reported chronic energy deficiency in rural Indian girls, with a BMI of 16.49.

Visweswara Rao *et al.* (1991) reported BMI of 15.2 to 17.8 in adolescent girls of twelve to fifteen years of age and belonging to low socio-economic groups.

The food intake of children from institutions is limited to that provided by the institution. Sharada (1997) reported that children in A.P. Social Welfare hostel had low fat diets with excessive amounts of starchy cereals. Their diets were grossly deficient in iron.

A profile of institutionalized children by Sharma *et al.* (1991) showed that only 80.3 per cent of RDA for calories and 78.4 per cent for protein were met by their diets. The girls of thirteen years age had chronic energy deficiency as indicated by their Body Mass Index of 16.17.

Sreedevi (1994) found the diets of children in Ashram schools in Kurnool to be high in cereals, just adequate in pulses and poor in fruits and vegetables. Sarojini and Ratnakumari (1989) had also found a similar pattern of diet in the Ashram schools in Vizianagaram. The diet did not provide adequate nutrients.

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MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The study was planned to assess the effect of supplementation of Deacidified Deodourised Red Palm oil, iron and vitamin C on the vitamin A and iron status of adolescent girls. A detailed description of the procedures followed for conducting the study is given in this chapter under various headings.

3.1 SELECTION OF LOCATION

Krishi High School located in Acharya N.G. Ranga Agricultural University campus, Rajendranagar of Ranga Reddy district was selected for conducting the experiment. The selected school was at a convenient distance from the Department of Foods and Nutrition, Rajendranagar where the analysis of samples was done during the study.

3.2 SELECTION OF SAMPLE

The criteria adopted for selecting of the sample was low serum levels of retinol and haemoglobin in adolescent girls belonging to the age group 13-15 years. Willingness to participate in the study was also one of the criteria.

Thirty girls were selected and their blood samples were analysed to estimate the retinol and haemoglobin levels. Among them 15 subjects with depleted levels of retinol and haemoglobin were selected to conduct the study. All the 15 subjects were oriented to the purpose of the study, the criteria of selection and study design.

3.3 ASSESSMENT OF NUTRITIONAL STATUS BY ANTHROPOMETRY

Heights and weights of all girls were measured before and after the study.

3.3.1 Height : Height of the girls was measured using a height rod with 0.1 cm sensitivity. The subject was made to stand on a plain surface and height was recorded by holding the chin tightly.

3.3.2 Weight : Weight of the subjects was measured with an electronic balance with 0.1g sensitivity. While measuring care was taken to ensure girls had minimum clothing.

3.3.3 Body mass index : Body mass index was calculated using the heights and weights of the subjects adopting the following formula (Lazarus *et al.*, 1996).

$$\text{Body mass index} = \frac{\text{Weight in kg}}{\text{Height in mt}^2}$$

3.4 DIETARY ASSESSMENT

Dietary assessment was conducted to assess the general meal pattern and intake of protective foods (β-carotene,vitamin A and iron rich foods) using a food frequency schedule.

The food frequency schedule was developed to elicit information on general meal pattern consumption of protective foods by frequency and quantity at a time (iron, β-carotene, Vitamin A, Vitamin C).

3.5 PROCUREMENT OF MATERIAL

Synthetic iron tablets (Rishab Chemical Industries, Indore) and Ascorbic acid tablets (Alphine pharmaceuticals, Hyderabad) were obtained from a local medical shop and Deacidified Deodorised Red Palm Oil was supplied courtesy M/s. Global Nutrition Products Pvt. Ltd., Ahmedabad.

Chemicals used were of high purity. HPLC grade solvents Methanol, Acetonitrile, Chloroform were procured from Qualigens Ltd. Standards used for analysis, that is Retinol, Retinyl acetate and β-carotene were procured from Sigma chemical Co., St. Louis, USA, Haemoglobin kits were procured from Monozyme India Ltd., Hyderabad.

Blood samples were drawn by intravenous puncture in the field and were immediately brought to the laboratory. The samples were centrifuged at 2000 rpm and the separated serum was transferred from centrifuge tubes to vials for haemoglobin estimation. 20 μl of blood was taken on to Whattman No.1 filter papers and later analysed using Cyanmethemoglobin method.

3.6 PREPARATION OF PLACEBO SAMPLES

Ingredients :	Besan	-	20 g (Seived)
	Sugar	-	8 g (powdered)
	Vanaspati	-	4 g

Procedure

20 g of Besan was roasted on a frying pan. 8 gms of powdered sugar and 4 g of vanaspathi were added to it and were mixed well. The balls were prepared when it was hot.

3.7 PREPARATION OF DDRPO LADDU

A standardised RPO Laddu receipe (Saritha Mahapatra, 1995) was used in the preparation of DDRPO Laddus.

Ingredients :	Besan	-	20 g (Seived)
	Sugar	-	8 g (powdered)
	DDRPO	-	4 g

Procedure

20 g of Besan was roasted on a frying pan. 8 gms of powdered sugar and 4 g of DDRPO were added to it and were mixed well. The balls were prepared when it was hot.

3.8 INTERVENTION

The intervention proposed in the investigation are:

Baseline

Blood collection and Analysis

30 days of placebo

Blood collection and Analysis

45 days of placebo and iron

Blood collection and Analysis

45 days of DDRPO laddu and iron

Blood collection and Analysis

45 days of DDRPO laddu, iron and Vitamin C

Blood collection and Analysis

Placebo was given twice a week to the girls for a period of 30 days and blood was analysed for retinol, haemoglobin and β-carotene.

For the next 45 days, placebo and iron tablets were given twice a week. Each iron tablet consisted of 200 mg of dried ferrous sulphate which is approximately equivalent to 60 mg of ferrous iron. Blood was analysed after 45 days for Retinol, β-carotene and Haemoglobin.

In the third phase of the study, laddu prepared with DDRPO and iron tablets were given twice a week for a period of 45 days. Each laddu consisted of 4 g of DDRPO equivalent to 1200 μg β-carotene thereby supplying 2400 μg/week. Blood samples were analysed after 45 days for Retinol, β-carotene and haemoglobin.

Lastly laddu prepared with DDRPO supplying 2400 μg β-carotene/ week, iron tablets supplying 120 mg of ferrous iron/week and vitamin C tablets were given twice a week for 45 days and blood samples were analysed for retinol, β-carotene and haemoglobin. Each vitamin C tablet consisted of 100 mg of Ascorbic acid.

3.9 ESTIMATION OF HAEMOGLOBIN BY CYANMETHEMOGLOBIN METHOD

Haemoglobin pipette was calibrated before use. 20 μl of blood was obtained from 2 ml blood drawn from each subject for retinol and β-carotene analysis. Before drawing blood into the pipette, the usual precaution of avoiding undue pressure and squeezing was taken. Blood was transferred onto Whatman No.1 filter paper for the estimation of Haemoglobin.

3.9.1 Estimation of Haemoglobin

The blood stained portion of the filter paper was cut out and transferred into a test tube with 5 ml drabkins reagent. After 30 min, when the entire blood was extracted into drabkins reagent, the absorbance of the solution was read in a spectrophotometer at 540 nm. The readings were taken after ensuring the total extraction of blood into the solution. An aliquot of CMG standard and test sample were taken for reading absorbance.

3.9.2 Calculations

$$\text{Haemoglobin in gm/dl} = \frac{\text{Abs. of T}}{\text{Abs. of S}} \times \frac{251}{1000} \times 65$$

Where,

251 = The dilution Factor

1000 = to correct mg/dl to gm/dl.

3.9.3 Principle of Cyanmethemoglobin method

$Hb + K_3\ (Fe\ (CN)_6 - Hb^+ + K_4\ (Fe(CN)_6)$

$Hb^+ + CN$ .... Hb+ CN-
(Methemoglobin) (Cyanmethemoglobin)

In the method, when blood was dissolved in drabkins reagent containing potassium cyanide and potassium ferricyanide, haemoglobin is converted to Cyanmethemoglobin (Hb CN). The absorbance of the solution was then measured in spectrophotometer at a wavelength of 540 nm.

Readymade drabkins reagent was obtained and used for this purpose.

3.9.4 Composition of drabkins reagent

Potassium ferricyanide	-	0.2 g
Potassium cyanide	-	0.05 g
Sodium bicarbonate	-	1.0 g
Water	-	1 lit.

3.10 SIMULTANEOUS DETERMINATION OF SERUM RETINOL AND β-CAROTENE

Serum retinol and β-carotene levels of the selected 15 girls were assessed using HPLC method. Two ml of blood was drawn from each subject using a disposable syringe. Analysis was done once before supplementation, later again 30 days after giving placebo supplement, and next 45 days after the iron and placebo supplementation. Again analysis was conducted 45 days after the iron and DDRPO laddu supplement and finally after 45 days of iron, DDRPO laddu and vitamin C supplement.

3.10.1 Collection and preservation of blood samples

For assessment of vitamin A nutritional status, venous blood was collected into a 2 ml disposable syringe and was transferred to centrifuge tubes. The tubes were then centrifuged at 2000 rpm for 5 minutes. Serum was separated and stored at -20°C for estimation of vitamin A.

Serum vitamin A and β-carotene were simultaneously determined by High performance liquid chromatography technique (HPLC) (Sai Jyothirmai, 1997).

The HPLC system used was Shimadzu LC-6A, with a UV detector SPD-10A, isocratic pump LC-6A, shimpac column, CLC-ODS (M) C18, 25 cm x 4.6 mm, Rheodyne injection value, SCL-6A system controller and CR-6A recorder. The principle of the procedure followed is given below.

3.10.2 Principle

The vitamins are extracted with a suitable organic solvent and an aliquot of the organic phase is injected on to a normal or reversed phase HPLC column followed by an eluting solvent of suitable polarilty. Retinol which is eluted as a sharp peak within 2-3 min is detected by a sensitive UV detector set at 325 nm, β-carotene which is also eluted as a sharp peak within 8-13 mts is detected by sensitive UV detector set at 450 nm. Retinol and β-carotene are quantified by use of peak area ratios relative to an internal standard Retinyl acetate or other appropriate analogs.

Reagents used

100% Absolute alcohol	(Ethanol) - Fluka
Retinyl acetate standard	(Sigma Chem. Co., St.Louis, USA)
Retinol standard	(Sigma Chem. Co., St.Louis, USA)
B-carotene standard	(Sigma Chem. Co., St.Louis, USA)
Hexane	Qualigens, HPLC grade
Methanol	Qualigens, HPLC grade
Chloroform	Qualigens, HPLC grade
Acetonitrile	Qualigens, HPLC grade

3.10.3 Procedure

100 μl of serum, 80 μl of ethanol and 40 μl of Internal Standard solution in ethanol (0.04 to 0.06 μg of RA/ml) were taken in a glass centrifuge tube and mixed vigorously on a vortex mixture for 30 seconds. To this 1000 μl of HPLC grade hexane was added and the contents were mixed vigourously for 60 seconds on a vortex mixer until the bottom layer was thoroughly extracted. The contents were centrifuged at a rate of 2000 rpm for 5 minutes and 500-700 μl of the upper hexane layer was carefully transferred to the small test tube. Hexane was evaporated under nitrogen. Care was taken such that nitrogen did not condense the contents. The remaining

lipid residue was dissolved in 50 μl of methanol. The test tube was gently swirled and tapped to ensure the complete solubility of the lipid residue. An aliquot of 20 μl of the solution was injected on to the HPLC system and eluted with filtered acetonitrile, chloroform and methanol (70:20:10).

Chromatographic conditions

Column	-	25 cm x 4.6 m, Shim pak.
Mobile phase	-	Aceto nitrile : Chloroform : Methanol (70:20:10) by volume
Flow rate	-	2.0 ml/min.
Pressure	-	68 kg/cm^2
Detector wave-length	-	325 for Retinol
		450 for β-carotene
Detection sensitivity	-	0.005
Temperature	-	Ambient
Recorder	-	10 mu. km/min

Elution time

Retinol	-	2.1 min.
Internal standard (Retinyl acetate)	-	2.5 min.
β-carotene	-	8.5 min.

3.11 PREPARATION OF STANDARD RETINOL, RETINYL ACETATE, β-CAROTENE (STOCK SOLUTIONS AND STANDARD CURVES)

A stock standard retinyl acetate solution in ethanol was prepared by dissolving about 1 mg of retinyl acetate in 10 ml of ethanol, determining the concentration in a dilution of aliquot in ethanol by use of E1% 1cm at 325 nm of 1510, and then the stock standard was diluted appropriately with ethanol. Retinol standard and β-carotene standards were also prepared similarly and concentrations were calculated using an MEC of 1845 and 2500 respectively.

By use of an internal standard, losses due to incomplete extraction, inaccurate aliquots, oxidation etc. were automatically corrected, provided that the internal standard has physical and chemical properties sufficiently similar to retinol, was suitably separated from retinol on HPLC, does not coincide with other 325 nm absorbing materials in serum and is not converted to retinol under the assay conditions. Similarly β-carotene also does not coincide with other 450 nm absorbing materials in serum.

A precisely known amount of internal standard was added to the aliquot of plasma for determining the relative extraction efficiency and detector response of retinol/β-carotene and the internal standard, a standard curve was plotted in which the ratio of peak areas were plotted against the retinol/retinyl acetate concentration.

Unknown plasma retinol concentrations were calculated from the above standard curve after correction of per cent loss due to internal standard.

3.12 STATISTICAL ANALYSIS

One way analysis of variance (ANOVA) was conducted to assess difference between means using the method described. Paired t-test was performed to assess differences between height, weight and body mass index.

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RESULTS

CHAPTER IV

RESULTS

The present study was undertaken to evaluate the effect of supplementation of Deacidified, deodourised red palm oil (DDRPO), iron and vitamin C on the vitamin A and iron status of adolescent girls.

The results are presented as follows:

4.1 Details of subjects selected
4.2 Anthropometry
4.3 Food frequency
4.4 Haemoglobin levels
4.5 Serum Retinol levels
4.6 Serum β-carotene levels

4.1 Details of subjects selected

The details of the subjects selected are given in the following table

Table 4: Details of subjects selected

Parameters	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15
Age in years	13.7	13.11	14.11	13.10	14.1	14.2	14.7	14.10	14.6	13.8	13.7	14.6	13.4	13.8	14.5
Sex	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f
Class in which studying	IX	IX	X	X	X	X	X	X	IX	IX	IX	IX	IX	X	X
Height	153.3	144.1	158.2	147.0	155.1	151.5	165.7	151.0	154.6	156.0	153.7	162.7	157.3	162.0	156.0
Weight	44.2	35.8	52.6	34.8	42.2	41.2	46.4	47.4	43.4	36.8	41.2	52.4	42.0	49.8	41.5

The subjects were all between the age group of 13.4 years and 14.11 years. None of the subjects were obese and almost all of them had normal weight and height.

4.2 ANTHROPOMETRY

Anthropometric measurements, weight, height and body mass index were recorded both at the beginning and end of each supplementation period (Individual values are given in Appendix 2).

Table 5: Changes in anthropometric parameters after supplementation

Parameter	Before	After
Height (cm)	155.21±5.51	155.34±5.52
Weight (cm)	43.44±5.50	44.04±5.05
Body mass index (kg/mt²)	17.97±1.72	18.20±1.49

From the above table it is evident that there is no significant difference in the weights, heights and body mass index before and after the supplementation periods. All the three parameters were not significant at both 5% and 1% levels of significance (P>0.05).

4.3 FOOD FREQUENCY

The details of consumption of protective foods is given in Appendix 6.

It is observed from the consumption patterns of the subjects that the intake of protective foods is inadequate in almost all the subjects. Among green leafy vegetables, most of the subjects consume them once a week and none of them consume green leafy vegetables which are good sources of iron and vitamin A daily. Also there is a small chunk of subjects who consume green leaves rarely. The intake of other vegetables and roots and tubers is either once or twice a week. Vegetables like carrot which are good sources of vitamin A are rarely consumed. Milk and milk products are consumed daily by all the subjects but the amount consumed is less than the required dietary allowances. Fleshy foods which are good sources of non-haem iron are consumed once a week or once in two weeks by all the subjects. Five of the subjects do not consume any meat and meat products. Fruits like Banana and Guava are consumed frequently by all the subjects. Seasonal fruits like Mango, Pomogranate and Amla which are rich sources of vitamin A and vitamin C are consumed in small amounts during the season. Iron rich foods like jaggery and rice flakes are consumed frequently (2 or 3 times a week) by most of the subjects.

Table 6: Mean haemoglobin, serum retinol and β-carotene of subjects in different experimental periods

Period	Haemoglobin (g/dl)	Retinol (μg/dl)	β-carotene (μg/dl)
Baseline	10.8673±2.24[b]	16.2207±3.87[a]	0[a]
Placebo (30 days)	8.7707±1.54[a]	12.8240±2.73[ab]	0[a]
Placebo + iron (45 days)	9.2353±0.90[a]	10.6193±4.63[b]	0[a]
DDRPO + Iron (45 days)	12.2820±1.56[c]	22.2273±6.67[c]	16.1887±22.24[b]
DDRPO + iron + vitamin C(45 days)	13.0573±0.75[c]	25.1953±4.77[c]	30.9600±15.48[c]

* Values are expressed as Mean±SD* Alphabets in superscript indicate significant differences ($P<0.05$)

4.4 MEAN HAEMOGLOBIN

Table 6 gives the mean haemoglobin levels of the subjects at baseline and during placebo; placebo+iron; DDRPO + iron; DDRPO + iron + vitamin C supplementation periods. It is observed that the initial haemoglobin levels were significantly higher than the placebo and placebo+iron supplementation periods. There is no significant difference between the placebo and placebo+iron supplementation period. Results showed that the initial haemoglobin levels were significantly lower than after supplementation with DDRPO + iron and DDRPO + iron + vitamin C. There was no significant difference between these two experimental periods. The DDRPO + iron and DDRPO + iron + vitamin C levels were significantly higher than placebo and placebo + iron supplementation levels (individual values are reported in Appendix 4).

4.5 MEAN SERUM RETINOL

Mean serum retinol levels at baseline and during placebo + iron and DDRPO supplementation periods were found to be significantly different from each other. Serum retinol levels fluctuated from 10.6193 to 25.1953 during the study period. There was a significant decrease in serum retinol levels during the placebo and placebo + iron supplementation when compared to Baseline levels. The serum retinol values during placebo supplementation showed similarities with both baseline and placebo + iron supplementation periods. There was a significant increase in the serum retinol levels during the DDRPO + iron and DDRPO + iron + vitamin C supplementation periods when compared to baseline, placebo and placebo + iron supplementation periods. There was no significant difference between DDRPO + iron and DDRPO + iron + vitamin C supplementation periods (individual values are reported in Appendix 3).

4.6 MEAN SERUM β-CAROTENE

With respect to serum β-carotene values (Table 6), there was a significant increase observed during DDRPO + iron and DDRPO + iron + vitamin C supplementation periods. The baseline, placebo and placebo + iron supplementation periods were similar with respect that no serum β-carotene was detected during these periods (individual values are reported in Appendix 5).

DISCUSSION

CHAPTER V

DISCUSSION

Iron deficiency anaemia and hypovitaminosis A have been known to mankind and studied in detail for the last few centuries. The symptoms and causes of these deficiencies have been clearly described and the vulnerable segments of the populations identified. The treatment is simple and effective - administration of iron and vitamin A either through food sources or as a supplement. Yet, inspite of all this knowledge, iron deficiency anaemia and vitamin A deficiency persists all over the world, their prevalence being more in socially and economically deprived population groups.

In India, a recent FAO/WHO report (1993) indicates that 45% of adult men, 65% of adult women, 77% of children of 1 to 5 years age and about 65% of pregnant women are anaemic. As much as 63% (NIN, 1993-94, 1994-95) to 73.7% (Chaturvedi *et al.*, 1996) of adolescent girls in India have been reported to be anaemic. Surveys in different parts of India have shown that about 5-10 of children have clinical signs of vitamin A deficiency (Reddy, 1991a). Severe forms of the disease involving cornea are seen most frequently in children in the age group of 1-5 years (Reddy, 1991b).

Adolescent girls are a vulnerable, though often neglected group. They represent a crucial segment of the country's population. They will not only contribute to nearly half of the adult female population in the next few years, but even more importantly, they will usher in the next generation (Venugopal, 1989). The high prevalence of anaemia and vitamin A deficiency in adolescent girls in India indicates that adverse repercussions would be seen not only on the girls themselves, but also on their future offspring. Yet very few studies on anaemia and vitamin A deficiency in this age group are available.

Inspite of adolescence being a crucial period and care during this period being sure to yield rich dividends in terms of improved health of both present and future generations, adolescent health care has been on a low priority in both developing and developed countries (Brabin and Brabin, 1992). In countries like India, health care focuses mostly on children below five years and on pregnant women.

Hence in the present study, adolescent girls were selected with an aim to contribute information towards resolving their anaemic status and vitamin A deficiency. The present study was taken up to observe the effect of supplementation of DDRPO, iron and vitamin C on the vitamin A and iron status of adolescent girls. The findings from the study are discussed under the following headings.

- Growth and nutritional status
- Haemoglobin levels in subjects during different supplementation periods

- Serum retinol levels in subjects during different supplementation periods
- Serum β-carotene levels in subjects during different supplementation periods
- Effect of iron supplementation alone on iron and vitamin A status of subjects
- Effect of iron and DDRPO supplementation on iron and vitamin A status of subjects
- Effect of iron, DDRPO and vitamin C on iron and vitamin A status of subjects
- Overall impact of integrated supplementation of the three nutrients on iron and vitamin A status of the subjects

5.1 GROWTH AND NUTRITIONAL STATUS

The anthropometric indices like height, weight and Body Mass Index of the study population at baseline and during the end of the study was assessed. No statistical differences were observed among the subjects.

One of the objectives of the present study was to observe the effect of supplementation on vitamin A and iron status. To assess the dietary sources of iron and vitamin A of the sample studied, information of the protective food consumption pattern of the subjects was collected. Results of the study indicated that the recommended intake of 100 g of green leafy vegetables a day was not met. In a study on Tamil adolescent girls in Hyderabad, Mythili (1980) observed that the consumption of cereals, pulses, vegetables and fruits by them was inadequate inspite of their availability. A study conducted by the National Institute of Nutrition (1994) in Hyderabad showed that adolescent girls from high income families also suffered from chronic energy deficiency. Iron intake of the subjects was only 23.3 per cent of the RDA. In this study, it was observed that adolescent girls perceived expensive foods to be better than green leafy vegetables.

Milk, which is a good source of vitamin A was consumed in small quantities, only in tea or coffee. Curds was consumed by most of the subjects in a dilute form i.e., butter milk. Buttermilk cannot provide enough carotenes as it is a very dilute form of curds. The low intake of green leafy vegetables and milk and milk products is indicative of a low intake of vitamin A and iron in the subjects.

Vijayalakshmi (1997) found the intake of pulses, milk and milk products, fleshy foods and green leafy vegetables to be low among adolescent girls in rural Ranga Reddy district of Andhra Pradesh. As a result only 37.8 per cent of the protein requirement and 47.6 per cent of iron requirement were met by their diets. The mean BMI of these subjects was 17.1, showing chronic energy deficiency. Thimmayamma (1982) studied the milk consumption pattern of different socio-economic groups and reported that differences were observed in the milk intake of upper, middle and low income groups.

In the present study though fruits were frequently consumed by the subjects, they were taken in very small quantities only during the season. Other protective foods like eggs and fleshy foods were consumed once in a week or once in two weeks. Pusphpamma *et al.* (1982) noted that the nutrient intake of rural adolescent population in Andhra Pradesh was low in all foods except cereals. The intake of vitamin A and C were the most inadequate. Narsinga Rao *et al.* (1991) reported that even though impressive production of fruits and vegetables was observed in Andhra Pradesh, they are inadequate to meet the minimal nutritional needs of our populations. Less frequent consumption of dark green leaves, fruits or eggs was reported in < 3 years children by Lisa *et al.* (1991).

In a study carried out in preschool children of urban slums of Hyderabad, it was reported that the daily intake of nutrients was far below the requirement, ranging from 60 to 100 µg whereas the recommended level was 300 µg (Reddy *et al.*, 1991). Lower intake of nutrients such as vitamin A, B-complex, iron, calcium and vitamin C by preschool children was also reported by several workers (Madhyasta *et al.*, 1988) in costal India, Roberts *et al.* (1984) in children following vegetarian diets, Pepping *et al.* (1989) in Tanzanian children of 4-9 years old.

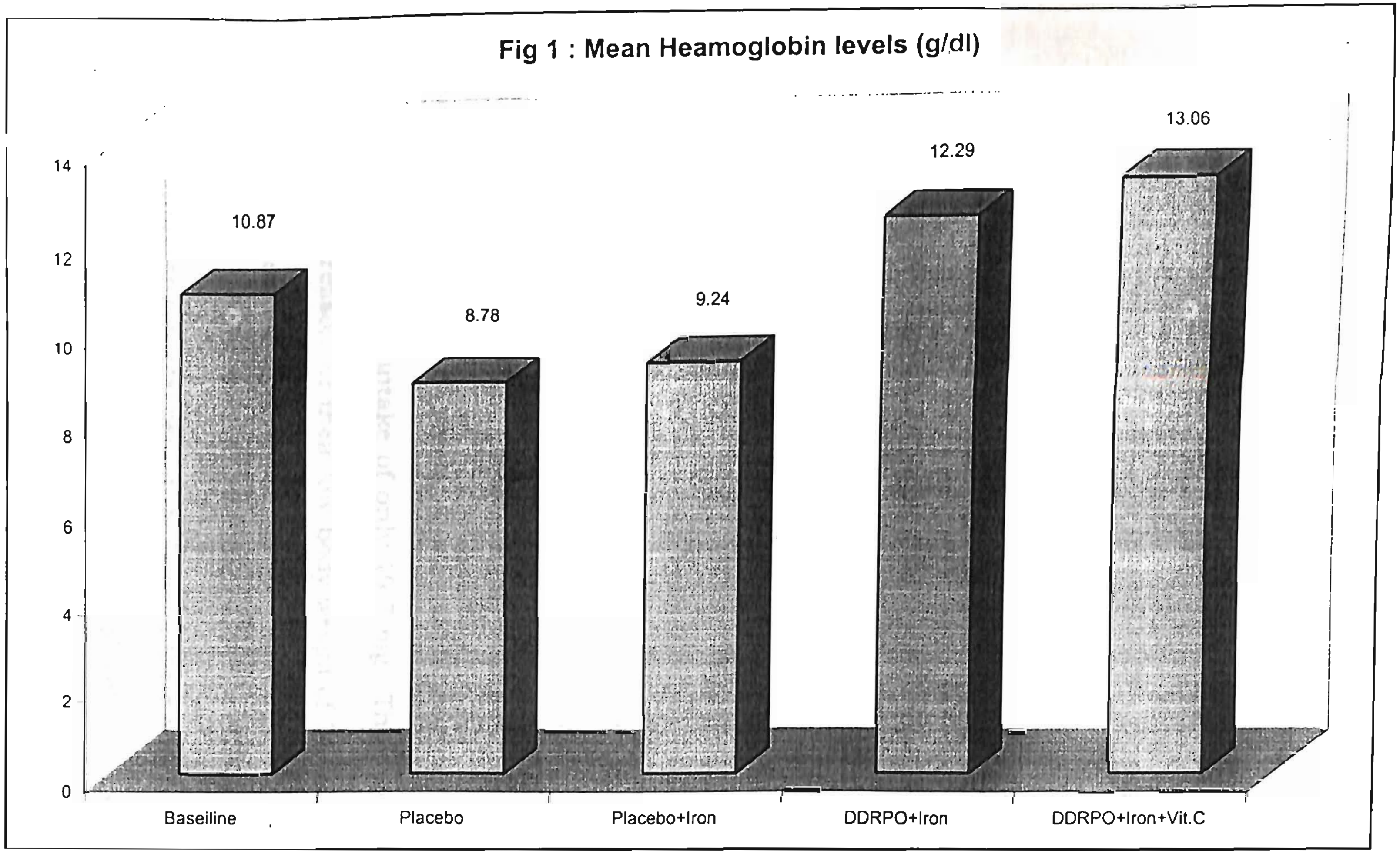


Fig 1 : Mean Heamoglobin levels (g/dl)
0
2
4
6
8
10
12
14
10.87
8.78
9.24
12.29
13.06
Baseiline
Placebo
Placebo+Iron
DDRPO+Iron
DDRPO+Iron+Vit.C

The pattern of protective food consumption as seen in this study indicates that the only vitamin A and iron rich sources are green leafy vegetables, milk and fleshy foods but its intake is far below the requirement in all the subjects studied.

5.2 HAEMOGLOBIN LEVELS IN SUBJECTS DURING DIFFERENT SUPPLEMENTATION PERIODS

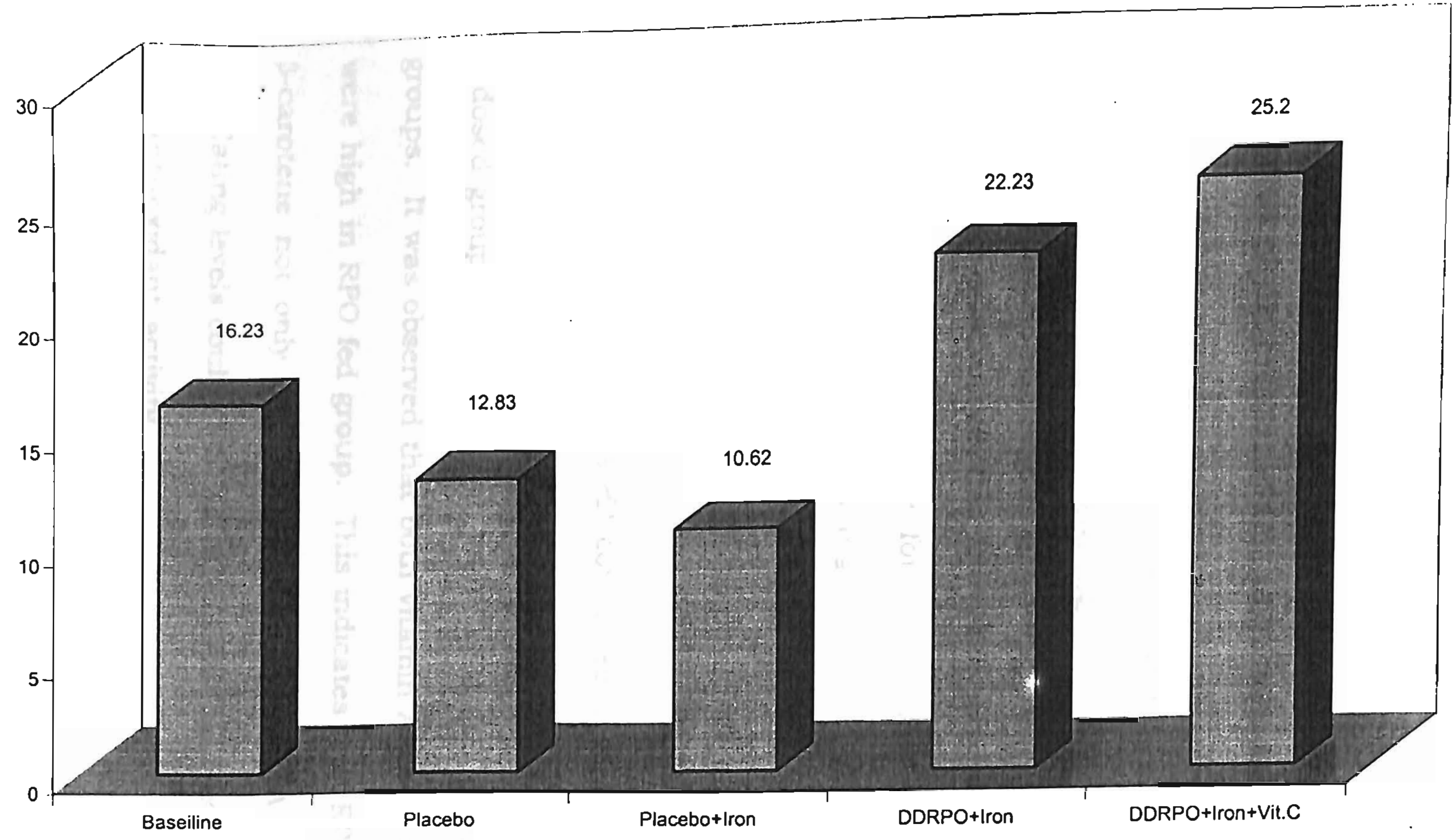
In the present study, the haematological status of all the subjects supplemented with iron twice weekly improved significantly. At first sight there was a significant decrease in the haemoglobin levels during the placebo period (8.77 g/dl). The immediate decrease in the haemoglobin levels from 10.86 g/dl to 8.77 g/dl can be attributed to various factors like poor dietary intake of iron, poor absorption or may be due to other factors. One of them could be that the subjects had just returned to school after summer vacation and so the haemoglobin levels might have gone down. Narsinga Rao (1989) found 13-15 years old adolescent girls in Andhra Pradesh to have a daily iron intake of only 19.7 mg. Their poor food intake could be the reason for their low body weight of 34.7 kg as compared to a reference weight of 47.8 kg.

After the placebo period (8.77 g/dl) there was a slight increase in the levels when supplemented with placebo + iron for 45 days (9.23 g/dl). However, this increase in the haemoglobin levels was not statistically significant. Though the subjects were supplemented with ferrous sulphate tablets there was no significant increase in the haemoglobin levels. Izak *et al.* (1973) studied the effect of giving either 100 mg of elemental iron or 300 μg of folic acid or a combination of both. The study clearly demonstrated that a combination of iron and folic acid is superior to just iron or just folic acid, in improving haemoglobin levels. Sood *et al.* (1975) studied prevalence of anaemia among pregnant women in two centres - Delhi in North India and Vellore in South India. The results clearly showed that a daily dose of 120 or 240 mg of iron together with folic acid and B12 was superior to other dosages. In the present study the decrease in haemoglobin levels could be due to lack of folic acid and B12 or vitamin C.

With respect to DDRPO + iron supplementation, the haemoglobin levels have increased significantly (12.28 g/dl) and during the DDRPO + iron + vitamin C supplementation, there was further increase in the levels (13.05 g/dl) though not significant when compared to the DDRPO + iron supplementation period. Therefore, the results indicate that there was significant rise in the haemoglobin levels during the DDRPO + iron supplementation and it remained stable during the DDRPO + iron + vitamin C supplementation period. This indicates that vitamin A and vitamin C have enhanced the haemoglobin levels.

Hodges (1978) found that vitamin A supplementation has a positive effect on the iron utilisation in the body. Similar results

Fig 2 : Mean Serum Retinol levels (μg/dl)



were obtained by Meija *et al.* (1979); Meijia and Arroyave (1982); Rekha, Y. (1983); Vijayalakshmi and Devadas R P (1987). Several studies have demonstrated the enhancing effect of ascorbic acid on the absorption of non-haem iron (Sayees *et al.*, 1973; Bjorn Rasmussen and Hallberg, 1974; Hallberge *et al.*, 1986, 1989).

5.3 SERUM RETINOL LEVELS IN SUBJECTS DURING DIFFERENT SUPPLEMENTATION PERIODS

With respect to serum retinol, it was observed that there was a significant difference for each supplementation period. DDRPO supplementation showed an increase in serum retinol values which were significantly higher than the other supplementation periods.

Manorama *et al.* (1996) conducted a comparative study on school children fed RPO snacks for one month with massive vitamin A dosed groups. Serum retinol levels increased significantly in both groups. It was observed that both vitamin A and β-carotene levels were high in RPO fed group. This indicates that RPO is supplying β-carotene not only for conversion to vitamin A but these high circulating levels could come of use for its other biological functions like anti-oxidant activity.

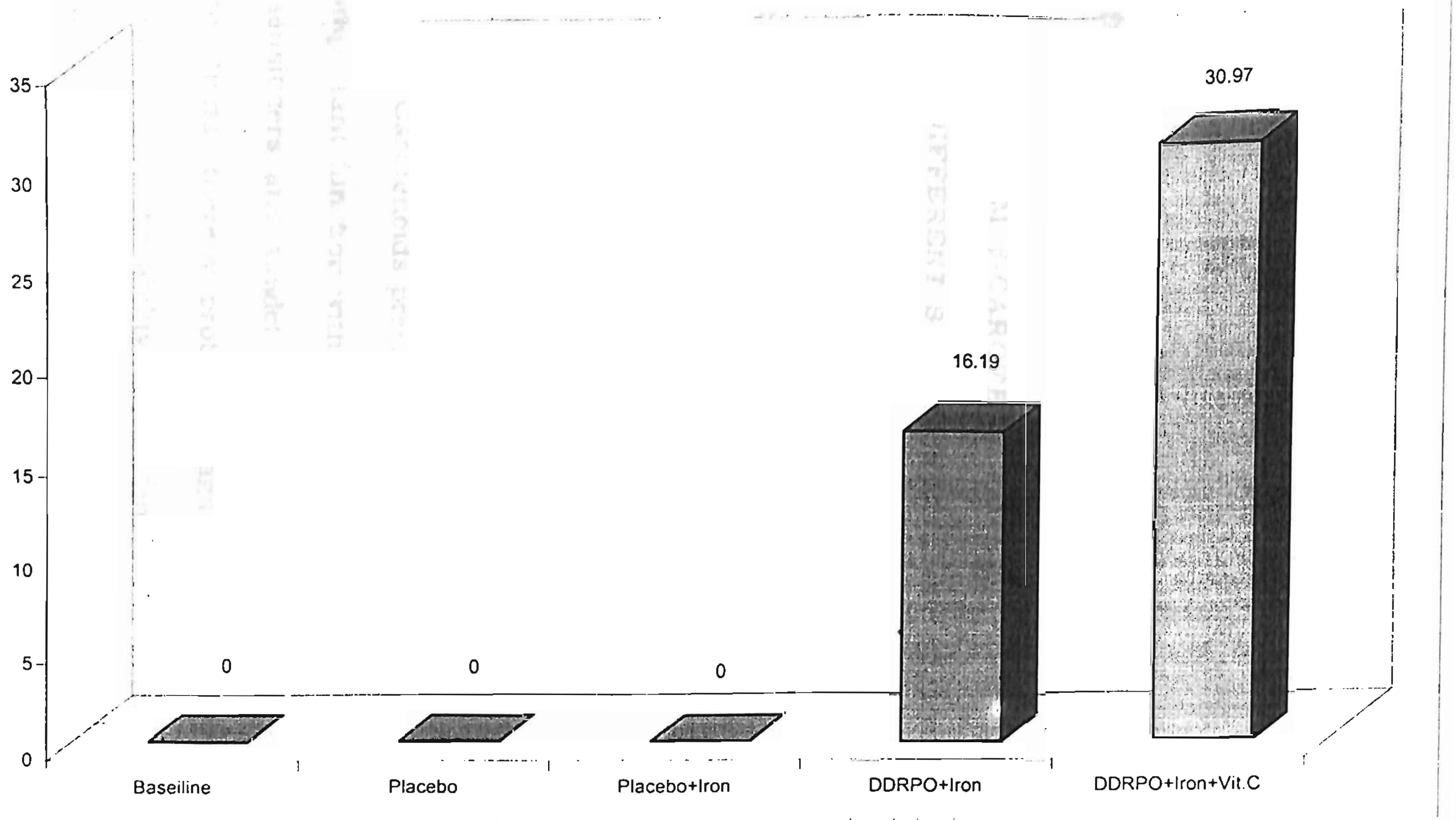
It has been reported (Olson, 1986) that ingestion of large amounts of β-carotene and other provitamin A carotenoids over a significant period of time will improve vitamin A status.

Indian school children fed on supplementary food prepared with RPO for 60 days had significant increases in serum retinol levels as well as increased liver retinol store suggesting the ready bio-availability of β-carotene (Manorama *et al.*, 1996).

Studies have also indicated that improved vitamin A status reduces severe morbidity and mortality from infectious diseases among children from areas where vitamin A deficiency is endemic. Vitamin A deficiency results in widespread alterations in immunity including pathological alterations in mucosal surfaces, impaired antibody responses to challenge with protein antigens, changes in lymphocyte subpopulations and altered T and B cell function. Vitamin A and its metabolites were termed as immune enhancers which restore the integrity and function of mucosal surfaces, and were postulated to have potential applications in therapy for some infectious diseases. Olson (1986) also stated that in large doses, vitamin A and other retinoids show dramatic chemopreventive effects in animals against some forms of cancer. The dietary intake of fruits and vegetables, and fruits rich in carotenoids has been associated with a lower incidence of some types of cancer (Ong and Chytil, 1983).

In the present study, the serum retinol levels at baseline and during placebo and placebo + iron decreased significantly. This could be due to poor dietary intake of vitamin A sources from foods. Pushpamma *et al.* (1982) noted that the intake of rural adolescent

Fig 3 : Mean Serum β–Carotene levels (μg/dl)



population in Andhra Pradesh was low in all foods except cereals. The intake of vitamin A and C were the most inadequate.

Hence, it can be concluded from these observations that though serum retinol levels of the subjects were much lower than the normal value (20 μg/dl) initially, after supplementation with DDRPO, their serum retinol levels increased significantly.

5.4 SERUM β-CAROTENE LEVELS IN SUBJECTS DURING DIFFERENT SUPPLEMENTATION PERIODS

The results comparing serum β-carotene levels during different supplementation periods showed a significant increase in β-carotene levels during DDRPO supplementation period in comparison to Baseline, placebo and placebo + iron supplementation periods. Absolutely no β-carotene was detected at baseline and during placebo and placebo + iron periods.

Carotenoids present in abundance in RPO could perform an important function with respect to their capacity to quench triplet sensitizers and singlet oxygen (O_2) and inhibition of free radicle reactions, thereby protecting against harmful photosynthesized oxidations. The ability of carotenoids to deactivate the reactive chemical species such as singlet oxygen, triplet photochemical sensitizers and free radicles have been gaining a lot of attention with the main focus on β-carotene.

Manorama *et al.* (1992) tested RPO as a source of β-carotene in repleting liver reserves of vitamin A in children. The results clearly indicated that β-carotene in RPO could build up vitamin A stores in children and were comparative to that of vitamin A given to children.

Manorama *et al.* (1992) proved that RPO, apart from providing calorie density to the diet is also the richest natural source of β-carotene, containing 550 μg/g of total carotenes and 375 μg/g of β-carotene. The results of the present study are in line with above studies.

5.5 EFFECT OF IRON SUPPLEMENTATION ALONE ON IRON AND VITAMIN A STATUS OF SUBJECTS

With respect to iron supplementation alone, there was an overall increase in the haemoglobin levels by the end of the study but no significant increase in vitamin A was found due to iron supplementation alone. In an iron supplementation study by Chwang *et al.* (1988) moderately anaemic school children given 2 mg/kg body weight of iron per day for 12 weeks were found to have a 35.7% increase in haemoglobin. Liu *et al.* (1995) also reported a 33.3% increase in the haemoglobin level of moderately anaemic preschool children supplemented with iron for twelve weeks. Studies on mildly anaemic pregnant women (Charoenlarp, 1988) showed that they took 10 weeks of supplementation with 120 mg of iron per day to reach normal levels.

In this study, the subjects were supplemented with iron for 20 weeks twice weekly. In the begining at baseline, the mean haemoglobin levels were observed to be 10.87 g/dl and during placebo it was decreased to 8.77 g/dl and with iron supplementation it increased to 9.23 g/dl. After supplementation of DDRPO, the haemoglobin levels reached 12.28 g/dl and thereafter to 13.05 g/dl after supplementing with DDRPO, iron and vitamin C. There was no significant improvement in vitamin A status during iron supplementation alone. Vitamin A status could have been low due to poor dietary intake.

Wintrobe (1981) reported that twenty one days of supplementation was sufficient to bring about a big shift (48-61%) from moderate to mild anaemia. Thereafter, the tempo of shift from mild to normal slowed down. This was to be expected as subjects with values of haemoglobin nearer to normal take a longer period to show an increase in haemoglobin levels. As haemoglobin regeneration occurs absorption decreases.

It is reported (Stephenson, 1995) that cumulative daily doses rapidly reduce the intestinal absorption of iron. Iron deficient persons, may absorb 30-40% of the iron given on the first day, but this rapidly decreases on the following days to an absorption of 3-6%. The twice weekly dosage schedule is similar to the time it takes for the intestinal mucosal cells to turn over in humans. By administering a dose of iron once in 3-4 days, the cells that were located with iron from the previous dose would have been shed and iron absorption would increase.

Other investigators (Ridwan *et al.*, 1996; Gross *et al.*, 1994) had showed that intermittent iron supplementation had greater biological efficiency over daily iron supplementation. However all of them found the haemoglobin levels of daily supplemented subjects to be higher (though not statistically significant) over the weekly supplemented ones.

5.6 EFFECT OF IRON AND DDRPO SUPPLEMENTATION ON IRON AND VITAMIN A STATUS OF SUBJECTS

The results of this study indicate that vitamin A supplementation can improve the hematological condition of adolescent girls. Iron studies have accurately investigated the effect of intervention with vitamin A on improvement of iron metabolism. Meija and Arroyave (1982) evaluated a vitamin A fortification program and observed an improvement of the iron status. MohanRam *et al.* (1977) observed that intervention with vitamin A led to increased haemoglobin concentrations.

In an experiment involving a vitamin A deficient group of otherwise healthy volunteers, an association between iron metabolism and hypovitaminosis A was established. The experimental group developed anaemia that was refractory to medicinal iron but responsive to vitamin A (Hodges *et al.*, 1978). The same workers described significant correlations between serum retinol levels and haemoglobin among non-pregnant, non-lactating women by reevaluating data from a nutrition survey. An evaluation of the data resulting from a national program of vitamin A fortification conducted in Guatemala showed that the indices of iron status improved although iron intake did not significantly change during the period of fortification (Meija *et al.*, 1977; Mejia and Arroyave, 1982).

In the present study, it was seen that there was no significant increase in haemoglobin levels when supplemented with iron alone, but when supplemented with both DDRPO and iron for 45 days, there was a significant increase in the haemoglobin levels from 9.23 g/dl to 12.28 g/dl. Djoko Sucharno *et al.* (1993) observed the influence of vitamin A and iron supplementation in anaemic pregnant women and concluded that maximum haemoglobin was achieved with both vitamin A and iron supplementation (12.78 g/L), with one-third of the response attributed to vitamin A (3.68 g/L, 2.03 to 5.33) and two-thirds to iron (7.71 g/L, 5.97 to 9.45). After supplementation, the proportion of women who became non-anaemic was 35% in the vitamin A supplemented group, 68% in the iron-supplemented group, 97% in the group supplemented with both and 16% in the placebo group. Improvement in vitamin A status may thus contribute to the control of anaemia.

Mejia *et al.* (1977) observed that after 6 months of vitamin A fortification, indices of the iron status had improved. Because the levels of total iron - binding capacity increased and the levels of ferritin decreased, it seems that after fortification there was an increase in the availability of serum iron by depletion of the iron stores for hematopoiesis. After 2 years of vitamin A fortification an overall significant improvement of all iron nutritional indices was observed. A plausible explanation for this phenomenon is that vitamin A enhances both hematopoiesis and the availability of serum iron by depletion of the iron stores.

In a recent study by Richard *et al.* (1992) it was observed that vitamin A supplementation helps mobilize hepatic stores of iron and may enhance hematopoiesis in children with vitamin A deficiency. Another recent experiment by Layrisse *et al.* (1998) demonstrated that vitamin A prevented the inhibiting effect of polyphenols and phytates on iron absorption. It was also demonstrated that vitamin A had the same effect on iron absorption as phytase. The observations made in the present study are in agreement with the available literature.

5.7 EFFECT OF IRON, DDRPO AND VITAMIN C ON IRON AND VITAMIN A STATUS OF SUBJECTS

There was a significant increase in both iron and vitamin A status of the subjects when supplemented with iron, DDRPO and vitamin C. Derman *et al.* (1980) and Rathee and Pradhan (1980) showed that ascorbic acid increases iron availability. Ascorbate and iron are metabolically inter-related. High dietary intake of either nutrient was found to modify tissue iron distribution (Smith and Bidlack, 1979). Hunt J R *et al.* (1990) reported that in iron-depleted women consuming a diet with predicted poor iron availability, ascorbic acid supplementation enhanced body iron retention for 5.5 weeks.

Vitamin A status might have improved due to the supplementation with DDRPO which has already been proved in several studies. There was a significant improvement in iron status during DDRPO + iron supplementation period. There was no significant difference in the iron status during DDRPO + iron and DDRPO + iron + vitamin C supplementation periods. This indicates that though vitamin C is a good enhancer in iron absorption, it did not help to bring out much significant increase in the haemoglobin levels which already reach the normal range. West (1996) observed that iron is probably absorbed only in the ferrous form, so elemental iron has to be oxidised and ferric iron reduced to ferrous iron before absorption. Haeme iron is absorbed to a much greater extent than non-haeme iron. In iron deficient persons, the absorption of haeme iron is about 35% and non-haeme iron about 25% when enhancers are abundant. The corresponding figures in iron replete subjects are 15% and 2%. Enhancers and inhibitors exert their effect only on the absorption of non-haeme iron. The author further stated that dietary ascorbic acid, meat, acidic and fermented foods enhanced the absorption of non-haeme iron. Phytates and phenolic compounds present in cereals and in tea/coffee are inhibitors of non-haeme iron absorption. However their action is counteracted upon by increasing dietary ascorbic acid. Therefore in the present study ascorbic acid must have helped in improvement of iron status to some extent only because prior to ascorbic acid supplementation only, there was significant increase in haemoglobin levels. This could have be due to vitamin A supplementation.

Recent experiments by Layrisse *et al.* (1998) demonstrated that vitamin A prevented the inhibiting effect of polyphenols and phytates on iron absorption. It was also demonstrated that vitamin A had the same effect on iron absorption as phytase. The results of present study are in agreement to the available literature on both vitamin A and ascorbic acid in improvement of iron absorption.

5.8 OVERALL IMPACT OF INTEGRATED SUPPLEMENTATION OF THE THREE NUTRIENTS ON IRON AND VITAMIN A STATUS OF THE SUBJECTS

With respect to the impact of integrated supplementation of the three nutrients on iron and vitamin A status of the subjects, it can be observed from the results that iron and vitamin A levels in all the subjects have improved from the baseline levels to a normal levels. The improvement in serum-retinol levels and β-carotene levels was due to the effect of supplementation with DDRPO. The improvement in haemoglobin levels was mostly due to vitamin A and partly due to ascorbic acid because with DDRPO supplementation, the normal haemoglobin levels were achieved in all the subjects within 45 days. Later with both vitamin A and Ascorbic acid supplementation there was not much further improvement in the haemoglobin levels (12.28 g/dl to 13.05 g/dl), showing a non-significant increase. So the improvement in haemoglobin levels could be attributed to vitamin A and ascorbic acid. There is no literature available on the integrated supplementation of all the three (vitamin A, iron and vitamin C) nutrients together to support this conclusion.

With respect to vitamin A and iron supplementation, the serum retinol and haemoglobin levels have reached normal levels and also β-carotene levels were improved. This result would not have been achieved if the subjects were supplemented with only iron and vitamin C. Vitamin C would have improved only the iron status of the subjects invariably. But with DDRPO and iron supplementation, haemoglobin, serum retinol and β-carotene levels have improved. Moreover Northropclewes *et al.* (1996) showed that vitamin A status of target groups should be an important consideration when carrying out iron supplementation programs, especially in places where infections are highly prevalent.

Furthermore, the study showed that vitamin A may have a protective effect during iron supplementation. The protective effect of vitamin A during iron supplementation as described has not been reported before although increased mobilization of iron after vitamin A supplementation is widely recognized. Vitamin A is well known for its anti infective properties apart from helping in iron absorption.

Saskia *et al.* (1996) observed that food based approaches to combat deficiencies of micronutrients such as iron and vitamin A deserved great attention because they were likely to be sustainable in the long term, and the intake of other nutrients will increase simultaneously.

The results of the present study are supported by research literature and have shown that DDRPO can be used as a unique source of vitamin A in any supplementation programme and that vitamin A with its anti-infective properties helps in iron absorption apart from bringing about an increased serum β-carotene levels.

In conclusion, red palm oil has a beneficial effect in bringing about significant increase in haemoglobin and serum β-carotene levels. The minor components present in the oil have many beneficial effect. Hence, its use as a source of vitamin A in combating vitamin A deficiency as well as anaemia could be considered.

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SUMMARY AND CONCLUSIONS

CHAPTER VI

SUMMARY AND CONCLUSION

Vitamin A deficiency and anaemia prevalence is high all over the world and more so in the socially and economicaly backward population groups. Pregnant and non-pregnant women, young children and adolescent girls are particularly vulnerable to nutritional anaemia and vitamin A deficiency disorders. So far the focus has been on the treatment and prevention of anaemia and vitamin A deficiency in pregnant women and young children. More recently the benefits of nutritional care, including vitamin A deficiency and anaemia prevention, on adolescent girls have been recognised.

Adolescence is a crucial period of development. The growth spurt of adolescence and the onset of the menstural cycle impose the burden of higher iron and other micronutrient requirements on adolescent girls. Added to this the dietary iron intake and vitamin A intake is generally poor, both qualitatively and quantitatively. Thus, in India most of the adolescent girls are suffering from various micronutrient deficiencies. Since adolescent girls are the mothers to be, their poor nutrient status would definitely have a negative impact on the future generations. Therefore it is essential that the major problems like a vitamin A deficiencies and anaemia in adolescent girls are to be addressed urgently. Both, treatment of existing micronutrient deficiencies and prophylaxis to prevent them are necessary.

Changes in dietary habits and fortification of foods with vitamin A and iron are important measures to combat vitamin A deficiency and anaemia. However, they are expensive and are long term measures. Therefore in this study, Red palm oil which is the richest natural source of carotenoids was used as a source of vitamin A and supplementation wth ferrous sulphate tablets which has been shown to improve haemoglobin levels within a short time span was used as a source of iron. Ascorbic acid tablets were used as a source of Vitamin C.

Keeping adolescent girls in view, the study was designed to determine the effect of supplementation of Red palm oil, iron and vitamin C on the vitamin A and iron status. The study was conducted in a school situated in the Acharya N.G. Ranga Agricultural University campus, Hyderabad. Based on their initial haemoglobin and vitamin A levels, 15 girls with depleted levels of haemoglobin and serum retinol were included in the study. All the subjects were later supplemented with placebo for 30 days; placebo + iron tablets for 45 days; DDRPO laddu + iron tablets for the next 45 days and DDRPO laddu + iron tablets + vitamin C tablets for the last 45 days. Blood samples were collected by an intravenous puncture at the end of each supplementation period for the determination of serum retinol, serum β-carotene and haemoglobin levels. Anthropometric data was recorded prior to and after the experiment. Information regarding general meal pattern and intake of protective foods was collected.

Findings of the study

- Anthropometric data showed that the subjects on the whole had better heights, weights as well as body mass index, but there was no significant difference in the heights, weights and body mass index before and after the supplementation periods.

- Dietary intake of the nutrients observed from the consumption patterns of the subjects indicated that the intake of protective foods was inadequate in almost all the subjects.

- There was a significant decrease in the haemoglobin levels during placebo and placebo + iron supplementation perods when compared to baseline levels. Later there was a steady significant increase in the haemoglobin levels during the DDRPO and vitamin C supplementation periods indicating that iron supplementation alone could not bring about a significant increase in the haemoglobin levels but vitamin A supplementation and vitamin C supplementation along with iron could bring about a desirable increase in the haemoglobin levels. Vitamin C supplementation could bring about an increase in the haemoglobin levels but maximum increase in the haemoglobin levels was attained during vitamin A supplementation period itself. This indicates that vitamin A supplement apart from improving the serum retinol and serum β-carotene levels also brought about a significant improvement in iron status which could not have been achieved only with iron and vitamin C supplementation.

* Results of the study indicate a significant overall increment in the serum retinol and serum β-carotene levels. The serum retinol levels decreased during the placebo and placebo + iron supplementation periods which could be due to poor intake and absorption of vitamin A. β-carotene, was not detected at baseline and during the placebo and placebo + iron supplementation periods. Later during the DDRPO supplementation periods there was a significant increase in both serum retinol and serum β-carotene levels indicating that Red palm oil is a very good natural source of vitamin A.

* On the whole, integrated supplementation with all the three nutrients i.e., vitamin A, iron and vitamin C could bring about a significant improvement in vitamin A and iron status of adolescent girls.

Vitamin A which is well known for its antiinfective properties also helps in mobilizing hepatic stores of iron and may enhance increased haemoglobin levels in adolescent girls with vitamin A deficiency and anaemia. This study provides further evidence of a causal association between Vitamin A and iron metabolism. In areas where vitamin A deficiency is endemic, periodic vitamin A supplementation programs can also improve iron status of the population apart from an improvement in serum retinol and serum β-carotene status. Red palm oil can be effectively used as a source of vitamin A and β-carotene.

Based on these findings, Red palm oil and Iron supplement could be provided to all adolescent school girls twice in a week for a continuous period of 90-120 days in a year. This could resolve any vitamin A deficiency and anaemia present, as well as help in building vitamin A and iron stores in the body. Since school girls are a captive group they would be easily available for a continuous programme of supplementation. Moreover the school teachers who are in daily contact with them could be entrusted this job. If implemented, the impact may be seen both on the physical growth and scholastic performance of the girls; it would also help in building up vitamin A and iron reserves in their bodies which would help them in their future role as mothers.

Future long term studies on periodic supplementation of adolescent girls intermittently during the year could provide more concrete evidence for the supportive and beneficial role of RPO in addition to other micronutrients like Vitamin C and Iron, on the positive health of adolescent girls in their vulnerable pre-reproductive age.

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APPENDICES

APPENDIX - I

FOOD FREQUENCY SCHEDULE

DIETARY INFORMATION

NAME:

*GENERAL MEAL PATTERN OF RESPONDENTS

Breakfast	Lunch	In between snacks	Dinner

***CONSUMPTION OF PROTECTIVE FOODS BY FREQUENCY AND QUANTITY AT A TIME (IRON, B-CAROTENE/VITAMIN A, VITAMIN C)**

S.No.	Name of the food stuff	FREQUENCY OF CONSUMPTION (quantity at a time (g))					
		Daily	Thrice a week	Twice a week	Once a week	Rarely	Nil
1.	Green leafy vegetables						
	Amaranth						
	Cabbage						
	Drumstick leaves						
	Ponnagani leaves						
	Spinach						
	Tamarind leaves						

Contd..

S.No.	Name of the food stuff	FREQUENCY OF CONSUMPTION (quantity at a time (g))					
		Daily	Thrice a week	Twice a week	Once a week	Rarely	Nil
2.	Other vegetables						
	Bitter gourd						
	Drumstick						
3.	Roots & Tubers						
	Carrot						
	Beetroot						
	Sweet potato						
	Potato						
4.	Milk						
	Cows milk						
	Buffaloe milk						
	Curds						
	Egg						
5.	Other fleshy foods						
	Fish						
	Meat						
	Sheep liver						

Contd..

S.No.	Name of the food stuff	FREQUENCY OF CONSUMPTION (quantity at a time (g))					
		Daily	Thrice a week	Twice a week	Once a week	Rarely	Nil
6.	Fruits						
	Banana, Ripe						
	Dates						
	Guava						
	Jackfruit						
	Mango, ripe						
	Papaya ripe						
	Pomogranate						
7.	Miscellaneous						
	Jaggery						
	Rice flakes						
	Roasted bengalgram						
	Til						
	Amla						

APPENDIX 2

GENERAL INFORMATION AND ANTHROPOMETRY

S.No.	Name	Age (years)	HEIGHT (cms)		WEIGHT (kgs)		BODY MASS INDEX (kg/mt^2)	
			Before the study	After the study	Before the study	After the study	Before the study	After the study
1.	Ch. Indira	13.7	153.3	153.3	44.2	42.6	18.64	17.97
2.	D.Kalpana	13.11	144.1	144.1	35.8	37.4	16.27	17.00
3.	M.Madhavi	14.11	158.2	158.4	52.6	53.0	21.04	21.20
4.	K.Sumalatha	13.10	147.0	147.0	34.8	39.2	15.81	17.81
5.	K.Vinila	14.1	155.1	155.1	42.2	44.8	17.58	18.66
6.	B.Subhashini	14.2	151.5	151.5	41.2	42.0	18.31	18.66
7.	T.Rekha	14.7	165.7	165.7	46.4	48.2	17.05	17.72
8.	R.Krishna Priya	14.1	151.0	151.2	47.4	45.0	20.78	19.73
9.	G.Pushpa	14.6	154.6	154.6	43.4	43.0	18.31	18.14
10.	S.Harisha	13.8	156.0	157.0	36.8	37.4	15.1	15.20
11.	S.Sangeetha	13.7	153.7	153.7	41.2	40.8	17.60	17.43
12.	C.Nisha	14.6	162.7	162.7	52.4	53.6	20.00	20.45
13.	N.Priyanka	13.4	157.3	157.3	42.0	43.8	17.07	17.59
14.	M.Kalpana	13.8	162.0	162.0	49.8	49.2	19.0	18.77
15.	G.Ramani	14.5	156.0	156.0	41.5	40.6	17.07	16.70
	Mean		155.21	155.34	43.44	44.04	17.97	18.20
	S.D.		5.5102	5.5225	5.50	5.05	1.72	1.49

APPENDIX - 3

SERUM RETINOL LEVELS (ug/100 ml)

Sample	Baseline	Placebo 30 days	Placebo + iron 45 days	RPO laddu + iron 45 days	RPO laddu + iron + vitamin C 45 days
01	17.3	10.79	24.23	29.74	21.63
02	16.85	15.52	14.05	28.58	21.31
03	12.39	10.64	6.80	22.02	25.48
04	14.34	11.93	8.77	22.19	31.16
05	16.89	13.12	6.76	20.17	32.39
06	15.00	10.68	12.70	26.19	29.22
07	24.72	11.37	6.20	24.25	29.97
08	12.92	12.23	7.70	25.49	26.22
09	14.70	11.81	7.67	31.20	25.11
10	20.16	21.24	9.87	29.30	24.88
11	16.64	15.67	6.40	18.88	24.42
12	19.11	12.45	9.43	21.66	23.79
13	8.63	12.70	13.68	15.86	29.92
14	12.59	10.12	15.23	9.28	15.85
15	21.07	12.09	9.80	8.60	16.58
Mean	16.2207	12.8240	10.6193	22.2273	25.1953
S.D.	3.8774	2.7335	4.6337	6.6705	4.7737

ANOVA

Source of variation	DF	Sum of squares	Mean squares	F-Ratio	CD (0.05)
Treatments	4	2285.6563	571.4141	23.77	3.55525
Error	70	1668.9727	23.8425		
Total	74	3954.6289			

Coefficient of variation (CV) : 3.567024

APPENDIX - 4

HAEMOGLOBIN (gm/dl)

Sample	Baseline	Placebo 30 days	Placebo + iron 45 days	RPO laddu + iron 45 days	RPO laddu + iron + vitamin C 45 days
01	10.72	7.89	8.00	10.48	11.52
02	7.39	7.69	10.82	11.05	12.22
03	11.03	10.43	9.91	13.88	13.06
04	11.84	11.72	9.9	13.61	13.95
05	12.87	7.67	8.90	11.85	13.72
06	8.21	9.18	8.20	12.28	13.41
07	5.21	5.57	10.30	8.09	12.24
08	11.61	8.25	8.20	12.08	13.00
09	9.69	8.48	8.80	11.05	12.80
10	12.83	8.66	8.60	13.68	13.98
11	12.39	8.22	8.90	12.52	12.89
12	12.13	10.28	8.10	12.45	12.19
13	13.24	7.08	10.30	13.61	13.69
14	12.94	9.68	10.00	14.02	13.74
15	10.91	10.76	9.60	13.58	13.45
Mean	10.8673	8.7707	9.2353	12.2820	13.0573
S.D.	2.2479	1.5407	0.9007	1.5684	0.7552

ANOVA

Source of variation	DF	Sum of squares	Mean squares	F-Ratio	CD (0.05)
Treatments	4	207.8066	51.9517	21.61	1.128966
Error	70	168.2949	2.4042		
Total	74	376.1016			

Coefficient of variation (CV) : 6.99269

APPENDIX - 5

B-carotene (ug/dl)

Sample	Baseline	Placebo 30 days	Placebo + iron 45 days	RPO laddu + iron 45 days	RPO laddu + iron + vitamin C 45 days
01	-	-	-	43.27	51.83
02	-	-	-	10.93	9.29
03	-	-	-	2.29	42.54
04	-	-	-	0.41	18.39
05	-	-	-	0.25	46.41
06	-	-	-	0.56	16.74
07	-	-	-	7.60	47.40
08	-	-	-	1.99	24.72
09	-	-	-	7.49	40.17
10	-	-	-	2.19	34.50
11	-	-	-	55.12	60.14
12	-	-	-	31.63	20.52
13	-	-	-	21.96	21.14
14	-	-	-	54.45	13.42
15	-	-	-	2.69	17.19
Mean	0	0	0	16.1887	30.9600
S.D.	0	0	0	22.2408	15.4895

ANOVA

Source of variation	DF	Sum of squares	Mean squares	F-Ratio	CD (0.05)
Treatments	1	1636.4395	1636.4395	4.94	13.61493
Error	28	9280.9082	331.4610		
Total	29	10917.3477			

Coefficient of variation (CV) : 1.294861

APPENDIX 6

FOOD INTAKE

NAME OF THE FOOD STUFF	FREQUENCY OF CONSUMPTION IN SUBJECTS											
	Daily		Thrice a week		Twice A week		Once A week		Rarely		Nil	
	No.of subjects	Qty. (g) M.Q/C	No.of subjects	Qty. (g) M.Q/C	No.of subjects	Qty. (g) M.Q/C	No.of subjects	Qty. (g) M.Q/C	No.of subjects	Qty. (g) M.Q/C	No.of subjects	Qty. (g) M.Q/C
GREEN LEAFY VEGETABLES												
Amaranth	0	-	2	90	4	134	6	95.5	3	51.6	0	-
Cabbage	0	-	3	35	1	45	8	36	2	42	1	-
Drumstick leaves	0	-	0	-	0	-	3	45	10	50	2	-
Ponnagani leaves	0	-	0	-	0	-	0	-	0	-	0	-
Spinach	0	-	0	-	5	51	7	45.7	3	47.5	0	-
Tamarind leaves	0	-	0	-	0	-	0	-	11	35.5	4	-
OTHER VEGETABLES												
Bittergourd	0	-	1	75	0	-	10	30	2	25	2	-
Drumstick	0	-	0	-	0	-	8	73.75	3	71.6	0	-

Contd..

NAME OF THE FOOD STUFF	FREQUENCY OF CONSUMPTION IN SUBJECTS											
	Daily		Thrice a week		Twice A week		Once A week		Rarely		Nil	
	No.of subjects	Qty. (g) M.Q/C	No.of subjects	Qty. (g) M.Q/C	No.of subjects	Qty. (g) M.Q/C	No.of subjects	Qty. (g) M.Q/C	No.of subjects	Qty. (g) M.Q/C	No.of subjects	Qty. (g) M.Q/C
ROOTS AND TUBERS												
Carrot	0	-	0	-	3	40	8	56.5	4	45	0	-
Beet root	0	-	0	-	0	-	0	-	15	38	0	-
Sweet potato	0	-	0	-	0	-	0	-	11	80.5	4	-
Potato	0	-	4	55.5	5	68.5	4	70	2	77.5	0	-
MILK												
Cows	2	105	0	-	0	-	0	-	0	-	0	-
Buffaloe	13	157.6	0	-	0	-	0	-	0	-	0	-
Curds	12	90.4	2	90	0	-	0	-	1	75	0	-
Egg	1	100	0	-	7	100	2	100	0	-	5	-
OTHER FLESHY FOODS												
Fish	0	-	0	-	0	-	4	97.5	6	102.5	5	-
Meat	0	-	0	-	1	80	7	71.4	2	77.5	5	-
Sheep liver	0	-	0	-	0	-	1	35	9	40.5	5	-

NAME OF THE ʼOD STUFF	FREQUENCY OF CONSUMPTION IN SUBJECTS											
	Daily		Thrice a week		Twice A week		Once A week		Rarely		Nil	
	No.of subjects	Qty. (g) M.Q/C	No.of subjects	Qty. (g) M.Q/C	No.of subjects	Qty. (g) M.Q/C	No.of subjects	Qty. (g) M.Q/C	No.of subjects	Qty. (g) M.Q/C	No.of subjects	Qty. (g) M.Q/C
FRUITS RDA												
Banana, Ripe	2	100	6	100	3	100	2	100	2	100	0	-
Dates	0	-	0	-	0	-	0	-	14	16	1	-
Guava	2	45	5	76.5	3	50	4	75	1	80	0	-
Jack fruit	0	-	0	-	0	-	0	-	13	75	2	-
Mango ripe (seasonal)	4	85.5	5	120.5	2	88.5	4	85	0	-	0	-
Papaya, ripe	0	-	0	-	2	60	4	75	6	80.8	3	-
Pomogranate (seasonal)	0	-	0	-	5	68.5	3	80.5	7	73.5	0	-
MISCELLANEOUS												
Jaggery	0	-	2	28	4	22	7	18	2	15	0	-
Rice flakes	1	100	3	87.7	2	90	6	89.1	3	85	0	[illegible]
Roasted Bengalgram	2	37.5	8	42.6	3	40.5	2	47.5	0	-	0	-
Til	0	-	3	10.5	4	15.5	2	15	6	12.5	0	-
Amla (seasonal)	2	20	2	12.5	3	15.5	4	16.25	4	17.5	0	-

M.Q/C = Mean quantity/consumption