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RESEARCH ARTICLE

Effect of Beta Carotene from Dehydrated Drumstick Leaf Powder on the Haematological Indices of Non-Pregnant Non-Lactating Young Women Aged 18 – 25 Yrs (Preliminary Trials)

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ABSTRACT

Low bioavailability of iron is the primary cause of anemia in Indian diets which are chiefly vegetarian and are comprised of inhibitors such as phytates and polyphenols. Several *in vitro* studies have demonstrated good antioxidant potential in green leafy vegetables which are rich sources of β–carotene, iron and other micronutrients.

Aim: To investigate the efficiency of beta carotene from shade dehydrated drumstick leaf (DDL) powder on the hematological indices of young women.

Methods: Twenty unmarried MIG women (18-25 y), consuming a typical Gujarati meal were purposively selected for the study and randomly divided into control and the experimental group (n=10 in each group). The later group received DDL powder (4g/day, 692 mcg beta carotene) for 25 days. Data on their SES, dietary habits, anthropometry, reproductive health and hematological indices were elicited.

Results: Reveal a positive shift of anemic girls to the non- anemic category, a mild increase in Hemoglobin, a favorable change *in* the hematological indices and an increase in the normocytic normochromic cells.

Conclusions: The study indicates that vitamin A/ beta carotene from DDL had a protective effect on iron availability from a typical Gujarati diet containing iron inhibitors. Further studies on effect on vitamin A and beta-carotene on anemic and non-anemic women for a longer duration are warranted.

Key words: Drumstick leaves, Beta Carotene, Hematological Indices.

INTRODUCTION

Adolescence is characterized by a large growth spurt and the acquisition of adult phenotypes and biologic rhythms. During this period, iron requirements increase dramatically in both boys and girls as a result of the expansion of the total blood volume, the increase in lean body mass and the onset of menses in young females.

"Hidden hunger", a lack of essential micronutrient is associated with decreased work capacity, fatigue and low concentration among adolescents. The majority of the Indian population subsists on suboptimal and imbalanced diets. Poverty remains a major cause of hunger and malnutrition and this situation is further aggravated by the rapid growth of population, unhealthy environment, and lack of education [1].

While adequate iron nutrition is achievable on a vegetarian diet, it must provide iron sources such as pulses and enhancers of absorption (vitamin C and fish or poultry, if acceptable). Temporary, unskilled vegetarianism may result in insufficient absorbed iron. Apart from inadequate dietary iron intake, poor bioavailability of dietary iron from foods like cereals, legumes and other vegetable foods which constitute a majority of Indian diets appear to be a major factor leading to iron deficiency in India [2]. Hence the challenge is to improve the bioavailability of iron by amalgamation of enhancers of iron in the vegetarian diets.

Vitamin A deficiency may cause anemia as a result of altering the absorption, storage, release or

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transport of iron to the marrow [3]. Over the past 30 years the metabolic connection of vitamin A and iron in affecting risk of anemia, and the potential of vitamin A to enhance effects of iron or alone to alleviate part of the burden of anemia have been the areas of intense research, review and program interest [4,5].

Vitamin A and B-carotene form a complex with iron keeping it soluble in the intestinal lumen and preventing the inhibitory effect of phytates and polyphenols on iron absorption [6].

Since Indian vegetarian diets contain fairly good amounts of phytates and polyphenols which inhibit iron absorption, it was found worthwhile to assess the effect of vitamin A (from plant source) in anemic/nonanemic girls consuming typical Indian diets.

Moringa Oleifera (drumstick leaves) with a total carotene content of 40,000 µg and beta carotene 19000 µg/ 100g FW exhibiting bioavailability equivalent to synthetic vitamin A [7,8] and can be acceptability incorporated in traditional Indian diets [9,10,11,12] were selected for the present study as a source of vitamin A.

The major objective of the study was to assess the effect of beta carotene from dehydrated drumstick leaf powder on the hematological indices of non-pregnant non-lactating young women 18 – 25y.

MATERIALS AND METHODS

Selection of the subjects:

The subjects were postmenarcheal, non-pregnant non-lactating young unmarried girls aged 18 – 25 yrs belonging to middle income group from free living population consuming typical Gujarati, (Western Indian) diets. Both the subjects and their parent's written consent to participate in the study were sought. Thirty subjects who were willing to participate in the study were age matched and randomly divided into experimental and control groups. Experimental group was given 4gm of shade dehydrated drumstick leaf (DDL) powder (as per the procedure described in our earlier paper Nambiar *et al*, 2003) daily for a period of 25 days. The supplementation was done everyday in the morning by the principal investigator. Thus each subject in the experimental group received 3217.3 mcg beta carotene, 1543.4 mg total phenols and 11.5 g fiber per day.

Data and sample collection

Pre-tested questionnaires were used to elicit the data on SES and reproductive health of the subjects. Data on the anthropometric indices (weight and height) was collected. BMI was calculated using the formula given by WHO 2004. All the subjects were observed for presence of clinical signs and symptoms of anemia that included fatigue, pallor, breathlessness and flat nails. The dietary intake of the subjects was assessed using food frequency method and 24hr dietary recall. The nutrient content of food consumed by each subject was calculated using the food composition tables given by Nutritive Value of Indian Foods [13]. The food and nutrient intake was compared with the RDA given by ICMR and percent adequacy was calculated. The Hematological indices were assessed before and after supplementation in the Laboratory. The F-620 semi-automated hematology analyzer was used for estimating the hematological indices which comprised of Hb, RBC counts, MCV, MCHC, PCV, differential counts, WBC platelets and RBC morphology as described in our earlier paper [14].

Statistical analysis

The data were analyzed using the statistics analysis package of Microsoft Excel. Frequency distributions and percentages were calculated for all the parameters. Mean and standard errors were calculated for all parameters that were expressed as numerical. Paired't' test was used to assess the difference between the means of the same group before and after the study period. Chi-square test was used to test the differences between the frequency distribution

RESULTS AND DISCUSSION

Though the initial sample size was 30, due to dropouts, the results are presented for 20 subjects (E=10, C=10).

Socio – Economic Status

The Socio – economic background of the families at the baseline were mapped out and shown in (**Table 1**). The average age of the subjects was 21.4±2.6 yrs. Families were essentially nuclear in nature in experimental (90%) and control group (70%), and the sample had a family size between 5–7 members. Disparity was seen in the economic and educational status of the women.

Reproductive health

(**Table 2**) estimates the reproductive health of the subjects which is one of the confounding variables of anemia. Among all the individuals only one of the subjects reported family history of anemia. The Gynecological data of the mothers included the number of children and menstruation pattern. The mean number of children was reported as 3 per family. Ninety percent of the mothers in the experimental and 70% in the control groups had attained menopause. No major complications on

reproductive health were reported by any of the subjects. Majority of the subjects had attained menarche at the age of 13 – 16 yrs with 90% in experimental group and 100% in control group. The average number of days of menstruation was 4 – 6 days and blood loss during that period was normal in both the groups. It is estimated that blood loss during menstruation leads to 30 mg loss of dietary iron and 3 mg of absorbed iron per day [14]. In adolescents, the amount of iron moving from one compartment to another is likely to be modified slightly on the basis of body size and the onset of menses in the female portion of the adolescent population [15].

Anthropometric profile

The mean height and weight of the subjects at baseline were 156.8±5.3cm and 45.6±6.2 kg respectively. The ideal weight for height ranged from 54.4 – 58.1 kg (**Table 3**) thus indicating that the weight and height of the subjects were below the standards given by ICMR, 1996.

Similar results were seen in the BMI data [16], wherein only 40% in experimental and 80% in control group could be classified as normal. Thirty percent of women were classified as "moderately underweight" in the experimental group with one subject as "severely malnourished" with BMI>16.

A low Body Mass Index of < 18.5 is a risk factor for poor pregnancy outcome. Fifty percent of the adult population in India and 53.4% of the children is reported to have a Body Mass Index below normal [17]. The nutritional requirements of adolescent are influenced primarily by the normal event of puberty and simultaneous spurt of growth. Puberty is an intensely anabolic period with increase in height and weight, alteration in the body composition resulting from increased lean body mass and change in the quantity and distribution of fat and enlargement of many organ systems. Adolescent are particularly susceptible to iron deficiency anemia in view of the increased need for dietary iron for hemoglobin and myoglobin synthesis during the rapid period of growth when blood volume and muscle mass are increasing.

According to the recent National Family and Health Survey of India, women and men suffer a dual burden of overnutrition and undernutrition. More than one-third of women are too thin, while 13% are overweight or obese. In all, nearly half of married women are either underweight or overweight. One-third of men are too thin, and 9% are overweight or obese. Overweight and obesity are even more common in the cities, among highly educated adults, and among adults from the wealthiest households. The states with the largest percentage of overweight women and men are Punjab, Kerala, and Delhi [1].

Clinical profile

Clinical signs and symptoms of anemia (weakness, flat nails, pale nails and skin, breathlessness) were obvious in both the group of girls. Ninety percent of the women in experimental group had flat and pale nails. Overall, breathlessness and pale skin was seen in 30% of the subjects.

Nutrient intake of the typical Gujarati diet consumed by the subjects

The nutrient intake data of typical Gujarati diet consumed by the subjects revealed that all the essential macro as well as micro nutrients were consumed in low amounts by the subjects. The calorie intake of all the subjects was approximately only 1316 Kcal per day. The per capita daily energy and protein intake was 61-65% RDA. The percent calorie from Carbohydrate, protein and fat were only 63%, 9% and 28% respectively. The intake of ascorbic acid (60.1% RDA), beta carotene (46.5% RDA) was woefully inadequate. The daily intake of iron was 32.33% the RDA highlighting widespread dietary inadequacy of iron.

Adolescents often have chaotic eating patterns that do not conform to dietary recommendations. Many *adolescent girls* try to control their weight and inadvertently limit iron intake. Estimates suggest that about 25-50 per cent girls become anemic by the time they reach menarche. Other factors such as gender discrimination in intra-household food allocation and early marriage leading to early pregnancy also aggravate anemia. A survey conducted by National Nutrition Monitoring Bureau [18] indicates that the daily intake of most foods in Indian households, except for cereals and millets, is much below the recommended dietary allowances (RDA).

Since the dietary data reveals nutrient inadequacy, other nutritional deficiencies such as folic acid, vitamin B_{12}, vitamin C or vitamin A may also adversely affect hematopoiesis and lead to anemia [19]. Iron balance is the difference between iron retention and iron requirements and has been well described over the past 50 years [20]. The retention of iron, frequently called the absorbed iron, is the product of iron intake and the bioavailability of that dietary, supplemental or contaminant iron. The excess iron that accumulates beyond that necessary for the daily requirement is stored

within the core of the ferritin molecule. This stored ferritin iron is then available for cellular iron needs should dietary intake fall below the organ needs. When this negative iron balance persists for a period of time, the iron stores are depleted and the iron supply to the essential iron pools of the body is diminished. Functional consequences then result from insufficient iron-dependent functioning for oxygen transport, oxidative metabolism, nuclear metabolism and gene transcription. Clinical sequelae to this poor iron status include anemia, poor immune function and decreased work performance. Poor fetal outcomes may occur if iron deficiency occurs in the first trimester of pregnancy [20].

Biochemical Parameters

The average blood parameters of the subjects at the baseline given in (**Table 4**). Most of the subjects had Hemoglobin levels below the reference range (85%), merely 15% were normal. Similar results are shown by the NFHS III survey, which reports that more than half of women in India (55%) are anemic, and anemia among women has increased slightly in the past seven years. Anemia also increased for pregnant women during that period. Even though men are much less likely than women to be anemic, anemia levels in men are still unacceptably high (24%) [1].

The present study reveals that half of the subjects had MCV, MCH, PCV, MCHC and Erythrocyte counts below the reference age.

There are three stages in the development of iron deficiency anemia. The first stage is characterized by depletion of iron stores as reflected by a decline in SF concentration. The second phase, iron-deficient erythropoiesis, is characterized by a decrease in TS and an increase in EP concentration. The final stage of iron deficiency is characterized by a reduction in the concentration of Hb in RBCs. The use of multiple indices of iron status provides a more accurate measure of iron status than any single index [21].

Lower serum retinol concentration has also been associated with lower iron indices. For example, significant correlations between the concentrations of serum retinol, serum iron and Hb, packed cell volume, MCHC and TS in adolescent girls in urban Bangladesh were reported [22].

Impact of DDL on the hematological indices

Distribution of anemia before and after supplementation is shown in (**Fig 1**) Overall the prevalence of anemia before supplementation was 80% in experimental group and 90% in control group. There was a marginal non significant increase in the hemoglobin levels of the subjects (10.4 to10.8 g/dl) receiving DDL powder. The shift of subjects from moderate to mild anemia was 10% in the experimental group. The percentage of subjects normal increased from 20% to 30% after supplementation. No change was observed in the control group (**Table 5**). The rise in Hemoglobin levels were 0.4g/dl in experimental group and less than 0.1g/ dl in control group.

There was increase in PCV, MCV, MCH and MCV by 0.7 %, 2.5 fl, 1.4pg and 1% respectively though not significant. The RBC counts reduced though it was only 0.1mm/cm in the experimental group unlike 0.4mm/cm in the control group. Other hematological indices showed a positive trend other than the WBC as there is reduced resistance to infections in anemia.

The trend in morphology of RBC after supplementation is shown in (**Fig 2**). Fifty percent subjects in the experimental group were normocytic and normochromic i.e. normal peripheral blood cells before supplementation, which increased to 80% after supplementation. About 40% were hypochromic (pale cells) and microcytic (smaller than the average RBC) which reduced in half of the subjects (20%) after supplementation. Round Macrocytes was seen in one of the subjects, disappeared after supplementation unlike the control group where it increased in 2 subjects only.

Vitamin A is required for the effective utilization of iron and for maintaining normal Hb concentration [23]. Clinical trials carried out in populations of children and women of reproductive age have tended to show improvement in indices of iron metabolism and erythropoisis in response to vitamin A interventions, despite variation in nutritional risk and in the dosage, frequency and duration and form of supplementation [24, 25]. Although a postulated mechanism includes a chelating effect of vitamin A to protect iron from the inhibitory effects of phytates, polyphenols, and tannic acid in the gut, there is not sufficient evidence in vivo for this effect [26, 27]. Significant correlations between concentrations of serum retinol and Hb and serum iron, indicating a possible relationship between vitamin A status and the use of iron for haematopoiesis were reported [28].

This randomized control trial though conducted for a short duration, showed that there is very small positive shift in the hematological indices

after supplementation of drumstick leaves, though consistent effect was not seen. Further studies on effect on vitamin A and beta-carotene on anemic and non-anemic women for a longer duration are underway.

Table 1: Socio – economic profile of the subjects at baseline

Socio – Economic Characteristics	Experimental Group (n = 10)		Control Group (n = 10)	
	N	%	N	%
Age				
18 – 20yrs	3	30	5	50
20 – 25yrs	7	70	5	50
Type of family				
a)nuclear	9	90	7	70
b) joint	1	10	3	30
c) extended	0	0	0	0
Family size				
3 – 5	3	30	3	30
5 – 7	6	60	7	70
Above 7	1	10	0	0
Family income (Rs)				
<9000	2	20	2	20
10,000 – 19,000	2	20	1	10
>20,000	6	60	7	70
Fathers Education				
Primary	2	20	1	10
Secondary	1	10	1	10
Higher secondary	2	20	2	20
Graduation	4	40	6	60
Post graduation	1	10	0	0
Mothers education				
Primary	4	40	2	20
Secondary	1	10	2	20
H.Sc	4	40	0	0
Graduation	1	10	5	50
Post graduation	0	0	1	10

Table 2: Reproductive histories of the subjects

Characteristics	Experimental Group (n = 10)		Control Group (n = 10)	
	N	%	N	%
Family history				
Mother				
Father	1	10	0	0
Gynaecological history of mother				
No. of children (mean)	3	30	3	30
Menstruation pattern				
Regular	1	10	3	30
Irregular				
Menopause	9	90	7	70
Menstruation pattern of subject				
Age of menarche				
11 – 13 yrs	1	10	0	
13 – 16 yrs	9	90	10	100
No. days				
<3 days	0	0	2	20
4 – 6 days	9	90	8	80
Above 6 days	1	10	0	
Menstruation pattern				
Regular	12	100	12	100
Irregular	0	0	0	0
Loss of blood				
Less	3	30	0	0
Normal	6	70	8	80
Heavy	1	10	2	20

Table 3: Categorization of the subjects according to BMI

Group	Mild Under Weight (17 – 18.4)		Moderate Under Weight (16 – 16.9)		Severe Under Weight <16		Normal (18.5–24.9)		χ^2
	N	%	N	%	N	%	N	%	
Experimental group (n = 10)	2	20	3	30	1	10	4	40	17.6***
Control group (n = 10)	1	10	1	10	0	0	8	80	

*(WHO 2000 and 2004) *** Significant at 1% level*

Table 4: Haematological Indices of the subjects

S. No	Blood parameter	Reference range	Normal		Below reference range		Above reference range	
			n	%	n	%	N	%
1	Hemoglobin	12 – 14 g/dl	3	15	17	85	-	-
2	RBC count	4.4 – 6.6 m/cm	10	50	7	35	3	15
3	Packed Cell Volume	34 – 54 %	11	55	9	45	-	-
4	Mean Cell Volume	76- 96fl	7	35	9	45	4	20
5	Mean cell Hemoglobin	26-32 pg	6	30	10	50	4	20
6	Mean Cell Hemoglobin	30 – 36%	17	85	3	15	-	0
7	concentration							
8	White Blood Cells	4,000 – 11,000/ cmm.	19	95	1	5	0	0
9	Platelet count	150,000 – 450,000/ cmm	20	100	0	0	0	0

Table 5: Effect of supplementation on hematological indices at baseline-end line

Variable	Experimental group		Control group	
	Pre	Post	Pre	Post
Hemoglobin				
Mean ± SD	10.4±2.3	10.8±2.2	11.01±0.2	11.05±0.1
Mean ± SE	10.4±0.75	10.8±0.7	11.01±0.2	11.05±0.1
't' – value	-0.9161^{NS}		-0.5345^{NS}	
RBC counts				
Mean ± SD	4.3±0.69	4.2±0.61	4.22±0.6	3.96±0.5
Mean ± SE	4.3±0.2	4.2±0.1	4.22±0.2	3.96±0.1
't' – value	0.7673^{NS}		1.9390^{NS}	
Packed Cell Volume				
Mean ± SD	32.7±4.8	33.4±4.5	34.4±2.2	33.4±2.0
Mean ± SE	33.4±1.3	33.2±1.2	34.9±0.7	33.4±2.0
't' – value	-0.7483^{NS}		2.3664^{S}	
Mean Cell Volume				
Mean ± SD	77.8±16.3	80.3±14.6	83.3±10.3	86.1±9.6
Mean ± SE	77.8±5.5	80.3±4.6	83.3±3.2	86.1±3
't' – value	-0.7483^{NS}		-1.6404^{NS}	
Mean cell Hemoglobin				
Mean ± SD	24.7±7.1	26.1±6.4	26.7±4.05	27.1±1.2
Mean ± SE	24.7±2.2	26.1±2	27.1±1.2	28.7±1.2
't' – value	-0.97167^{NS}		-2.1439^{NS}	
Mean Cell Hemoglobin Concentration				
Mean ± SD	31.5±3.09	32.05±2	32±1.1	33.06±1.3
Mean ± SE	31.5±0.9	32.05±0.8	32.1±0.3	33.06±0.4
't' – value	-1.1692^{NS}		-2.7007^{NS}	
White Blood Cells				
Mean ± SD	6490.6±1467	6350.33±1621	7000±1727	6920±1844
Mean ± SE	6490.6±464	6350.33±512	7000±546	6920±583
't' – value	0.4392^{NS}		0.2579^{NS}	

Figure 1: Categorization of anemic women (%) according to their hemoglobin levels

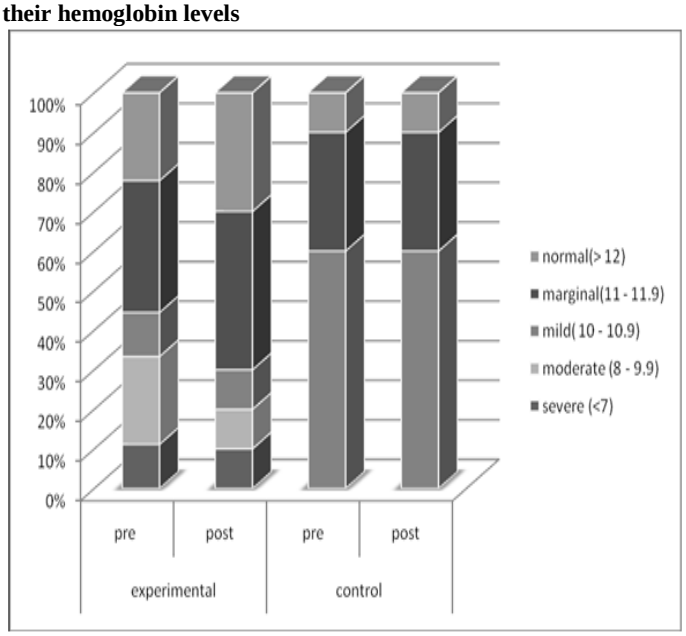
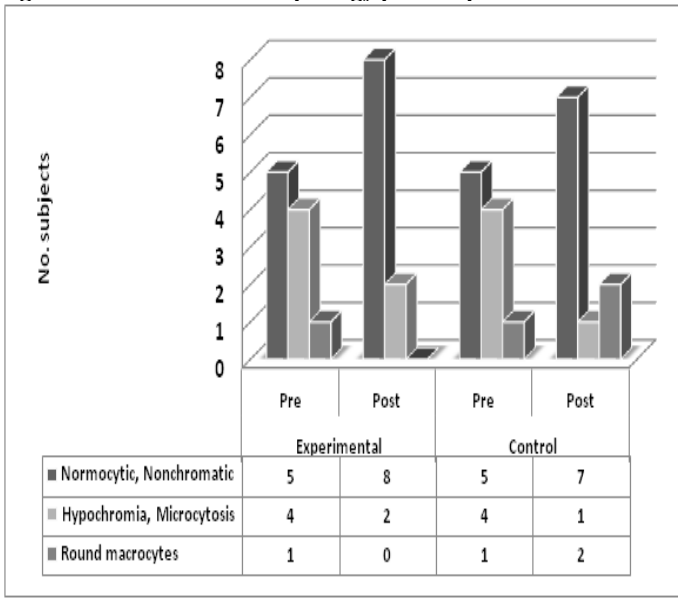


Figure 2: Trend in RBC morphology pre and post intervention



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