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CASE STUDY

Effect of Drumstick Leaves supplementation on Hematological Indices of young girls (16-21 years)

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ABSTRACT

Background: Drumstick leaves (DL) with its high beta carotene content (19690 mcg/100g) along with vitamin C from lemon juice may have a positive impact in the mobilization of stored iron and increase hemoglobin levels of anemic subjects. **Aim:** To determine the effect of DL and vitamin C supplementation on hematological indices of young girls (16-21 years). **Method:** Based on pallor, 100/700 girls studying in first and second year the Faculty of Family and Community Sciences were selected and assessed for hematological Indices (Hb, Hct, PCV, MCV, MCHC) and red cell morphology and were divided into Group A (329 RE of Beta carotene from DL rich recipes (boiled mung/desi chana/kabuli chana) and 5.85 mg Vitamin C as lemon juice, n=21), Group B (329 RE from the above DL recipes, n=20) and Group C (recipes without DL leaves, n=21) for 45 days respectively. **Results:** Post supplementation data revealed 28.6% reduction of anemia in Group A, by followed by 5% in group B and 4.7% in group C. There was a positive change observed in red cell morphology (normocytic normochromic) in Group A (18%) and B (2.6%) respectively. Similar results were seen for RBC, MCV and MCHC. Group B which received drumstick leaves showed small positive change in MCH showing a causal association between VA and iron metabolism. **Conclusions:** The study emphasizes that anemia is still prevalent even in young adult women of the middle and high-income groups of urban Vadodara (54%). It also shows a strong association between vitamin C from lemon juice and iron and a causal association between VA available from DL and iron metabolism.

Keywords: Anemia, Vitamin A, Beta Carotene, Drumstick leaves, Young girls, Hematological Indices.

INTRODUCTION

Women and adolescent girls are at high risk of micronutrient malnutrition especially Iron Deficiency Anemia and Vitamin A Deficiency. Globally the most important cause of anemia is believed to be iron deficiency due to inadequate dietary intake, physiologic demands of pregnancy & rapid growth & iron losses due to parasitic infection. Other prevalent causes of anemia include malaria, chronic infection and nutritional deficiencies of vitamin A, folate & Vitamin B_{12}. [1] Vitamin C is a well known enhancer of iron bioavailability. Several researchers have reported relationship between Iron and vitamin A. Hodges et al and Mejia & Arroyave [2,3] established an association between iron metabolism & hypovitaminosis A. Anemia provoked by VAD resembles Hypochromic anemia. Vitamin A binds with iron during digestive process and forms a complex that acts as a chelating agent, thus blocking the effect of hydroxyl radicals present in phytates and polyphenols in flour and tea / coffee respectively [4]. After supplementation with Vitamin A, hematologic evidence and measures of iron status have improved [5, 6]. Vitamin A can play a role in improving utilization of available iron in young girls. [7]

Drumstick leaves (Moringa oliefera) is reported to contain alkaloids, flavonoids [8] anthocyanins, proanthocyanidins and cinnamates and is highly reputed in folklore and traditional system of medicine as a remedy for variety of ailments. Drumstick leaves (*Moringa oleifera*) with a beta carotene (a precursor of vitamin A) content of 19690mcg/100gm FW is acceptable in

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traditional Indian diets both in fresh as well as dry forms, has a good bioavailability in fresh and dry forms may thus be useful in improving utilization of iron especially from high phytate Indian diets.[9-13]

With this hypothesis in mind, the present study was planned. The broad objective was to study the effect of Drumstick Leaves (*Moringa oleifera*) with or without vitamin C rich lemon juice supplementation along with traditionally consumed high phytates recipe on the hematological indices of young anemic/non anemic girls.

MATERIALS AND METHODS

***Study area*:** The study was conducted in the Faculty of Family and Community Sciences of The M. S. University of Baroda, Vadodara, Gujarat.

***Study design*:** Out of the 700 girls (16-19y) screened for pallor, 100 positive subjects were selected and divided into 3 groups. These girls were supplemented with 30gm cooked (1 cup) of boiled mung/desi chana/kabuli chana (phytates 201-265 mg percent), with or without freshly blanched drumstick leaves or lemon juice for a period of 45 days during the lunch hour.

However due to drop outs the final data is presented for 62 subjects. Group A subjects received freshly blanched drumstick leaves in boiled mung/desi chana/kabuli chana along with 200 ml of lemon juice containing 329 RE of Beta carotene from DL and 5.85 mg Vitamin C as lemon juice, (n=21), Group B received freshly blanched drumstick leaves in boiled mung/desi chana/kabuli chana containing 329 RE beta carotene (n=20) and Group C received boiled mung/desi chana/kabuli chana recipes without DL leaves (n=21) respectively. Pre-post estimations for hematological indices were done after 45 days and after 3 months to assess the wash out effect of the supplementation.

Product Development Sensory evaluation and standardization: Drumstick leaves obtained from drumstick trees situated in university campus, Vadodara, were selected as a food source of beta carotene for supplementation. Different levels of freshly blanched drumstick leaves were tried: 6-25 gm per 30 gm raw weight i.e. one serving of each recipe namely kabuli chana, desi chana and mung. All recipes were prepared fresh and presented to the judges for sensory evaluation. The final test was conducted with incorporation of 20 gms fresh DL in each recipe and was selected for the study. Details of product development and organoleptic evaluations are described in our earlier paper. [14]

Baseline data: Baseline data were collected on the socioeconomic status, clinical examination for anemia, anthropometry (weight, height, BMI) and dietary intake (24-h DRM and FFQ methods).

***Biochemical Estimation*:** Pre-post analysis was done on the hematological indices to assess the impact of supplementation. Semi-automated hematology analyzer F-620 was used for in vitro diagnosis of Red Blood Cell (RBC/Hb), Hemoglobin Concentration (HGB), Hematocrit (HCT) and Mean Corpuscular Volume (MCV). Hemoglobin was measured using Sysmax Lysing reagent; HCT was measured electronically; The MCV is calculated from HCT and the RBC. A thin blood film was used to study the Red Cell Morphology of the erythrocytes, using Leishman's stain.

***Statistical Analysis*:** The data was entered and analyzed using Micro Soft Excel. Means and standard errors were calculated for the entire parameters. Chi square, Paired't' and Independent't' tests were performed to test the differences between the control and experimental groups.

RESULTS

***Baseline Data*:** Data on family type reveal that 79% girls belonged to nuclear families and 77.3% has an average family size of 3-6 members. The mean age of menarche was 14 y. Anthropometric and FFQ data revealed no significant differences between the weight, height or BMI or in the consumption of iron or vitamin A rich foods among the 3 groups. Out of the total 700 girls of FY and SY, 100 girls were selected on the basis of pallor which accounts for 14% prevalence. Based on hemoglobin estimations 54% were found to be anemic (Hb<12g/dl).

Out of these 39% were mildly anemic, 14% moderately anemic and 1% severely anemic. Almost 87% girls had lower Hematocrit (%) than normal, 73% had lower MCHC, 49% had lower MCH followed by 40% had lower RBC counts and 20% had lower MCV.

Almost 29% girls had abnormal morphology which included 16% Microcytic Hypochromic, 8% Normocytic Hypochromic and 5% Macrocytic. When the Hb levels were compared with the red cell morphology (RCM) it was seen that a 14% of anemic girls had a normal RCM indicating that they were in initial stages of

IDA which can be easily reverted to normal by appropriate food based approaches.

IMPACT OF DRUMSTICK LEAVES SUPPLEMENTATION: The mean intake after supplementation showed an increase in the total energy intake (201, 149 and 133 Kcal); protein intake (7.6, 7.55 and 6.98 gms); Beta Carotene intake (3980.5, 3960.5 and 42.5 mcg); vitamin C (51.85, 46 and 2 mg) and iron intake (1.5, 1.49 and 1.35 mg) of all the three groups Group A, B and C respectively. The change in hemoglobin levels of subjects after supplementation is shown in (**Table 1**).

Table 1 Changes in Hemoglobin Levels after Supplementation of 45 days (PI) and after a gap of 3 months (PII)

Mean H Levels	A (n=21)	B (n=20)	C (n=21)	Total (n=62)
Initial	10.67±0.31	11.09±0.21	10.92±0.253	10.893±1.22
PI	11.33±0.37	11.18±0.31	11.29±0.27	11.26±0.188
PII	11.58±0.36	11.22±0.28	11.59±0.43	11.46±0.21
Difference (PI-I) (PII-I)	(+0.66) (+0.91)	(+0.09) (+0.13)	(+0.37) (+0.67)	(+0.36) (+0.57)
Paired 't'	2.462*	0.305	1.478	2.345*

** $p < 0.05$ Hb = haemoglobin*

There is significant difference seen which is mainly contributed by Group A which was supplemented by Drumstick leaves recipes and lemon juice, Group B and C do not show any significant change though there is a little change in their final hemoglobin levels. Post supplementation data revealed 28.6% reduction of anemia in Group A by followed by 5% in group B and 4.7% in group C (**Table 2**).

Table 2 Comparison of prevalence of anemia after 45 days of supplementation

Hemoglobin level (g/dl)	Group A %	Group B %	Group C %
Initial			
≥ 12	19.04 (4)	25 (5)	23.8 (5)
< 12	80.95 (17)	75 (15)	76.1 (16)
(Mild) 10-11.9	76.47 (13)	87(13)	75 (12)
(Moderate) 7-9.9	17.64 (3)	13 (2)	25 (4)
(Severe) <7	5.88 (1)	-	-
Final			
≥ 12	48 ↓ (10)	30 (6)	28.57 (6)
<12	52.38 ↑ (11)	70 (14)	71.42 (14)
(Mild) 10-11.9	55 (6)	71 (10)	86 (12)
(Moderate) 7-9.9	45 (5)	29 (4)	14 (2)
(Severe) <7	-	-	

Figures in Parenthesis indicate number of subjects (A=Drumstick Leaves+Lemon Juice, B= Drumstick Leaves, C=Plain Recipe)

Table 3 Red cell morphology of subjects after supplementation of 45 days (pi) and after a gap of 3 months (pii)

Red Cell Morphology	A			B			C		
	Initial	PI	PII	Initial	PI	PII	Initial	PI	PII
Normal									
Normocytic Normochromic	57.1 12)	75 (16)	76.47 (13)	65 (13)	66.6(14)	73.3 (11)	66.6(14)	66.6 (14)	83.3 (10)
Abnormal									
Macrocytic	4.76(1)	-	-	10 (2)	-	-	-	-	-
Normocytic Hypochromic	19 (4)	-	-	5(1)	-	-	4.76(1)	47.6 (1)	-
Microcytic Hypochromic	19(4)	25(5)	23.6 (4)	20(4)	33.3(6)	26.6(4)	28.5(6)	28.5(6)	16.6 (2)

Figures in Parenthesis indicate number of subject (A=Drumstick Leaves+Lemon Juice, B= Drumstick Leaves, C=Plain Recipe)

These results highlight the fact that vitamin C as well as beta carotene rich supplements have brought about a positive change in the anemic girls. In Group B there was shift of 5% these results are encouraging and support the fact that vitamin A helps in the metabolism of iron as this group received 329 RE as beta carotene supplement. Considering the fact that both the pulses contained enough inhibitors of iron as phytic acid (201-265 mg percent, [15] even a 5% increase in Hb after 45 days of supplementation is a positive result and emphasizes that vitamin A from drumstick leaves would have helped in preventing inhibitory effects of phytates. In Group C, there was shift of 4.7% which received plain recipes.

Table 3 reveals that the trend of RBC, MCV and MCHC remain same after supplementation. In case of PCV only in group C, normal PCV shifted to abnormal. In case of MCH

in group B, girls shifted from abnormal MCH to normal. In group A, a positive change is noted with the Normocytic normochromic NN shifts from 57% to 75% after supplementation. In Group B NN shifts from 65 to 66.6 and in Group C, there was no change seen after supplementation, but after a gap of 3 months a better condition was seen as most of them started taking iron tablets realizing their anemic condition. Though the increase of normal subjects in group B is not as high as that in Group A, the results are encouraging and support the fact that vitamin A helps in the metabolism of iron.

CONCLUSIONS

Freshly blanched drumstick leaves showed a mild positive relationship in the improvement of anemia. The study also emphasizes that anemia is still prevalent even in young adult women of the middle and high-income groups of urban Vadodara (54%). It also shows a strong association between vitamin C from lemon juice and iron and a causal association between VA available from DL and iron metabolism.

DISCUSSION

The beta carotene from drumstick leaves though theoretically high, may not have produced equivalent amounts of vitamin A activity. The release of carotenoids from the food matrices, their solubilization in the gut and their absorption into intestinal mucosal cells are critically important processes. [16] Thus the study could have given better results if dehydrated DDL tablets with increased dosage, would have been used as we did to study their impact on hyperlipidemic patients. [17] The main effect of vitamin A is to maintain adequate levels of iron in plasma to supply the different body tissues including the bone marrow with proper amounts of this essential mineral which may be the mechanism by which the hemopoeitic tissue becomes flavored with more available iron Although deficiency of iron in the diet is regarded as the most important factor in the etiology of nutritional anemia certain human and animal studies have shown that supplementation of iron with vitamin A may increase the iron status as measured by hematological indices like hemoglobin and hematocrit. [18] A significant association of serum retinol with hematocrit, serum iron and serum ferritin has been reported by [7] in a cross sectional study of children in north east Thailand. Supplementation with vitamin A alone has significantly increased hemoglobin, hematocrit, plasma ferritin in children, [19-20] and pregnant women. [21-22]

The presence of vitamin A increased iron absorption up to twofold for rice, 0.8-fold for wheat and 1.4-fold for corn. Beta -carotene increased absorption more than threefold for rice and 1.8-fold for wheat and corn, suggesting that both compounds prevented the inhibitory effect of phytates on iron absorption. [23]

The supplement received by Group B was 663.4 RE, obtained from Beta-carotene, which is the most abundant provitamin A in foods. Approximately 10-50% of the total β-carotene consumed is absorbed in the gastrointestinal tract and within the intestinal wall is partially converted into vitamin A. Beta carotene from the drumstick leaves is also not toxic, so it is considered a safe source of vitamin A, thus need to be promoted not only as a source of vitamin A to combat VAD but also to prevent IDA in the communities. Also the low cost and the easy availability, fast growth and ease of cultivation enhance its uses. The Social Forestry Schemes of India also promotes this tree plantation by providing free samplings. The results of the present study have highlighted another importance of these leaves besides the ones already recorded. [24-25]

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