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RESEARCH PAPER

Effect of consumption of dehydrated green leaf mixture on the hemoglobin levels of the anemic adolescent girls

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ABSTRACT : The present study was designed to investigate the efficacy of leaf mixture (LM) on the hemoglobin (Hb) levels of anemic adolescent girls. The LM was prepared by the combination of the leaf powders of four under utilized leaves of beetgreens (*Beta vulgaris*), carrot(*Daucus carota*), cauliflower (*Brassica oleracea*) and turnip (*Brassica rapa*) in the ratio of 1:2:1:1, respectively. The study was done in a Randomized Experimental Setting on the adolescent girls of poor communities residing in the urban slums of Jaipur city. The baseline investigations included measurement of height weight and Hb levels. The Hb levels of the anemic girls were measured every month for a period of four months of intervention and were compared with the Hb levels of the control group. The results revealed a significant improvement in the Hb levels of experimental group as compared to the control group ($P \leq 0.01$).

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Micronutrient deficiencies are still a mjor public health problem in the world today with an estimated 2.5 to 5 billion people affected (Rush, 2000). Anemia of iron deficiency is the major micronutrient deficiency that affects the youth particularly in developing countries. The world's adolescent population (age 10-19) years is estimated to stand at more than 1 billion, yet adolescents remain a largely neglected, difficult to measure and hard to reach population in which the needs of adolescent girls in particular are often ignored (Barbin *et al.*, 2001).

Adolescence is a time of intense physical, psychosocial and cognitive development. Increased nutritional needs at this juncture relate to the fact that adolescents gain upto 50 per cent of their adult weight, more than 20 per cent of their adult height, and 50 per cent of their adult skeletal mass during this period. The iron needs are high in adolescent girls because of the increased requirements for expansion of blood volume associated with the adolescent growth spurt and the onset of menstruation (WHO, 2008).

Various programmes and policies to combat IDA is in place but the need of the hour is the Food Based Strategy, a preventive and comprehensive strategy that use food (*i.e.* whole, refined, processed, fortified or a combination) as a tool to overcome micronutrient deficiencies. The objective of the food based strategy is to ensure that

people get micronutrients in sufficient quantity through their daily diets (WHO, 2002).

From time immemorial green leaves (natural foods) are considered as the rich source of iron. There are various leaves which are rich in iron but are discarded or are used as animal fodder due to unawareness and ignorance. The need is to identify such leaves and to use them for human consumption, especially in the developing countries where people are entrapped in poverty and the diet is pre-dominantly cereal based. Propogation and promotion of less utilized green leafy vegetables is thus, essential (Chakraborty and Ghosh, 2002).

In the present study four such leaves of beetgreens (*Beta vulgaris*), carrot (*Daucus carota*), cauliflower (*Brassica oleracea*) and turnip (*Brasica rapa*), were identified and their mixture (LM) was used to elevate anemia in the adolescent girls of low socio-economic status.

RESEARCH METHODOLOGY

The study was conducted in the urban slums of Jaipur city- India. The subjects were postmenarcheal, nonpregnant girls aged 14 -18 y. Both the subjects' and their parents' written consent to participate in the study was sought and all eligible girls were screened for anemia. Initial screening for anemia was carried out with a B-Hemoglobin Analyzer (HemoCue) on a finger-prick capillary blood sample. A total of 120 adolescent girls, identified as anemic (hemoglobin < 12.0 g/dL) were then invited to provide a venous blood sample (baseline sample) on a prefixed date and their hemoglobin concentration was measured again with a cyanmethemoglobin method. The girls who were found to be anemic on the basis of the baseline blood sample were recruited for the study. The girls with severe anemia (hemoglobin < 8.0 g/dL) were excluded from the study and referred to the hospital. The girls with any sign of chronic infection or metabolic disorder were also excluded from the study.

A pre coded, pre-tested and semi structured interview schedule was used to collect information about the subject. The baseline data on subjects was collected by interviewing the adolescent girls' themselves. Information on age, gender, number of siblings, family size, income pattern of the family, basic amenities available, age at menarche and age at marriage was recorded on the baseline form.

'Twenty four hour recall' method was used for dietary assessment. On the day of interview adolescent subjects were asked to recall the previous day's food intake in terms of household measures, which was converted into the measurements of standardized utensils and entered into the questionnaire. Information was also collected, on the raw amount of food used for cooking the family meal, the total volume of cooked food and the volume of food consumed by the subject. The Twenty four hour recall was done on two consecutive days during the initiation and termination of the study and the means of the two recalls were taken and were compared with the RDA.

Preparation of leaf mixture :

The leaves of beetroot, carrot, cauliflower and turnip were collected from the strategic market locations of Jaipur city. The leaves were washed thoroughly and were dried in sun till they were brittle to touch. The selected dried leaves were then powdered homogeneously and were mixed in the ratio of 1:2:1:1, respectively to form the leaf mixture for the intervention. The prepared leaf mixture was analysed for its biochemical composition using standard methods of AOAC (2000). The nutritive value of the leaf mixture is presented in the Table A.

Table A : Biochemical composition of the leaf mixture

Moisture(%)	6.37±0.10
Dry matter (%)	93.63±0.10
Ash (%)	17.17±0.23
Acid insoluble ash (%)	2.09±0.35
Crude fat (%)	4.38±0.02
Crude protein (%)	25.77±0.10
Crude fibre (%)	8.49±1.20
Carbohydrate (%)	44.16 ±1.36
Energy (Kcal/100g)	319.23±5.20
Calcium (%)	3.77 ±0.15
Phosphorus (%)	0.50 ±0.01
Magnesium (%)	0.49 ±0.00
Iron (mg%)	77.10 ±0.00
Zinc (mg%)	2.52 ±0.00
Copper (mg%)	0.89 ±0.00
Manganese (mg%)	0.49± 0.00

The data are mean value ± standard deviation (SD) of four replicates.
Values expressed as % dry weight.

Haemoglobin estimation :

The diagnosis of iron deficiency Hemoglobin concentration was measured with a cyanmethemoglobin method. Adolescent girls were examined to detect the prevalence and severity of anaemia. The schedule followed was adopted from classification suggested by Jelliffee (1989).

Table B : Classification of Hb levels in terms of anaemia

Hb level (g/dl)	Grade of anaemia
≥12	Normal (non-anaemic)
11.9-10	Mild
9.9-7	Moderate
<7	Severe

Intervention period :

The selected 120 girls were randomly divided into two groups Group 1 (experimental) and group 2 (control) comprising of 60 girls in each group. Intervention period was of four months (120 days).

20g leaf mixture (LM) providing 15 mg iron and fulfilling ½ of the day's requirement of iron was provided to sixty adolescent anaemic subjects of group 1 for a period of four months while the sixty anaemic adolescent girls of group 2 did not receive any supplementation during the study period but were supplied with the leaf mixture once the study was culminated.

The subjects in the experimental group were dewormed prior to intervention by Albandazole tablets (Zentel tablets containing 400 mg Albandazole).

Everyday the homes of the subjects were visited and the pouch of LM was given and the previous day's pouch was taken back from them. A monthly calendar was maintained and a tick was marked on each date when they consumed the supplement and a cross when they forgot to consume or did not consume it. The visits were generally done at the time of food preparation to check whether they are incorporating it in their chapaties or not and sometimes they were also guided to use it in food preparations other than chapaties. Some times group meetings of the subjects were fixed at one place and the subjects were made to interact with one another to share their experiences. Sharing of experiences and the mutual interactions motivated the subjects who were not taking the LM on a regular basis. However, almost all the subjects were taking the LM regularly as the taste of the LM was like any other green leafy vegetable.

Haemoglobin estimations of all the subjects (experimental and control) was done every month to see the improvement in the haemoglobin levels of the experimental group and for the comparison of the change in haemoglobin with that of the control group to determine the impact of leaf mixture.

Iron status :

WHO (2002) classification was used for interpreting the results of haemoglobin estimations.

Statistical analysis :

The data was analysed using SPSS version 13.0. The significance of difference between means of groups was studied by applying Duncans multiple range tests (Duncan,1955).

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Profile of the family :

The infrastructure of the household in which the adolescent girls lived is drawn from the family structure, literacy level, living conditions, availability of basic amenities, occupational pattern and the income of the families.

The study sample constituted of 120 adolescent girls from 80 families. All the 80 families covered were Hindus. There was dominance of nuclear families in the sample under study. More than half (87.5%) of them were residing in a nuclear family as against 12.5 per cent who continued to live in a joint family (Table 1).

It seems that because of the economic pressures of the urban life more and more families are breaking into smaller units. As can be seen from the Table 1 of the family could be categorized as large, having more than 6 members. The family size ranged from 2-6 members, with an average of 4.5 ± 0.6 members per family.

A glimpse into the age structure of the families revealed that the members were fairly young. The majority of the family members were in the age range of 15-55 years. Quite a number of families were just a husband and wife and their two to three children. The families were migrated from native village in search of a better

income.

The families were quite aware that large families will create a burden to their shoulders so most of the young couples had adopted family planning measures and thus, had a small family.

Housing :

The families by and large were residing in rented pucca houses. The rent paid by a family varied from Rs. 550 to Rs. 750 excluding the amount paid for water and electricity which ranged between Rs. 300 to Rs. 400. The rent of the house depended on the number and size of the rooms. All the families had a single room to live in. Only 10 per cent families had a separate kitchen while others were using one corner of the room for cooking. The houses in the slum area were in a row and some houses had an advantage of some open space in front of their houses which they referred to as *Gallery*. They were using that *Gallery* as their cooking space. The housewives took special care towards the cleanliness of their house/ room. The housewives were illiterate but most of them were working as maids in the affluent society which had created awareness in them about the importance of cleanliness and sanitation and they were using that knowledge at their houses too.

Basic amenities :

In the area under study the families did not have water connection at their houses and they were dependent on the community tap, but the good part was that it had a 24 hour water supply and thus, did not create any problem to the families residing in the community.

Special care and attention was observed by the housewives in storing fresh water for drinking and cooking purposes, by proper cleanliness of the containers and storage area, but their hard work was all lost as the collection source was inhabited by mosquitoes breeding on the stagnated water. Due to lack of proper drainage

Table 1 : Socio-economic profile of the families **(n=80)**

Characteristics	Details	Number	Per cent
Type of family	Nuclear	70	87.5
	Joint	10	12.5
No. of family members	2-4	38	47.5
	5-7	42	52.5
	8-10	-	-
	Average family size	4.5±0.6	-
Religion	Hindu	80	100
	Muslim	-	-
	Christian	-	-
Literacy status of the subject	Non-illiterate	52	65
	Can read and write	8	10
	Primary	14	17.5
	Middle	12	7.5
Monthly income	≤ 2100	-	-
	2100-4500	62	77.5
	4500-7500	18	22.5
	7500 and above	-	-
	Average monthly income	3700±858.44	-
	Average share by adolescent girl in the family income	1263.75±263.45	-
Age at menstruation	11-12yrs	34	42.5
	13-14yrs	46	57.5
	>14	-	-

± SD

system there was water logging giving rise to squalid conditions unfit for hygienic living.

The unhygienic and unsanitary surroundings of the slum dwellers in the present study were reflected by the open area and *Nallas* used for defecation. Though the slum area had four flush latrines which were common for all the families but as most of the members in the family were working and were in a hurry to leave for their work places they preferred to defecate in the open than to waste time for waiting for their turns near the toilets. The latrines were mostly used by the ladies.

Literacy level :

As evident from Table 1 more than half of the subjects (65%) received no education at all. Acquiring education till middle level was observed among only 7.5 per cent subjects. 17.5 per cent of the subjects barely managed to finish their primary level. The rest 10 per cent were even not able to finish their primary level. The girls were asked to work for their families at very early ages and sending them to school was considered as waste of time and money. The girls started accompanying their mothers to their work places at the tender age of ten years and help them to finish their work fast which allows them to work at more and more houses and thus, earning more for the family. As the girls were trained and attained full efficiency they were sent to work individually. All the bargaining for their salaries was being done by the mothers. Even the girls were not interested in studying and they too considered it as waste of time. Instead of going to school they preferred to work and earn money which they wanted to save for their marriage.

Income pattern of the families :

The employment status of the working males revealed that earning money was a tough task for the majority. Most of them were working as labourers, white washers, thread cutters in a factory and were engaged in a glass factory near by on daily wages. There was no conformity of the job and had to struggle on a day to day basis to provide food to their families. The other avenues for employment included working in a ration shop, stitching, or working in an export import factory or any private concern for a meager monthly income.

Their housewives and daughters working as maid servants in the households was a real help to them. The working males used to admit that without their help they would not be able to survive in the city. They said that these days most of the people in the affluent society require assistance in their household chores and most often their housewives get the job easily and even earn more than what they were earning, so they never felt small in helping their housewives in their household chores and looking after the children. In most of the families there was more than one earning member. All the females of more than 10 years of age were working and earning for their families either by accompanying their mothers or by working individually.

As apparent from the Table 1 the mean total household income of the families was Rs. 3700, the flow of income into the families ranged from Rs. 2500 to Rs. 5500. Looking at the contribution of the adolescent girls' into the family income, one finds that their monthly average contribution was Rs. 1200 to 1300.

Profile of the subject :

Majority (57.5%) of the girls had menarche between the age of 13 to 14 years. 60 per cent of the subjects were vegetarian and 40 per cent were ovo-vegetarian. 40 per cent consumed tea, 31 per cent consumed both coffee and tea whereas 30 per cent of the subjects consumed coffee alone. Milk was consumed by 15 per cent of the adolescents.

Nutrient intake :

Twenty four hour recall method was used for the dietary assessment. The recall was done for the two consecutive days before the initiation of the study and the means of the two recalls was taken and translated into nutrient intake. It was basically done to justify that the diet pattern of the subjects in both the groups was homogeneous in terms of nutrients.

The computed food intake of the experimental and control groups were compared with the recommended dietary allowances suggested by ICMR (2004). In both the groups experimental and control, the mean intake of green leafy vegetables, roots and tubers, fruits, meat sugar and jaggery fats and oils, meat, fish and egg were found to be deficient.

It was evident that the consumption of iron rich food was low in both the groups, which was responsible for the occurrence of anaemia. Low overall dietary intake was one of the most important factors responsible for iron deficiency in both the groups.

It is evident from the Table 2 that three macronutrients namely energy, protein and fat were not met through the diet. Protein is essential for the proper production of haemoglobin and red blood cells. Except for the nutrients niacin and thiamin, the mean intakes of the subjects were deficient in all the other nutrients. The deficient intake of iron rich foods by the girls was reflected in their iron nutriture. The adolescent girls consumed less quantity of iron when compared to RDA leading to a deficit of 51 per cent in group 1 and 48 per cent in group 2.

Effect of leaf mixture supplementation on :

Nutritional status of the adolescent girls :

The nutritive value of the 20g LM provided to the girls is presented in Table 3.

A look at the mean nutrient intake after supplementation revealed a significant increment in iron and calcium intakes in the experimental group. The better increment in these two nutrients can be attributed to the iron and calcium content of the LM provided to the experimental group. Initially, their was a deficit consumption of 51 per cent and 32 per cent for iron and

Table 2 : The mean nutrient intake of the selected adolescent girls

Nutrients	ICMR RDA 2004	Amount consumed by group 1	% Deficit/ surplus	Amount consumed by group 2	% Deficit/ surplus
Energy (Kcal)	2060	1779	-13	1868	-9
Protein (g)	65	50	-23	60.3	-7
Fat (g)	22	20	-9	22.9	+4
Ca (mg)	600	403.6	-32	464.5	-22
Iron (mg)	28	13.6	-51	14.3	-48
Retinol (mcg)	600	506.4	-15	520.0	-13
Thiamine (mg)	1.0	1.1	+10	1.2	+20
Riboflavin (mg)	1.2	0.9	-0.25	0.8	-33
Niacin (mg)	14	16.0	+14	15.3	+9
Vitamin C (mg)	40	39	-2.5	38.0	-5
Pyridoxine (mg)	2.0	0.80	-60	0.73	-63
Folic acid (mcg)	100	39.9	-60	34	-66
Vitamin B_{12} (mcg)	1.0	0.66	-34	0.71	-29

Group 1: Experimental (n=60); Group 2: Control (n=60)

Table 3 : Nutritive value of leaf mixture per 20 g

Nutrients	Nutritive value per 20 g
Moisture (%)	1.27 g
Ash	3.43g
Crude fat	0.876g
Crude protein	5.15g
Crude fibre	1.69g
Carbohydrate	8.83g
Energy	63.84 kcal
Calcium	754 mg
Phosphorus	100 mg
Magnesium	98 mg
Iron	15.42 mg
Zinc	0.50 mg
Copper	0.17
Manganese	0.09 mg

calcium, respectively in group 1 when compared with the RDA while after supplementation the iron intake was comparable to the RDA and there was a surplus supply of calcium. The intake of iron and calcium did not improve in the control group. Thus, through the results it could be inferred that the improvement registered in the nutrients in the experimental group was because of the LM supplementation as the basis diet structure of both the groups was more or less similar. The increment was because of LM supplementation, could also be confirmed by comparing the intakes of macronutrient which remained the same in both the groups even after the supplementation as the leaves are not a rich source of energy, protein, fat and carbohydrates. The improvement in iron and calcium status in both the groups is depicted graphically in Fig. 1 and 2, respectively.

Clinical status :

Clinical symptoms of the subjects before and after supplementation are exhibited in Table 4. Clinical symptoms of anaemia among the selected adolescent girls decreased significantly in the experimental group. Fatigue, giddiness, pallor of conjuctiva, headache and dimness of vision were more prevalent in group 1. These symptoms decreased remarkably after supplementation. In the control group symptoms like giddiness, fatigue and headache were reduced but the effect was lesser compared to that observed on LM supplemented group. As the intakes of iron in the experimental group improved after supplementation so were the clinical signs and symptoms of anaemia. There was no change in the dietary intakes of the control group, thus, no changes in the clinical symptoms were observed. The results have thus,

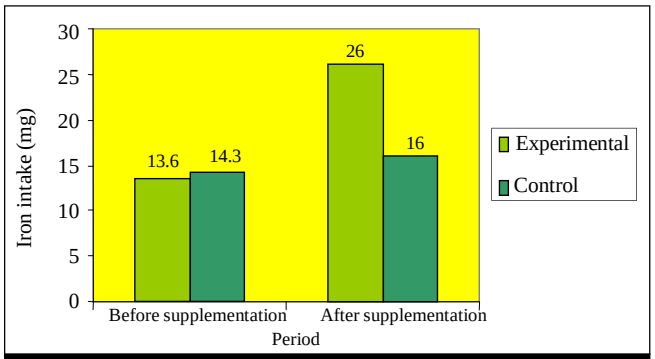

Fig. 1 : Effect of LM supplementation on iron intake

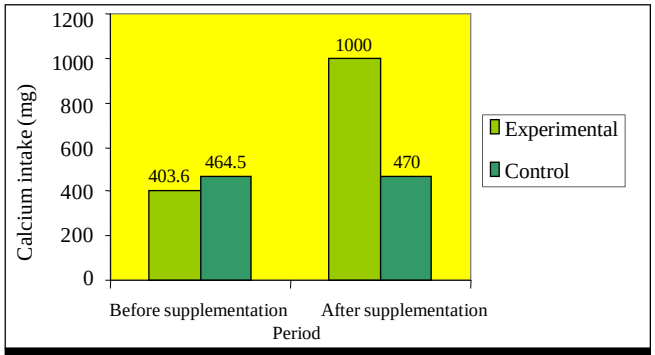

Fig. 2 : Effect of LM supplementation on calcium intake

Table 4 : Clinical status of the adolescent girls

Clinical signs	Group 1 (n=60)		Group 2 (n=60)	
	Before	After	Before	After
Fatigue	45	27	48	42
Giddiness	57	24	51	36
Headache	45	15	30	24
Weight loss	3	3	3	3
Dim vission	12	6	15	15
Pallor conjuctiva	45	15	42	42
Cheilosis	24	12	24	24
Loss of appetite	39	15	30	30
Dry hair	6	6	3	3
Pallor of skin	30	15	27	27
Pallor of nails	45	15	24	24
Koilonychia	-	-	-	-
Dental caries	15	15	24	24

Group 1: Experimental; Group 2: Control

been correlated well with the nutrient intakes and confirm the miraculous effect of the green leaf mixture.

Changes in haemoglobin :

The mean Hb per cent increase in group 1 and group 2 over the period of four months was 10.53±1.51 and 9.27±1.00, respectively. The difference in the mean haemoglobin per cent in the two groups was significant (p<0.001). The change in mean Hb per cent of the two groups is depicted in Fig 3.

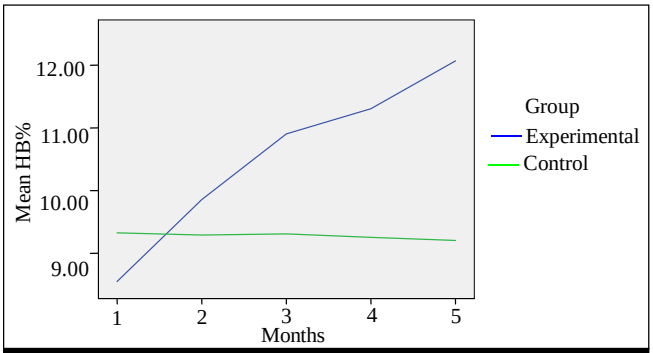

Fig. 3 : Effect of LM supplementation on the Hemoglobin (%)

Table 5 represents the effect of LM on the Hb per cent of the experimental group over the period of four months as compared to the control group. The mean Hb per cent of group 1 was 8.5 initially which increased to 12.07 per cent showing an increase of 42 per cent in four months, whereas, the initial Hb per cent of control group was 9.32 per cent which dropped down to 9.20 per cent showing a decrease of 1.28 per cent in four months. There was a direct relationship between the duration of supplementation and the rise in Hb per cent. The difference in Hb per cent of the period of four months among the experimental group was also significant (p<0.001).

No significant difference (p>0.05) in Hb per cent among control subject was seen during this period. They started with the initial Hb per cent of 9.32 and fluctuated at this level only.

On classifying the subjects according to different categories of anaemia (Table 6 and 7) it was observed that initially 95 per cent subjects in group 1 and 55 per cent in group 2 suffered from severe to moderate anaemia.

Table 5 : Hemoglobin statuses of the adolescent girls

	Hemoglobin per cent						
	Initial	After 1 mo	After 2 mo	After 3 mo	After 4 mo	SEM	P value
Group 1 (n=60)	8.5 [a]	9.8 [b] (15.29)	10.9 [c] (28.23)	11.3 [c] (32.94)	12.07 [d] (42)	0.20	0.00
Group 2 (n=60)	9.32 [a]	9.21 [a] (-1.18)	9.31 [a] (-0.10)	9.25 [a] (-0.75)	9.20 [a] (-1.28)	0.22	0.99

Values with different superscripts letters within a row differ significantly (P<0.05) by Duncans multiple range test.
Group 1: experimental; Group 2: Control
Figure in parentheses represent per cent increase in haemoglobin values from the initial values

Table 6 : Frequency distribution of subjects according to severity of anaemia in experimental group

WHO classification (%)	Category	Group 1 (n=60)				
		Initial	After 1 month	After 2 months	After 3 months	After 4 months
<7	Severe anaemia	-	-	-	-	-
7-9.9	Moderate anaemia	57 (95)	24 (40)	12 (20)	3 (5)	-
10-11.9	Mild anaemia	3 (5)	36 (60)	36(60)	42 (70)	21(35)
≥12	Normal	-	-	12(20)	15(25)	39(65)

Figures in parentheses represent per cent

Table 7 : Frequency distribution of subjects according to severity of anaemia in control group

WHO classification (%)	Category	Group 2 (n=60)				
		Initial	After 1 month	After 2 months	After 3 months	After 4 months
<7	Severe anaemia	-	-	-	-	-
7-10	Moderate anaemia	33 (55)	36 (60)	36 (60)	36 (60)	36 (60)
10-12	Mild anaemia	27 (45)	24 (40)	24 (40)	24 (40)	24 (40)
>12	Normal	-	-	-	-	

Figures in parentheses represent per cent.

There was a continuous shift of anaemic patients towards normalization in LM supplemented group (Group 1) while the severity of anaemia remained the same in control group (Group 2). By the end of the intervention period almost all the subjects in the experimental group were able to attain the normal values of haemoglobin with a per cent increase of 42 per cent whereas the haemoglobin per cent in the control group decreased slightly (-1.28%).

The subjects in the LM supplemented group were able to attain the normal values in the first two months of intervention only as the rate of iron absorption is more in severe cases. As the severity of anaemia declined the rate of absorption also declined due to this the subjects in normal category showed minimum per cent increment. The rate of iron absorption is dependent on the severity of the anaemia. Thus, it could be concluded that the leaf mixture showed a positive impact on the haemoglobin levels of the subjects in experimental group and can be used as a remedy to cure iron deficiency anaemia. Similar results were reported by (Ibrahim *et al.,* 2001; Akubugwo *et al.*, 2007; Singh *et al.*, 2007; Darkwa and Darkwa, 2013) on the researches done on various green leafy vegetables strengthening the importance of dehydrated green leafy vegetables.

In dehydration, the moisture content of the food is reduced and the growth of micro-organisms in the dried food is retarted (Kowsalya and Vidhya, 2004). Thus, Dehydration of vegetables is generally done either for preserving the perishable raw commodity against deterioration or to reduce the cost of packaging (Gupta and Prakash, 2011). However in the present study dehydration of the green leafy vegetables was done with an objective to transform them into concentrated source of micronutrients.

Dehydrated green leafy vegetables are a rich source of micronutrients and their impact to combat micronutrient deficiency has been reprted by other researchesrs too (Mathur *et al*., 1989; Maharaj and Sankat, 1996; Mundra and Mathur, 2000; Motey and Lele, 2003; Gupta and Prakash, 2011).

Most developing countries depend on starch based foods as the main staple food for the supply of both energy and protein. This accounts in part for micronutrient deficiency which prevails among the populace recognised by Food and Agriculture Organisation (Ladiji *et al.*, 1995). In most of the topical countries where the daily diet is dominated by starch staple foods, dehydrated vegetables are cheapest and most readily available source of important proteins, vitamins, minerals and essential amino acids as dehydration make the vegetables a concentrated source of nutrients (Lakshmi and Vimla, 2000; Chu *et al.,* 2002; Pattan and Devi, 2014).

Conclusion :

The green leaf mixture was a concentrated source of micronutrients and was able to improve significantly the iron status of anemic adolescent girls.

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