Impact of Trianthema portulacastrum linn (*saravallai*) incorporated dhal powder on the blood iron status of anemic adolescent girls

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Abstract*- Introduction**: Adolescence is considered as a nutritionally critical period of life. Anemia is the most prevalent nutritional problem worldwide and it is mainly caused due to iron deficiency. India has the world's highest prevalence of iron deficiency anemia among women, with 60 to 70 percent of the adolescent girls being anemic. *Trianthema portulacastrum Linn* is a herb used in ayurvedic medicine. Different parts of *Trianthema portulacastrum Linn*, are traditionally used as analgesic, stomachic, laxative, treatment of blood disease, anemia, inflammation, and night blindness. **Objective**: To assess the efficacy of "*Saravallai* dhal powder" (*Trianthema portulacastrum linn)* in improving the blood iron profile of anemic adolescent girls**. Materials and Methods:** *Saravallai* dhal powder (SDP) was prepared from *Trianthema portulacastrum Linn (Saravallai) leaf* Powder" and dhal powder (Bengal gram, Roasted Bengal gram , Black gram and Groundnut (1:1:1:1) were mixed in 1:1 proportion. Energy (Computed), moisture, ash, fiber, carbohydrate (difference method), protein, iron, and calcium were estimated in triplicates. Initially blood hemoglobin was estimated for one hundred girls. Eighty anemic adolescent volunteers were chosen out of hundred (control group n=40 and experimental group n=40) and 20g of *saravallai* dhal powder (SDP) was supplemented to the experimental group for 90 days. **Result:** The SDP contained energy (1885.47±74.39kJ), carbohydrate (58.89±2.21g%), protein(23.74±0.53g), fat(7.94±2.52g%), crude fiber 18.6±0.52g%), ash (12.3g±0.2g), calcium (589.33±8.14mg), iron(30.13±2.40mg), Zinc (0.9±0.10mg), carotene (544.66±28.30µg), thiamine and riboflavin (0.32±0.02mg, 0.1±0.02mg). The girls' blood iron parameters**(**Hb(12.92±1.24),RBCcount(4.30±0.41),PCV(38.77±3 .74),SI(65.67±32.62),TIBC(391±45.39), Transferrin Saturation(20.9±7.60) and SF(30.45±14.99)**)** improved considerably after supplementation. **Conclusion:** *Saravallai* ***(*Trianthema portulacastrum Linn****) dhal* Powder improves blood iron parameters. T. portulacastrum has been proved to possess strong anthelminthic activity in vivo. The beneficial effects of this underutilized green to tackle anemia, at a much lower cost without any side effects.

I. Introduction

Adolescent girls are at a high risk for anemia and malnutrition. According to WHO adolescent age group is between 10-19 years (Siva *etal*,2016)[1].Inadequate nutrition during adolescent can have serious consequences throughout the reproductive years of life and beyond. Anemia is the most prevalent nutritional problem worldwide and it is mainly caused due to iron deficiency. Although it involves all age groups and sex, the adolescent girls are more vulnerable to it. The pre-pregnancy anemic status of adolescent girls is crucial as it has long term intergenerational consequences

In 2008 WHO reported that 24.8% of the world's population is affected by anemia, of whom 42% were pregnant women, 30% non-pregnant women, and 47% were preschool children. A 2011 WHO study estimated global anemia prevalence to be 496 million of non-pregnant women and 32.4 million of pregnant women aged 15 - 49 years (WHO 2014)[2].

In India the prevalence of anemia among adolescent girls were 56 percent (Aguayo *et a*l 2013)[3] and according to Lancet Global Health (2013)[4] 29 and 38 percent of non-pregnant and pregnant women aged 15–49 years are anemic due to increased iron demand, menstrual blood loss, infection ,worm infection etc (Kumari *et al* 2017)[5].Even a much earlier study by ICMR (District Nutrition Project) in 16 districts of 11 states, on prevalence of anemia in non-pregnant adolescent girls (11-18 years) showed rates as high as 90.1 percent with severe anemia (Hb<7 g/dl) in 7.1 per cent (Teoteja and Singh, 2002)[6].Rajaratnam *et al.*, (2002)[7] had opined that anemia among rural girls of Tamilnadu is also high as in other parts of the county.

Hence it is imperative to plan intervention programs that would increase the blood iron status among adolescent girls through various programs. While Long term approaches include **dietary improvement** for increasing the iron content of the diet by including iron-rich foods such as green leafy vegetables (GLVs), and cooking in iron pots; **enhancing iron bio-availability** in the existing diets by including foods rich in iron absorption promoters such as ascorbic acid and animal foods such as fish and meat; **promotion of home/kitchen gardening** to increase the availability of common iron rich food such as green leafy vegetables and increasing iron intake through **fortification** The Short term approaches are direct **supplementation** either weekly or daily. Side effects associated with the allopathic drugs and the resultant chemophobia have prompted research into traditional health care system throughout the world (Fulzele *et al*., 2002)[8]. World Health Organization (WHO) has recommended the use of traditional health and folk medicine systems as they are

proved to be more effective in correcting the health problems hence worldwide a renewed interest is seen in tapping the traditional and easily feasible strategies to prevent anemia. **Rationale:** Different parts of ***Trianthema portulacastrum Linn,*** (***Saravallai)*** are traditionally used as analgesic, stomachic, laxative, and for treatment of blood disease. This study with the indigenous green leafy vegetable will help to reaffirm the beneficial effects of the underutilized greens to tackle anemia which is one of the major public health problems of our country, at a much lower cost without any side effects.

Objectives:

- To estimate the nutrients present in the newly developed " ***Saravallai*** (*Trianthema portulacastrum linn)* dhal powder"
- To assess the efficacy of "***Saravallai*** dhal powder" (*Trianthema portulacastrum linn)* in improving the blood iron profile of anemic adolescents.

II. Materials and Methods

The study was conducted in two phases and Phase **I** consisted of formulation of "*Saravallai* Dhal Powder" (SDP) and **Phase II** was on the impact of SDP on the blood parameters of the anemic volunteers

Phase I

(i)Preparation of *saravallai* powder

Trianthema portulacastrum Linn (Saravallai) the red colored form (*Lal Sabuni*), a deep rooted perennial spreading leaves found as a weed in abundance in rural area of *Dharmapuri* district were chosen. Fresh, healthy and disease free *saravallai* greens were collected. They were identified and authenticated by the taxonomist at the Department of Botany, PSG College of Arts and Science, Coimbatore, India. The selected under-utilized plant (*Saravallai*) were weighed, cleaned and washed with sufficient water to remove foreign organic matter. The leaves of ***saravallai*** were completely dried in shade, pulverized in a mixer grinder, sieved (using 60 mesh) repeatedly to get a free flowing fine power, packed in HDPE covers and stored in air tight containers so as to retain its potency and to prevent moisture entry.

(ii) Preparation of dhal powder

Bengal gram dhal, Roasted Bengal gram dhal, Black gram dhal and Groundnut (dehusked) were cleaned ones and roasted for five to seven minutes at medium heat (70^{o}C). All the above items were ground separately in a (butterfly brand) blender to get a coarse textured powder then they were mixed in a 1:1:1:1 proportion and stored in HDPE covers and kept in air tight container.

(iii) Formulation of *saravallai* dhal powder and sensory evaluation

The "*saravallai* leaves powder" and the "dhal powder" were mixed in 1:1 proportions to formulate *Saravallai* dhal powder (SDP). Organoleptic evaluation of the SDP was carried out using a score card developed exclusively for this purpose with the help of taste panel members. The panelist indicated their preferences on a five point hedonic scale for each of the sensory attributes.

The "*saravallai* dhal powder" was estimated in triplicates for mineral and vitamin content as per the standard Association of Official Analytical Chemists (AOAC) methods. The actual amount of iron that is available to the body from the *Saravallai* Dhal Powder was estimated in the laboratory in duplicates using the "Invitro iron availability" procedure of Rao and Prabhavathi (1978)[9] and Govindaraj *et al.*,(2007)[10] as "Bioaccessibility" (or biological availability) is defined as the proportion of nutrient in the food that can be absorbed and utilized and is the key to nutrient effectiveness.

Phase II

Islamiah Women's Arts and Science College, Vaniyambadi in Vellore district was chosen for conducting the study. This area was selected because the entire sample could be obtained with fewer variations in the social, economic, cultural aspects and also food habits **One hundred** girls volunteered for the study**.** The study participants were informed about the study knew the composition of the products and participated voluntarily. The Ethical clearance certificate was forwarded by Medical Joint Director, Vellore, and approved by Chief Medical officer at Vaniyambadi Government Hospital, Vellore district, Tamil Nadu, India. Initially blood hemoglobin was estimated for all the **one hundred girls** who were willing to undergo blood test and eat *saravallai* dhal powder continuously for 90 days. Blood was drawn using hygienic disposal syringes and transferred to the tubes that contained the anticoagulants and transported to the laboratory in cold condition for the determination of hematological parameters..Among them (n=40) who had hemoglobin above 12g percent were designated as control group. Girls whose hemoglobin below 12g percent (n=40) were considered as anemic as per WHO (2001)[11] guidelines and designated as experimental group. Twenty girls were omitted from the study, because some of them did not want any more blood test to be done and few others had minor illness such as fever, and still a few others were not willing to eat *saravallai* dhal powder.

Dietary intake data was collected from all the volunteers (n=80 by Twenty four hour diet recall. For determining the quantities standard cups and spoons were used. The food intake of the respondents for three consecutive work days was recorded and their nutrient intake was computed of using the nutrient content of foods as given by Rao *et al.*, (2010)[12] after calculating the raw equivalents. The difference between the actual mean iron intake of the selected girls and the ICMR dietary recommendation (2010)[13] for iron was calculated. The quantity of *saravallai* dhal powder that could be consumed per day by the adolescents was ascertained. Based on these two criteria twenty grams *saravallai* dhal powder was packed in separate HDPE covers and supplied to the experimental group (n=40) volunteers every day. They were instructed to consume the same at lunch time with a glass of water. They were requested to avoid drinking coffee or tea immediately after consuming *saravallai* dhal powder. The supplementation was carried out for a period of 90 days immediately after anthelminthic therapy with 400 mg single-dose of albendazole as per the advice of the medical practitioner since anthelminthic therapy combined with iron supplementation enhances Hb response to iron supplementation (Stoltzfus and Dreyfus, 1997)[14]. The blood analysis was done before and at the end of the supplementation

period. The control group girls were instructed to follow their regular diet without skipping.

Hemoglobin percentage, red blood corpuscle count and packed cell volume were done for the eighty sub-sample. From this eighty sample a further smaller sample of ten each from Control and Experimental group were drawn and their serum iron, serum total iron binding capacity, Transferrin saturation and serum ferritin were estimated due to high cost of these tests.

III. Results and Discussion

The total mineral quantity,(Table-I) expressed as ash content was 12.3g±0.2g percent in the SDP. While Agashe et al., (2015)[15] and Hussain et al., (2010)[16] had reported an ash value of 13.0 and 10.15±0.6g percent in *Trianthema portulacastrum* (*saravallai*) on dry weight basis, Gupta et al., (2004)[17] found an ash value of 2.29g in fresh leaves. The SDP value also near the former quoted value

Table-I
Mineral and vitamin content of *saravallai* dhal powder

Nutrients	Mean	Standard Deviation
Ash(g)	12.3	0.2
Calcium(mg)	589.33	8.14
Iron(mg)	30.13	2.40
Zinc(mg)	0.90	0.10
Magnesium(mg)	112.00	5.29
β -carotene (µg)	544.66	28.30
Thiamine(mg)	0.32	0.02
Riboflavin(mg)	0.10	0.02
Vitamin-C(mg)	29.38	1.19

The **calcium** content of *Saravallai* Dhal Powder was high at 589.33±8.14mg percent. Whereas Shivhare et al., (2012)[18],Hussain et al.,(2010) and Gupta et al., (2004) reported a much lower value of calcium (0.3mg percent (fresh) 311ppm (dry) and 52mg/100g(fresh) in *Trianthema portulacastrum* leaves respectively.

The **iron** content of *Saravallai* dhal powder was 30.13±2.40 mg percent. Patricia *et al.*,(2014)[19], Khan *et al.*,(2013)[20], Shivhare *et al*., (2012)[21], Hussain et al.,(2010) and Gooneratne and Kumarapperuma (2004)[22], Gupta *et al*., (2004) reported a value of 30.87±0.16(Hibiscus sabdariffa),45.80 ±0.01mg (Vigna unguiculata), (6.44±0.4mg/100g (*Trianthema portulacastrum* -dry leavas), 50 ppm (T.*portulacastrum* -fresh leaves), 1185ppm (T.*portulacastrum*-dry leaves) 6.7 ± 1.5 mg/100g(T.*portulacastrum* -fresh leaves), and 4.16mg/100g (fresh *Trianthema portulacastrum* leaves) respectively.

The dry powder of SDP (T.*portulacastrum*) contained 0.9±0.10mg percent of **zinc**. Khan *et al*.,(2013), Shivhare *et al*.,(2012), and Gupta *et al.*, (2004) reported a much lower value of 0.20 ± 0.02, 30.0ppm and 0.46 mg/100g zinc from their study on T.*portulacastrum* (*Bishkhapra).The saravallai* dhal powder prepared for the current investigation had 112.00±5.29mg percent of **magnesium**. Shivhare *et al.*, (2012) and Gooneratne and Kumarapperuma (2004) reported a value of 0.2 percent (Dry powder) and 153mg/100g (fresh) magnesium in *Trianthema portulacastrum* While the former value is lower than the present finding, the latter reported value is higher.

The vitamin **β–carotene**, content was 544.66±28.30µg percent in the SDP. The values quoted by Khan *et al*.,(2015) Shivhare *et al*.,(2012), Gooneratne and Kumarapperuma (2004) and Gupta *et al*., (2004) for β-carotene, 0.81mg/g, 2.3, 4.0 and 4.0mg/100g respectively are higher than the present finding.

Thiamine and riboflavin content of the *Saravallai* Dhal Powder were 0.32±0.02mg and 0.1±0.02mg. While Gupta *et al*., (2004) reported lower thiamin (0.1mg percent) content (*T. portulacastrum*-fresh leaves), Khan *et al*.,(2013) reported a higher value for riboflavin (2.02mg/g) for the same *T. portulacastrum*. Ascorbic acid content of the *Saravallai* dhal Powder of the current investigation was 29.38±1.19mg.

The contaminant **lead** and anti-nutrient factors such as **phytic acid and oxalates** were 0.21±0.01, 1.08±0.05 and 1.43-±0.13mg percent in the prepared *saravallai* dhal powder.

A **phytate** content of 17.25 ±0.00 (Vigna unguiculata) to 86.45 ±0.10 (Hibiscus sabdariffa) and 2.02mg percent (*Trianthema portulacastrum*) was observed by Patricia *et al*.,(2014) and Gupta *et al*., (2004). Udousoro, Ekop and Udo(2013)[23], reported the phytate content in untreated *Gongronema latifolium (Utazi leaf), Vermonia amygdalina* (Bitter leaf), *Ocimum canum sims* (Curry leaf), *Heinsia crinata (*Bush apple leaf) *and Talinum triangulare* (Waterleaf) grown in Nigeria as 40.01,33.34,41.27,41.91 and 43.81mg/100g. However these values are higher than the SDP value.According to Patricia et al., (2014) the oxalate content ranged from 780.00 ±0.00 (Amaranthus hybridus) to 1310±78.00mg (Hibiscus sabdariffa) percent, but Naik, Jammuna and Nayak (2013)[24] quoted only 40.5mg percent of oxalate in *Peucedanum graveolens*. While Pattan and Usha devi (2011)[25] reported 45 (*Gynandropsis pentaphylla*) to 275mg (*Brassica oleracea*) percent of oxalate, Joshi Mathur (2010)[26] had reported an oxalate content of 9.49±0.14 (Beet greens), 0.56±0.22 (Carrot greens), 0.32±0.84

(Cauliflower greens) and 2.26±0.06mg (Turnip greens) percent. Udousoro, Ekop and Udo(2013), reported the oxalate content in untreated *Gongronema latifolium* (Utazi leaf), *Vermonia amygdalina* (Bitter leaf), *Ocimum canum sims* (Curry leaf), *Heinsia crinata* (Bush apple leaf) and *Talinum triangulare* (Waterleaf) grown in Nigeria as 132.00,246.40, 246.40,193.60 and 184.80mg/100g respectively. While some of these values are above the present finding in *Saravallai* Dhal Powder, some others are lower.

(i)Bio accessible iron

Bioavailability (or biological availability) is the key to "nutrient effectiveness". This is defined as the proportion of the nutrient in food that can be utilized and absorbed (Ballot *et al*., 1987)[27] and Gilloly *et al*., 1983)[28].

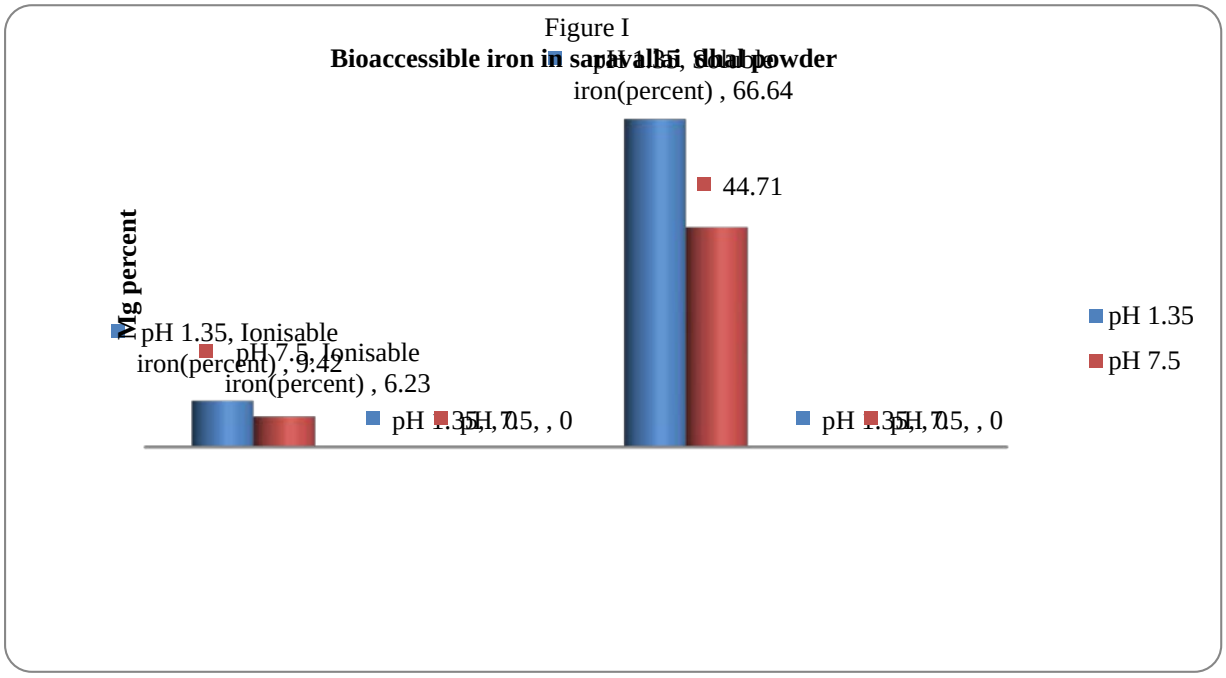
The total iron content of *saravallai* dhal powder was 30.13 ±2.40 mg/100g.

It could be observed from (Figure I) that ionisable iron is considerably lower than the soluble iron at both the pH i.e. 1.35 and 7.5. The percentage of Ionizable (9.42) and soluble (66.64) iron at acidic pH (pH 1.35) were better than the percentage of ionisable iron (6.23) and soluble (44.71) at alkaline pH (pH 7.5), hence the Ionizable iron at pH 1.35 was 2.84 ± 0.0 and it was 1.88±0.0 mg/100g at pH 7.5. The soluble iron was 20.08 ±0.0 and 13.47 ±0mg/100g at pH 1.35 and pH 7.5.

According to Rao and Prabhavathi (1978) the Ionizable and soluble iron at pH 7.5 can be directly correlated with percent in-vivo iron absorption and the highest correlation with physiological availability in humans could be observed with ionisable iron at pH 7.5.

It is clear from the foregoing discussions that the experimental group girls' intake of nutrients at the baseline survey was highly inadequate. To bridge the gap between inadequate intake, they were supplemented with 20 grams of *saravallai* dhal powder. After supplementation their intake of nutrients improved considerably.

Figure I



(ii)Blood iron status of the selected girls

(i)Hemoglobin

Hemoglobin is the conjugated protein containing four heme groups and is the oxygen carrying pigment of erythrocytes. It is evident from the table- II that the control group girls' (who did not receive any supplementation) Hemoglobin showed not much of a change and it remained at 12.1 ±0.16 g percent, whereas the

Table II
Mean blood iron parameters of selected sub-samples

Blood parameters	Normal values	Control group n=40			Experimental group n=40		
		Mean±SD					
		SV	EV	P values	BS	AS	P values
Hemoglobin (g percent)	11.7-15.5*	12.21 ±0.02	12.1 ±0.16	0.0007	10.44 ±1.52	12.92 ±1.24	0.0001
Red blood corpuscle count (mc/cumm)	4.2 - 5.4**	4.07 ±0.06	4.03 ±0.05	0.0008	3.51 ±0.54	4.30 ±0.41	0.0001
Packed cell volume (percent)	35-45*	36.63 ±0.61	36.3 ±0.48	0.0007	31.62 ±4.86	38.**77** ±3.74	0.0001
		Control group n=10			Experimental group n=10		
Serum iron (µg/dl)	50-170*	27.65 ±0.47	27.39 ±0.46	0.0010	23.78 ±2.69	65.67 ±32.62	0.0025
Total iron binding capacity (µg/dl)	250-425*	383.05 ±17.08	378.27 ±16.10	0.0006	459.10 ±47.94	391±45.39	0.0001
Transferrin saturation (percent)	15-50*	25.20 ±0.79	25.30 ±1.16	0.8589	9.8 ±6.81	20.9 ±7.60	0.0005
Serum ferritin (ng/ml)	10-120**	23.09 ±2.82	22.6 ±2.71	0.1668	10.68 ±9.29	30.45 ±14.99	0.0001

SV-Start Value EV-End value
BS-Before supplementation AS-After supplementation # Calculated values
*Burtis and Ashhood (2008)
**Srilakshmi (2014)

experimental group girls' Hemoglobin increased by 2.48 g percent and it was 12.92±1.24g percent at the end of 90 days supplementation of *saravallai* dhal powder. It could be observed that the mean hemoglobin of experimental group girls which was below the WHO (2001) standard of 12g percent for defining anemia before supplementation; improved and it came within the standard range after supplementation. While Lande's (2014)[29] spirulina incorporated chappati improved by 0.8 to 1.1 percent, Divya and Choudhary's (2014)[30] whey guava beverage also enhanced Hb (Before supplementation 9.50 to 10.24 and AS 11.06 to 11.42). Talha's (2014)[31] millet mix-nurti dense biscuits to 11-13yrs anemic children (hb <11g/dl) increased the hemoglobin by 1.66g/dl and 0.7g/dl in moderate and mild anemia. Naik, Jamuna and Nayak (2013) reported in their study an increase in hemoglobin from 9.8 to 11.8g percent in the experimental group girls after feeding *shepu* greens incorporated product for 90 days. Agte, Jahagirdar and Chiplonkar(2006)[32] investigated the effect of GLV as a natural fortificant of multiple micronutrients through a human trial and reported that three weeks' supplementation of GLV with more oil resulted in significant increase of plasma β–carotene (51percent) and Hemoglobin (nine percent) and they concluded that using 100 g GLV/day with 10 g oil could be a single moderate strategy for supplementation of iron, β–carotene, ascorbic acid and zinc. . In the present study the Hemoglobin (AS) of experimental group girls was weakly positively correlated (r = 0.20) with iron intake.

(ii) Red Blood Corpuscle Count

The **erythrocyte count** of the control group girls was almost same from the start date (4.07mc/cumm±0.06) till the study completion date (4.03mc/cumm±0.05). In the experimental group girls the erythrocyte count which was sub-normal at 3.51±0.54mc/cumm improved to 4.30 mc/cumm±0.42 after supplementation and this value was much better than even the control group girls' red blood cell count. When compared to the standard red blood corpus cell count as given by WHO (2001)except the experimental group before supplementation values (3.51mc/cumm±0.54) rest all were within the normal range of 4-5mc/cumm, however even in the control group the red blood corpuscle count was just above the normal values which could be termed as "low normal". The normal red blood corpus cell count as given by Srilakshmi(2014)[33] is 4.2 to 5.4mc/cumm. When compared to this standard; even the control group's red blood corpus cell count was lower than the standard. The experimental group's mean erythrocyte count was very much lower than the standard values before supplementation.

(iii) Packed Cell Volume

The mean **hematocrit** value of the control group girls was 36.63 ±0.61 and 36.3±0.49 percent in the beginning and at the end of the study. The experimental group participants' mean packed cell volume which was 31.62 ±4.86 percent raised to 38.77±3.74 percent after the supplementation with *saravallai* dhal powder for ninety days. However all the above mean values were

below the standard hematocrit (Srilakshmi 2014) values of 40-50 percent .It could be observed that even in the control group girls, the cell volume was lower even though the red blood corpuscle count was just normal. Burtis &Ashwood (2008)[34], WHO(2001) and Mahan &Stump (2000)[35] had quoted a reference value of 35-45,36 and 37-47 percent hematocrit value. When the present study's PCV values were compared with the above given standard values, the control groups PCV (before and after) values were within the standard of the first author. The experimental groups' PCV values were lower than all the above three authors' reference values before supplementation, however after the completion of the intervention the PCV had actually improved and this value was just few points above the standard value as given by Burtis and Ashwood (2008).The test of significance values obtained for PCV of the control group (P=0.0007) and experimental group (P=0.0001) indicated a significant difference between the base line and final values.

(v) Serum Iron

The serum iron is the amount of circulating iron that is bound to transferrin. The serum iron of the control group girls sub-samples (n=10) was 27.65 ±0.47 and27.39±0.46 µg/dl before and after the study period. The experimental groups' mean serum iron was 23.78±2.69 µg/dl before supplementation, however after the intake of *saravallai* dhal powder for a period of ninety days, the serum iron increased by 41.89 µg/dl. Burtis &Ashwood (2008), Mahan &Stump (2000) and had quoted the reference values of 50-170 and 50-175 µg/dl serum iron. When the present study's values were compared with the above given cut off values, the control groups' serum iron (before and after study period) were below the standard values. The experimental groups' serum iron values were lower than the above two authors' reference values before supplementation, however at end of the study, the serum iron had actually improved to 65.67±32.62 µg/dl (Experimental group) and had come within the normal range given above and could be termed as "Low normal". It has to be noted here that the volunteers who were designated as control group had their Hemoglobin just above the borderline and not very high. The low serum iron in this group indicates that even the control group needs an intervention to improve their serum iron status. There was a significant difference between the beginning and end of the study period's serum iron values in both the control (P=0.0001) and experimental groups (P=0.0025).

(vi) Total Iron Binding Capacity

Total iron binding capacity(TIBC) is a measurement of the potential for plasma to bind ferric ion and it depends on the number of free binding sites on the plasma iron transport protein i.e. transferrin (Burtis and Ashwood, 2008). In the present study the control group girls' total iron binding capacity was 383.05±17.08 µg/dl whereas in their experimental counterparts it was higher at 459.1±47.94µg/dl at beginning of the study. When compared to the reference values (250-425µg/dl) of Burtis and Ashwood, (2008) and Mahan and Stump,2000 (250-450 µg/dl), the control group's TIBC values was within the reference standards , but the experimental group's baseline value (459.1 ±47.94 µg/dl) was much above the normal range. According to Mahan and Stump, (2000) the increased total iron binding capacity indicates iron deficiency, hence it could be opined that the present study's experimental group girls have iron deficiency. At the end of the study the control group's total iron binding capacity (378.27 ± 16.1 µg/dl) was more or less similar to that of the initial values, whereas the experimental group's TIBC had decreased by 68 µg/dl and stood at 391.0±45.39 µg/dl and this values was well within the reference range of Burtis and Ashwood,(2003) and Mahan and Stump(2000).The statistical tool 'T' test for total iron binding capacity of the control group (P=0.0006) and experimental group (P=0.0001) indicated a significant difference between the base line and final values.

(vii) Transferrin Saturation

Transferrin saturation is the ratio of Serum iron and Total iron binding capacity multiplied by 100. It is an extremely sensitive indicator of functional iron depletion. The mean transferrin saturation values of the control group girls from the beginning (25.2±0.78 percent) to the end (25.3±1.15 percent) of the study did not show much variation. However in the experimental group girls before supplementation it was quite low to 9.8 ±6.81 percent at the beginning of the period, but when the supplementation period was over it had risen to 20.9 ± 7.60 percent. These values were compared to the reference standard of Mahan &Stump,(2000) and Burtish &Ashwood (2008) which was 15-50 percent. As per this value the mean transferrin saturation values of the experimental group was very much lower indicating that the iron bound to "transferrin" the plasma protein that transports iron from gut wall to tissues was very much lower. It could also be seen that even in the control group the transferrin saturation was in the lower border of the normal transferrin saturation range. Among the selected samples (n=10) there was no statistically significant difference (P=0.8589) in the transferrin saturation values between the two values of control (Before and after study period). In experimental group the transferrin saturation differed significantly (P=0.0005) before and after supplementation.

viii) Serum Ferritin

Ferritin is a storage protein that sequesters the iron normally gathered in liver, spleen and marrow and its concentration is directly proportional to the amount of ferritin inside the storage cell and indirectly proportional to the amount of iron present in the cell (Mahan and Stump, 2000). The mean serum ferritin of control group girls was 23.09 ±2.82 ng/ml before commencement of the study and 22.6 ±2.71 ng/ml on completion of study. Although these values are within the normal range of 12 to 150ng/ml and 10 to 120ng/ml as given by Mahan and Stump, (2000) and Burtis and Ashwood, (2008) respectively, they are "low normal" values. This reaffirms that the control group girls also have low iron store. In the experimental group girls before the supplementation of *saravallai* dhal powder the serum ferritin was just 10.68 ± 9.28 ng/ml. After supplementation it had increased to 30.45ng/ml±14.99 and this value was even higher than the control groups serum ferritin values. According to Burtis and Ashwood, (2008) plasma ferritin concentration decline very early in the development of iron deficiency long before changes are observed in blood hemoglobin concentration. RBC size or Serum iron concentration serves as a very sensitive indicator of iron deficiency i.e., uncomplicated by other concurrent diseases. From this it could be stated that the ferritin level is in compromised level

not only in experimental girls but also in the control group girls. The paired 'T' test done for the base line and final values for serum ferritin of the control (n=10) and experimental groups-(n=10) separately were (0.1668) and (0.0001). By conventional criteria the former value (0.1668) is considered to be not statistically significant but the latter value (0.0001) is extremely significant. Several independent studies undertaken by Kavitha and Radhika(2001)[36], Radhaisri and Muthlaxmi (2001)[37], Anuradha and Sangeetha (2001)[38], Thirumani Devi and Uma (2001)[39] and Sengar *et al*,(2000)[40] on wheat grass juice (containing 3.3mg of iron and 13.14 mg of ascorbic acid per 100ml), hibiscus flower extract, soya malt, spirulina and Ber fruit supplementation to the anemic adolescent girls reported significant increase in hemoglobin, serum iron and serum ferritin respectively.

From the foregoing discussions, it is clear that the blood iron parameters of the experimental group had shown considerable increment after ninety days of *Saravallai* Dhal Powder supplementation. The additional iron supplied through SDP was 6.02 mg percent, but the total iron received by the experimental group girls worked out to 12.67mg/day which includes an amount of 6.65±1.68mg that they received from their regular food intake. Although the total iron intake in the experimental girls was only 50 percent ICMR (2010) dietary recommendations (26mg/day) still it had helped to improve the blood iron parameters.

Several reasons could be put forth for this **improved blood iron status.**

The **first** and the foremost is that the percentage of ionizable iron (6.24) and soluble (44.71) iron at (pH 7.5) in SDP used for supplementation was better and according to Rao and Prabhavathi (1978) the ionizable and soluble iron at pH 7.5 can be directly correlated with percent in-vivo iron absorption and the highest correlation with physiological availability in humans could be observed with ionizable iron at pH7.5.

Secondly the most commonly accepted and affirmed iron absorption inhibitors such as phytic acid (1.08±0.05mg) and oxalates (1.43-±0.13mg) were very much low and in the prepared *saravallai* dhal powder. Hence as such the iron from this *saravallai* green has better bioaccessibility. Earlier studies by Agte, Jahagirdar and Chiplonkar ,(2006) Das, Raghuramulu and Rao (2005)[41] Fahey(2005)[42] and Nambiar & Seshadri(2001)[43] Chiplonkar *et al.*,(1999)[44] on green leafy vegetables had also reported that green leafy vegetables although has a lower amount of total iron(1.82 to 3.76mg/100g),it has a high ionisable iron from 30. 22 to 52.13 percent and GLV-based meals will increase gross as well as bioavailable iron intake which will help in meeting daily requirements of iron, hence can be effectively used to treat anemia.

Thirdly T. portulacastrum has been proved to possess strong Anthelminthic activity in vivo (Hussain et al., 2011)[45], thus, justifying their effectiveness in improving the iron status among the selected girls.

Fourthly the human body tightly regulates the absorption of iron such that there is an inverse relationship between iron status and absorption(Kalasuramath *et al.*, 2015)[46] and several studies have shown that more iron is absorbed in an iron deficient state than in an iron repletion state (Walczyk *et al*.,2008)[47]. All the above reasons could have collectively helped to increase blood parameters.

IV. Conclusion

Anemia is high prevalence among adolescent girls. The finest way to prevent anemia is inclusion of GLV based foods in regular diet . In this way supplementation of Saravallai Dhal Powder to anemic adolescent girls, their blood iron parameters improved considerably. Because the SDP had better bioaccessible ion , anthelminhic effects and low anti nutrient content.

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