Triticum aestivum (Wheatgrass) formulation: An alternate treatment for the patients with Thalassemia

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SUMMARY

We have developed the tablet formulation of *Triticum aestivum* (wheatgrass) and investigated clinically, its effects in patients suffering from b-thalassemia (major) at K. T. Children Hospital, Civil Hospital, Rajkot. The tablets (wheatgrass powder 250 mg.) were given 3 times in a day for 9 months. Blood samples were collected at the start, after 6 months and 9 months and analyzed for various biochemical and hematological parameters. Treatment with wheatgrass formulation for 9 months produced significant decrease in hemoglobin, total RBC, eosinophil and reticulocyte counts. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were significantly increased was significantly increased. The serum ferritin was also significantly decreased. There was no influence on serum magnesium, serum iron and Thiobarbituric acid reacting substances (TBARS). Our data indicate that treatment with wheatgrass on patients with ß-thalassemia (major) may have beneficial effects in the form of a decrease in ineffective erythropoiesis, stimulation of hemoglobin synthesis in RBC, decrease in iron load and decrease in eosinophil count.

Key words: Triticum aestivum; Wheatgrass tablets; Thalassemia; Clinical trial

INTRODUCTION

Thalassemia is one of the most common groups of genetic blood disorder. Countries like Italy, Greece and Cyprus have the highest frequency of Thalassemia cases in the world. There are an estimated 240 million carriers of thalassemia in the world. India has the largest pool of numbering around 30 million (Ambekar *et al.*, 2001). The thalassemias ($\propto$ and β) are characterized by impaired production of one or more polypeptide chains of globin. Any of the four polypeptides (α, β, γ, ∂) that occur in normal hemoglobin may be involved. The fact that there are only two genes for the beta chain of hemoglobin makes ß-thalassemia simpler to understand. The β-globin gene is present, but produces little β-globin protein (Jandl, 1982; Steinberg, 1988; Weatherall, 1997). The synthesis and accumulation of excess normal β-globin chain within the red cell, lead to the formation of unstable aggregates, which upon oxidation, due to oxidative stress generated by iron overload, may precipitate and cause cell membrane damage. These deformed cells undergo premature destruction either in the bone marrow (extravascular hemolysis) or the peripheral circulation

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(intravascular hemolysis) (Festa, 1985). Management of ß-thalassemia major (*Cooley's Anemia*) requires patients to have life-long regimen of regular blood transfusions coupled with iron chelation therapy. Blood transfusion produces on long term, serious and unavoidable side-effects because with each unit of blood transfused 200 to 250 mg of iron gets deposited in the heart, liver, pancreas and other glands in the body. This may lead to heart failure, cirrhosis of liver, diabetes mellitus and malfunctioning of other glands. Generally, regular blood transfusions and iron chelation treatment with desferrioxamine are initiated early in life. Therefore, the patients and their families have to sustain regular treatment throughout their childhood, adolescent, and adult years (Zurlo *et al.,* 1989; Olivieri *et al.,* 1994). Data indicate that dietary magnesium supplementation improves some of the characteristic cellular function abnormalities of b-thalassemia intermedia (De Franceschi *et al.,* 1998).

Thus, in thalassemia, there is a vicious cycle of iron overload leading to oxidative stress with consequent increase in hemolysis and increase in blood transfusion requirement causing further iron overload and its toxicities on other organs. Replenishing magnesium and antioxidants by administration of wheatgrass can break this vicious cycle. A recent report by Marwaha *et al.* (2004) has provided an evidence to support this hypothesis wherein in a pilot study it was reported that the administration of wheatgrass significantly reduces blood transfusion requirement in thalassemic patients. Further, fresh wheatgrass has been proposed to be used as a juice, which is prepared in a mixer/blender with addition of little water followed by filtration through a cloth (Marwaha *et al.,* 2004). In a chronic disease like thalassemia, the drug treatment is of long duration, may even be for years. In such a circumstances the factor of patient compliance becomes very important. Outcome of the therapy will largely depend upon regular supply (round the year and in all seasons) and acceptability of the drug by patient. As a pharmaceutical scientist,

preparation of a suitable dosage form is prime area of research in the development of new drug formulations. Preparation of wheatgrass tablets was a challenge for us. We attempted to prepare tablets of the shade dried wheatgrass powder and clinically evaluated for the effects in the patients with thalassemia.

MATERIALS AND METHODS

Preparation of dried wheatgrass powder

For preparation of dried wheatgrass powder (to be used for manufacturing of Wheatgrass tablets) different drying techniques were used such as freeze-drying, spray drying and shade drying. In freeze drying technique, fresh wheatgrass was frozen to sub-zero temperature and subsequently subjected to low-temperature heating (5°C - 10°C) in vacuum to evaporate crystallized water content. Dried wheatgrass was then milled to obtain powder. In spray-drying technique, fresh wheatgrass was pressed in a hydraulic press to obtain juice. The juice was then sprayed in aerosol form through a nebulizing nozzle in a conical vessel from top. A hot air counter current (55°C) was passed from bottom of the vessel. The nebulized juice settled on the bottom of the vessel in the form of powder. In shade-drying technique, fresh wheatgrass was dried at room temperature in a dark room. The dried wheatgrass after 3 - 4 days of drying period was powdered in a mill.

Preparation of wheatgrass tablets

Wheat grains of *Triticum durum* variety were acquired and sown in plain soil without using any type of fertilizer. The wheatgrass was grown under specially constructed shades. The grass was harvested after 8 days from sprouting and shade-dried in well-ventilated dark rooms for 4 days. Dried wheatgrass was powdered in a mill. Tablets of wheatgrass were manufactured in a pharmaceutical tablet unit by adding suitable binders and excipients

Clinical studies on wheatgrass formulation

The clinical trial of wheatgrass on β-thalassemia was carried out at K. T. Children Hospital working under Civil Hospital, Rajkot. Necessary permission for conducting the clinical trial was obtained from the Institutional ethical committee of the hospital. Thalassemic patients, visiting K. T. Children Hospital regularly for blood transfusion and registered at the hospital were enrolled for the trial, after taking informed consent. Twenty patients suffering from β-thalassemia (major) whose age ranged from 8 to 20 years were included in the trial. The patients of this group were given wheatgrass tablets with dosage regimen of 2 tablets (wheatgrass powder 250 mg). 3 times in a day for 9 months. Blood samples were collected at the start, after 6 months and at the end of period of clinical trial (i.e. 9 months period).

The samples were analyzed for hemoglobin content by using standard method (Sahli's Hemoglobinometer) while, Total RBC count, PCV, Mean corpuscular volume (MCV), MCH, Mean corpuscular hemoglobin concentration (MCHC), Total WBC count, Neutrophil count, Lymphocyte count, Eosinophil count, Basophil count and Total lymphocyte count were recorded by using automatic cell counter – Model KX-21 – Sysmex. We also estimated Serum iron, Serum magnesium, Serum ferritin and Thiobarbituric acid reacting substances (TBARS). The Serum magnesium was estimated by Calmagite Dye method using standard kit obtained from Ranbaxy India. Transferrin-bound iron was estimated spectrophotometerically by the method of using standard kit obtained from Ranbaxy India at 560 nm.

Serum Ferritin was estimated using Access Immunoassay method using a Beckman Coulter automatic analyzer, Lipid peroxidation product (MDA) was estimated by thiobarbituric acid reaction method (Ohkawa *et al.,* 1979).

Statistical analysis

The results were using student's paired *t* test. The value of p less then 5% ($P \leq 0.05$) was considered as statistically significant.

RESULTS

The demographic data of the patients has been given in Table 1.

After 9 months treatment with wheatgrass, Hb g % and total RBC count in normal-range subgroup were significantly decreased (Table 2). There was no alteration in the other group. The decrease in reticulocyte count was highly significant. Reticulocyte count in abnormal-range subgroup was significantly decreased (before treatment: 10.8 ± 2.55, after treatment: 2.8 ± 1.21). Decrease in reticulocyte count after wheatgrass treatment is an indication of increase in tissue oxygenation and improvement in erythropoiesis. MCV in normal-range subgroup was significantly decreased (before treatment: 86.26 ± 1.06, after treatment: 77.02 ± 2.58) and that in abnormal-range subgroup was significantly increased (before treatment: 77.63 ± 0.94, after treatment: 82.99 ± 1.74). MCHC in abnormal-range subgroup was significantly increased (before treatment: 28.85 ± 1.06, after treatment: 32.63 ± 1.06) (Table 2) (Fig. 1). Positive changes in MCV and MCHC reflect correction of both hypochromia and microcytosis, thus exhibiting reversal of abnormal condition in thalassemia.

After 9 months treatment with wheatgrass, eosin

Table 1. Profile of patients in the treatment group <β-thalassemia (major)>

Total number of patients	20
Patients below 12 years age	8
Patients above 12 years age	12
Patients with S. Ferritin below 2,500 (low iron load)	7
Patients with S. Ferritin above 2,500 (high iron load)	13
Patients with spleen	13
Patients without spleen (spleenectomized)	7
Male/Female	12/8

Table 2. Effect of wheatgrass on patients with β-thalassemia (major) with respect to changes in parameters related to erythropoetic activity

Parameter	Normal Value	Subgroup	Number of patients	Before treatment with wheatgrass	After treatment with wheatgrass
Hb gm %	M : 13.5 - 18 F : 12 - 16	Normal	3	12.87 ± 0.10	10.77 ± 0.36[*]
		Abnormal	17	8.68 ± 0.54	8.83 ± 0.43
RBC m/cmm	M : 4.5 - 6.5 F : 4.2 - 5.4	Normal	8	4.55 ± 0.13	3.74 ± 0.20[**]
		Abnormal	12	3.08 ± 0.21	3.28 ± 0.19
Reticulocyte%	0.1 - 2	Normal	16	1.06 ± 0.06	1.00 ± 0.00
		Abnormal	4	10.80 ± 2.55	2.80 ± 1.21[*]
MCV cu mic	82 - 92	Normal	6	86.26 ± 1.06	77.02 ± 2.58[*]
		Abnormal	14	77.63 ± 0.94	82.99 ± 1.74[*]
MCH pic gm	27 - 31	Normal	6	28.29 ± 0.16	26.73 ± 0.48[*]
		Abnormal	17	24.96 ± 0.56	26.34 ± 0.49
MCHC %	32 - 36	Normal	12	33.83 ± 0.51	32.60 ± 0.42
		Abnormal	8	28.85 ± 1.06	32.63 ± 1.06[*]

[*]Significantly different from initial value ($P < 0.05$).
[**]Significantly different from initial value ($P < 0.01$).
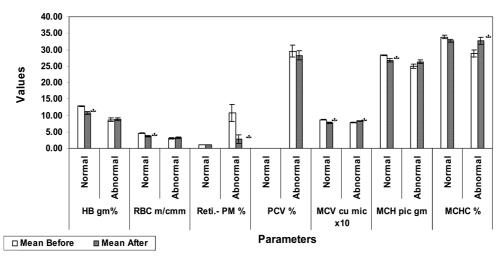

Fig. 1. Effect of Wheatgrass on patients with β-thalassemia (major) with respect to changes in parameters related to erythropoetic activity.
[*]Significantly different from initial value ($P < 0.05$)
[**]Significantly different from initial value ($P < 0.02$)

count (Eosin %) in abnormal-range subgroup was significantly decreased (before treatment: 3.22 ± 1.14, 0.33 ± 0.22) (Table 3) (Fig. 2). Reversal of eosinophilia may be an indicator of detoxifying or antiallergic property of wheatgrass.

After 9 months treatment with wheatgrass, serum ferritin was significantly decreased (before treatment: 6,846 ± 1,222, after treatment: 3,877 ± 676)

indicating decrease in iron load. In absence of substantial rise in hemoglobin content, the only possible explanation for decreased iron load is increased excretion of iron. Treatment with wheatgrass had no influence on serum magnesium, serum iron and TBARS (Table 4) (Fig. 3). These, data indicate that although wheatgrass treatment increases hemoglobin content of RBC in

Table 3. Effect of wheatgrass on patients with β-thalassemia (major) with respect to changes in parameters related to natural defense mechanism of the body

Parameter	Normal Value	Subgroup	Number of patients	Before treatment with wheatgrass	After treatment with wheatgrass
Platelet	1.5 - 4.5	Normal	11	273182 ± 12107.5	285091 ± 29245.2
		Abnormal	2	199,000 ± 126572.1	258000 ± 154149.3
WBC/cmm	4000 – 10000	Normal	12	6008.3 ± 485.84	5769.2 ± 457.19
		Abnormal	8	13875.0 ± 2902.79	16057.1 ± 2033.11
Neutro %	60 - 70	Normal	7	62.14 ± 1.11	58.71 ± 2.63
		Abnormal	13	51.69 ± 2.04	54.23 ± 3.39
Lympho %	20 - 30	Normal	1	23.00 ± 0.00	43.00 ± 0.00
		Abnormal	19	40.00 ± 1.83	40.53 ± 2.00
Total lympho Count	800 - 3000	Normal	13	1963.85 ± 132.08	2293.92 ± 161.40
		Abnormal	7	5274.00 ± 916.94	6968.29 ± 1364.91
Eosin %	1.0 - 4.0	Normal	1	2.18 ± 0.36	0.64 ± 0.23
		Abnormal	9	3.22 ± 1.14	0.33 ± 0.22*

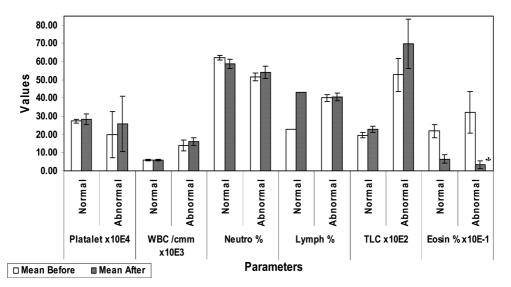*Significantly different from initial value (*P* < 0.05).

Fig. 2. Effect of Wheatgrass on patients with β-thalassemia (major) with respect to changes in parameters related to natural defense mechanism of the body.
*Significantly different from initial value (*P* < 0.05)

thalassemia, the mechanism of action is neither iron or magnesium supplementation nor replenishment of antioxidants.

After 9 months treatment with Wheatgrass, different parameters in patients of above 12 years age group showed significant changes. In this group, the hemoglobin content in normal range subgroup was significantly decreased (before treatment: 12.86 ± 0.1, after treatment: 10.77 ± 0.36). RBC count in normal range subgroup was significantly decreased (before treatment: 4.54 ± 0.14, after treatment: 3.71 ± 0.22). Reticulocyte count in abnormal range subgroup was significantly decreased (before treatment: 10.8 ± 2.55, after treatment: 2.8 ± 1.21). MCV in normal range subgroup was significantly decreased (before treatment: 86.73 ± 1.37,

Table 4. Effect of wheatgrass on patients with β-thalassemia (major) with respect to changes in parameters related to mechanism of action of wheatgrass

Parameter	Normal Value	Subgroup	Number of patients	Before treatment with wheatgrass	After treatment with wheatgrass
S. iron mic/dl	90 - 150	Normal	3	130.00 ± 12.25	274.00 ± 46.68
		Abnormal	14	225.51 ± 15.57	229.68 ± 22.34
S. ferritin ng/dl	12 - 140	Abnormal	16	6846 ± 1222	3877 ± 676[*]
S. mag meq/l	1.3 - 2.5	Normal	18	1.93 ± 0.10	1.92 ± 0.05
		Abnormal	1	3.54 ± 0.00	2.48 ± 0.00
TBARS × 10^5 M/cmm	1.3 - 2.5	Normal	0	-	-
		Abnormal	12	0.63 ± 0.05	0.60 ± 0.05

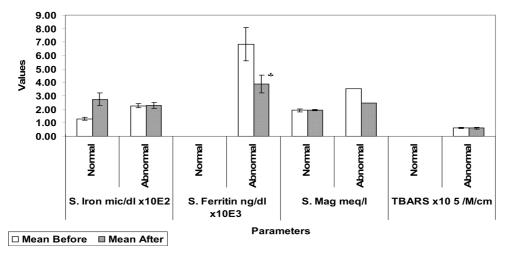[*]Significantly different from initial value ($P < 0.05$)

Fig. 3. Effect of Wheatgrass on patients with β-thalassemia (major) with respect to changes in parameters related to mechanism of action of Wheatgrass.
[*]Significantly different from initial value ($P < 0.05$)

after treatment: 76.2 ± 3.52). MCHC in abnormal range subgroup was significantly increased (before treatment: 28.43 ± 1.37, after treatment: 33.34 ± 1.28) (Table 5). Total lymphocyte count in normal range subgroup was significantly increased (before treatment: 1768.5 ± 151.04, after treatment: 2351.8 ± 157.97) (Table 6). In below 12 years age group, the serum iron content in normal range subgroup was significantly increased (before treatment: 149 ± 2.83, after treatment: 287 ± 7.78) (Table 7). Comparison of the effects of wheatgrass treatment in patients of both these age groups revealed that patients of above 12 years age group had experienced more beneficial effects of wheatgrass treatment compared

to the patients of below 12 years age group.

DISCUSSION

Results of the present study indicate haemopoietic suppression effect of wheatgrass. Hemoglobin in patients of normal-range subgroup, was found to be significantly decreased (before treatment: 12.87 ± 0.10, after treatment: 10.77 ± 0.36). RBC count in normal range subgroup was also significantly decreased (before treatment: 4.55 ± 0.13, after treatment: 3.74 ± 0.20). In the patients with normal hemoglobin content and RBC count in abnormal range the treatment did not produce any effect.

Table 5. Comparison of effects of wheatgrass tablets on patients of different age groups with β-thalassemia (major) with respect to changes in parameters related to erythropoetic activity

Parameter	Subgroup	Below 12 years age group		Above 12 years age group	
		Before	After	Before	After
Hb g %	Normal	-	-	12.86 ± 0.1	10.77 ± 0.36[**]
	Abnormal	9.13 ± 0.18	8.63 ± 0.41	8.43 ± 0.7	8.94 ± 0.62
RBC m/cmm	Normal	4.57 ± 0.0	3.93 ± 0.0	4.54 ± 0.14	3.71 ± 0.22[**]
	Abnormal	3.34 ± 0.23	3.28 ± 0.19	2.89 ± 0.3	3.28 ± 0.3
Reti.- PM %	Normal	1.0 ± 0.0	1.0 ± 0.0	1.08 ± 0.08	1 ± 0.0
	Abnormal	-	-	10.8 ± 2.55	2.8 ± 1.21[*]
PCV %	Normal	-	-	-	-
	Abnormal	27.85 ± 2.7	26.92 ± 1.36	30.19 ± 2.33	28.77 ± 1.88
MCV cu mic	Normal	85.30 ± 1.42	78.65 ± 2.9	86.73 ± 1.37	76.2 ± 3.52[**]
	Abnormal	74.53 ± 2.17	80.63 ± 4.2	78.87 ± 0.67	83.93 ± 1.66
MCH pic g	Normal	-	-	28.29 ± 0.23	26.73 ± 0.63
	Abnormal	25.61 ± 0.56	25.67 ± 0.94	24.61 ± 0.79	26.7 ± 0.53
MCHC %	Normal	34.36 ± 0.88	32.97 ± 0.36	33.56 ± 0.6	32.42 ± 0.59
	Abnormal	30.09 ± 0.01	30.48 ± 0.31	28.43 ± 1.37	33.34 ± 1.28[*]

[*]Significantly different from initial value ($P < 0.05$).
[**]Significantly different from initial value ($P < 0.01$).

Table 6. Comparison of effects of wheatgrass tablets on patients of different age groups with β-thalassemia (major) with respect to changes in parameters related to natural defense mechanism of the body

Parameter	Subgroup	Below 12 years age group		Above 12 years age group	
		Before	After	Before	After
Platelet	Normal	284250 ± 23495	221000 ± 9753	266857 ± 12884	321714 ± 39421
	Abnormal	-	-	258000 ± 154149	199000 ± 126572
WBC /cmm	Normal	6383 ± 536	6166 ± 740	5633 ± 781	5833 ± 343
	Abnormal	-	-	14025 ± 2639	14275 ± 2724
Neutro %	Normal	62.67 ± 2.18	62.33 ± 2.13	61.75 ± 1.02	56 ± 3.79
	Abnormal	50.33 ± 2.6	55.0 ± 0.82	52.1 ± 2.52	54 ± 4.4
Lymph. %	Normal	30 ± 0.0	40 ± 0.0	28.25 ± 1.52	34.5 ± 4.26
	Abnormal	42 ± 3.3	40 ± 2.15	44 ± 2.45	42.5 ± 2.5
Total lympho. Count	Normal	2276 ± 166	2345 ± 343	1768.5 ± 151.04	2351.8 ± 157.97*
	Abnormal	3744 ± 0.0	2970 ± 0.0	7505 ± 1483	6034 ± 960
Eosin %	Normal	1.75 ± 0.45	1 ± 0.35	0.8 ± 0.34	1 ± 0.3
	Abnormal	7 ± 2.12	1.5 ± 0.35	6.5 ± 1.06	0.5 ± 0.35

[*]Significantly different from initial value ($P < 0.05$).

Marwaha *et al.* (2004) showed that treatment with wheatgrass juice in thalassemia patients for a period of 6 months did not produce any effect but the treatment for 12 months produces a significant beneficial effect. In our study also, the transfusion requirements remained unchanged even after a period of 9 months of ingesting wheatgrass tablets. However, there was a significant improvement in some hematological parameters related to thalassemia. After 9 months treatment with wheatgrass, reticulocyte count in abnormal-range subgroup was significantly decreased. The reticulocyte is an

Table 7. Comparison of effects of wheatgrass tablets on patients of different age groups with β-thalassemia (major) with respect to changes in parameters related to mechanism of action of wheatgrass

Parameter	Subgroup	Below 12 years age group		Above 12 years age group	
		Before	After	Before	After
S. iron mic/dl	Normal	149 ± 2.83	287 ± 7.78[**]	124.33 ± 10.71	231 ± 57.91
	Abnormal	281 ± 32.51	258 ± 56.79	225.8 ± 13.9	221.84 ± 27.48
S. mag meq/l	Normal	1.85 ± 0.07	1.87 ± 0.04	1.95 ± 0.11	1.95 ± 0.07
	Abnormal	1.96 ± 0.57	1.89 ± 0.03	3.54 ± 0.0	2.48 ± 0
TBARS × 10^5 M/cm	Normal	-	-	-	-
	Abnormal	0.68 ± 0.09	0.45 ± 0.01	0.61 ± 0.08	0.66 ± 0.06

[**]Significantly different from initial value ($P < 0.01$).

immature erythrocyte. Normally, the reticulocyte count ranges from 1 to 2% and reflects daily replacement of 1% of red cell population of red cells (Adamson and Longo, 2001). The rate of reticulocyte release from the marrow into the peripheral circulation is governed primarily by the rate at which O_2 is being supplied to the tissues. A decrease in PO_2 (hypoxia) is recognized by the kidney, which is stimulated to release erythropoietin (Adamson and Longo, 2001). The chronic hypoxic condition in thalassemia, due to persistent low hemoglobin content of blood stimulates secretion of erythropoietin, which in turn increases ineffective erythropoiesis and reticulocyte count. Decrease in reticulocyte count after wheatgrass treatment is an indication of increase in tissue oxygenation and decrease in ineffective erythropoiesis.

Thalassemia is characterized by hypochromic microcytic anemic condition. After wheatgrass treatment MCV was significantly increased. MCV expresses the mean volume of each red cell. Similarly MCHC was also significantly increased. Positive changes in both these parameters reflect correction of both hypochromia and microcytosis, thus exhibiting reversal of abnormal condition in thalassemia. MCHC provides the mean concentration of hemoglobin in each red cell. It is most valuable in evaluating therapy for anemia because it takes in to consideration hemoglobin and hematocrit and not the RBC count. The MCHC reflects defects in hemoglobin synthesis (Adamson and Longo, 2001).

Increase in MCHC indicates rise in hemoglobin concentration in individual RBC. In the face of increased MCV this rise in MCHC signifies a substantial increase of hemoglobin in RBC. In our investigation the increase in MCHC i.e. increase in hemoglobin content of individual RBC was offset by haemopoietic suppression effect of wheatgrass i.e. decrease in number of RBC. Thus, the reduction in overall transfusion requirement was not observed after 9 months treatment with wheatgrass.

Increase in MCHC could be an indication of increased synthesis of hemoglobin in RBC. Since, total adult hemoglobin comprises of hemoglobin A_1 - $\propto_2\beta_2$ (97%), hemoglobin A_2 - $\propto_2\delta_2$ (2.5%) and hemoglobin - $\propto_2\gamma_2$ (0.5%), it follows that rise in MCHC was contributed by any of these three type of hemoglobin or in other words due to increased expression of β, γ or δ gene. It is known that RNA splicing mutations are fairly common and represent a large portion of all mutations resulting in beta thalassemia (Benz, 2001). Probability of increasing expression of a mutated gene is very low. It is also known that a critical control region of the delta globin gene (promoter) is known to be defective. It inhibits messenger RNA (mRNA) processing, resulting in only a small amount of Hb A2 (alpha2/delta2) production, which accounts for less than 3% of total Hb in adult RBC. Further, it has been found that stimulation or induction of fetal hemoglobin in thalassemia can improve the patient's clinical condition (Jones and Taylor,

1980). Thus, increase in MCHC after wheatgrass treatment may be an indication of induction of HbF in thalassemic patients.

Further, consequence of diminished beta-chain production is the formation of excess alpha chains that form tetramers and inclusion bodies because they are less soluble than normal Hgb. These inclusion bodies are lethal to developing erythroid precursors and are responsible for most of the severe clinical effects of thalassemia. The inclusion bodies cause the destruction of approximately 95% of red blood cell precursors prior to release into the circulation (i.e. intramedullary hemolysis), either as a consequence of arrest in the G1 phase of the cell cycle or by apoptosis during later phases of maturation (Festa, 1985; Weatherall, 1997). The only reasonable chance a red blood cell has for a somewhat normal morphology and lifespan occurs if significant amounts of non–beta-chain Hgb (e.g. gamma, delta) are available to bind the excess alpha chains. Thus, increased synthesis of gamma chain due to wheatgrass treatment can increase the life span of RBC, reduce severity of clinical picture and inhibit ineffective erythropoiesis in thalassemia. In our investigation, 90% patients taking wheatgrass treatment reported feeling of well-being increase in vitality and appetite, which is in concurrence with the forgoing discussion.

After 9 months treatment with wheatgrass, eosinophil count was significantly decreased. Eosinophilia is seen in allergic disorders. Thalassemic patients on regular blood transfusion therapy are subject to various allergens hence, eosinophilia is commonly found in thalassemic patients. Thus, reversal of eosinophilia may be an indicator of much acclaimed detoxifying or antiallergic property of wheatgrass.

It is well known that management of ß-thalassemia major (*Cooley's Anemia*) requires patients to have life-long regimen of regular blood transfusions coupled with iron chelation therapy (Modell, 1994; Cao *et al.,* 1997). Blood transfusion produces on long term, serious and unavoidable side effects because iron gets deposited in the heart, liver, pancreas and other glands in the body. This may lead to heart failure, cirrhosis of liver, diabetes mellitus and malfunctioning of other glands. Iron overload may be treated or prevented with a chelating agent capable of complexing with iron and promoting its excretion. At present, only two iron-chelating agents are available for clinical use in thalassemia viz. desferrioxamine (Desferal - given subcutaneously) and deferiprone (Kelfer - given orally) (Zurlo *et al.,* 1989; Olivieri *et al.,* 1994). Painful administration, muscle aches, arthralgia and high cost are major disadvantages of desferrioxamine while neutropenia, agranulocytosis and arthropathy, are noted side effects of deferiprone (Agarwal *et al.,* 1992; Al-Refaie *et al.,* 1992; Al-Refaie *et al.,* 1995; Cohen *et al.,* 2000). Thus, the search continues for an oral, safe, effective, easily available and economical iron chelating agent.

As wheatgrass contains substantial quantity of iron, which is contraindicated for thalassemic patients, the major apprehension expressed by patients and doctors, at the outset of this study, was the possibility of increase in iron load with wheatgrass treatment. The measurement of plasma or serum ferritin is the most commonly used indirect estimate of body iron stores (Brittenham *et al.,* 2001). In our investigation serum ferritin level, against all predictions, was significantly decreased at the end of 9 months treatment with wheatgrass. In absence of substantial rise in hemoglobin content, the only possible explanation for decreased iron load is increased excretion of iron. Thus, the most notable outcome of our investigation, apart from ascertaining the ability of wheatgrass to increase hemoglobin content, is the detection of its probable ability to decrease iron load in thalassemia. Wheatgrass may well be the long sought-after safe, oral and economical iron-chelating agent for thalassemic patients.

The deficiency of magnesium in serum or erythrocytes has also been reported in human β-thalassemia and that dietary magnesium

supplementation improves some of the characteristic cellular function abnormalities of β-thalassemia (De Franceschi *et al.*, 1998). Clinical data suggests that the iron-induced liver damage in thalassemia may play a major role in the depletion of lipid-soluble antioxidants (Livrea *et al.*, 1996). The synthesis and accumulation of excess normal globin chain (i.e. β-chain in α-thalassemia and α-chain in β-thalassemia), within the red cell, lead to the formation of unstable aggregates, which upon oxidation, due to oxidative stress generated by iron overload, may precipitate and cause cell membrane damage. These deformed cells undergo premature destruction either in the bone marrow (extravascular hemolysis) or the peripheral circulation (intravascular hemolysis) (Festa, 1985; Weatherall, 1997). Hence, logical mechanism of action of wheatgrass in thalassemia, at the onset of the clinical trial, was either replenishment of magnesium and/or antioxidants that can break this vicious cycle. In our study, treatment with wheatgrass had no influence on serum magnesium and TBARS. Both the parameters were included to investigate probable mechanism of action of wheatgrass. Thus, our data indicate that although wheatgrass treatment exhibits beneficial therapeutic effects in thalassemia, the mechanism of action is neither magnesium supplementation nor replenishment of antioxidants.

Results of clinical study indicate wheatgrass formulation to be one of the effective treatments for thalassemia. Although direct molecular studies are required to confirm, it is hypothesized that wheatgrass produces direct beneficial effect in thalassemia patients by stimulating hemoglobin synthesis in RBC. Wheatgrass was found to decrease significantly the iron load and suppress the ineffective erythropoiesis.

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