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Alterations in Biochemical and Hematopoiesis Markers in Healthy Human Adults Supplemented with Fresh Wheatgrass Juice

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ABSTRACT

Management of anemia due to chronic disease including cancer is of primary importance for improving the quality of life of cancer patients. Red blood cell (RBC) transfusions and erythropoiesis stimulating agents (ESAs) e.g. erythropoietin are the only options available for patients with cancer-related anemia when nutrition deficiencies are not identified. Supplementation of wheat grass juice (WGJ) has become a routine practice for overall health benefit and rectification of anemia without medical supervision. Therefore, a careful scientific evaluation of wheatgrass juice supplementation in the rectification of anemia was done in the present study. The circulating levels of various interleukins (1, 3 & 6), TNF-α, GMCSF & EPO were estimated before and after 14 days of supplementation in 30 healthy volunteers. The study revealed variations in serum level of IL 1(↓), 3(↓), 6(↑), TNF-α(↓), EPO(↓) and GM-CSF(↑) due to WGJ supplementation. Comparable level of serological, hematological and biochemical parameter is suggestive of its nontoxic nature at the same time it indicates marginal improvement in liver and renal function in healthy condition which favors its overall health benefits. However, a further confirmation study is required to investigate the role of WGJ supplementation in inducing erythroid progenitors in-vitro using hematopoietic stem cell (HSC) model before establishing its role as anemia rectifier.

KEYWORDS : Anemia, Wheat grass juice, Erythropoietin, interleukin.

INTRODUCTION

Mammalian erythropoiesis is normally regulated in vivo by erythropoietin (Epo), a glycoprotein hormone responsible for the differentiation of red blood cells (RBCs) and for the viability and proliferation of their progenitors. Malfunctioning of erythropoiesis leads to development of anemia in acute or chronic diseased condition including cancer. Development of blood cells is regulated by numerous growth factors and cytokines released by stromal cells, as well as T cells and mature leukocytes within the bone marrow. The specific regulatory functions of these cells and their role in hematopoiesis are to be yet elucidated.

Anemia gives rise to several symptoms such as exhaustion, respiratory distress, impaired concentration and fatigue. This leads to a decrease in mental and physical capacity, increases the stress associated to the treatment and influences the decision of patients to discontinue therapy. Many therapeutic options are available to treat anemia including supplementation of vitamins (Folic acid/vit-B12), Iron and phytopharmaceuticals e.g. wheatgrass juice and capsules. Red blood cell (RBC) transfusions and erythropoiesis stimulating agents (ESAs) e.g. erythropoietin infusion in patients with anemia due to chronic disease is a routine practice when nutritional deficiencies are not identified as causative factors.

Erythropoietic stimulation via mechanisms that do not involve EPO receptors would have specific advantages in underproduction anemias characterized by endogenous EPO concentrations that are elevated but ineffective. It has been previously reported that non-responsiveness to ESAs may be due to inadequate production of endogenous erythropoietin as a result of increased inflammatory serum cytokines (INF-γ, IL-1β, TNF-α) levels[1, 2]. Moreover, increased levels of some of these molecules have been correlated to the partial failure of exogenously administered erythropoietin in cancer patients by inhibit the release of iron from the reticuloendothelial system and thus inducing anemia [3-5]

In present study we scientifically evaluated the role of wheatgrass juice supplementation in the rectification of anemia. The alterations in circulating levels of various interleukins (1, 3 & 6), TNF-α, GMCSF, EPO and further laboratory & clinical parameters as a result of wheatgrass juice supplementation in healthy volunteers was assessed before and after supplementation.

MATERIALS & METHODS

Clinical study of wheatgrass juice supplementation on healthy volunteers.

Equal number of healthy males and healthy females (n=15) were recruited as volunteer for the study. None of the volunteers have any major illness in recent past. None of them were obese, under weight or anemic (Hb<12). All the volunteers were given wheat grass juice for 14days in the morning in empty stomach. The study was approved by institutional ethics committee and Informed consent was taken prior to recruitment in the study. The study was performed at Anand Multispeciality hospital pvt. ltd., Ahmedabad, Gujarat, India. Day 0 and day 14 blood samples were collected. In normal healthy female volunteers, supplementation was started on 5th day of menstruation cycle. Healthy volunteers were selected with no major illness in recent past with normal renal and hepatic function as verified by serum LFT and RFT. Phlebotomy was done by trained laboratory technician. 9ml blood in plain vacutainer (Red cap) 1) For Biochemistry and 4ml in EDTA vacutainer (Purple cap) for Haematology in both instances i.e. pre and post supplementation.

Hematology & Biochemistry Assays

Hematological parameters e.g. CBC (Complete Blood Count) were performed using "Mindray BC2800 automated hematology analyzer 3 part differential Cell Counter" and Biochemical assays e.g. LFT(Liver Function Test), RFT(Renal Function test) were done using standard kits from Biosystem Reagents and Instruments on fully automated A -15 Biosystem Biochemical Analyzer following manufacturer's instructions.

Estimation of Interleukins & cytokines by ELISA

Colorimetric Sandwich ELISA was used for estimation of serum IL-1, IL-3, IL-6 GMCSF, TNF-α, and EPO following manufacturer's instructions using quantitive ELISA kits (R&D System, USA). The optical density (O.D.) was read of each well within 15 minutes, using a microplate reader set to 450 nm. Wave length correction was set to 600 nm.

Statistical Analysis

The cytokine levels and all the laboratory parameters in the unsupplemented and supplemented sera were compared using student's t-test and p-value <0.05 was considered statistically significant.

RESULTS

The microbiological analysis of wheatgrass juice reported occasional *E. coli* sp. As no other harmful pathogenic organisms were found in the WGJ, it was declared safe for human supplementation. A total of 30 volunteers with equal representation from both sex with age range between 20-45 yrs were selected for the study as depicted in Table-1

Table-1: Profile of Volunteers in the present clinical study

Total number of Volunteers	30
Age range of Volunteers	20 to 45 years
Number of male Volunteers	15
Number of female Volunteers	15
Patients above 30 years age	10
Patients below 30 years age	20

Hematological parameters e.g. Hb, RBC, MCH, MCHC levels in both male and female healthy volunteers were slightly increased after 14 days of the wheat grass supplementation however the difference was not statistically significant. Similarly MCV (Mean corpuscular volume) and RDW (red blood cell distribution width) showed marginally significant increasing trend after 14 days of the wheat grass juice supplementation whereas WBC count showed reverse trend. However there was no significant change observed in PCV (Packed cell volume), MCH (mean corpuscular hemoglobin) and RDW parameters.

The biochemical profile reveals significant decrease in S. Bilirubin mainly due to indirect Bilirubin and increase in albumin reflects improved liver function. There was no remarkable change in the rest of the biochemical parameters in either sex due to supplementation as indicated by t-test.

Table-2: Effect of Wheatgrass juice on Healthy Volunteers with respect to changes in parameter related to Erythropoietic activity (n=30) for Male Volunteers

Test	Normal Value	Mean Value Before supp. (n=30)	Mean Value After supp. (n=30)	Statistical 't' test 'p value'
Hemoglobin	13.5- 18.0	13.89± 0.09	13.95± 0.10	0.802
Total RBC Count	4.7- 6.0	5.21± 0.03	5.30± 0.03	0.398
Hematocrit (P.C.V.)	42- 52	44.03± 0.28	43.93± 0.34	0.913
M.C.V.	78- 100	82.15± 0.73	83.53± 0.71	0.069
M.C.H.	32- 36	24.83± 0.27	26.04± 0.26	0.097
M.C.H.C.	32- 36	31.34± 0.09	31.63± 0.10	0.321
R.D.W.	11.5- 14.0	16.28± 0.09	16.64± 0.09	0.052
Total WBC Count	4000 - 10000	6966.67± 147.57	6693.33± 143.84	0.060
Differential WBC Count				
Polymorphs	50 - 60	56.80± 0.21	57.00± 0.20	0.748
Lymphocytes	20 - 40	35.93± 0.21	36.33± 0.19	0.541
Eosinophils	1-4	2.80± 0.06	2.87± 0.05	0.817
Monocytes	2-6	4.47± 0.11	3.80± 0.07	0.207
Basophils	0	0.00± 0.00	0.00± 0.00	
Platelet Count	1.5- 4.5	3.03± 0.04	3.02± 0.04	0.941

Table-3: Effect of Wheatgrass juice on Healthy Volunteers with respect to changes in parameter related to Erythropoietic activity (n=30) for Female Volunteers

Test	Normal Value	Mean Value Before supp. (n=30)	Mean Value After supp. (n=30)	Statistical 't' test 'p value'
Hemoglobin	11.5 - 15.5	12.47± 0.04	12.57± 0.04	0.491
Total RBC Count	4.2- 5.4	4.51± 0.02	4.62± 0.02	0.412
Hematocrit (P.C.V.)	37- 47	37.17± 0.06	37.45± 0.10	0.478
M.C.V.	78- 100	84.74± 0.15	85.09± 0.18	0.293
M.C.H.	32- 36	28.41± 0.08	28.62± 0.08	0.268
M.C.H.C.	32- 36	32.27± 0.07	33.02± 0.11	0.094
R.D.W.	11.5- 14.0	14.87± 0.08	15.15± 0.11	0.276
Total WBC Count	4000 - 10000	5873.33± 92.25	5866.67± 77.32	0.942
Differential WBC Count				
Polymorphs	50 - 60	56.40± 0.19	56.87± 0.17	0.666
Lymphocytes	20 - 40	37.53± 0.18	36.67± 0.16	0.377
Eosinophils	1-4	2.73± 0.05	2.93± 0.06	0.550
Monocytes	2-6	3.33± 0.06	3.53± 0.06	0.510
Basophils	0	0.00± 0.00	0.00± 0.00	
Platelet Count	1.5- 4.5	2.75± 0.03	2.90± 0.03	0.226

Table-4: Effect of Wheatgrass juice on Healthy Volunteers with respect to changes in parameter related to Biochemical activity (n=30) for Male Volunteers

Test	Normal Value	Mean Value Before supp. (n=30)	Mean Value After supp. (n=30)	Statistical 't' test 'p value'
S.G.P.T.	0.00 - 40.00	24.69±0.54	24.91±0.50	0.886
S.G.O.T.	0.00 - 40.00	24.14±0.35	25.51±0.30	0.411
alakaline Phosphatase	53.0 - 128.0	69.06±1.03	64.99±0.89	0.263
S.Bilirubin (Total)	0.3 to 1.0	0.54±0.01	0.44±0.01	0.076
S.Bilirubin (Direct)	0.0 to 0.3	0.25±0.00	0.25±0.00	0.827
S.Bilirubin (Indirect)	0.1 to 1.0	0.28±0.01	0.19±0.01	0.056
Total protein	6.0 to 8.3	7.23±0.02	7.04±0.02	0.049
Albumin	3.5 to 5.0	3.91±0.03	3.71±0.02	0.113
Globulins	2.5 to 2.8	3.32±0.01	3.33±0.03	0.960
A/G Ratio		1.18±0.01	1.14±0.02	0.578
Gamma G.T.	16.0 - 86.0	54.17±0.55	50.09±0.57	0.120
Blood Urea	15.00 - 39.00	30.67±0.35	26.90±0.29	0.010
Serum Creatinine	0.90-1.50	1.04±0.01	0.97±0.01	0.044
Sodium	135.0 - 145.0	138.88±0.10	140.92±0.11	<0.001
Potassium	3.5 - 5.5	4.21±0.02	4.13±0.02	0.420
Chlorides	95.0 - 105.0	98.20±0.09	99.07±0.13	0.109

Table-5: Effect of Wheatgrass juice on Healthy Volunteers with respect to changes in parameter related to Biochemical activity (n=30) for Female Volunteers

Test	Normal Value	Mean Value Before supp. (n=30)	Mean Value After supp. (n=30)	Statistical 't' test 'p value'
S.G.P.T.	0.00 - 40.00	24.91± 0.48	25.25±0.44	0.427
S.G.O.T.	0.00 - 40.00	22.61± 0.36	22.50±0.34	0.883
Alakaline Phosphatase	42.0 - 98.0	53.88± 0.52	54.31±0.50	0.113
S.Bilirubin (Total)	0.3 to 1.0	0.56± 0.01	0.47±0.01	0.014
S.Bilirubin (Direct)	0.0 to 0.3	0.23± 0.00	0.26±0.00	0.139
S.Bilirubin (Indirect)	0.1 to 1.0	0.32± 0.01	0.21±0.01	0.019
Total protein	6.0 to 8.3	6.81± 0.04	6.98±0.03	0.132
Albumin	3.5 to 5.0	4.19± 0.03	4.42±0.04	0.048
Globulins	2.5 to 2.8	2.62± 0.03	2.56±0.04	0.626
A/G Ratio		1.64± 0.02	1.83±0.04	0.123
Gamma G.T.	12.0 - 48.0	30.21± 0.62	29.49±0.62	0.487

Blood Urea	15.00 - 39.00	24.53± 0.30	24.40±0.27	0.905
Serum Creatinine	0.60 - 1.50	0.92± 0.01	0.92±0.01	0.899
Sodium	135.0 - 145.0	139.11± 0.13	139.53±0.15	0.397
Potassium	3.5 - 5.5	4.38± 0.03	4.31±0.02	0.604
Chlorides	95.0 - 105.0	99.60± 0.18	99.00±0.16	0.447

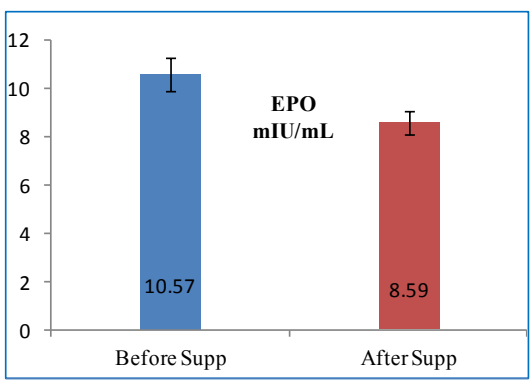
•P<0.05 were statistically significant

Estimation of Interleukins & cytokines by ELISA

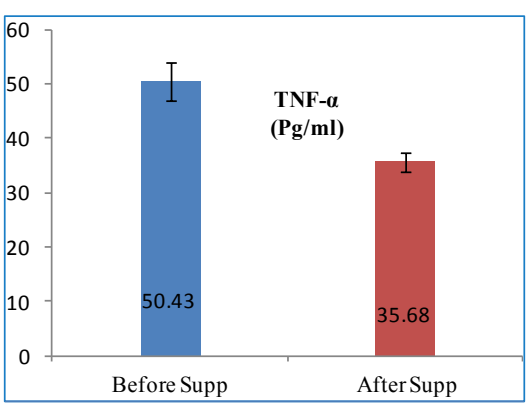
Figure-1(A-F) depicts mean values of serum EPO, TNF-α, IL-1, IL-3, IL-6 and GM-CSF levels before and after supplementation. There was significant increase in IL-6 & GMCSF level but there was significant decrease in EPO, TNF-α, IL-1 & IL-3 level in day 14 serum as compared to day 0.

(F) Alterations in GM-CSF level due to WGJ.

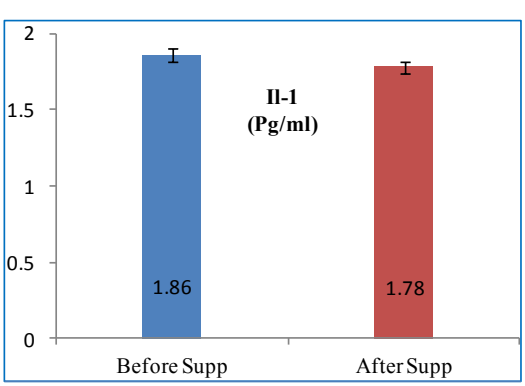
Figure- 1 Result of Cytokine study in Healthy Volunteers by ELISA method



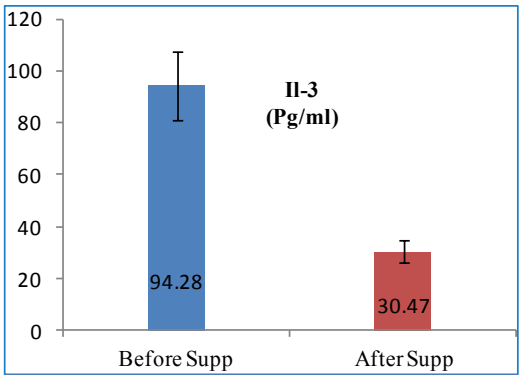
(A) Alterations in EPO level due to WGJ.



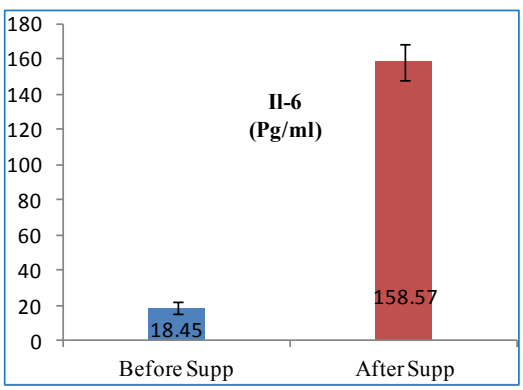
(B) Alterations in TNF-α level due to WGJ.



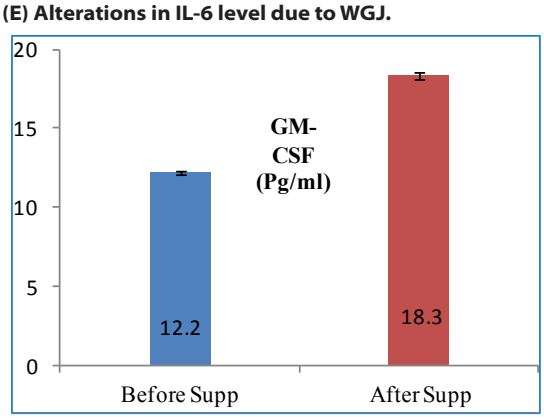
(C) Alterations in IL-1 level due to WGJ.



(D) Alterations in IL-3 level due to WG



(E) Alterations in IL-6 level due to WGJ.



(F) Alterations in GM-CSF level due to WGJ.

DISCUSSION

All cellular blood elements are derived from hematopoietic stem cells residing within the medulla of the bone (bone marrow) and have the unique ability to give rise to all of the various mature blood cell types and tissues. Development of blood cells is regulated by numerous growth factors and cytokines released by stromal cells, as well as T cells and mature leukocytes within the bone marrow. The specific regulatory functions of T cells and their role in hematopoiesis are to be yet elucidated. [6] The proliferation and self-renewal of these cells rely upon stem cell factor (SCF), a glycoprotein growth factors, which regulate the proliferation and maturation of the cells that enter the blood from the marrow and cause cells in one or more committed cell lines to mature and proliferate. Three more factors that stimulate the production of committed stem cells are called colony-stimulating factors (CSFs) and include granulocyte-macrophage CSF (GMCSF), granulocyte CSF (G-CSF) and macrophage CSF (M-CSF). These stimulate much granulocyte formation and are active on either progenitor cells or end product cells. [7]

Interleukin-6 (IL-6) is a multifunctional cytokine with a major role in inflammatory responses and is the primary inducer of acute phase proteins. IL-6 also regulates the proliferation and differentiation of he-

matopoietic cells from several lineages at different maturation stages. In synergy with IL-3, IL-6 stimulates proliferation of erythroid progenitors, megakaryocyte, and granulocyte-macrophage. This cooperation has also been shown in several in vivo model systems designed to evaluate the effect of IL-6 on the stem cell compartment.[8] Many a times the roles of IL6 in vitro and in vivo are contradicting. There are reports indicating that elevated IL6 level induces hepcidin expression which intern regulate iron absorption and metabolism leading to anemia. Finding of our current study reveal elevated IL-6 in response to WGJ supplementation. This may be one of the co-inducer of CFU-e in vitro, however it may also be the culprit in inducing mild decrease in Hb level as indicated in few volunteers possibly through hepcidin pathway.

Interleukin-1α and interleukin-1β are 17.5kDa peptides which exert a wide variety of biological activities. IL-1 has been shown to have profound stimulatory effects on hematopoietic cell growth both in vitro and in vivo. Although IL-1 alone does not induce the proliferation and differentiation of hematopoietic progenitor cells in vitro, IL-1 synergizes with the colony stimulating factors (CSFs) to promote the growth of bone marrow cells (BMCs) in soft-agar colony assays. Our result also corroborates with above findings and seems to be the causative factor for developing CFU-e in presence of supplemented sera. [9]

Interleukin 3 is a pleiotropic factor produced primarily by activated T cells that can stimulate the proliferation and differentiation of pluripotent hematopoietic stem cells as well as various lineage committed progenitors. IL-3 influences the proliferation and maturation of stem cells, as well as progenitors of granulocyte, macrophage and basophil lineages. Whereas GM-CSF functions as a growth factor for granulocyte, macrophage and eosinophil lineages. IL3 alone or in combination with other colony stimulating factors or Epo is probably useful in the reconstitution of bone marrow and in stimulating erythropoiesis. In the present study we obtained low IL3 and comparable EPO level in supplemented group suggestive of a functional pathway independent of EPO and IL-3 possibly involving GM-CSF.

GM-CSF can be produced by a wide variety of tissue types, including T cells, endothelial cells, macrophages, fibroblasts, many types of tumor cells, epithelial cells and mesothelial cells. In most of these tissues, inflammatory mediators, such as interleukin 1 & 6 and TNF-α are potent inducers of GM-CSF gene expression. [10]TNF-α is a monocyte derived cytotoxin that has been implicated in tumor regression, and cachexia. In vitro studies have shown that tumor necrosis factor (TNF-α) and interleukin-1 (IL-1) inhibit EPO mRNA synthesis. which indicates that hypoproliferative response of the marrow in cancer patients could be a cytokine- mediated phenomenon.[11] Because TNF-α exhibits anti tumor activity, so research has been conducted to determine the protein's effectiveness against certain forms of cancers. As we obtained low TNF-α level in supplemented group it eliminates the possibility of its tumoricidal use. Its role in anemia causation has been reported earlier. Elevated TNF-α, impairs HSC survival, homing and proliferation, or indirectly change the bone marrow microenvironment critical for HSC homeostasis and function, therefore contributing to development of anemia. Current study showed low TNF-α level possibly favoring the erythropoiesis in supplemented group.[12]

The present study favors supplementation of wheatgrass juice as nutritional supplement which may be useful in hepatic and renal function. Alterations in interleukins, TNF- α and GMCSF suggestive of its possible role in hematopoiesis which needs to be confirmed by invitro-assays using HSC model for CFU-e induction. Further role of elevated IL-6 need to be assessed with reference to hepcidin expression before establishing the role of wheatgrass juice as rectifier of anemia.

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