European Journal of Medicinal Plants
2(2): 113-124, 2012

SCIENCEDOMAIN *international*
www.sciencedomain.org

In vitro Studies of Iron Chelation Activity of Purified Active Ingredients Extracted from *Triticum aestivum* Linn. (Wheat Grass)

Priyabrata Das[1], Ashis Mukhopadhyay[1*], Suvra Mandal[2], Bikas Chandra Pal[3], Raghwendra Mishra[4], Debarati Mukherjee[4], Soma Mukhopadhyay[1], Jayasri Basak[1] and Manoj Kar[4]

[1]Netaji Subhas Chandra Bose Cancer Research Institute, 16A Park Lane, Kolkata-700016, West Bengal, India.
[2]National Research Institute for Ayurvedic Drug Development, Kolkata-700091, West Bengal, India.
[3]NIPER, Indian Institute of Chemical Biology, Jadavpur, Kolkata-700032, West Bengal, India.
[4]N.R.S Medical College & Hospital, Kolkata-700014, West Bengal, India.

Research Article

Received 6th January 2012
Accepted 27th January 2012
Online Ready 23rd February 2012

ABSTRACT

Objective: Seven to eight days germinated plants of *Triticum aestivum* (wheat grass) are a rich source of vitamin A, C, calcium, magnesium, phosphorus, potassium, sodium, sulphur, cobalt, zinc and protein. Traditionally the aqueous extract of *T. aestivum* was reported to be used as a health tonic in folk and ayurvedic medicine. We previously reported that aqueous extract of *T. aestivum* was found to reduce the blood transfusion requirement in iron overloaded Thalassemia and Myelodysplastic syndrome patients. Our objective was to extract and purify active ingredients from wheat grass and study their mode of action in stabilizing hemoglobin level in those patients.
Design and Method: Active ingredients of wheat grass were extracted and purified by cation exchange column chromatography followed by High Performance Liquid Chromatography. *In vitro* experiments with phenylhydrazine treated red blood cell hemolysate were carried out before and after treatment of purified fraction of *T. aestivum* to study iron chelating activity.
Result: Purified fraction of *T. aestivum* treated red blood cell showed significant inhibition

**Corresponding author: Email: molecularbiology.ncri@gmail.com;*

of free reactive iron production and formation of thio-barbituric acid reactive substances when compared to desferrioxamine treated hemolysate.
Conclusion: Iron chelating activity of purified fraction of *T. aestivum*, an inexpensive, easily available source, is very promising for further clinical trial and development of oral iron chelator drug for Thalassemia, Myelodysplastic syndrome and other iron overloaded diseases.

Keywords: Iron chelation; wheat grass extract; mugineic acid; thalassemia; myelodysplastic syndrome; HPLC; iron overload; oral iron chelator drug.

1. INTRODUCTION

Iron overload is a threatening measure for transfusion dependent Thalassemia patients because there is no mechanism for the excretion of excessive iron and accumulated iron is responsible for elevated oxidative stress in biological system. In transfusion dependent Thalassemia, iron is accumulated in different organs, mainly, heart, liver, spleen, pituitary etc. and produces dreadly damaging hydroxyl radicals in presence of superoxide or H_2O_2. Further, cellular constituents like lipid, protein, carbohydrate and nucleic acid are also damaged by this radical attack. To remove accumulated iron, chelation therapy is thus recommended for patients with iron overload (Brittenham et al., 1994; Olivieri et al., 1994; Olivieri and Brittenham, 1997).

For 30 years, desferrioxamine (DFO) has been used as a drug of choice for treatment of transfusional iron overload. DFO has been shown to be a generally safe and efficient mean of controlling body iron that can prolong survivals and prevent ameliorated organ dysfunctions. But DFO is poorly absorbed from gastro intestinal tract and rapidly eliminated from the circulation. So, prolonged parenteral infusion is thus needed. Effective therapy usually requires subcutaneous and intravenous administration by a portable infusion pumps for 9-12 hours daily (Cao et al., 1996; Gabutti and Piga, 1996; Giardina and Grady, 1995; Weatherall, 1993).

Although DFO has shown adequate control of body iron but inexpensive and oral therapy for iron chelation is highly demanded and thus an ideal chelator is eventually required to treat the patients with transfusional iron overload. The characteristics of such a compound can extrapolate the followings from the clinical requirements:

i) Oral administration
ii) Good tissue penetration
iii) Easy mobilization of iron chelator complex
iv) Inexpensive
v) Non-toxic
vi) Hexadentate binding of iron ions

Till date most of the efforts have been concentrated on “bi” and “tri-dentate” iron chelating agent that remain active after oral administration to develop substitute of DFO iron chelating therapy. However, partial ligands might exacerbate iron toxicity. A hexadentate nontoxic oral ligand is further warranted (Wiwanitkit et al., 2010).

The mechanisms of iron acquisition in plant kingdom (higher plant) have been grouped into strategy I (Jin, et al., 2009) and strategy II (Ma and Nomoto, 1993; Wiren and Khodr, 2000). The strategy II is, so far, only found in some graminaceous plants such as barley and wheat (Ma and Nomoto, 1993; Wiren and Khodr, 2000). It has been reported that *Triticum aestivum* (Wheat grass) plants can solubilize iron from soil by secretion of phytosiderophores (mugineic acid derivatives) in root and shoot parts and iron uptake has been proposed to be maintained by a putative transporter for the phytosiderophore-ferric complex without a reduction process.

Recent advances in our understanding of how graminaceous plants, grown in low iron containing soil, take up insoluble forms of iron from the rhizosphere and mobilize them in plant tissue are preliminary based on the identification of various transporters that are specific to metal-phytosiderophore complexes containing mugineic acid and deoxymugineic acid (Namba et al.,2010). The phytosiderophore-ferric complex has an octahedral six-coordinate structure. As the phytosiderophore binds to all six sites of iron completely it inactivates the free "iron" forming a "hexadentate" chelator (Wiren and Khodr, 2000) (Fig. 1.). Density functional theory methods combined with the polarizable continuum model have been employed by Kato et al. (2011) to understand the metal-chelating mechanism of phytosiderophore mugineic acid at an atomic level.

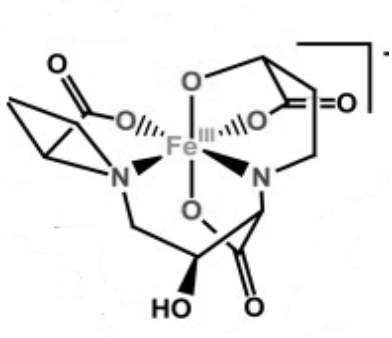

Fig. 1. Structure of phytosiderophore-ferric complex (hexadentate chelator) (Kato et al., 2011)

This natural phenomenon tempted us to utilize the aqueous extract of wheat grass (*Triticum aestivum* Linn.) containing phytosiderophores for chelating free "iron" for treatment of transfusional iron overload. On the basis of our preliminary findings we've reported that the aqueous extract of *T. aestivum* helps to stabilize the haemoglobin level, and thus reduces the blood transfusion requirement when fed to the iron overloaded Thalassemia and MDS patients (Mukhopadhyay et al., 2007, 2009).

7-8 Days germinated plants of *T. aestivum* are a rich source of vitamin A & C. It is also an excellent source of calcium, magnesium, phosphorus, potassium, sodium, sulphur, cobalt, zinc and protein. It is a superior detoxification agent compared to carrot juice and other fruits and vegetables. Dr. Erap-Thomas, associate of Ann Wigmore, observed that 15 pounds of wheat grass is the equivalent to 350 pounds of carrot, lettuce, celery and so forth (Bhatti, 2003). Traditionally the aqueous extract of *T. aestivum* was reported to be used as a health tonic in folk and ayurvedic medicine. Recently, it has been reported the extract of *T.aestivum* increases the haemoglobin level in different types of anemic patients clinically (Marwaha et al., 2004; Pole, 2006; Singh et al., 2010).

Till date, there is no report of mechanism of action of wheat grass juice for increasing haemoglobin level in anemia. So, in our present study we have tried to find out whether iron chelating components are present in the aqueous extract of wheat grass and whether the iron chelating activity is the actual mode of function of wheat grass juice in reducing the blood transfusion requirement in iron over loaded patients.

In the present study we have performed an *in vitro* model of over expressed non haem iron (Ferrali et al., 1992; Ramot et al., 2007) and chromatographically purified fractions from root and shoot extract of *T. aestivum* containing mainly mugineic acid derivatives were used to measure the iron chelating property and inhibition of lipid peroxidation activity on that *in vitro* model. Iron chelation property of our proposed compound was also estimated by inhibition study of deoxyribose degradation end product. All the experiments were done with purified fraction of *T. aestivum* (PFT) and also compared with DFO.

2. MATERIALS AND METHODS

Amberlite IR 120(H^+ form), Thiobarbituric acid, Deoxyribose, Phenylhydrazine (PHE), (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^+$), Trichloroethanoic acid, Xylenol orange, Ferrozine, Hydrogen peroxide were purchased from Sigma Chemicals. Desferrioxamine was purchased from Novartis Company, Switzerland.

2.1 Preparation of the Purified Fraction of the *T. aestivum* (PFT)

Plants (*T. aestivum*) were cultivated in calcareous soil at Darjeeling district, West Bengal, India and 7-13 days roots and shoots were collected. Five grams of roots and shoots of plants were crushed in 200 ml water and rectified spirit (1:1) mixture and kept for 48 hours for extraction. It is further filtered and passed through a cation exchange resin column (30X2.5 cm) filled with Amberlite IR 120(H^+ form) resins and eluted by NH_4OH (2M). The elution was concentrated to dryness by rotary evaporator (<40°C) to get a solid mass (PFT). It was used in the Biochemical studies.

The solid mass isolated from *T. aestivum* was found to contain a mixture of 3- hydroxy mugineic acid and 2'-deoxy-mugineic acid (Kawai et al., 1987), (Fig. 2A and 3). Following this method we have extracted the active ingredients from wheat grass by HPLC and analyzed them as the mugineic acid and its analogues. The analysis exhibited the presence of two major compounds (1 and 2) which are analogues of mugineic acid derivatives (Fig.2 B). Compound 1 was identified as 3- hydroxy- mugineic acid (3-HMA; relative percentage: 57.61%) and compound 2 was 2'-deoxy-mugineic acid (2'-DMA; relative percentage: 37.77%) (Fig 2B).

2.2 Preparation of RBC Hemolysate and Pre-Treatment with Purified Fractions of *T. aestivum* (PFT) and DFO for *in vitro* Experiment

Venous blood (5ml) was obtained from healthy donors (RBC: $4.59*10^6$/µl, HGB: 14.4 gm/dL) into blood collection tubes containing heparin as anticoagulant. The collected blood was centrifuged in 5000 rpm for 5 mins at 4°C. The buffy coat and plasma were removed and the erythrocytes were washed 3 times with 5 ml of 0.15M normal saline solution. Thereafter 500 µl packed cells had been taken out and diluted in 10 ml of 0.15M normal saline solution.

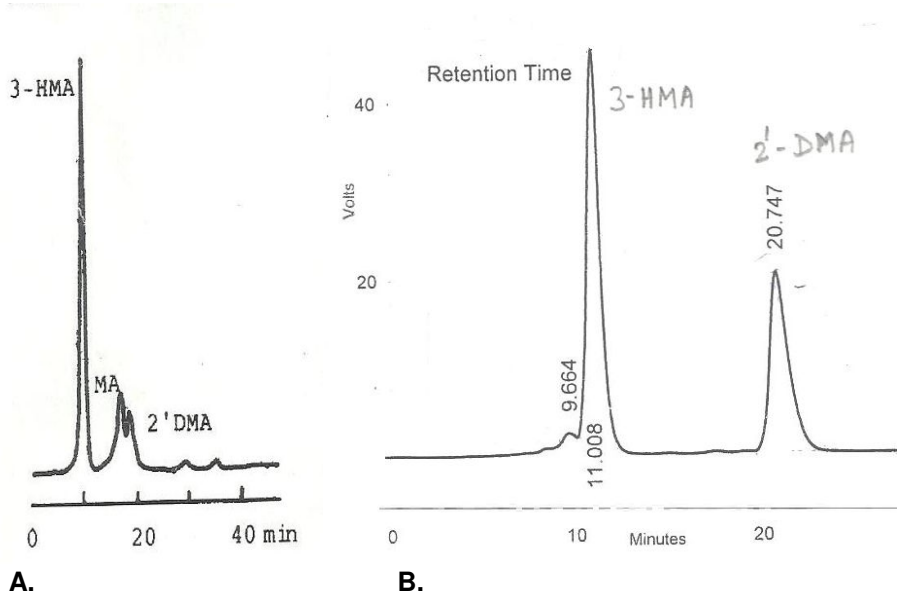

Fig. 2. HPLC analysis of (A) 3- HMA and 2'- DMA by Kawai et al., 1987; (B) Wheat grass root and shoot extract to show the presence of compound 1 (3- HMA) and compound 2 (2'- DMA) eluted at the same retention time.

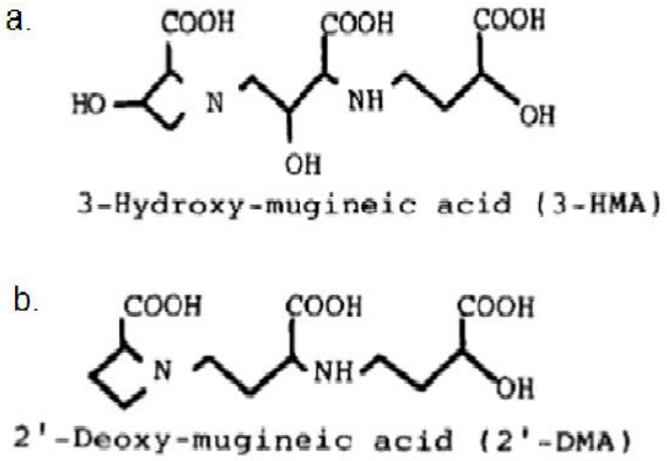

Fig. 3. Structure of (a) compound 1 (3- HMA) and (b) compound 2 (2'- DMA), extracted and purified from *T. aestivum* (Kawai et al., 1987)

The complete blood count of that diluted sample was measured by Sysmex KX-21, cell counter. The RBC concentration was $0.51*10^6$/ µl and hemoglobin (Hb) concentration was 1.7g/dL. 700 µl of erythrocyte suspensions were treated with different concentration (50-200 µg/ml) of PFT (mixture of purified compound 1 and 2) and DFO and each volume was made up to 900 µl by adding PBS. Similarly two samples were prepared by using 700 µl

erythrocyte suspensions and 200 µl Phosphate Buffered Saline (PBS) and all the samples were incubated at 37°C for 2 hours. After incubation 100 µl phenylhydrazine (0.005 mg/ml) was added in each tube except in one of the untreated samples, where 100 µl PBS was added. The last one will serve as the negative control. Then all the tubes were further incubated for another 2 hours at 37°C. Samples were then diluted for 5 times by distilled water for complete haemolysis, centrifuged at 14000 rpm for 20 minutes to precipitate the RBC membrane. Total Oxidant Status (TOS) (Erel, 2005), Thiobarbituric Acid Reactive Substances (TBARS) (Buege and Aust, 1976) and Free Reactive Iron (FRI) (Panter, 1994) of the PFT and DFO treated hemolysate were estimated following the standard protocols. All the experiments were repeated thrice and the mean of the results are mentioned here.

2.3 Inhibition of Deoxyribose Degradation Assay for Detecting Iron Chelating Activity

In performing the assay (Aruoma, 1994) the reaction mixture in a final volume of 1.2 ml contained 2.8 mM deoxyribose, 25 mM $FeCl_3$, 100 µM EDTA, 2.8 mM H_2O_2 in 10 mM phosphate buffer pH 7.4 and different concentration of PFT and DFO were added in it. Finally 100 µM ascorbate is added to start the reaction. After incubation at 37°C for 1 hour, 1ml of 1% TBA in 15mmol NaOH and 1ml of 2.8% TCA were added. The mixture was heated at 90°C for 30 minutes. Samples were cooled and the colour developed, is extracted in 2ml Butanol. Colour absorbtion was read at 532 nm. Percentage of inhibition was calculated in respect of the values of that of control.

2.4 Statistical Analysis

Statistical calculations for three sets of experiments have been done accordingly and all values were expressed as mean ± SEM (Standard Error of Mean). Statistical evaluation was calculated by one way ANOVA. For all comparisons, $p<0.05$ was considered as significant.

3. RESULTS

Mean results of three sets of all *in vitro* experiments have been mentioned below.

3.1 Deoxyribose Degradation Assay of the PFT and DFO

The result shows both PFT and DFO significantly inhibited the deoxyribose degradation in dose dependent manner (result is not shown here).

3.2 Total Antioxidant Status (TAS) of the PFT and DFO

Total Antioxidant Status of PFT and DFO were estimated according to the method of Re et al., 1999. Both DFO and newly isolated purified fraction showed no antioxidant activity.

3.3 Estimation of Free Reactive Iron

When normal erythrocytes were treated with phenylhydrazine, a significant level of ferrozine detected non haem iron was found, compared to control. We have treated this sample with different concentration of our newly isolated PFT and DFO before phenylhydrazine treatment. Result shows both the PFT and DFO can reduce the free reactive iron production

significantly at 100 µg/ml and 200 µg/ml concentration. However at 50 µg/ml concentration it does not show significant change, as shown in the Fig. 4.

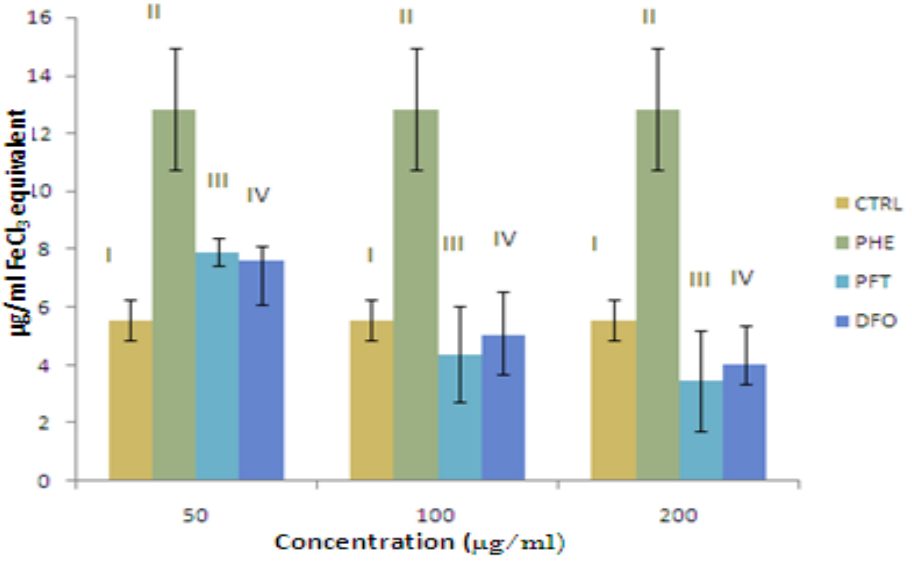

Fig. 4. The comparative study of iron chelating property of PFT and DFO by inhibition of Ferrozine detected non haem iron formation. (I) control haemolysate with no phenylhydrazine, DFO or PFT; (II) only phenylhydrazine treated control hemolysate with no DFO or PFT; (III) phenylhydrazine treated hemolysate with different concentration of PFT; (IV) phenylhydrazine treated hemolysate treated with different concentration of DFO.

Values represent means ± SEM for 3 sets of experiments.

3.4 Thiobarbituric Acid Reactive Substances (TBARS) Production Inhibition

Thiobarbituric acid reactive substances or TBARS level, an indicator of cellular damage mediated by the free radical injury, was found to be significantly increased in phenylhydrazine treated erythrocytes when compared to control. But when DFO and PFT were treated with various concentrations with RBC hemolysate before addition of phenylhydrazine, the level of TBARS was found to be significantly reduced in case of 200 µg/ml concentration of the PFT only. But no significant change was found in DFO at the same concentration, as shown in Fig. 5.

3.5 Effect of Total Oxidant Status (TOS) by DFO and the PFT

Different concentration of DFO and the PFT were treated with erythrocyte suspension before phenylhydrazine treatment. The level of TOS after haemolysis was estimated according to the method of Erel, 2005. The level of TOS in both DFO and PFT treatment are not significantly changed although the values were reduced in dose dependent manner, as shown in the Fig. 6.

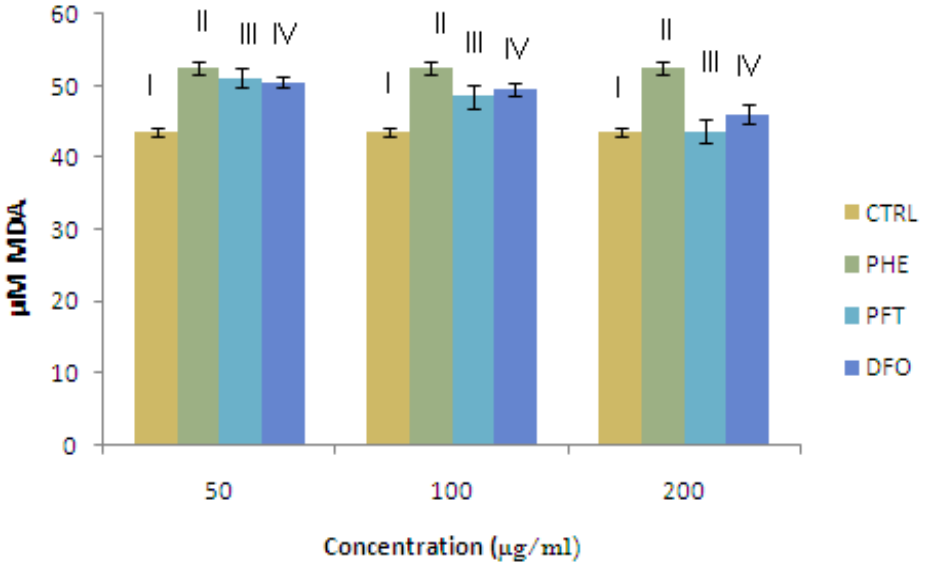

Fig. 5. The comparative study of TBARS production inhibition of PFT and DFO treatment. (I) control haemolysate; (II) phenylhydrazine treated control; (III) phenylhydrazine treated hemolysate with different concentration of PFT; (IV) phenylhydrazine treated hemolysate treated with different concentration of DFO.

Values represent means ± SEM for 3 sets of experiments.

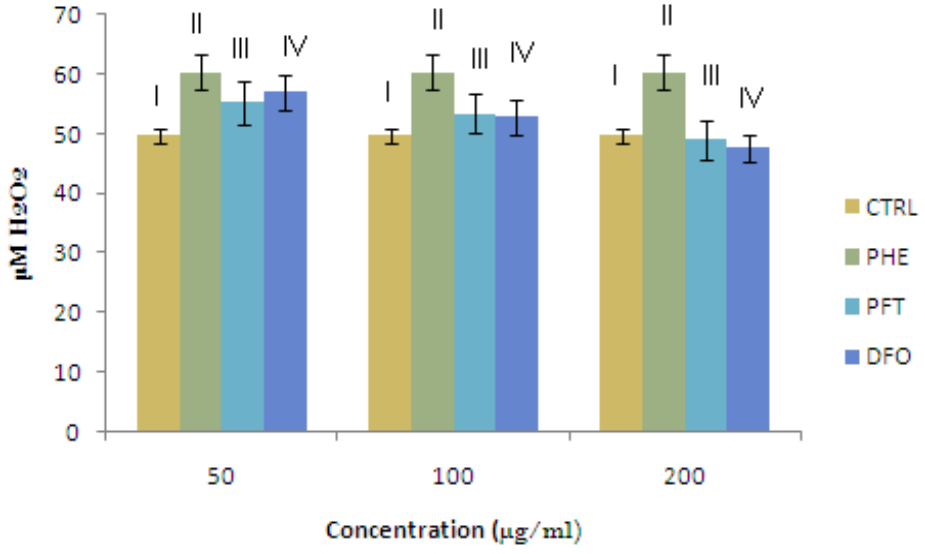

Fig. 6. The comparative study of TOS estimation in (I) control haemolysate, (II) phenylhydrazine treated control, (III) phenylhydrazine treated hemolysate with different concentration of PFT, (IV) phenylhydrazine treated hemolysate treated with different concentration of DFO.

Values represent means ± SEM for 3 sets of experiments.

4. DISCUSSION

T. aestivum is an important graminaceous plant species can acquire iron for physiological need from soil by release of phytosiderophores and by subsequent uptake of iron–phytosiderophore complexes. In this way they can compensate their physiological iron demand when cultivated in low iron containing calcareous soil. Thus enrichment of high quality of phytosiderophores may help the plant to protect chlorosis by chelating and concentrating trace amount of iron present in the soil.

Phytosiderophores are hexadentate ligands that co-ordinate iron (III) with their amino and carboxy groups and form iron (Fe) and phytosiderophore complexes (Ma and Nomoto, 1993; Wiren and Khodr, 2000). On the basis of the fact of iron-phytosiderophore complex formation in *T. aestivum*plant root and shoot, we developed a concept of iron chelation activity of root and shoot extract of *T. aestivum* when applied to the patients with iron overload.

Some investigators reported that oral intake of aqueous extract of *T. aestivum* may help to maintain hemoglobin level of different type of anaemic patients (Marwaha et al., 2004; Pole, 2006; Singh et al., 2010). One group of investigators reported the reduction of transfusion requirement of Thalassaemia patients (Marwaha et al., 2004) whereas the other group claimed that *T. aestivum* aqueous extract raises the foetal haemoglobin level (Reynolds, 2005) possibly by gene signaling and upregulating foetal haemoglobin level which is more stable compared to defective beta-thalassaemia haemoglobin. However, foetal haemoglobin is not a suitable transporter of oxygen in adult life as it has less cooperative activity and more oxygen affinity that inhibit foetal hemoglobin to release oxygen in cell. Thus there might be some other mechanism to stabilize the haemoglobin level by *T. aestivum* aqueous extract.

We reported for the first time that *T. aestivum* aqueous extract (crude extract) may contain iron chelating property and oral intake of whole wheat grass juice may be beneficial for iron overloaded diseases like Thalassaemia and in MDS (Mukhopadhyay et al., 2007, 2009).

In the present study we have extracted and purified the active ingredients from aqueous extract of *T. aestivum* by cation exchange chromatography. The white crystalline form of eluted compounds after rotary evaporation was found to contain predominantly 3 hydroxy-mugineic acid and 2 deoxy-mugineic acid which can form hexadentatephytosiderophore-iron complex (Ma and Nomoto, 1993; Wiren and Khodr, 2000).

We further studied in vitro, the iron chelating property of the components along with the same concentration of DFO on phenylhydrazine treated iron overloaded RBC model. Our results as depicted in Fig. 4, show that at high concentration (100 µg/ml and 200 µg/ml) active compounds isolated from *T. aestivum* (PFT) have more iron chelating activity than that of DFO, of same concentration. But at low concentration (50 µg/ml) DFO shows iron chelating activity in a slightly higher side. It is also interesting to note that the active compounds can penetrate erythrocytic membrane and chelate intra cellular non-haem iron like DFO because we had treated the intact RBC samples with DFO and PFT before haemolysis in our experiment.

Formation of the cellular damage marker TBARS is further found to be significantly inhibited when compared to the control and also with DFO at 200 µg/ml concentration. Moreover, our novel substances showed significantly higher inhibition property of lipid peroxidation at this concentration. These results clearly indicated that iron mediated hydroxyl radical formation

(by Fenton reaction) was inhibited by hexadentate chelating property of the active compounds of *T. aestivum* which finally reduced cellular damage and all the results were quite compatible with the known iron chelator DFO.

Total Oxidant Status (TOS) is a marker for pro-oxidant level of the cell (Erel, 2005). In the phenylhydrazine treated erythrocytes, iron is released from haemoglobin and show high prooxidant status possibly by formation of hydroxyl radical with non haem iron mediated Fenton reaction. The level of TOS was found to decline when dose dependent DFO and PFT were used. These findings directed us to conclude that the mugineic acid derivatives present in aqueous extracts of *T. aestivum* may act as iron chelator efficiently like DFO. The more advantage of this compound is that it can be taken up orally as our previous report (Mukhopadhyay et al., 2007, 2009) showed that nutritional drink of wheat grass juice does not have any toxic effect on patients and maintain the level of the Hb of iron overloaded diseases mainly transfusion dependent Thalassemia and MDS.

Total Antioxidant Status (TAS) of DFO and PFT were found to be insignificant when estimated by $ABTS^{+}$ method (Re, et al., 1999). But both the PFT and DFO are hexadentate iron chelators. Thus the iron chelating activity is predominantly thought to be responsible for protection of cellular damage.

DFO is a well known iron chelator. It is commonly used to remove the excessive iron, released in the patients of iron overloaded disorders like Thalassaemia and transfusion dependent MDS. But being very expensive, DFO cannot be affordable to all of the patients especially in developing countries. Moreover, medical paraphernalia is required for DFO treatment, where as the active compounds of wheat grass can be used as oral iron chelator and being very inexpensive, it can be affordable to all of the patients of different economic groups. No medical supervision is needed for this therapy.

In our preliminary study, we suggested 200 transfusion dependent Eβ, E and sickle Thalassaemia patients (Mukhopadhyay et al., 2007) and 20 transfusion dependent MDS (Mukhopadhyay et al., 2009) patients for consumption of whole wheat grass juice with mean ferritin level of 2250 ng/ml (Mukhopadhyay et al., 2007) and mean haemoglobin level of 6.2% (Mukhopadhyay et al., 2007) before treatment. But after 6 months of consumption we observed the increase of mean haemoglobin level (7.8%) and decrease of mean serum ferritin level (950 ng/ml) of those patients.

A group of investigators claimed that the structural resemblance of chlorophyll and haemoglobin is responsible for the stabilization of haemoglobin level in wheat grass juice treated Thalassaemia patients (Pole, 2006). But being a major constituent of green leafy vegetables, chlorophyll is consumed by us; so in that way wheat grass juice should not have any specialty to reduce the transfusion requirement of Thalassemia patients, as observed by us and other investigators (Marwaha et al., 2004; Mukhopadhyay et al., 2007, 2009).

In our present study the *in vitro* experiments clearly indicate the iron chelation property of the active compounds of wheat grass extracts and its iron chelation activity is significantly comparable with the efficient iron chelator DFO.

5. CONCLUSION

The present *in vitro* experiments clearly show the iron chelation property of the active components of *T. aestivum* extract and significant inhibition of free reactive iron production as well as formation of TBARS. These properties could be extended for further animal experiments and clinical studies towards the development of a new oral iron chelator for iron overloaded diseases.

ACKNOWLEDGEMENTS

We sincerely acknowledge Abhijit Chakraborty, Deboshree Majumdar, Swati Dasgupta, Sukanta Koner, Dr. S.B. Mandal and Dr. Amartya Pal for their kind co-operation and help. We are thankful to all the patients and other staffs of Netaji Subhas Chandra Bose Cancer Research Institute for their continuous support.

COMPETING INTERESTS

Authors have declared that no competing interest exists.

REFERENCES

Aruoma, O.I. (1994). Deoxyribose assay for detecting hydroxyl radical.Oxygen Radicals in Biological Spectrum, 233, 57-66.

Bhatti, S. (2003). Wheat Grass: A potential polycrest remedy. Germany.

Brittenham, G.M., Griffith, P.M., Neinhuis, A.W., McLlaren, C.E., Young, N.S., Tucker, E.E., Allen, C.J. Farrell, D.E., Haris, J.W. (1994). Efficacy of desferrioxamine preventing complications of iron overload in patients with Thalassemia major. N Eng J Med, 331, 567-573.

Buege, J.A., Aust, S.D. (1976). Lactoperoxidase-catalyzed lipid peroxidation of microsomal and artificial membranes.Biochim Biophys. Acta (BBA) - General Subjects, 444, 192-201.

Cao, A., Galaneilo, R., Rosateui, M.C., Argiolu, F., Virgiiiis, D. (1996). Clinical experience of management of Thalassemia, the Sardinian experience. SeminHematol, 33, 66-75.

Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. Clin Biochem, 38, 1103–1111.

Ferrali, M., Signorini, C., Ciccoli, L., Comporti, M. (1992). Iron release and membrane damage in erythrocytes exposed to oxidizing agent, phenylhydrazine, divicine and isouramil. Biochem J., 285, 295-301.

Gabutti, V., Piga, A. (1996). Results of long term iron chelating therapy. Acta Haematol, 95, 26-36.

Giardina, P.J., Grady, R.W. (1995). Chelation therapy in beta Thalassemia, the benefits and limitation of desferrioxamine. SeminHematol, 32, 304-312.

Jin, C.W., Du, S.T., Chen, W.W., Li, G.X., Zhang, Y.S., Zheng, S.J. (2009). Elevated Carbon Dioxide Improves Plant Iron Nutrition through Enhancing the Iron-Deficiency-Induced Responses under Iron-Limited Conditions in Tomato. Plant Physiol., 150, 272–280.

Kato, M., Izuka, S., Fujihara, T., Nagasawa, A., Kawai, S., Tanaka, T., Takayanagi, T. (2011). Electronic structure calculation study of metal complexes with a phytosiderophoremugineic acid. Inorganica Chimica Acta, 370, 304-310.

Kawai, S., Sato, Y., Takagi, S.I. (1987). Separation and determination of mugineic acid and its analogues by high-performance liquid chromatography. J Chromatography, 391, 325-327.

Ma, J.F., Nomoto, K. (1993). Two related biosynthetic pathways of mugineic acids in Gramineous plants. Plant Physiol, 102, 373-378.

Marwaha, R.K., Bansal, D., Kaur, S., Trehan, A. (2004). Wheat grass juice reduces transfusion requirement in patients with thalassemia major: a pilot study. Ind Ped, 41, 716-720.

Mukhopadhyay, S., Mukhopadhyay, A., Gupta, P.R., Kar, M., Ghosh, A. (2007). The role of iron chelation activity of wheat grass juice in blood transfusion requirement of intermediate Thalassaemia. ASH Annual Meeting Abstracts 110.

Mukhopadhyay, S., Basak, J., Kar, M., Mandal, S., Mukhopadhyay, A. (2009). The role of iron chelation activity of wheat grass juice in patients with myelodysplastic syndrome. J Clin Oncol, 27, 15. (ASCO Annual Meeting).

Namba, K., Kobayashi, K., Murata, Y. (2010). Mugineic acid derivatives as molecular probes for the mechanistic elucidation of iron acquisition in barley. Angewandte Chemic Int Edition, 49, 9956-9959.

Olivieri, N.F., Nathan, D.G., MacMillan, J.H., Wayne, A.S., Liu, P.P., MacGee, A., Martn, M., Koren, G., Kohen, A.R. (1994). Survival in medically treated patients with homozygous beta-thalassemia. N Eng J Med, 331, 574-578.

Olivieri, N.F., Brittennam, G.M. (1997). Iron chelating therapy and the treatment of thalassemia. Blood, 89, 739-761.

Panter, S.S. (1994).Release of iron from hemoglobin.Methods Enzymol, 231, 502-514.

Pole, S.N. (2006). Wheat grass juice in thalassemia. Ind Ped, 43, 79-80.

Ramot, Y., Koshkayev, A., Goldfrab, A., Yedgar, S., Barhtein, G. (2007).Phenylhydrazine as a partial model for β-thalassemia red blood cell hemodynamic properties. Br J Haematol, 140, 692-700.

Re, R., Pellegrini, N., Proteggente, A., Pannala, M, Yang, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med, 26, 1231-1237.

Reynolds, C. (2005). A DNA-technology-based cellular assay used to measure specific biological activity in a wheatgrass extract. J Australasian Int Med Assoc.

Sharma, R., Pancholi, S. (2010). Oral iron chelator: a new avenue for the management of thalassemia major. J Curr Pharma Res, 1, 1-7.

Singh, K., Pannu, M.S., Singh, P., Singh, J. (2010). Effect of wheat grass tablets on the frequency of blood transfusions in thalassemia major. Ind J Ped., 77, 90-91.

Weatherall, D.J. (1993). The treatment of thalassemia - slow progress and new dilemmas. N Eng J Med., 329, 887-879.

Wiren, N.V., Khodr, H. (2000). Hydroxylatephytosiderophores species possess an enhanced chelate stability and affinity for iron (iii). Plant Physiol, 124, 1149-1157.

Wiwanitkit, V. (2006). Quantam chemical analysis of the deferiprone-iron binding reaction. Int J Nano Med, 1, 111-113.