INVESTIGATION INTO BENEFICIAL EFFECTS OF *TRITICUM AESTIVUM* (WHEAT GRASS) IN IRON OVERLOAD COMPLICATIONS

P. R. Tirgar[1]*, T. R. Desai[2]

Authors' addresses

1. Pravin Rambhai Tirgar*
Department of Pharmacology,
R. K. College of Pharmacy,
Kasturbadham, Tramba,
Rajkot- 360020, Gujarat – India.

2. Tusharbindu Rameshchandra Desai,
R. K. College of Pharmacy,
Kasturbadham, Tramba,
Rajkot- 360020, Gujarat – India.

Pravin Rambhai Tirgar*
M: +91 9913110021
E-mail - tirgar_pr@yahoo.com

Summary

In medicine, iron overload disorders are diseases caused by the accumulation of iron in the body. Iron overload is one of the major causes of morbidity in all patients with severe forms of thalassemia. Excess iron in vital organs, even in mild cases of iron overload, increases the risk for liver disease (cirrhosis, cancer), heart attack or heart failure, diabetes mellitus etc. and in some cases premature death. Iron mismanagement resulting in overload can accelerate such neurodegenerative diseases as Alzheimer's, early-onset Parkinson's, Huntington's, epilepsy and multiple sclerosis.

Triticum aestivum, wheatgrass is a cereal grass of Gramineae (Poaceae) family has been traditionally used, to treat various diseases like cancers, diabetes, gastritis, ulcers, pancreas and liver problems, anemia, skin problems etc. Wheat grass is a rich source of phenolic and flavanoid content, which known for antioxidants and also possess chelating property.

The present study was planned to evaluate beneficial effects of wheatgrass in iron overloaded complications in animals. Water and methanol extracts of wheatgrass were found to be significant reduction in serum iron levels in iron overload rats compare to diseases control rat.

We observed that water and methanol extracts of *T. aestivum* strikingly reduce SGPT, SGOT, serum creatinine and serum creatinine kinase levels in iron overloaded rats compare to disease control rats and which was found to be very near to desferoxamine drug group which is a standard iron chelator use in treatment of iron overload in thalassemia.

In conclusion, our data suggest *Triticum aestivum* possess beneficial effect in iron over load disorders due to its iron chelation property.

Keywords: *Triticum aestivum,* iron overload, iron overload complications, chelating property.

Introduction

Iron is an absolute requirement for most forms of life, including humans and most bacterial species, because plants and animals all use iron; hence, iron can be found in a wide variety of food sources. The control of this necessary but potentially toxic substance is an important part of many aspects of human health and disease.

Iron is essential to life because of its unusual flexibility to serve as both an electron donor and acceptor. Iron can also be potentially toxic. Its ability to donate and accept electrons means that if iron is free within the cell, it can catalyze the conversion of hydrogen peroxide into free radicals. Free radicals can cause damage to cellular membranes, proteins, and DNA, a wide variety of cellular structures, and ultimately kill the cell. To prevent that kind of damage, all life forms that use iron bind the iron atoms to proteins. That allows the cells to use the benefits of iron, but also limit its ability to do harm. [1]

In medicine, iron overload disorders are diseases caused by the accumulation of iron in the body. Iron overload is one of the major causes of morbidity in all patients with severe forms of thalassemia, regardless of whether they are regularly transfused. A variety of other iron overload diseases are present. These are usually associated with chronic anemias. These are Thalassemia, sideroblastic anemia, abnormal red cell production (dyseryphropoiesis), iron overload secondary to IV therapy, chronic liver disease secondary to alcohol, porphyria cutanea tarda.[2]

Iron toxicity results when the amount of circulating iron exceeds the amount of transferrin available to bind it, but the body is able to vigorously regulate its iron uptake. Thus, iron toxicity from ingestion is usually result of extraordinary circumstances like iron tablet overdose rather than variations in diet. The type of acute toxicity from iron ingestion causes severe mucosal damage in gastrointestinal tract, among other problems.[3]

Excess iron in vital organs, even in mild cases of iron overload, increases the risk for liver disease (cirrhosis, cancer), heart attack or heart failure, diabetes mellitus, osteoarthritis, osteoporosis, metabolic syndrome, hypothyroidism, hypogonadism, numerous symptoms and in some cases premature death. Iron mismanagement resulting in overload can accelerate such neurodegenerative diseases as Alzheimer's, early-onset Parkinson's, Huntington's, epilepsy and multiple sclerosis. [3]

In **thalassemia major**, body mistakes anemia for iron deficiency and absorbs iron as much as 3-4 mg/day depending upon severity of anemia from the iron present in food. Absorption may increase up to 10 mg/day if iron tonics are administered to correct anemia. Iron absorbed from food adds to body iron stores. On an average each unit of blood contains 200-250 mg of iron. A patient receiving 30 units of blood/year receives 6gm of elemental iron annually. The human body is unable to excrete this extra iron from the body. Extra iron absorbed from digestive route & released from blood transfusion accumulates, gets deposited in liver, heart, kidney, endocrine glands and various organs resulting in poor functioning of these organs.[4]

Hemochromatosis - A most often hereditary blood disorder that causes body tissue to absorb and store too much iron. The disease (which is actually many diseases) has also been known to develop as a result of dietary iron intake in sufficient quantity. Its worst effects are preventable, by early diagnosis and treatment, but, if the patient is not found in time, it is crippling and potentially fatal. [5]

Modern science has already accepted the potential of herbs as a source of new bio-active constituents. There are numerous plants derived drugs of unknown chemical structure that have been found clinically useful in different alternative system of medicine including Ayurveda, Homeopathy and Unani system of medicine. The plants are rich reservoir of potential leads for drug discovery against various disorders.

Wheat, (Triticum species) a cereal grass of the Gramineae (Poaceae) family, is the world's largest edible grain cereal-grass crop. The wheat plant is an annual grass. In early growth stages the wheat plant consists of a much-compressed stem or crown and numerous narrowly linear or linear-lanceolate leaves. For over fifty years, researchers have known that the cereal plant, at this young green stage, is many times richer in levels of vitamins, minerals and proteins as compared to seed kernel, or grain products of the mature cereal plant.[6] [7]

Wheatgrass has been traditionally used, since ancient times, to treat various diseases and disorders. Presently, there are a number of wheatgrass suppliers, in almost all cities of India, supply fresh wheatgrass, on daily basis to their regular customers by home-delivery system for various ailments and as health tonic. Dr. Ann Wigmore, U. S. A. founder director of the Hippocrates Health Institute, Boston, U.S.A. was one of proponents of 'Wheatgrass Therapy'. Dr. Wigmore reported that "wheatgrass" used in her program contain abscisic acid and laetrile, both of which may have anti-cancer activity. It was also reported that young grasses and other chlorophyll-rich plants are safe and effective treatment for ailments such as high blood pressure, some cancers, obesity, diabetes, gastritis, ulcers, pancreas and liver problems, fatigue, anemia, asthma, eczema, hemorrhoids, skin problems, halitosis and constipation. [8]

Scientific reports on nutritional analysis of wheatgrass have been published frequently in various journals. These reports and chemical analyses undertaken reveal that wheatgrass

is rich in chlorophyll, minerals like magnesium, selenium, zinc, chromium, antioxidants like beta-carotene (pro-vitamin A), vitamin E, vitamin C, antianemic factors like vitamin B12, iron, folic acid, pyridoxine and many other minerals, amino acids and enzymes, which have significant nutritious and medicinal value. [9] [10] Clinically it was proved that different varieties of wheatgrass extracts are therapeutically used in treatment of anemia, thalassemia (major), cancer and bacterial diseases. [11]

There was a direct relation oberved between iron chelatory activity and phenolic content in plant. Some extracts with high phenol and flavonoid contents showed good chelating of Fe^{2+}. For example, *E. hirsutum* and *M. arvensis* that contained highest phenol and flavonoid contents showed the best chelating activity. [12]

Synthetic agents like desferrioxamine and deferiprone used for treatment of iron overload in thalassemia are accompanied by serious side effects and certain limitaions including need for parenteral administration, arthralgia, nausea, gastrointestinal symptoms, fluctuating liver enzyme levels, leucopenia, agranulocytosis and zinc deficiency and obviously the heavy cost. In addition, they are not suitable for use during pregnancy. The poor oral bioavailability, short plasma half-life and severe side effects of available chelators are still not optimal. [13] [14] Within this context and taking in consideration the relative paucity of iron chelating agents it is not surprising that clinical scientists put a great effort towards finding any potentially useful sources in order to obtain the maximum possible benefit with the least possible harm.[15] Compared to synthetic drugs, herbal preparations are frequently less toxic with fewer side effects. Therefore the search for more effective and safer treatment of thalassemia and other blood disorders has become an area of current research activity. For thousands of years, mankind has known about the benefit of drugs from nature. Plant extracts, for the treatment of various ailments, were highly regarded by the ancient civilizations. Even today, plant materials remain an important resource for combating illnesses.

In the light of foregoing discussion the present study is planned to isolate, characterize the iron chelator compound from Triticum aestivum for management of iron overload disorder. In order to narrow down work we are planned to find out biological evaluation of these iron chelator compound from Triticum aestivum responsible for these beneficial effects.

Materials and Methods

Certified samples of species of wheat *Triticum aestivum*, was acquired from Wheat Research Center, Gujarat Krushi University, Junagadh, Gujarat. The authenticity of these certified samples was also confirmed by comparing their morphological characters with description mentioned in different standard texts and floras.[16] These wheat varieties were grown in plastic trays as per standard procedure described by Wigmore, 1985. Dried powder (60#) of wheatgrass was subjected for extraction with acetone, methanol and water using soxhlet apparatus. Solid powder obtained from successive extraction of acetone, methanol and water were subjected for investigation into beneficial effects of iron overload complication in iron-dextran induce iron overload model.

During the study all animals were housed at ambient temperature (22±1^{0}C), relative humidity (55±5%) and 12h/12h light dark cycle. Animals had free access to standard pellet diet and water given *ad libitum*. The protocol of the experiment was approved by the institutional animal ethical committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Induction of iron overload

Male Sprague-Dawley rats initially weighing 150-200 g were used. The rats were given six i.p. injections of iron-dextran (12.5 mg/l00 g body wt.) evenly distributed over a 30 days of period that results in condition of chronic iron overload, which was very resemble to iron overload in thalassemia. Control rats were injected with an equal volume of dextran at the same time. [17] [18]

The experimental animals were divided into five groups, (n=6).

Group 1: Normal control received dextrose solution (NC)

Group 2: Disease control treated with iron dextran (12.5mg/l00g body wt.) (DC)

Group 3: Disease control treated with desferroxamine (40 mg/kg, p.o., per day) (DCD)

Group 4: Disease control treated with water extract of *T. aestivum* (100 mg/kg, p.o., per day) (DCWT)

Group 5: Disease control treated with methanolic extract of *T. aestivum* (100 mg/kg, p.o., per day) (DCMT)

All the studies were carried for a period of 30 days. During the study, blood samples and urine sample were collected on 15 and 30 days under fasting conditions and subjected for following parameters.

B. Estimation of serum iron [19]

Principle

Under acidic conditions (pH 4.5), iron (Fe^{+3}) bound to protein transferrin is released in presence of reducing agent, ascorbic acid, (Fe^{+3}) is reduced to (Fe^{+2}). The resulting product, Fe^{+2} forms a blue complex with 3-(2-pyridyl)-5, 6-bis-2-(5-furyl sulfonic acid)-1,2,4- triazine, disodium salt (Ferene®). The absorbance of the complex, measured using a bichromatic (600, 700 nm) endpoint technique, is directly proportional to concentration of transferrin-bound iron in serum.

Fe^{+3}- transferrin —› Fe^{+3} + Transferrin

2 Fe^{+3} + Ascorbic Acid —› 2 Fe^{+2} + Dehydroascorbic Acid + 2 H^{+}

Fe^{+2} + 3 Ferene® —› Fe^{+2}- Ferene®$_3$ complex

(absorbs at 600 nm)

Procedure:

Test conditions:

- Sample size - 50 µl
- Reagent 1 volume - 100 µl
- Reagent 2 volume - 25 µl
- Diluents size - 225 µl
- Test temperature - 37^{o} C
- Wavelength - 600 and 700 nm
- Type of measurement - bichromatic endpoint

Sampling, reagent delivery, mixing, processing and printing of results are automatically performed by fully automated biochemistry Dimension-Rx L Max-Dade Behring Analyzer.

Serum Glutamic Pyruvic Transaminase (SGPT) levels were estimated using fully automated biochemistry Dimension-Rx L Max-Dade Behring Analyzer. Serum Glutamate Oxaloacetate Transaminase (SGOT) levels were estimated using fully Semi-auto analyzer RA-50 Bayer co. Serum Creatinine and Creatine kinase (CKMB) levels were estimated using Span Diagnostics Ltd., India.

Histopathological study of major organ include heart, liver, kidney were carried out to study protective effects of methanolic extract of *T. aestivum* on iron overload induce complication on the animals.

All above mentioned parameter were estimated to find out in-vivo iron chelating property of various extracts of *Triticum aestivum* and to find out its beneficial effects in prevention of complication due to iron overload.

Results are presented as mean ± SEM. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test. Data were considered statistically significant at $P \leq 0.05$ and highly significant at $P \leq 0.001$. Statistical analysis was performed using Sigma stat statistical software.

All above mentioned parameter were estimated to find out in-vivo iron chelating property of various extracts of *Triticum aestivum* and therefore its beneficial effects in prevention of complication due to iron overload.

Results

Serum iron levels

I.P. injections of iron-dextran (12.5 mg/l00 g body wt.) evenly distributed over a 30 days period that results in condition of chronic iron overload, which was very resemble to iron overload in thalassemia. Control rats were injected with an equal volume of dextran at the same time showed normal level of urine iron in rat.

There was significantly increase in serum iron (6099 ± 252 µg/dl) level in iron overloaded rats compare to normal control rats (203 ± 17 µg/dl). Iron overloaded rats suggest chronic iron overload conditions which are very resemble to iron overload in thalassemia. There were significantly reduction in serum iron levels in desferroxamine group (2876 ± 281 µg/dl) standard iron chelator and water extracts (3510 ± 264 µg/dl,) and methanol extract (4636 ± 142 µg/dl) of *T. aestivum* compare to disease group at the end of 15 days treatment period. These data suggest water and methanol extracts have effectiveness in reduction of iron overload which may be benefits in iron overload disorders as desferroxamine. (Table 1)

Table 1: Beneficial effect of various extracts of *T. aestivum* on urine iron level on iron over loaded rats.

Parameters	NC (n=6)		DC (n=6)		DCD (n=6)		DCWT (n=6)		DCMT (n=6)	
	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
Serum Iron µg/dl	203 ± 17	221 ± 31	6099 ± 252*	6640 ± 291*	2876 ± 281#	2193 ± 128#	4636 ± 142#	4293 ± 132#	3510 ± 264#	3173 ± 202#

Values are expressed as Mean ± S.E.M,*- significantly different from normal control ($p < 0.05$), # - significantly different from diseases control ($p < 0.05$),

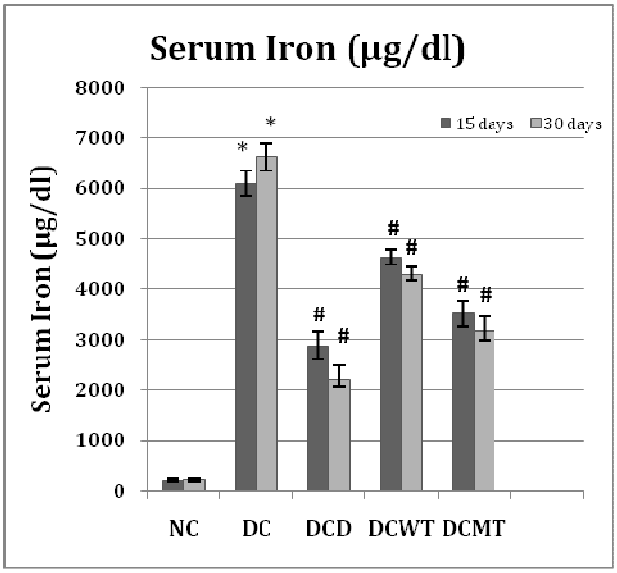


Figure 1: Beneficial effect of various extracts of *T. aestivum* on urine iron level on iron over loaded rats.

Beneficial effects of wheatgrass on iron overload complications

There were significant increases in SGPT and SGOT levels in diseases control group which indicate liver damage because of iron overload in body. Treatment with water and methanol extract of wheatgrass produces significant reduction in these enzymes levels indicating protective effect of extract in liver complications due to iron overload. It was observed that serum creatinine and creatinine kinase levels were found to be significantly increase in iron overloaded rats. While methanol and water extracts treated animals showed reduces in these enzyme level which indicates these extracts prevent damage of vital organs like kidney and heart respectively in iron overload complications. (Table 2)

Table 2: Beneficial effect of various extracts of *Triticum aestivum* on iron over complications on major organ heart, liver and kidney.

Parameters	NC (n=6)		DC (n=6)		DCD (n=6)		DCWT (n=6)		DCMT (n=6)	
	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
SGOT µg/l	46.28± 5.2	48.03± 3.4	170.9 ± 11.3*	191.6± 12.0*	117.3± 5.7#	105.9± 5.6#	148.0± 6.5#	144.5± 3.8#	132.4± 8.8#	126.4± 5.7#
SGPT µg/l	12.8 ± 2.5	12.4 ± 1.9	101.9 ± 8.7*	160.4± 14.6*	77.20± 11.0#	63.8 ± 5.2#	95.65± 6.9#	128.1± 5.7#	81.9 ± 5.8#	89.1 ± 10.3#
Serum creatinine mg/dl	0.67 ± 0.08	0.63 ± 0.63	1.76 ± 0.08*	1.90 ± 0.06*	1.31 ± 0.11#	1.12 ± 0.09#	1.59 ± 0.05#	1.49 ± 0.04#	1.45 ± 0.07#	1.33 ± 0.09#
Creatine kinase (CKMB) µg/l	91.8 ± 8.76	80.7 ± 10.3	398.2 ± 23.7*	446.0 ± 12.1*	219.0 ± 23.8#	189.2 ± 15.4#	335.7 ± 17.9#	325.7 ± 14.4#	316.5 ± 11.5#	271.4 ± 16.0#

Values are expressed as Mean ± S.E.M

***- significantly different from normal control ($p < 0.05$)**

 # - significantly different from diseases control ($p < 0.05$)

Group 1: Normal control received dextrose solution (NC)

Group 2: Disease control treated with iron dextran (12.5mg/l00g body wt.) (DC)

Group 3: Disease control treated with desferoxamine (40 mg/kg, p.o., per day) (DCD)

Group 4: Disease control treated with water extract of *T. aestivum* (100 mg/kg, p.o., per day) (DCWT)

Group 5: Disease control treated with methanol extract of *T. aestivum* (100 mg/kg, p.o., per day) (DCMT)

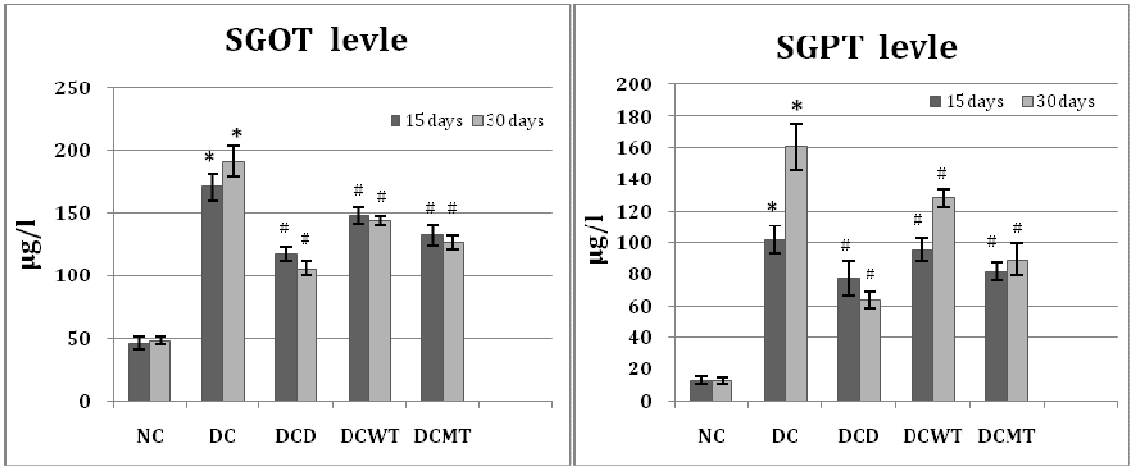


Fig 2: Beneficial effect of various extracts of *Triticum aestivum* on iron overload induces liver complication.

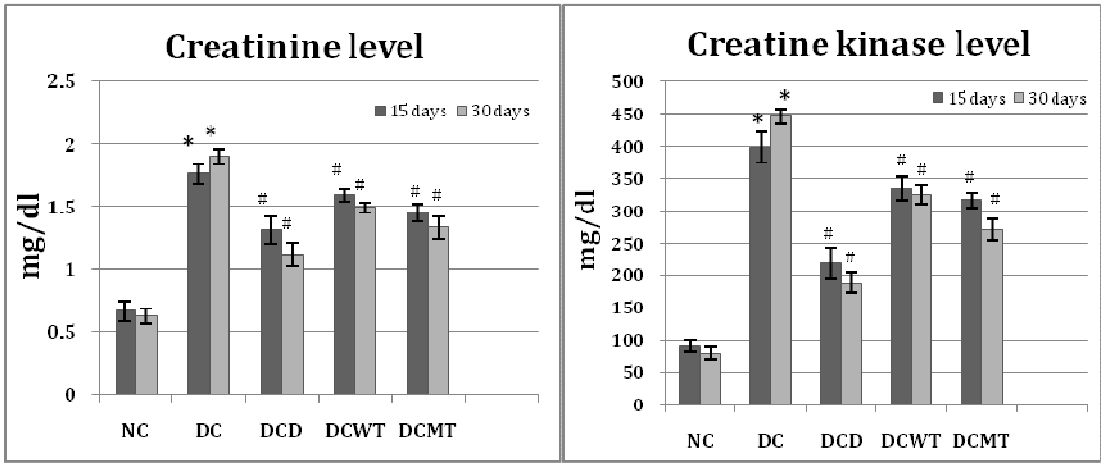


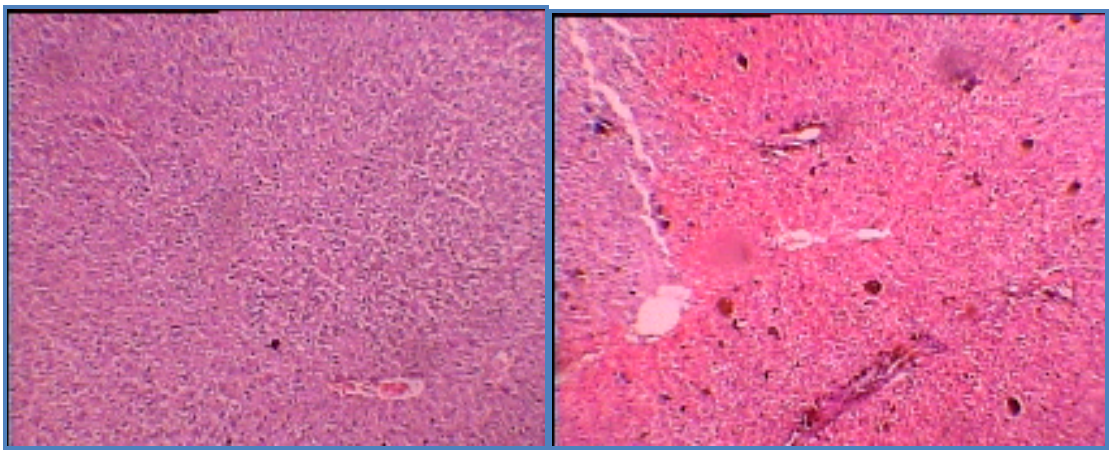
Fig3: Beneficial effects of various extracts of *Triticum aestivum* on iron overload induce kidney and cardiac complications.

Values are expressed as Mean ± S.E.M
***- significantly different from normal control (p < 0.05)**
 # - significantly different from diseases control (p < 0.05)
Group 1: Normal control received dextrose solution (NC)
Group 2: Disease control treated with iron dextran (12.5mg/l00g body wt.) (DC)
Group 3: Disease control treated with desferoxamine (40 mg/kg, p.o., per day) (DCD)
Group 4: Disease control treated with water extract of *T. aestivum* (100 mg/kg, p.o., per day) (DCWT)
Group 5: Disease control treated with methanol extract of *T. aestivum* (100 mg/kg, p.o., per day) (DCMT)

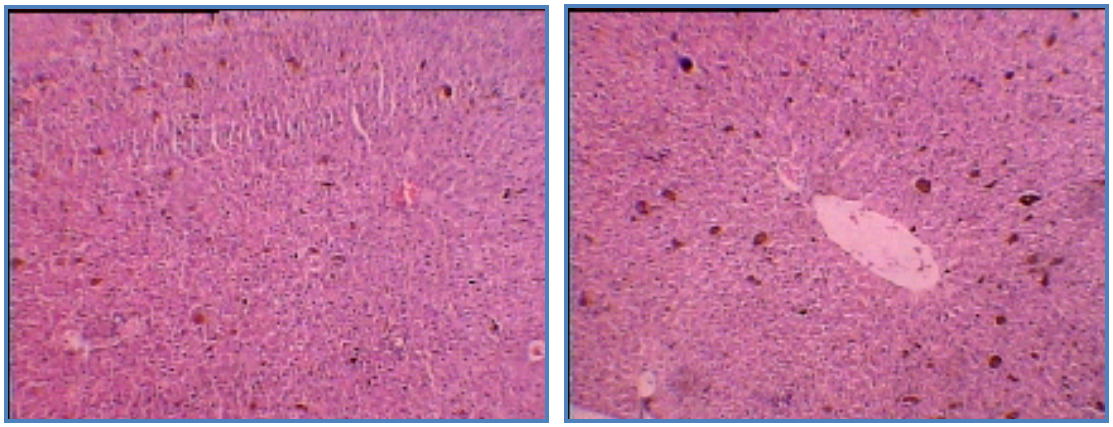
Beneficial effects of wheatgrass on Iron overloaded Liver complications

Hepatotoxicity is the most common finding in iron over-load because liver is the main recipient of the excess iron. [20] The liver of control rats showed a normal structure (Fig. 4), which was influenced by the administration of chronic iron dextran. Iron dextran treated rats show loss of architecture, fibrosis and fatty infiltration (Fig 4). After chronic iron administration, there was heavy iron deposition in all of the hepatocytes and Kupffer cells were observed. Also the trabecular structure of the lobules was slightly or distinctly blurred and hepatocytes and necrotic cells were observed.

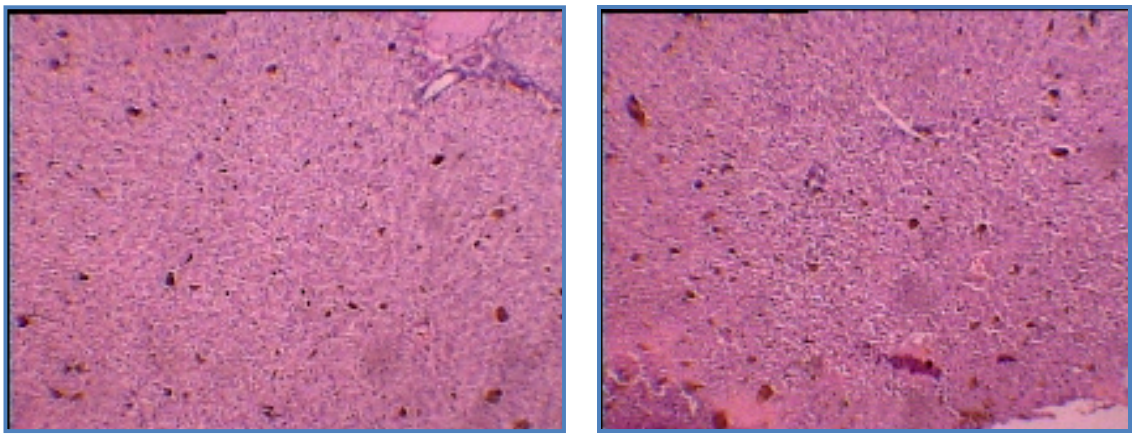
Chronic treatment with desferoxamine and water, methanol and acetone extracts of wheatgrass shows reduced iron pigmentation, minimal pleomorphism, vaculation, fibrosis, less disarrangement and degeneration of hepatocytes. While degree of protection was found to be minimal with acetone extracts. (Fig. 4)



a. Normal control b. Iron overloaded Diseases control



c. iron overload rats treated with desferoxamine d. iron overload rats treated with water extract



e. iron overload rats treated with methanol extract f. iron overload rats treated with acetone extract

Fig 4: Protective effects of various extracts of wheatgrass on iron overloaded liver complications.

Beneficial effects of wheatgrass on Iron overloaded Kidney complications

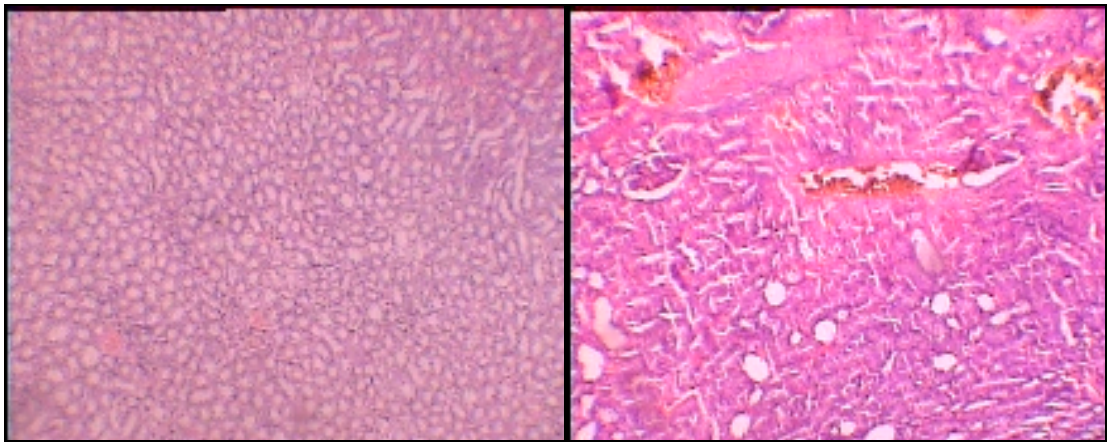
It was reported that an iron-deficient diet or iron chelators prevent the development of tubulointerstitial disease and renal functional deterioration in nephrotoxic serum nephritis. [21]

Baliga et al. have demonstrated that cytochrome P45012 and, more specifically, cytochrome P450 2B1, an isozyme present in the glomerulus, are sources of catalytic iron that participate in glomerular injury.[21]

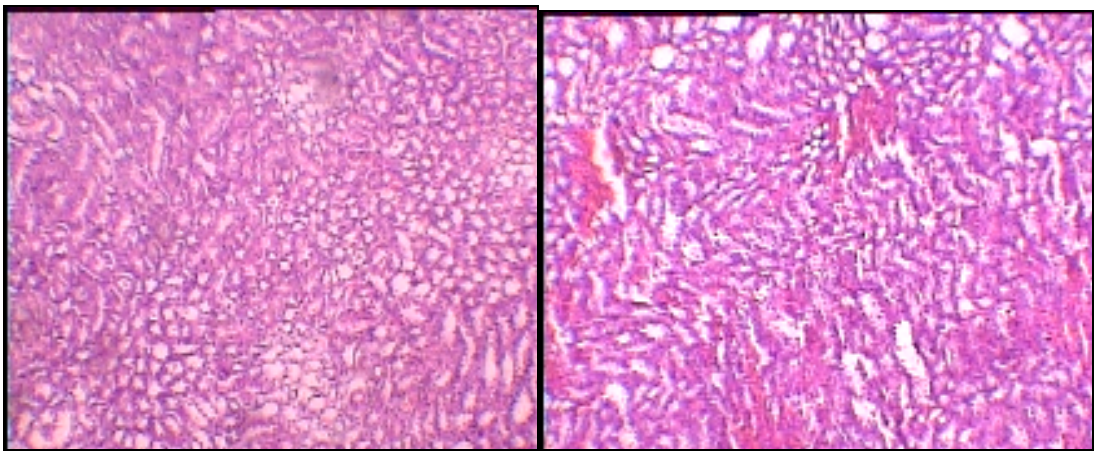
The evidence reviewed suggests the possibility of using iron chelators to halt the progression of kidney disease. Lin et al. have shown that chelation therapy with ethylenediaminetetraacetic acid (EDTA) in patients with chronic renal insufficiency results in a reduced rate of decline in the glomerular filtration rate. [22]

Normal structure of the cortex and medulla was observed in the kidney of normal control rats (Figs 6.2.9). The animals exposed to chronic iron dextran showed damage of renal tubules and glomeruli. Hypertrophy of epithelial cells and degeneration of epithelia of renal tubules with infiltration of mononuclear cells, dilation of glomerul and mononuclear cell infiltrates were evident in all diseases control rats. (Fig 5). Pathological changes in kidney ultra structure (injured brush-border microvilli and swollen proximal convoluted tubular cells) were observed when iron dextran.

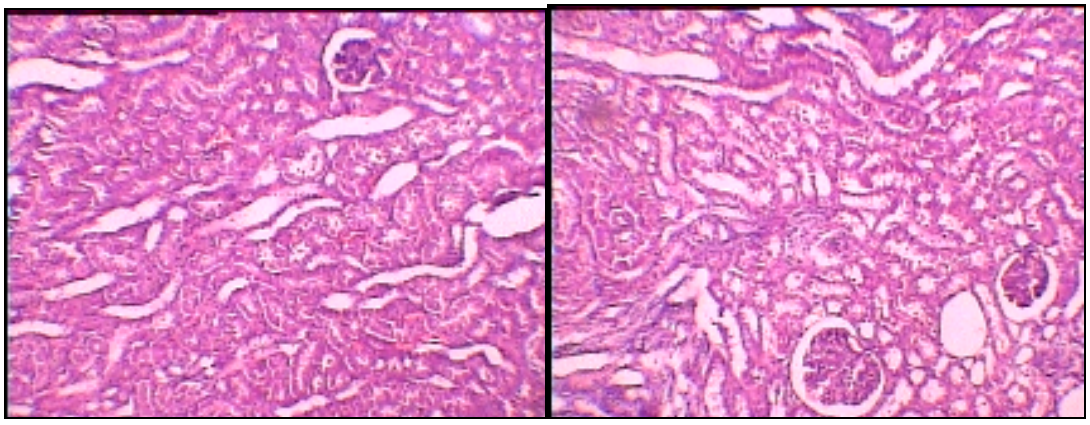
Histology of liver in iron overload rat treated with desferoxamine, a iron chelator, showed reduced damage of renal tubules and glomeruli. Pathological changes was also prevented by desferoxamine. Our result suggest protective effects of methanol, water and acetone extracts of wheatgrass in iron overload kidney complications as it reduced damage of kidney ultra structure (injured brush-border microvilli and swollen proximal convoluted tubular cells). Protective effects found much better in methanol extract and less in acetone extract. (Figs 5)



a. Normal control - kidney **b. Diseases control – kidney**



c. Diseases control - with desferoxamine **d. Diseases control – with methanol extract**



e. Diseases control – with water extract **f. Diseases control– with acetone extract**

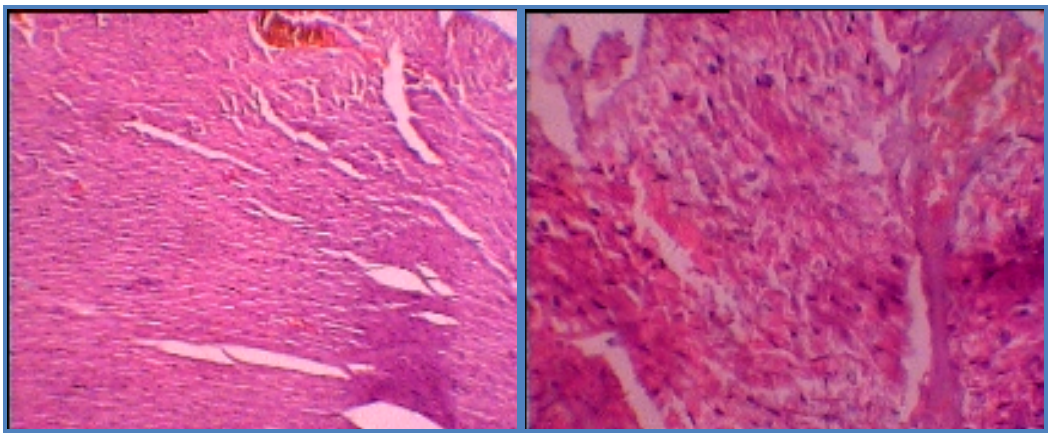
Fig 5: Protective effects of various extracts of wheatgrass on iron overloaded kidney complications

Beneficial effects of wheatgrass on Iron overloaded cardiac complications

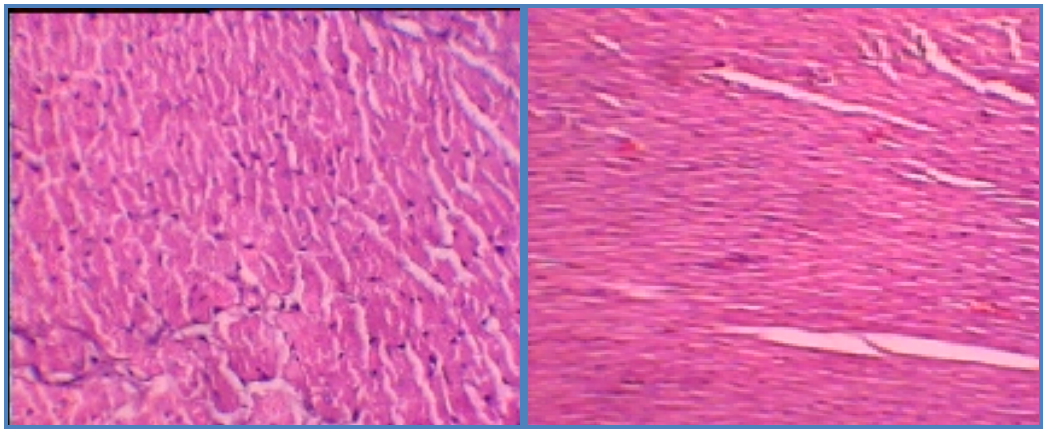
Iron-overload cardiomyopathy is a common cause of CV death worldwide in subjects in their second and third decades of life. [2] Indeed, iron-overload cardiomyopathy is the most important determinant of survival in European, [23] North American, [2] (and Chinese [4] patients with thalassemia major. Long-term follow-up studies in beta-thalassemia patients have established that the level of cardiac iron accumulation correlates directly with both the occurrence of heart disease and mortality, [24] while in patients with primary haemochromatosis, CV disease also contributes significantly to their mortality and morbidity. [5]

Hearts from rat injected chronically with iron displayed extensive interstitial fibrosis and myocyte vacuolar degeneration with mild inflammatory infiltrate compared to placebo (Figure 6) there was vascular hemorrhage and hypertrophy observed in iron overload rats compared to placebo.

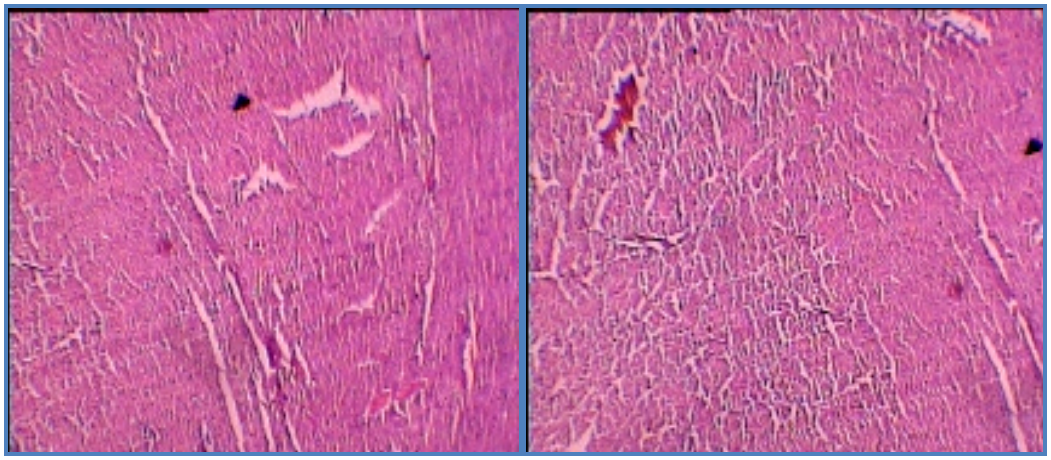
Treatment with desferoxamine and extracts of methanol and water of wheatgrass showed protective effects on myocytes as well as reduces fibrosis and hypertrophy of myocytes. Vascular hemorrhages were also found to be reducing in iron overloaded rates treated with methnoal and water extracts. Treatment with acetone extracts was produce less protective effects. (Figure 6)



a. Normal control **b. Diseases control**



c. Diseases control with desferoxamine **d. Diseases control with methanol extract**



e. Diseases with water extract **f. Diseases with acetone extract**

Fig 6: Protective effects of various extracts of wheatgrass on iron overloaded cardiac complications.

Discussion

Iron is essential to life because of its unusual flexibility to serve as both an electron donor and acceptor. Iron can also be potentially toxic. Patients with chronic anemias such as thalassemia, require regular blood transfusions in order to improve both quality of life and survival. Humans are unable to eliminate the iron released from the breakdown of transfused red blood cells and the excess iron is deposited as hemosiderin and ferritin in the liver, spleen, endocrine organs and myocardium. The accumulation of toxic quantities of iron causes tissue damage and leads to complications such as heart failure, endocrine abnormalities like diabetes, hypothyroidism, liver failure and ultimately early death. [15, 25, 26]

There was significantly decrease in serum iron, serum ferritine level in methanol and water extract treated animal compared to normal group. Rate of reduction of serum iron was very near to standard desferoxamine iron chelator drug which is used to reduce iron overload in thalassemia patients. Rate of reduction was found higher in methanol extract compare to water extract.

Comparison of effects at 15 and 30 days of treatment with wheat grass reveals that more beneficial effects of Wheatgrass therapy was found at 30 days as compared to 15 days treatment of wheat grass.

There were significant reduction in SGPT, SGOT, serum Creatinine and serum creatineine kinase enzyme level were observed with treatment of methanol and water extracts, which suggest protective effects of iron overload liver, kidney and cardiac complications.

Our histopathology study also suggests protective effects of wheatgrass on iron overload complications on liver, kidney heart.

In conclusion our finding suggest *Triticum aestivum* have protective effects in iron overload disorders and its complications on liver, kidney and heart. Possible mechanism is because of its iron chelating property.

References

1. Andrews NC. Disorders of iron metabolism. *New England Journal of Medicine*. Related correspondence, published in NEJM 342(17):1293-1294, Apr 27, 2000.
2. Weatherall DJ, Clegg JB. Thalassemia—a global public health problem. Nat Med 1996;2:847-9.
3. Zurlo MG, De Stefano P, Borgna-Pignatti C, et al. Survival and causes of death in thalassaemia major. Lancet 1989;2:27-30.
4. Li CK, Luk CW, Ling SC, et al. Morbidity and mortality patterns of thalassaemia major patients in Hong Kong: retrospective study. Hong Kong Med J 2002;8:255-60.]
5. Niederau C, Fischer R, Purschel A, Stremmel W, Haussinger D, Strohmeyer G. Long-term survival in patients with hereditary hemochromatosis. Gastroenterology 1996;110:1107-
6. Kohler, G. (1953), the unidentified vitamins of grass and alfalfa. Aug. 8, Feedstuffs.
7. Schnabel, C. (1935), the biologic value of high protein cereal grasses. Paper presented to the biologic section of the Am. Chem. Soc. in New York, April 22.
8. Wigmore, A. (1985), the wheatgrass Book. Avery Publishing Group. Wayne, New Jersey.
9. Kohler, G. (1944), the effect of stages of growth on the chemistry of the grasses. J. Biol. Chem. 152: 215-223.
10. Hamilton, E., Whitney, E., Sizer, F. (1988) Nutrition: Concepts and Controversies. 4th edn, West Publishing Co., St. Paul, Minn.
11. Desai TR and Goyal RK, Investigation Into The Mechanism Of Action And Effects of Triticum Aestivum (Wheat) Grass, 2005.
12. Ebrahimzadeh MA, Fereshteh Pourmorad and Ahmad Reza Bekhradnia, 'Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran,' African Journal of Biotechnology Vol. 7 (18), pp. 3188-3192, 17 September, 2008.
13. Hebbel RP, Leung A, Mohandas N (1990). Oxidation-induced changes in microheological properties of the red cell membrane. Blood, 76:1015-1022.
14. Grinberg LN, Rachmilewitz EA, Kitrossky N, Chevion M (1995). Hydroxyl radical generation in ß-thalassemia red blood cells. Free Rad. Biol. Med. 18: 611-615.
15. Loukopoulos D (2005). Combined therapy with deferiprone and desferroxamine in thalassemia major. Hematol. J. 90: 1305-1305.
16. Percival, J. (1974) In: *The wheat plant*. Duckworth, U K, pp 55-59
17. Addison GM, Beamish MR, Hales CN, *et al*. An Immunoradiometric Assay for Ferritin in the Serum of Normal Subjects and Patients with Iron Deficiency and Iron Overload. *J Clin Pathol* 1972;25:326-9.
18. Studies of an oral iron chelator: British journal of hematology, 1990, 75,274-277
19. Hachiro et al., Modification of Fully Automated Total Iron-binding Capacity (TIBC) Assay in Serum and Comparison with Dimension TIBC Method, *Clinical Chemistry* 48: 1565-1570, 2002;

20. Zurlo MG, De Stefano P, Borgna-Pignatti C, et al. Survival and causes of death in thalassaemia major. Lancet 1989;2:27-30.
21. Baliga R, Ueda N, Shah SV, Kidney iron status in passive Heymann nephritis and the effect of iron deficient diet, J Am Soc Nephrol, 1996;7:1183–8.
22. Lin J-L, Lin-Tan D-T, Hsu K-H, Environmental lead exposure and progression of chronic renal diseases in patients without diabetes, N Engl J Med, 2003;348:277–86.
23. Olivieri NF. The beta-thalassemias. N Engl J Med 1999;341:99-109.
24. Brittenham GM, Griffith PM, Nienhuis AW, et al. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. N Engl J Med 1994;331:567-73
25. Taher A, Isma'eel H, Cappellini MD (2006). Thalassemia intermedia revisited. Blood Cells Mol. Dis. 37: 12-20.
26. Rund D, Rachmilewitz E (2005), thalassemia. N. Engl. J. Med. 353: 1135-1146.