Health is not merely absence of a disease. Rather, health is defined as positive state of well being in which, the harmonious development of physical and mental capacities of the individual lead to the enjoyment of a rich and full life. Health involves, primarily, the application of medical science for the benefit of individual and society. Health is thus, a vital part of a concurrent and integrated program of development of all aspects of community life. Considering the importance of health, WHO and UNICEF jointly organized an international conference on Primary Health Care at Alma Ata, USSR from 6th to 12th September, 1978 and took a momentous decision to achieve “Health for All” by the year 2000 AD. Now, we are entering in the year 2007, but the ‘Health for All’ by the year 2000 still continues to be a vision. There are various diseases like tropical diseases, herpes, AIDS, cancer, diabetes, thalassemia, certain blood disorders etc. for which the cure is yet to be found.

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. This allows the cells to use the benefits of iron, but also limit its ability to do harm (Andrews NC 1995). Most well-nourished people have 4 to 5 grams of iron in their bodies. Of this, about 2.5 g is contained in the hemoglobin needed to carry oxygen through the blood, and most of the rest is contained in ferritin complexes that are present in all cells, but most commonly in bone marrow, liver, and spleen. The liver’s stores of ferritin are the primary physiologic source of reserve iron in the body (Schrier SL 2005). The human body needs iron for oxygen transport. That oxygen is required for the production and survival of all cells in our bodies. Human bodies
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tightly regulate iron absorption and recycling. Iron is such an essential element of human life, in fact, that humans have no physiologic regulatory mechanism for excreting iron (Schrier and Bacon 2005).

In medicine, iron overload disorders are diseases caused by the accumulation of iron in the body. Iron toxicity results when the amount of circulating iron exceeds the amount of transferrin available to bind it. The type of acute toxicity from iron ingestion causes severe mucosal damage in gastrointestinal tract, among other problems. 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 thalassemia, sideroblastic anemia, abnormal red cell production (dyserythropoiesis), iron overload secondary to IV therapy, chronic liver disease secondary to alcohol, porphyria cutanea tarda. Iron overload can be inherited (genetic) or acquired by receiving numerous blood transfusions, getting iron shots or injections, or consuming high levels of supplemental iron. Some of the genetic disorders that result in iron overload include are hereditary hemochromatosis (all types), African iron overload, sickle cell disease, thalassemia, X-linked sideroblastic anemia, enzyme deficiencies (pyruvate kinase; glucose-6-phosphate dehydrogenase) and very rare protein transport disorders aceruloplasminemia and atransferrinemia. None of these conditions should be confused with polycythemia vera (PV), which is not an iron disorder, but a condition where the bone marrow produces too many blood cells (red, white and platelet). People with PV have abnormally high hemoglobin and are at risk for a stroke and progressing to acute myelogenous leukemia (AML).

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. Synthetic agents like desferrioxamine and deferiprone used for the treatment of iron overload in thalassemia are accompanied by serious side effects and certain limitations 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 (Hebbel et al., 1990; Grinberg et al., 1995; Kukongviriyapan et al., 2008). 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. The poor oral bioavailability, short plasma half-life and severe side effects of available chelators are still not optimal (Filburn et al., 2007; Rachmilewitz et al., 1979; Livrea et al., 1996). 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 (Loukopoulos, 2005; Ebrahimzadeh et al., 2007).

For thousands of years, mankind has known about the benefits of drugs from nature. Plant extracts like wheatgrass juice have been highly regarded for their curative effects by ancient civilizations. Even today, plant materials remain an important resource for combating illnesses. WHO has approved the use of traditional medicines as a part of health programme. To pursue research in these systems of medicine, several USA agencies and institutions such as FDA and National Institute of Health have setup separate wings. According to the WHO survey 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for the primary health care needs. In almost all the traditional medicine, the medicinal plants play a major role and constitute the backbone of the traditional medicine. The potential of plant as a source for new drugs is yet to be unexplored systematically. Among the estimated 250,000-400,000 plant species, only 6% have been studied for biological activity and about 15% have been investigated phytochemically (Verpoorte et al., 1998; Cragg et al., 1997; Balandrin et al., 1985). India has an ancient heritage of traditional medicine. Materia Medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products. Indian traditional medicine is based on various system including Ayurveda, Siddha and Unani. With the emerging interest in the world to adopt and study the traditional system and to exploit their potentials based on
different healthcare systems, the evaluation of the rich heritage of the traditional medicine is essential. Further, treatment with, one such traditional herbal drug viz. Wheatgrass, on patients with \(\beta\)-thalassemia (major), has been reported to have beneficial effects by decreasing iron overload (Desai et al., 2005). Hence, in the present project, we planned to investigate iron chelating potential of wheatgrass and its various extracts, in iron overload condition.

Modern science has already, accepted the potential of the 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 a rich reservoir of potential leads for drug discovery against various disorders. Almost, half of the useful drugs today used for various diseases, are derived from natural sources. Only less than two percent of all the plants, available on the earth, have been subjected to pharmacological investigations. Research on the medicinal herbs can offer useful drugs, in time to come, for the treatment of chronic diseases like asthma and diabetes etc. The global market of herbal drugs is increasing very rapidly and it is expected to touch the $5 trillion by end of 2005 (Pharma Business, 2000). The recent development of the science of phyto-pharmaceuticals has generated new enthusiasm in herbal drug research to discover new medicines (Patel and Saluja, 2002). Looking at the dire need of a new safe and economical iron chelating molecule, we resolved to isolate probable active constituent of wheatgrass, responsible for its possible chelating activity.

Wheat \((Triticum\) species) a cereal grass of the \textit{Gramineae} (Poaceae) family, is the world's largest edible grain cereal-grass crop. Wheat has been a food crop for mankind since the beginning of agriculture. For over fifty years, researchers have known that the cereal plant, at this young green stage, is many times richer in the levels of vitamins, minerals and proteins as compared to seed kernel, or grain products of the mature cereal plant (Schnabel 1940). The young germinated plant is a factory of enzyme and growth activity. In the early stages of growth they store large amounts of vitamins and proteins in the young blades. After jointing stage, the nutritional level in the leaves drops rapidly while the fiber content increases rapidly (Kohler 1944). Agriculturally, important species of
Wheatgrass has been traditionally used, since ancient times, to treat various diseases and disorders. Presently, there are 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 a health tonic. Dr. Ann Wigmore, USA, founder director of the Hippocrates Health Institute, Boston, USA, was one of the proponents of the ‘Wheatgrass Therapy’. Dr. Wigmore claimed that wheatgrass is a safe and effective treatment for ailments such as high blood pressure, some cancers, obesity, diabetes, gastritis, ulcers, anemia, asthma and eczema. Scientific reports on nutritional analysis of wheatgrass have been published frequently in various journals (Kohler 1953, Hamilton et al., 1988, Laboratory Analyses 1989). These reports and the 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, phenol and flavonoid which have significant nutritious and medicinal value (Wigmore 1985). Since, iron overload induces considerable oxidative stress and wheatgrass is known to contain significant amount of antioxidants, we decided to investigate antioxidant benefits of wheatgrass in iron overload condition.

Platelets are made in the bone marrow similar to other cells in blood such as, white blood cells and red blood cells. Platelets originate from megakaryocytes which, are large cells found in the bone marrow. Platelets, in general, have a brief 7 to 10 days life in blood, after which they are removed from the blood circulation. The number of platelets in the blood is referred to as the platelet count and is normally between 150,000 to 450,000 per micro liter of blood. Platelet counts less than 150,000 are termed thrombocytopenia (Maton et al, 1993). There are a wide range of botanical sources and wide range of active constituents that might ultimately contribute to haemostatic action, including essential oils, flavonoids, saponins, and alkaloids. The possible mechanisms of action of the hemostatic herbs include: increasing the production of platelets, promoting the ability of platelets to aggregate when there is blood leakage, decreasing capillary permeability, contracting peripheral blood vessels,
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Inhibiting autoimmune attack against platelets. Dr. Wigmore in her programme the “wheatgrass” made several clinical trials on wheatgrass and reported that plants are a safe and effective treatment for anemia and various bleeding disorder like hemophilia and thrombocytopenia. These effects should be expected to be observed within a few days of administering the herbs (Wigmore 1985). In this context, we decided to investigate beneficial effects of wheatgrass in treatment of thrombocytopenia and other bleeding disorders.

The immune system is a remarkably effective structure that incorporates specificity, inducibility and adaptation. Failures of host defense do occur, however, and fall into three broad categories: immunodeficiencies, autoimmunity and hypersensitivities. The immune system is involved in the etiology as well as pathophysiological mechanisms of many diseases. Modulation of the immune responses to alleviate the diseases has been of interest for many years and the concept of ‘Rasayana’ in Ayurveda is based on related principles (Sharma P 1983). Indian medicinal plants are a rich source of substances which are claimed to induce paraimmunity, the non-specific immunomodulation of essentially granulocytes, macrophages, natural killer cells and complement functions (Sainis 1997). Ayurveda, the Indian traditional system of medicine, lays emphasis on promotion of health concept of strengthening host defenses against different diseases (Thatte 1986). Dr. Wigmore’s opinions are based on her experiences. A few clinical studies, have verified that some disease conditions can be benefited by the use of wheatgrass. Remarkably, a relatively large number of studies indicate that food factors and nutrients found in wheatgrass may provide beneficial in immunological disorders. In the light of foregoing discussion, we made an attempt to assess immunomodulatory potential of wheatgrass, using various animal models.
In nutshell the objectives of the present project were –

1. To carry out pharmacognostic studies of Triticum aestivum (Wheatgrass).
2. To carry out phytochemical studies of Triticum aestivum and its various extracts.
3. To evaluate iron chelating activity of various extracts of Triticum aestivum.
4. To isolate iron chelating compound from extract of Triticum aestivum, using column chromatography.
5. To evaluate iron chelating activity of isolated compound of Triticum aestivum, using pre-clinical study.
6. To investigate anti-oxidant property of Triticum aestivum in iron overload condition.
7. To investigate therapeutic benefit of Triticum aestivum in thrombocytopenia.
8. To investigate immunomodulatory activity of Triticum aestivum.

In our investigation, certified samples of wheat viz. Triticum aestivum, was acquired from the Wheat Research Center, Gujarat Krushi University, Junagadh, Gujarat. Adequate quantities of unpolished wheat grain were soaked overnight in water, and were grown in plastic trays filled with soil, on the next day. Trays were watered adequately every day, for 8 days. On 9th day the wheatgrass was harvested. For characterization, wheatgrass was subjected to microscopic study, which included transverse sections, surface preparations and powder study, using high-resolution microscope.

In conformation with the description in literature; the leaves were mainly near glabrous, auriculate, with blades narrowly to broadly linear; broad to narrow; 2–20 mm wide; flat; without cross venation (Percival 1974). Observations in microscopic studies of different species, also confirmed characteristics reported
in literature (Percival 1974). In transverse section, the wheatgrass leaf showed 1. elaborate epidermis with characteristic stomata and trichomes 2. green assimilating parenchyma, 3. conducting vascular bundles and 4. Longitudinal strands of fibrous stereome or supporting tissue. The upper surface of the leaf showed a series of longitudinal ridges or ribs, the lower surface being almost flat. At the summit of each ridge was a single row of elongated thick-walled and pitted cells alternating with hairs. The trichomes or hairs were always unicellular and very much in length and stoutness. On the leaves of *T. aestivum*, ample numbers of hair were present. Stomata were observed at the base of the ridge arranged in single or double lines. Each stoma on the leaf consisted of four cells; the two guard cells being narrow, with specially thickened walls round the stomata pore and thin-walled widely dilated ends. Pores of the stomata were seen to be in communication with large intracellular cavities in the mesophyll, called lacune. The ratio of the number of stomata on the upper and lower epidermis respectively was about 10:7, the number on the upper surface examined being 7000 per square centimeter. In the furrow between two ridges was a band of three to seven rows of motor cells. vascular bundles were collateral, with the xylem towards the upper surface of the leaf and the phloem bellow. In surface preparation, trichomes or hairs of various lengths were found scattered along the rows at more or less at regular intervals. The pharmacognostic characteristics observed in our study were in confirmation with that reported in the literature.

Successive solvent extracts of wheatgrass with petroleum ether, chloroform, acetone, methanol and water were prepared, employing soxhlet apparatus. Highest extractive value was that of water extract (5.3 %), followed by acetone (4.3 %), methanol (4.1 %), chloroform (3.4 %) and petroleum ether with lowest extractive value of 2.8 %.

There is a direct relation between iron chelatory activity and the contents of phenolic and flavonoids in some extracts as reported by Ebrahimzadeh et al., 2008. Hence, the extracts obtained from successive solvent extraction process were subjected to shinoda and FeCl₃ tests to determine the presence of phenolics, tannins and flavanoids. Methanol and water extracts showed presence
of phenolics, tannins and flavanoids, while petroleum ether, chloroform and acetone extracts had no phenolic and flavanoids components.

We also made attempt for in-vitro quantitative determination of phenolic contents and iron chelating property of various extracts of wheatgrass using Folin-Ciocalteau (FC) and Dinis et al. 1994, methods respectively. In methanol and water extracts 506.92 ± 16.36 and 198.5 ± 10.61 µg Gallic acid equivalent of phenol content were detected. Phenolic content of methanol extract was found to be higher compared to water extract. In-vitro iron chelating activities of EDTA, desferoxamine and both extracts were found to be increased with increase in their concentration with highest activity at concentration of 2 mg/ml. The chelating activity of methanol extract was found to be significantly higher compared to water extract.

Intraperitoneal injections of iron-dextran (12.5 mg/100 g body wt.) evenly distributed over a 30 days period on Sprague dwaley rats resulted in condition of chronic iron overload (serum iron - 6099 ± 252 µg/dl). Control group rats injected with an equal volume of dextran showed normal level of iron (serum iron - 203 ± 17 µg/dl). There was significant increase in serum ferritin level in iron overloaded group rats (1.13 ± 0.07 mg/dl) compared to normal control group rats (4.83 ± 0.51 mg/dl). All the studies were carried out for a period of 30 days. Blood, urine and fecal samples were collected on 15th and 30th days under fasting conditions and were subjected for various biochemical parameters.

After 15 days of treatment, there were significant reductions in serum iron and ferritin levels in desferoxamine group (serum iron - 2876 ± 281µg/dl, serum ferritin - 2.74 ± 0.42 mg/dl). There were significant reduction in serum iron and ferritin levels after treatment with water extract group (serum iron - 3510 ± 264 µg/dl, serum ferritin - 3.32 ± 0.19 mg/dl) and methanol extract group (serum iron - 4636 ± 142 µg/dl, serum ferritin - 3.97 ± 0.29 mg/dl) of wheatgrass compared to disease group. Treatment with acetone extract did not significantly reduce serum iron or ferritin levels (serum iron - 5222 ± 314µg/dl, serum ferritin - 4.64 ± 0.38 mg/dl) compared to disease control.
No changes were observed in urine and faces iron in iron overloaded group rats (urine iron – 69.2 ± 7.7 µg/dl, faces iron – 9.17 ± 2.5 µg/dl) and placebo group (urine iron – 26.2 ± 6.4 µg/dl, faces iron – 2.83 ± 0.3 µg/dl). There was significant increase in urine iron and faces iron levels in desferoxamine group (urine iron - 422.5 ± 79.1 µg/dl, faces iron - 31.0 ± 5.3 µg/dl), water extract group (urine iron - 256.0 ± 32.6 µg/dl, faces iron - 17.67 ± 2.1 µg/dl) and methanol extract group (urine iron - 296.5± 33.8 µg/dl, faces iron - 30.33 ± 2.5 µg/dl) compared to iron overloaded group rats (urine iron - 69.2 ± 7.7 µg/dl, faces iron - 9.17 ± 2.5 µg/dl). Increase in urine and fecal excretion of iron in rats treated with water and methanol extracts of wheatgrass indicate iron chelating property of wheatgrass that was comparable to desferoxamine. Similarly there were beneficial effects observed after 30 days treatment period with wheatgrass in iron overloaded rats group. Treatment of acetone extracts did not produce any significant increase in urine iron (94.0 ± 11.9 µg/dl) and faces iron (12.0 ± 1.01 µg/dl) levels compared to diseases control group. These data suggest effectiveness of water and methanol extracts in reduction of iron overload in rats by increase iron excretion in urine and faeces.

Excess iron in vital organs, even in mild cases of iron overload, increases the risk for liver disease (cirrhosis, cancer), kidney diseases, heart attack or heart failure, diabetes mellitus etc. and in some cases, premature death.

There were significant increases in SGPT (101.9 ± 8.7 µg/l) and SGOT (170.9 ± 11.3 µg/l) levels in iron overloaded group as compared to normal control group (SGPT – 12.4 ± 1.9 µg/l, SGOT - 46.28 ± 5.2 µg/l). After treatment with water and methanol extracts of wheatgrass there was significant reduction in these enzyme levels (water extract SGPT – 95.65 ± 6.9 µg/l, SGOT - 148.0 ± 6.5 µg/l; methanol extract SGPT – 81.9 ± 5.8 µg/l, SGOT - 132.4 ± 8.8 µg/l) indicating protective effects of extracts in liver complications due to iron overload.

Serum creatinine and creatinine kinase levels were significant increased in iron overloaded group rats (serum creatinine- 1.76 ± 0.08 mg/dl and creatinine kinase- 398.2 ± 23.7 µg/l) as compared to placebo group (serum creatinine- 0.67 ± 0.08 mg/dl and creatinine kinase- 91.8 ± 8.76 µg/l). Methanol and water extracts treated animals showed reduction in levels of these enzymes (water
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extract, serum creatinine- 1.59 ± 0.05 mg/dl and creatinine kinase- 335.7 ± 17.9 µg/l; methanol extract, serum creatinine- 1.45 ± 0.07 mg/dl and creatinine kinase- 316.5 ± 11.5 µg/l) indicating that these extracts prevent damage to vital organs like kidney and heart in iron overload complications.

Results of histopathological study of liver suggested that chronic treatment with desferoxamine and water; methanol and acetone extracts of wheatgrass reduce iron pigmentation, pleomorphism, vaculation, fibrosis, disarrangement and degeneration of hepatocytes as compared to iron overloaded group animals. The degree of protection was found to be minimal with acetone extract group. Histological findings of kidney (injured brush-border microvilli and swollen proximal convoluted tubular cells) in iron overloaded group rats suggested protective effects of methanol, water and acetone extracts of wheatgrass in iron overload kidney complications. Protective effects were much better in methanol extract treated group and lesser in acetone extract treated group. Treatment with desferoxamine and methanol and water extracts of wheatgrass showed protective effects on myocytes and fibrosis. Vascular hemorrhages were also reduced in iron overloaded group rats treated with methanol and water extracts. Treatment with acetone extract produced less protective effects as compared to water and methanol extracts.

Accumulation of iron in body leads to suppression of bone marrow resulting in reduction of total and differential leucocytes counts. This was observed in iron overloaded rats of disease control group (3.89 ± 0.24 10^3/πl) as compared to placebo group (5.98 ± 0.41 10^3/πl). After 15 and 30 days treatment with desferoxamine (4.01 ± 0.23 10^3/πl), water extract (4.93 ± 0.27 10^3/πl) and methanol extract (4.53 ± 0.32 10^3/πl) of wheatgrass in iron over loaded rats total WBC count was significant increased. The increase in leukocyte count indicates that wheatgrass may have stimulating effect on bone marrow and also on synthesis of all types of leucocytes. Treatment with acetone extract in iron overload rats did not produce any significant increase in total and differential leucocytes counts as compared to iron overloaded group rats. These results indicate beneficial effect of wheatgrass on immune system.
Iron overloaded group rats exhibited significant decrease in Hb count (10.78 ± 0.84 gm/dl) and RBC count (6.77 ± 0.29 m/cmm) as compared to normal control group rats (Hb-14.57 ± 0.61 gm/dl, RBC- 8.65 ± 0.49 m/cmm) suggesting toxic effect of iron excess on Hb and RBC synthesis. 15 days treatment with desferoxamine (Hb-13.5 ± 0.51 gm/dl, RBC- 7.86 ± 0.58 m/cmm), water extract (Hb-13.83 ± 0.31 gm/dl, RBC- 7.69 ± 0.52 m/cmm) and methanol extract (Hb-14.03 ± 0.68 gm/dl, RBC- 8.13 ± 0.87 m/cmm) in iron over loaded rats produced significant increase in Hb levels and RBC counts. The increase in RBC count indicates that wheatgrass may have stimulated haemopoietic process while rise in hemoglobin content indicates stimulation of hemoglobin synthesis in individual RBC. Treatment with acetone extract in iron overload rats did not produce any significant increase in Hb level (10.67 ± 0.68 gm/dl) and RBC count (7.13 ± 0.44 m/cmm) as compared to iron overloaded rats. Over all, there was improvement in Hb level and RBC count after treatment with wheatgrass extracts after 15 and 30 days in iron overload rats.

Similarly, treatment with methanol extract (699.6 ± 38.6 10³/πl) and water (793.6 ± 43.5 10³/πl) extract of wheatgrass, significantly increased platelet counts in iron over loaded group rats as compared to diseases control group rats (530.2 ± 32.6 10³/πl). Acetone extract did not produce any significant increase in platelet count (590.5 ± 59.5 10³/πl) as compared to disease control group. These data indicate beneficial effect of wheatgrass in platelet deficiency disorders.

In iron overload condition, oxidative stress is ultimately involved in dysfunction of vital organs including cardiovascular system (Shinar and Rachmilewitz, 1990; Hebbel et al., 1990; Grinberg et al., 1995). Antioxidant and other supportive therapies protect RBC against oxidative damage (Kukongviriyapan et al., 2008; Filburn et al., 2007). Also, a higher rate of LDL oxidation in thalassemia patients is, due to a lower concentration of vitamin E and C in the LDL particles. Enrichment with vitamins E and C was effective in preventing LDL oxidation in patients with thalassemia (Rachmilewitz et al., 1979; Livrea et al., 1996). Iron chelators mobilize tissue iron by forming soluble, stable complexes that are then excreted in the feces and/or urine. Chelation therapy reduces iron-related complications and thereby improves quality of life and overall survival (Shinar and Rachmilewitz, 1990; Hebbel et al., 1990).
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In our study, at the end of 30 days treatment periods, liver homogenates of iron overloaded group rats showed significant increase in OFRs, MDA level (2.56 ± 0.18 nmoles/mg protein) and decrease in SOD (0.73 ± 0.07 units/min/mg protein), catalase (0.98 ± 0.14 units/min/mg protein) and glutathione levels (7.22 ± 1.75 µgm/mg protein) compared to normal group rats (MDA- 0.74 ± 0.11 nmoles/mg protein, SOD- 1.42 ± 0.1 units/min/mg protein, Catalase- 4.59 ± 1.11 units/min/mg protein, GSH- 12.69 ± 1.04 µgm/mg protein). Treatment with methanol and water extracts of wheatgrass significantly reduced MDA level (1.24 ± 0.06 and 1.16 ± 0.08 nmoles/mg protein) in the rat liver homogenates. There were significant improvements in GSH, SOD and catalase levels in iron overloaded group rats treated with methanol (SOD- 1.29 ± 0.13 units/min/mg protein, Catalase- 3.47 ± 0.17 units/min/mg protein, GSH- 10.8 ± 0.7 µgm/mg protein) and water extracts (SOD- 1.35 ± 0.11 units/min/mg protein, Catalase- 3.78 ± 0.69 units/min/mg protein, GSH- 11.82 ± 0.51 µgm/mg protein). No significant changes were observed in MDA, SOD, catalase and glutathione levels in acetone extract treated group. These data indicate strengthening of antioxidant defense by wheatgrass in iron overload condition.

Since, the results, obtained so far, revealed maximum chelating activity in methanol extract group, we decided to process the methanol extract further, for the purpose of isolation of active iron chelator constituent.

Column chromatography fraction 71-76 eluted using methanol: water: acetone: glacial acetic acid (1:0-80:0.5:0.1) solvent system followed by concentration yielded brown crystals. On recrystallization with methanol produced needle shaped crystals of the active compound (PI$_1$), having melting point of 215-218 °C. The identity of the isolated compound PI$_1$ was confirmed by comparing the R$_f$ value 0.682 on TLC plate. The isolated compound gave black colored spot on spraying 5 % ferric chloride solution on TLC plate suggesting phenolic nature.

The isolated iron chelator compound was subjected to LCMS and IR spectroscopic analyses, for its molecular characterization. The compound was found to be aromatic in nature containing phenolic group.
The total phenolic content in isolated compound from methanol fraction of *Triticum aestivum*, using Folin-Ciocalteau (FC) method, was found to be $434.14 \pm 28.02$ µg Gallic acid equivalent of phenol. In-vitro iron chelating activity of isolated compound was compared with standard iron chelator drug, desferoxamine at 1.0 mg/ml concentration level. % inhibition of complex formation between Fe$^{2+}$ -ferrozine were found $61.18 \pm 5.37$ in desferoxamine and $30.27 \pm 2.98$ in isolated compound PI.

Intraperitoneal injections of iron-dextran (12.5 mg/100 g of body wt.), evenly distributed over a 2 days period, resulted in condition of acute iron overload, in SD rats. Control group rats injected with an equal volume of dextran, showed normal serum level of iron. At the end of day 2, urine samples were collected and analyzed for iron content. No significant changes in urine iron levels were observed in iron overloaded rats ($34.25 \pm 3.8$ µg/dl) and normal control rats ($26.2 \pm 6.4$ µg/dl). 2 day’s treatment with desferoxamine (urine iron- $108.75 \pm 7.4$ µg/dl) and isolated compound (urine iron- $62.21 \pm 9.4$ µg/dl) in iron overloaded rats produced significant increase in urine iron levels compared to diseases control rats. The chelating power or efficacy of the isolated compound was 34.5% compared to that of desferoxamine.

For evaluation of effects of wheatgrass in thrombocytopenia, busulfan was used to induce experimental thrombocytopenia. Busulfan is an alkylating agent with myeloablative properties and activity against non-dividing marrow cells and possibly, non-dividing malignant cells. Busulfan solution, at concentration of 10 mg/ml in polyethylene glycol, was prepared and infused in wistar rats at doses of 25 mg busulfan/kg body weight each, at 1, 5, 10 and 15 days of interval produced pancytopenia with significant reduction in platelet count mimicking severe bleeding conditions as found in thrombocytopenia.

Disease control group rats which, received busulfan showed significant reduction in Hb ($8.1 \pm 0.75$ gm/dl) and RBC count ($5.4 \pm 0.5$ m/cmm) compared to normal healthy group rats (Hb- $10.9 \pm 0.98$ gm/dl, RBC- $6.8 \pm 0.89$ m/cmm) indicating anemia in iron overloaded rats. Treatment with fresh wheatgrass juice (Hb- $10.5 \pm 1.1$ gm/dl, RBC- $6.7 \pm 0.59$ m/cmm), methanol extract (Hb- $10.2 \pm 1.2$ gm/dl, RBC- $6.1 \pm 0.47$ m/cmm) and acetone extract (Hb- $9.7 \pm 0.89$ gm/dl, RBC-
6.3 ± 0.7 m/cmm) of wheatgrass, produced significant increase in Hb levels and RBC counts in diseased rats. Decrease in blood Hb level and RBC count in rats was significantly prevented by treatment with fresh juice, methanol and acetone extracts of wheat grass. Thus, wheatgrass seems to help improve blood purification and also, to increase hemoglobin level and RBC count near to normal.

In disease control group rats which received busulfan, there was significant reduction in platelet count (523 ± 46 10^3/πl) compared to normal healthy group rats (905 ± 82 10^3/πl) indicating thrombocytopenia. Treatment with fresh wheatgrass juice (804 ± 72 10^3/πl), methanol extract (761 ± 58 10^3/πl) and acetone extract (708 ± 63 10^3/πl) produced significant increase in platelet count as compared to disease control group rats. Decrease in platelet count in these rats was significantly prevented by treatment with fresh wheatgrass juice, methanol and acetone extracts of wheatgrass.

Disease control group rats which received busulfan showed significant increase in bleeding (190 ± 18 sec) and clotting time (390 ± 35 sec) as a result of reduction in platelet counts compared to normal healthy group rats (bleeding time- 80 ± 12 sec, clotting time- 130 ± 22 sec) indicating hemophilia and thrombocytopenia in animals. Treatment with fresh wheatgrass juice (bleeding time- 98 ± 13 sec, clotting time- 150 ± 23 sec), methanol extract (bleeding time-106 ± 17 sec, clotting time- 196 ± 24 sec) and acetone extract (bleeding time-125 ± 15 sec, clotting time- 214 ± 30 sec) produced significant reduction in bleeding and clotting time in disease suffering rats. Increases in bleeding and clotting time in rats were significantly prevented by treatment with fresh juice, methanol and acetone extract of wheatgrass. Thus wheatgrass seems to help in reducing bleeding and clotting time, near to normal.

Disease control group rats which received busulfan showed significant reduction in total WBC and differential WBC counts compared to normal healthy group rats. Treatment with fresh wheatgrass juice, methanol extract produced significant increase in total WBC counts and differential WBC counts, in busulfan induced pancytopenic rats. Treatment with acetone extract did not produce significant increase in total WBC counts. Disease control group rats showed
pancytopenia (reduction in all blood cells count) compared to normal healthy control group rats. Treatment with fresh wheatgrass juice and different extracts showed increase in WBC counts compare to disease control group.

For investigation of beneficial effects of wheatgrass on immune system, reduction in cyclophosphamide-induced neutropenia and carbon clearance test, were used in our study. Cyclophosphamide belongs to nitrogen mustard subclass of alkylating agents and acts as an immunosuppressive agent by causing alkylation of DNA, in turn by interfering in DNA synthesis and function. It is also used extensively as an immunosuppressant (Thatte UM et al., 1987). Administration of cyclophosphamide (200 mg/kg, sc) produced a decrease in neutrophil count in all groups. Water extract of *Triticum aestivum* decreased neutrophil count significantly compared to control group.

There was significant increase in neutrophil adhesion to nylon fibres and increase in macrophage induced phagocytosis in carbon clearance test along with reduction in cyclophosphamide induced neutropenia. The methanol extract of wheatgrass was more effective than the water extract. The increase in adhesion of neutrophil to nylon fibres indicates migration of cells from blood vessels and the number of neutrophils reaching the site of inflammation (Shinde UA et al., 1999). Increase in neutrophil adhesion to nylon fibres may be due to up regulation of β2 integrins that are present on surface of neutrophils through which; they adhere firmly to nylon fibres. Hence, it can be inferred that wheatgrass causes stimulation of neutrophil migration towards the site of inflammation. Results of the present study also suggest that wheatgrass may stimulate cell mediated immunity.

Carbon clearance test was carried out to evaluate effect of drugs on the reticuloendothelial system (RES). It is a diffuse system of phagocytic cells, comprising of fixed tissue macrophages and mobile macrophages. The phagocytic cells in this system comprise of mononuclear phagocyte system (MPS). Macrophages are the major differentiated cell in MPS. Cells of the RES and MPS are known to be important in the clearance of particles from bloodstream. When colloidal ink containing carbon particles is injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is governed by an
exponential equation (Das M et al., 1998). Water and methanol extracts of wheatgrass showed significant increase in phagocytic index. Hence, we infer that wheatgrass may stimulate the reticuloendothelial system.

Thus, our investigation indicates beneficial effects of wheatgrass in iron overload diseases, immunocompromised conditions and thrombocytopenia. We have also, isolated a new iron chelator compound from wheatgrass. Further characterization as well as detailed toxicological and clinical studies of this new iron chelator molecule, may provide a new chemical entity for better management of iron overload diseases like thalassemia.