2. INTRODUCTION

The disease-preventive and health-promotive approach of ‘Ayurveda’, which takes into consideration the whole body, mind and spirit while dealing with the maintenance of health, promotion of health and treating ailments, is holistic and finds increasing acceptability in many regions of the world. Ancient Ayurvedic physicians had developed certain dietary and therapeutic measures to arrest / delay ageing and rejuvenating whole functional dynamics of the body system. 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 an absolute requirement for most forms of life, including humans and most bacterial species, because plants and animals all use iron. 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 (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 common 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 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. Iron overload is major problem found in thalassemia major patients. In untransfused patients with severe β-thalassemia, abnormally regulated iron absorption results in increases in body iron burden that may, depending on the severity of erythroid expansion, vary between 2 and 5 grams per year (Pippard et al., 1997, Pootrakul et al., 1988). Regular transfusions may double this rate of iron accumulation. Although most clinical manifestations of iron loading do not appear until the second decade of life in inadequately chelated individuals, evidence from serial liver biopsies in young patients indicates that the deleterious effects of iron are mediated much earlier. After approximately one year of transfusions, iron is deposited in parenchymal tissues, where it may cause significant toxicity as compared to that within reticuloendothelial cells (Hershko et al., 1998). As iron loading progresses, the capacity of serum transferrin, the main transport protein of iron, to bind and detoxify iron may be exceeded. Thereafter, the non-transferrin-bound fraction of iron within plasma may promote generation of free hydroxyl radicals, propagators of oxygen-related damage (Hershko et al., 1998). The effectiveness of an iron-chelating agent depends in part on its ability to bind non-transferrin bound plasma iron over sustained periods of time, thereby ameliorating iron-catalyzed toxicity of free radicals.

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; Mahmoudi et al., 2007; Pourmorad et al., 2007).

Despite the advances of combinatorial chemistry, natural products remain a major source of innovative therapeutic agents for numerous ailments including infectious diseases, cancer, lipid disorders, thalassemia, and several blood disorders. Further, they serve as lead compounds for drug development and are proven to be invaluable biochemical tools for the identification of novel biological targets. 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 ß-
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 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 Triticum include - Triticum aestivum, Triticum durum and Triticum dicoccum. 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, U. S. A. founder director of the Hippocrates Health Institute, Boston, U.S.A. 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 increased oxidative stress and wheatgrass is known to contain considerable 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, 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 (Wigmore 1985). These effects should be expected to be observed within a few days of administering the herbs. *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. *Hence, 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 study of *Triticum aestivum*.
2. To carry out phytochemical studies of *Triticum aestivum* (Wheatgrass) and it’s various extracts.
3. To evaluate iron chelating activity of various extracts of *Triticum aestivum*.
4. Isolation of 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*. 