CHAPTER 1

ABSTRACT
Wheat grass has been traditionally used, since ancient times, to treat various diseases and disorders. Wheat Grass cleanses and detoxifies the body and purifies the blood. It is a natural anti-oxidant, that supports the body during chemotherapy, radiation treatments and removes toxic metabolites, supports weight loss, digestion and immune system, helps to overcome nutrition deficiencies, helps to improve natural immune system, helps to resolves foul odors of breath and sweat, helps in blood purification and to balance hemoglobin production, helps in combating thalassemia and anemia, resolves digestion related problems such as constipation, acidity, piles, colitis, ulcers, diabetes, kidney malfunction etc., acts as an effective energizer and health rejuvenator, helps to overcome skin disorders and to improve skin / muscle tone.

Dr. Ann Wigmore, Founder & Director of Hippocrates Health Institute, Boston, was one of the proponents of the ‘Wheat Grass Therapy’. She utilized the chlorophyll present in the wheat grass as a body cleanser, rebuilder and neutralizer of toxins. She claimed that wheat grass is a safe and effective treatment for ailments such as high blood pressure, cancer, obesity, diabetes, gastritis, ulcers, anemia, asthma and eczema (Wigmore 1985, Marwaha 2004). Scientific reports on nutritional analysis of wheat grass have been published frequently in various journals (Hamilton 1988, Ben-Ayre 2002). These reports revealed that wheat grass was rich in chlorophyll, minerals like magnesium, selenium, zinc, chromium, antioxidants like beta carotene (pro vitamin A), vitamin E, vitamin C, anti-anemic factors like vitamin B12, iron, folic acid, pyridoxine and other minerals, amino acids and enzymes, which exhibited significant nutritious and medicinal value.

Wheat grass is an effective natural supportive in cancer. Wheat grass contains eleven times the calcium of cow's milk, five times the iron of spinach, four times the vitamin B1 of whole wheat flour, seven times the vitamin C in oranges and an
Abstract

abundance of vitamin B12, 80 mcg per hundred gram. But the therapeutic benefits of grass go beyond its vitamins and minerals. Wheat grass contains vitamins including B12, many minerals and trace elements, including selenium, and almost all amino acids. It also contains the hormone Abscisic acid, the antioxidant enzyme Superoxide Dismutase (SOD), a novel isoflavonoid and vitamin E succinate (VES) and over 30 other enzymes, the antioxidant enzyme cytochrome oxidase, laetrile, and a lots of nutrients. Several reports present proved the effectiveness of Abscisic acid in cancer. Abscisic acid is a naturally occurring compound in plants. It is a sesquiterpenoid (15-carbon) which is partially produced via mevalonic pathway in chloroplasts and other plastids. There are no reports to evaluate percentage presence of abscisic acid and laetrile in Wheat grass species like Triticum dicoccum, Triticum durum and Triticum aestivum and its anticancer activity, which may be due to abscisic acid and laetrile. Hence the present investigation was aimed to study the anti-proliferative activity of three species of wheat grass.

The study was conducted with the objectives: (a) To evaluate percentage presence of abscisic acid and laetrile in Triticum dicoccum, Triticum durum and Triticum aestivum using HPLC technique. (b) To estimate anti proliferative activity of one of the Triticum species by in-vitro microculture tetrazolium (MTT) assay. (c) To study clinical efficacy of wheat grass tablets as a supportive treatment in leukemia patients (in-vivo).

Certified samples of three major species of wheat viz, Triticum dicoccum, Triticum durum and Triticum aestivum were acquired from Wheat Research Center, Gujarat Krushi University, Junagadh (Gujarat). Wheat grass was grown using these samples and powder was prepared, which was used for phytochemical study. Wheat grass tablets were purchased from market (Herbal Hills Wheat grass Tablets, Manufactured by Herbal Hills, Lonavala Industrial Estate, Maharashtra, India) for clinical trials.
The phytochemical study was performed to evaluate percentage presence of abscisic acid and laetrile in *Triticum dicoccum*, *Triticum durum* and *Triticum aestivum* using HPLC technique at Amneal Pharmaceuticals (I) Pvt. Ltd. Ahmedabad. (India)

The MTT assay is colorimetric assay for measuring the activity of enzymes that reduce MTT to formazan dyes, giving a purple color. A main application allows assessing the viability (cell counting) and the proliferation of cells (cell culture assays). It can also be used to determine cytotoxicity of potential medicinal agents and toxic materials, since these agents would stimulate or inhibit cell viability and growth. The MTT assay was performed at Despande Laboratories, Bhopal.

The clinical study of wheat grass tablet on patients of leukemia cancer was carried out at Bharat Cancer Research Center, Surat. Necessary permission for conducting the clinical study was obtained from the Chetna Ethics Committee. Objective is to study clinical efficacy of wheat grass tablets as a supportive treatment in leukemia patients who were subjected to chemotherapy. The clinical efficacy was assessed: (a) By comparing Side Effect Index between 2 leukemic patients’ groups (Side Effect Index included headache, nausea, vomiting, bone pain, fever, skin rash, hair loss, mouth ulceration, anorexia, loss of weight and overall life quality as determined from patient and assessment by a physician), (b) By comparing changes in laboratory parameters (Complete Blood Count, Alkaline Phosphatase, SGOT, SGPT and Blood Urea Nitrogen) between two leukemic patients’ group.

A total of thirty patients of clinically diagnosed with leukemia cancer, who were subjected to chemotherapy treatment, age ranged between 20-70 years, who meet all the inclusion criteria and none of the exclusion criteria, based on history and clinical examination were recruited in the study during the screening visit (V1: Day 0). All the patients signed the informed consent before participating into study. The thirty patients were divided into 2 groups; Group-I (ALONE): were
including 15 patients and were kept on chemotherapy treatment alone. Group-II (WITH WHEAT GRASS): were including 15 patients and were kept on chemotherapy treatments and wheat grass tablet as a supportive treatment. The patients of Group-II were given wheat grass tablet with dosage regimen of 2 tablets (Wheat grass powder 500 mg), 3 times a day for 270 days (9 months). Patients of both groups were instructed to visit the facility at every month.

On day 0, the blood samples of the patients with leukemia cancer were taken to check the blood laboratory parameters. Patients recruited into study after the assessment of eligibility criteria. Instructions were given by physician about the dose administration and subsequent visits. On every visit, blood samples of the patients were taken to check effect of wheat grass tablet on blood laboratory parameters. Patients were asked about experienced adverse events and improvement in lifestyle. Improvement in lifestyle were assessed by physical well-being, social/family well being, emotional well being and functional well being. The blood samples were analyzed for Complete Blood Count, Alkaline Phosphatase, SGOT, SGPT and Blood Urea Nitrogen (BUN) at outside laboratory.

The results were presented as mean ± SEM. Statistical difference between the means of various groups were evaluated using Student’s paired ‘t’ test. Data were considered as statistically significant at ‘P’ value of 5 % (P ≤ 0.05).

The results of phytochemical analysis of wheat grass species revealed that three species contained the abscisic acid and laetrile. The amount of abscisic acid and laetrile was varied in three species. The wheat grass species *Triticum aestivum* (14.13 %) contained maximum amount of abscisic acid as compared to *Triticum durum* (8.59 %) and *Triticum dicoccum* (3.84 %). The amount of laetrile was highest in *Triticum aestivum* (9.10 %) as compared to *Triticum durum* (5.63 %) and *Triticum dicoccum* (2.95 %).
Abstract

MTT assay result of wheat grass species revealed that *Triticum aestivum* and *Triticum durum* exhibited significant cytotoxic activity.

The results of the clinical efficacy study of wheat grass tablets revealed that wheat grass tablet improved the patient’s lifestyle and decreased the adverse event incidence. It was found that administration of the wheat grass produced healthier blood levels while receiving the chemotherapy thus *decreasing* the need for blood building medications.