CHAPTER 7

DISCUSSION
The greatest power of any country is its healthier society. Food and medicines are playing important role in building up healthier society. Wheat grass was used as a food and medicine since long time in India as well as all-over world. Wheat grass juice is the most powerful and safest healing aid. Wheat grass can help to get more energy to be active, more confidence in the ability to stay healthy, and more hours alert and awake each day to accomplish the goals in life. Wheat grass contains at least 13 vitamins (several are antioxidants), including B12, many minerals and trace elements, including selenium, and 20 amino acids. It also contains the hormone abscisic acid (also called as dormin), the antioxidant enzyme SOD (Superoxide Dismutase) and over 30 other enzymes, the antioxidant enzyme cytochrome oxidase, laetrile and a whole lot of other nutrients.

Leukemia is a cancer that starts in the tissue that forms blood. Most blood cells develop from cells in the bone marrow called stem cells. A stem cell matures into either a myeloid stem cell or a lymphoid stem cell. A myeloid stem cell matures into a myeloid blast. The blast can form a red blood cell, platelets, or one of several types of white blood cells. A lymphoid stem cell matures into a lymphoid blast. The blast can form one of several types of white blood cells, such as B cells or T cells. Most blood cells mature in the bone marrow and then move into the blood vessels. Blood flowing through the blood vessels and heart is called the peripheral blood. In leukemia, the bone marrow makes abnormal white blood cells. The abnormal cells are leukemia cells. Unlike normal blood cells, leukemia cells don't die when they should. They may crowd out normal white blood cells, red blood cells, and platelets. This makes it hard for normal blood cells to do their work. The types of leukemia can be grouped based on how quickly the disease develops and gets worse. Leukemia is either chronic (which usually gets worse slowly) or acute (which usually gets worse quickly).

There are many options for treatment of leukemia like chemotherapy, radiation therapy, and stem cell transplant. The treatment duration is usually long and very
Chemotherapy treatment produces lots of side effects and also affects the lifestyle of patients. Hence, the present investigation was aimed to study the clinical efficacy of wheat grass tablets as a supportive treatment in leukemia patients, who were subjected to chemotherapy treatment.

Wheat species may differ from one another both morphologically and genetically. *Triticum* species can be placed in three groups, *Triticum dicoccum*, *Triticum durum* and *Triticum aestivum*. *Triticum aestivum* is the most widely grown wheat species in India. In the present investigation, three major species of wheat grass were acquired from the Wheat Research Center, Gujarat Krushi University, Junagadh, Gujarat. These wheat species were grown in plastic trays as per the standard procedure. Wheat grass powder was prepared by shade-drying. In this technique, fresh wheat grass was dried at room temperature in well-ventilated dark room. The dried wheat grass after 3-4 days of drying period was powdered in mill.

Wheat grass contains chlorophyll, protein, over 80 different enzymes, the essential fatty acids linolenic acid and linoleic acid and a wide range of vitamins and minerals including all of the B vitamins, vitamins A, C, E and K, selenium, iron and calcium, but Abscisic acid and laetrile are more important for cancer treatment than any other active ingredients in wheat grass. Dr. Wigmore reported that the "wheat grass" contained Abscisic acid and laetrile, both of which may have anti-cancer activity. In 1979, Dr. Chiu Nan Lai, suggested that wheat grass may have cancer-preventive properties.

There are several reports which 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 the mevalonic pathway in chloroplasts and other plastids.
Therefore, it was decided to evaluate percentage of Abscisic Acid and laetrile in *Triticum dicoccum*, *Triticum durum* and *Triticum aestivum* (wheat grass species) using HPLC technique.

The results of phytochemical analysis of wheat grass species revealed that the three species contained the Abscisic acid and laetrile. The amount of Abscisic acid or laetrile was varied in the three species. The wheat grass species *Triticum aestivum* contained maximum amount of Abscisic acid (14.13%) 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 %).

Based on the results of phytochemical study, the anticancer activity of wheat grass species might be due to abscisic acid and laetrile (Desai T R 2005, Desai T R 2011). Hence it was decided to study the anti-proliferative activity of the three species of wheat grass using the in-vitro method of MTT assay.

The MTT Assay is a cell viability test. In metabolic active cells, the enzyme succinate dehydrogenase breaks down MTT to purple blue formazan particles. All viable cells treated with MTT turn purple blue in colour. All treated cells, that have died, are unable to break down the MTT and therefore, their colour does not change. The rate of colour change, which is a measure of the amount of formazan particles, can be measured by reading the absorbance using a plate reader. The absorbance was read using a plate reader that assigned a numerical value to each well of a 96 well plate including the blank well. The viability was expressed as percentage of control. An MTT assay was performed to evaluate the antiproliferative activity of the three wheat grass species.

Three cancer cell line: A549, U937, HL60 and one normal cell line “Vero” were used for MTT Assay. The juices were diluted to give concentration range of 100-
0.005 µM concentration in water and evaluated against cancer cell lines (A549, HL-60, U-937) and one normal cell line (Vero). Methotrexate, Abscisic acid or laetrile was used as a reference compound and treated in same manner. Cells were grown in micro titer plates and to it various amount of compound was added. After incubation period, MTT labeling mixture was added and allowed to incubate for 4 hours. The solubilizing solutions were added and plates were read on microplate reader using wavelength of 550 nm. After 24 hr, the cytotoxicity data was standardized by determining absorbance and calculating the correspondent chemical concentration.

MTT assay result of wheat grass species revealed that *Triticum aestivum* and *Triticum durum* showed significant cytotoxic activity against HL60, A549 and U937 cell lines with IC50 below 10µM. *Triticum aestivum* was found to have highest cytotoxic activity (Anti-proliferative activity) as compared to other two species. Abscisic acid showed potential cytotoxic activity against A549 (IC50: 688.395nM), U937 (IC50: 537.189nM) and HL60 (IC50: 464.984nM). The potency of Abscisic acid against U937 (IC50: 537.189nM), HL60 (IC50: 464.984nM) and A549 (IC50:688.395nM) was higher than that of reference compound Methotrexate, U937 (IC50: 776.247nM), A549 (IC50:1479.10nM) and HL60 (IC50: 2345.81nM). The potency of *Triticum aestivum* was slightly lower than the potency of Abscisic acid, but it was greater than reference compound (Methotrexate). The potency of *Triticum aestivum* against U937 (IC 50: 684.873nM) and A549 (IC50: 2237.82nM) was higher than that of *Triticum durum*, U937 (IC50: 17269.83nM), A549 (IC50: 10695.22nM). Laetrile and *Triticum durum* showed lower cytotoxic activity than *Triticum aestivum* and Abscisic acid. Against A549, U937 and HL60 cell lines, *Triticum dicoccum* was found inactive by MTT assay. Against Vero cell lines, all the compounds showed very lower or no cytotoxicity (IC50 above 10µM).
Cytotoxic Activity

Abscisic acid > Triticum aestivum > Methotrexate > Triticum durum > Laetrile > Triticum dicoccum

The anticancer activity of the Abscisic acid may be probably due to production hyperpolarization condition on plasma membrane through a decrease of intracellular Na+ and K+. Such phenomenon is produced in cancer cells by mediation of ion channel and activation of the signaling g-protein pathway. Abscisic acid aborting sustained depolarization in malignant tissue may produce a change in the configurational state of cell from damage to a normal state. Additionally, a positive polarization of hCG outer layer accomplished through a removal of electrons may permit immune system cells coming close to cancer cells for destruction.

Based on phytochemical analysis and MTT assay, it was revealed that Triticum aestivum contained the highest amount of Abscisic Acid and possessed the highest cytotoxic activity in MTT assay.

Leukemia is a type of cancer of the blood or bone marrow characterized by an abnormal increase of white blood cells. Clinically and pathologically, leukemia is subdivided into a variety of large groups. Acute leukemia is characterized by a rapid increase in the numbers of immature blood cells. Crowding due to such cells makes the bone marrow unable to produce healthy blood cells. Immediate treatment is required in acute leukemia due to the rapid progression and accumulation of the malignant cells, which then spill over into the bloodstream and spread to other organs of the body. Acute forms of leukemia are the most common forms of leukemia in children. Chronic leukemia is characterized by the excessive build up of relatively mature, but abnormal white blood cells. Typically taking months or years to progress, the cells are produced at a much higher rate than normal cells, resulting in many abnormal white blood cells in the blood.
Whereas acute leukemia must be treated immediately, chronic forms are sometimes monitored for some time before treatment to ensure maximum effectiveness of therapy. Chronic leukemia mostly occurs in older people, but can theoretically occur in any age group.

Myelotoxicity induced by chemotherapy may become life-threatening for cancer patients. Neutropenia may be prevented by granulocyte colony-stimulating factors (GCSF), and epoetin may prevent anemia, but both cause substantial side effects and increased costs. According to non-established data, wheat grass juice may prevent myelotoxicity when applied with chemotherapy. The present investigation was planned to study the clinical effectiveness of wheat grass tablets as a supportive treatment in leukemia patients who were subjected to chemotherapy.

The clinical study of Wheat grass tablet on patients of leukemia cancer was carried out at Bharat Cancer Research Centre, Surat. Ethics committee approval was taken before conducting the clinical trial. Total thirty leukemic patients were enrolled into study, who was subjected to chemotherapy treatment. In the prospective control study, thirty patients with leukemia and subjected to chemotherapy were divided into two groups. Fifteen patients of group-I (ALONE) were kept on chemotherapy treatments alone. Fifteen patients of Group-II (WITH WHEAT GRASS) were kept on chemotherapy treatments and wheat grass tablets as a supportive treatment. The patients of this group were given wheat grass tablets 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. Patients were asked about experienced adverse events and improvement in lifestyle. The improvement in lifestyle was assessed by physical well-being, social/family well-being, emotional well-being and functional well-being. The clinical efficacy was assessed by comparing Side Effect Index (It 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) between and by comparing changes in
laboratory parameters (Complete Blood Count, Alkaline Phosphatase, SGOT, SGPT and Blood Urea Nitrogen).

Treatment of wheat grass along with chemotherapy significantly reduce the severity of nausea, vomiting, bone pain, fever, skin rash, hair loss, mouth ulceration, anorexia and loss of weight as compare to chemotherapy standard treatment. Overall lifestyle of leukemia patients was improved with supportive treatment of wheat grass.

The results of the clinical efficacy study of wheat grass tablets revealed that wheat grass tablets improved the patient’s lifestyle and decreased the adverse event incidence. The results supported the results of pilot study conducted by Bar-Selaa G et al. (2007), in that study; side effects related to wheat grass juice were minimal, including worsening of nausea in six patients, causing cessation of Wheat grass Juice intake. It was found that Wheat grass Juice taken during FAC chemotherapy (5-fluorouracil, doxorubicin, cyclophosphamide chemotherapy) reduced myelotoxicity, dose reduction and need for Granulocyte colony-stimulating factor (GCSF) support, without diminishing efficacy of chemotherapy.

Blood samples of leukemia patients were collected at the start of study and during every visit to assess the effect on laboratory parameters. The parameters recorded were haemoglobin, RBC, WBC, Platelets, Alkaline Phosphatase, SGOT, SGPT and BUN.

The haemoglobin count was increased significantly in group of patients treated with wheat grass tablets (8.07 ± 0.94 gm/dL) than the group of patients treated with alone chemotherapy (6.5 ± 1.53 gm/dL). It may be induction of haemopietic effect of wheat grass. Wheat grass supportive treatment increased the RBC count and platelets count and decreased the WBC count, alkaline phosphatase, SGOT, SGPT and BUN.
Results of the clinical study indicated that wheat grass tablets may be one of the effective supportive treatments for leukemia in term of reducing the adverse event and improving the patient lifestyle. It was found that the wheat grass tablets helped to produce healthier blood levels while receiving the chemotherapy thus decreasing the need for blood building medications.