CHAPTER 3

REVIEW OF LITERATURE
Wheat, a cereal grass of the *Gramineae (Poaceae)* family and of the genus *Triticum* and its edible grain, is the world's largest cereal-grass crop. It has been a food crop for mankind since the beginning of agriculture. The Middle East is probably the area of origin, and it was spreaded throughout Europe not later than the Stone Era. Historians believed that it has been growing since Paleolithic times and cultivated since 6000 years. Its status as a staple is second only to rice. The reason for its popularity is that, unlike other cereals, wheat contains a high amount of gluten, the protein that provides the elasticity necessary for excellent bread making. Although over 30,000 varieties of wheat exist, the two major types are bread wheat and durum wheat. Global production of wheat is approximately 600 million tons; with international trade approximately 100 million tons annually. Wheat is Asia's second most important staple and has been growing much faster than rice. Wheat provides one-fifth of total developing country food supply, up from 15 % in the early 1970s. In 1992-94, developing countries accounted for 45 % of world wheat production (551 million tons) and 46 % of world wheat area (219 million hac).

### 3.1 PHARMACOGNOSY AND PHYTOPHARMACOLOGY OF WHEAT GRASS / TAXONOMICAL DETAILS:

- **Kingdom:** Plantae – Plants
- **Subkingdom:** Tracheobionta – Vascular plants
- **Superdivision:** Spermatophyta – Seed plants
- **Division:** Magnoliophyta – Flowering plants
- **Class:** Liliopsida – Monocotyledons
- **Subclass:** Commelinidae
- **Order:** Cyperales
- **Family:** Poaceae – Grass family
- **Genus:** *Triticum* L. – wheat
- **Species:** *Triticum Aestivum*
The wheat plant is an annual food grass. It is mainly grown as a winter annual in milder climates, with seeding in the fall and harvest from June through August depending on the length of the winter. In areas with rigorous winter climates, it is mainly spring seeded. Planting is as early as soil can be worked, and harvest is in late summer and early fall. In early growth stages, the wheat plant consists of a much-compressed stem or crown and numerous narrowly linear or linear-lanceolate leaves. Leaves are mainly near glabrous. Buds in the leaf axils below the soil surface grow into lateral branches termed tillers. From both the main crown and the tillers, elongated stems develop later and terminate in a spike or head in which the flowers, and finally the seeds or grains develop. Stems of wheat reach from 18 inches to 4 or more feet in height depending on kind and growing conditions.

**Macroscopy** (*Triticum aestivum*)

*Triticum aestivum* is a bisexual plant with bisexual spikelets and hermaphrodite florets. Culm nodes are hairy, or glabrous. Culm internodes are solid, or hollow. Leaves are auriculate with blades narrowly to broadly linear. Leaves are 2–20 mm wide, flat, without cross venation and persistent. Inflorescence is a single elongated spike. Rachides are hollow. Spikelets are solitary, distichous and sessile. Female-fertile spikelets are 9–16 mm long, laterally compressed and disarticulating above the glumes. Rachilla is prolonged beyond the uppermost female-fertile floret. The rachilla extension is incomplete florets. Hairy callus is absent. Callus is very short and blunt. Glumes are two and more or less equal in size. They are shorter than the adjacent lemmas and lateral to the rachis; without conspicuous tufts or rows of hairs. Lower glume is 5–11 nerved. Upper glume is 5–11 nerved. Spikelets are usually with incomplete florets. The incomplete florets are distal to the female-fertile florets. The distal incomplete florets are usually 1, or 2 in number. Female-fertile florets have lemmas similar in texture to the glumes. Awns when present are much shorter than the body of the lemma or much longer than the body of the lemma. Lemmas are hairy or hairless but scabrid. Palea is present and is relatively long, entire or apically notched. Palea
keels are somewhat winged. Lodicules are membranous and ciliate. 3 stamens are present. Anthers are not penicillate with 2–4.5 mm length. Ovary is hairy. Styles are free to their bases. Stigmas are 2 in number and white in color. Fruit is free from both lemma and palea. It is medium sized or large i.e. up to 11 mm long, ellipsoid, longitudinally grooved, compressed dorsiventrally and with hairs confined to a terminal tuft. Hilum is long and linear. Embryo is large to small. Endosperm is hard; without lipid and contains only simple starch grains. Embryonic leaf margins are meeting. Seedling has a short mesocotyl and a tight coleoptile. First seedling leaf has a well-developed lamina. The lamina is narrow and erect (Desai T R 2005).

**Microscopy (Triticum aestivum)**

Leaf-blade epidermis has conspicuous costal zonation. Papillae are absent. There are long-cells of similar wall thickness. Mid-intercostal long-cells are rectangular and fusiform, having markedly sinuous walls. Microhairs are absent. Stomata are common with 63–69 microns length. Subsidiaries are parallel-sided or dome-shaped. Guard cells are overlapped by the interstomatals. Intercostal short-cells are common (e.g. *Triticum polonicum*) or absent or very rare. Crown cells are present. Costal zones have short cells. Costal short-cells are predominantly paired (*Triticum polonicum*) or neither distinctly grouped into long rows nor predominantly paired. Costal silica bodies are horizontally elongated.

Transverse section of leaf blade shows parenchymatous cells without a suberised lamella. Mesophyll has non-radiate chlorenchyma. Leaf blade has distinct and prominent abaxial ribs of more or less constant size. Midrib is conspicuous with one bundle only or has a conventional arc of bundles. The lamina is symmetrical on either side of the midrib. Bulliform cells are present in discrete and regular abaxial groups in the furrows). Many of the smallest vascular bundles are unaccompanied by sclerenchyma. Combined sclerenchyma girders are present (rarely) or are absent. Sclerenchyma is all associated with vascular bundles.
Different species of wheat

Wheat species differ from one another both morphologically and genetically. *Triticum* species can be placed in three groups, according to whether their body cells contain 14, 28 or 42 chromosomes. The basic haploid number being 7, these groups are described as Diploid, Tetraploid and Hexaploid respectively. Diploid species include *Triticum boeoticum* (Wild Eincorn - most ancient variety of wheat) and *Triticum monococcum* (Eincorn). *Triticum boeoticum* was growing in Southwestern Asia before the advent of agriculture. *Triticum monococcum* is now grown to a limited extent in the mountainous region of Yugoslavia, Asia Minor and North Africa. Diploid species can be readily crossed to yield Tetraploid group. Tetraploid species include *Triticum dicoccum* (Emmer wheat, local variety - DDK) and *Triticum durum* (Durum wheat or Macaroni wheat, local varieties - Bansí, Kathia, Khandwa, Raj 1555). *Triticum dicoccum* is one of the most ancient of cultivated cereals. It was formerly grown in the United States for feed on a limited acreage but now has substantially disappeared from cultivation. It is grown to a limited extent in the nilgiri hills and the neighboring areas and is preferred for the preparation of suji or rawa. *Triticum durum*, next important species to *Triticum aestivum*, is used mainly for the manufacture of semolina which is made into macaroni, spaghetti and related products. Although high in gluten, *Triticum durum* is not good for baking. Instead, it is often ground into semolina, the basis for excellent pasta, such as spaghetti and macaroni. It is grown to a considerable extent in parts of Gujarat and central peninsular India. It is preferred for preparation of vermicelli or sewian. When crossed with Diploid species, Tetraploid species yield Hexaploid group. Hexaploid species include *Triticum aestivum* (common wheat, bread wheat, local varieties - Sharbati, Lal kanak, Lok1, GW 273). *Triticum aestivum* is the most evolved and widely cultivated of all wheat species. It is high in protein (10-17 %) and yields flour rich in gluten, making it particularly suitable for yeast breads. It is also preferred for the preparation of biscuits, cake and pastry manufacture. In India, *Triticum aestivum* is the most widely grown wheat species.
The principal cultivated grasses are the cereal grains—wheat, rice, corn, barley, oats, rye and millet (Baker 1978). Various researchers have known that the cereal plant, at this young green stage, contains many times the level of vitamins, minerals and proteins found in the seed kernel, or grain product of the mature cereal plant. The nutrient content of these grasses varies with their stage of growth and growing conditions, rather than with the species of cereal grass (Kahn 1985). 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, the nutritional level in the leaves drops rapidly while the fiber content increases rapidly. The jointing stage is that point at which the internodal tissue in the grass leaf begins to elongate, forming a stem. This stage represents the peak of the cereal plant's vegetative development (Kohler 1944); factors involved in photosynthesis and plant metabolism would be expected to increase up to this stage. Sucrose, the simple carbohydrate found in table sugar, is the primary molecule from which all organic (carbon containing) molecules are formed in the plant (Duffus and Duffus 1984). At the appropriate times and rates, sucrose is converted into amino acids (which make up all proteins), complex carbohydrates, lipids (fats), and nucleic acids (DNA and RNA). The degree of conversion of sugars to specific complex nutrients is dependent on the activity levels of specific enzymes in the plant. Enzyme activity levels are dependent on the plant's growth stage. Chlorophyll, protein, and most of the vitamins found in cereal grasses reach their peak concentrations in the period just prior to the jointing stage of the green plant. Although this period lasts for only a few days, cereal grasses which are consumed, as food supplements should be harvested precisely during this stage of the wheat or barley plant's development. After the jointing stage, the stem forms branches and continues to elongate. The chlorophyll, protein, and vitamin contents of the plant decline sharply as the level of cellulose increases. Cellulose, the indigestible plant fiber, provides structural stability for the growing stem.
Nutritional analysis of wheat grass

Scientific reports on nutritional analysis of wheat grass have been published frequently in various journals (Kohler 1953, Hamilton et al., 1988, Laboratory Analyses 1989). Also, several reputed companies involved in growing and selling of wheat grass have published analyses of wheat grass. As is evident from Table 1, wheat grass is a rich source of chlorophyll, various minerals like iron, magnesium, calcium, phosphorus, antioxidants like beta carotene, insoluble dietary fibers while being low in fat content.

Table – 1 Nutritional analysis of wheat grass

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Value</th>
<th>Constituent</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>1.959 gm</td>
<td>Calories</td>
<td>21 Cal</td>
</tr>
<tr>
<td>Carbohydrates total</td>
<td>2.09 gm</td>
<td>Moisture</td>
<td>95 gm</td>
</tr>
<tr>
<td>Ash</td>
<td>0.0489 gm</td>
<td>Magnesium</td>
<td>24 mg</td>
</tr>
<tr>
<td>Selenium</td>
<td>&lt;1 ppm</td>
<td>Potassium</td>
<td>147 mg</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.33 gm</td>
<td>Phosphorus</td>
<td>75.2 mg</td>
</tr>
<tr>
<td>Calcium</td>
<td>24.2 mg</td>
<td>Sodium</td>
<td>10.3 mg</td>
</tr>
<tr>
<td>Iron</td>
<td>0.61 mg</td>
<td>Vitamin A</td>
<td>427 IU</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>0.08 mg</td>
<td>Vitamin B2</td>
<td>0.13 mg</td>
</tr>
<tr>
<td>Vitamin B3</td>
<td>0.11 mg</td>
<td>Vitamin B5</td>
<td>6.0 mg</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>0.02 mg</td>
<td>Vitamin B12</td>
<td>&lt;1 mcg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>3.65 mg</td>
<td>Vitamin E</td>
<td>15.2 IU</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>29 mcg</td>
<td>Biotin</td>
<td>10 mcg</td>
</tr>
<tr>
<td>Dietary Fiber (total)</td>
<td>&lt;0.1 gm</td>
<td>Lecithin, food</td>
<td>&lt;0.03 gm</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>42.2 mg</td>
<td>Choline</td>
<td>92.4 mg</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>260 mg</td>
<td>L-Arginine</td>
<td>135 mg</td>
</tr>
</tbody>
</table>

All above constituents are present in per 100g juice. Data based on scientific laboratory analysis by Irvine Analytical Laboratories Inc., Irvine, CA, USA
Medicinal uses of wheat grass

In today’s fast lifestyle and fast-food world, deficiency of any or many of these biochemical factors could easily occur culminating into a disease or disorder. For example, as reported by Altura and Altura (1995), ‘It is now becoming clear that a lower than normal dietary intake of magnesium can be a strong risk factor for hypertension, cardiac arrhythmias, ischemic heart disease, atherogenesis and sudden cardiac death. Deficiency in serum magnesium is often associated with arrhythmias, coronary vasospasm and high blood pressure’. Wheat grass juice, being a rich source of magnesium, may replenish the deficiency of magnesium and improve the clinical picture.

Conversely, a disease state may cause deficiency of a nutrient. According to Rude (1993), ‘A large body of evidence demonstrates the prevalence and adverse clinical consequences of magnesium deficiency in patients with diabetes mellitus. It would be prudent for physicians who treat these patients to consider magnesium deficiency as a contributing factor in many diabetic complications and in exacerbation of the disease itself. Repletion of the deficiency or prophylactic supplementation with oral magnesium may help avoid or ameliorate such complications as arrhythmias, hypertension, and sudden cardiac death and may even improve the course of the diabetic condition’. Regular intake of wheat grass juice could correct the secondary magnesium deficiency and thus, may be helpful in averting long-term clinical complications of diabetes mellitus.

The movement for the human consumption of wheat grass began in the western world in the 1930 and was initiated by Charles F. Schnabel, known as the father of wheat grass (Anderson 1986). He said ‘Fifteen pounds of wheat grass is equivalent to 350 pounds of the choicest vegetables.’ Later Wigmore (1940) healed herself of cancer from the weeds she found in vacant lots in Boston. She began a study of natural healing modalities—and with the help of a friend, Dr. Earp Thomas, she found that there are 4700 varieties of grass in the world and all are good for man.
Dr. Wigmore reported that the “wheat grass” 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 a 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, body odor and constipation (Wigmore 1985).

Dr. Wigmore’s opinions are based on her experiences with her guests at Hippocrates. A few clinical studies have verified that some disease conditions can be benefited by the use of wheat grass. Remarkably, a relatively large number of studies indicate that food factors and nutrients found in wheat grass may provide relief from many of the conditions claimed by Ann Wigmore.
3.2 PATHOPHYSIOLOGY AND TREATMENT OF CANCER

Cancer is a group of diseases characterized by unregulated division and spread of cells. The cancerous cells may occur in liquids, as in leukemia. Most, however, occur in solid tumors that originally appear in various tissues in various parts of the body. By their original locations they are classified into various types of cancer, such as lung, colon, breast, or prostate cancer. Localized tumors can be removed by surgery or irradiation with high survival rates. As cancer progresses, however, it metastasizes – invading the surrounding tissues, entering the blood stream, spreading and establishing colonies in distant parts of the body. Only a third of patients with metastasized cancer survive more than five years. Invasive distensions spreading crab-like from a tumor in the breast were described by Hippocrates. From the crab, *karkinos* in Greek and *cancer* in Latin came the name of the disease and the name of its inducing agents, carcinogens (Myers 1989, Dervan 1999).

**Etiology of Cancer:**
A cancer, or neoplasm, is thought to develop from a cell in which the normal mechanisms for control of growth and proliferation are altered. Current evidence supports the concept of carcinogenesis as a multistage process that is genetically regulated (Brander 2002, Kyle 2004, Cheung 2005, Jemal 2007). The first step in this process is *initiation*, which requires exposure of normal cells to carcinogenic substances. These carcinogens produce genetic damage that, if not repaired, results in irreversible cellular mutations.

This mutated cell has an altered response to its environment and a selective growth advantage, giving it the potential to develop into a clonal population of neoplastic cells. During the second phase, known as *promotion*, carcinogens or other factors alter the environment to favor growth of the mutated cell population over normal cells. The primary difference between initiation and promotion is that promotion is a reversible process. Because it is reversible, the promotion phase
may be the target of future chemoprevention strategies, including changes in lifestyle and diet. At some point, however, the mutated cell becomes cancerous \textit{(conversion} or \textit{transformation}). Depending on the type of cancer, 5 to 20 years may elapse between the carcinogenic phases and the development of a clinically detectable cancer. The final stage of neoplastic growth, called \textit{progression}, involves further genetic changes leading to increased cell proliferation. The critical elements of this phase include tumor invasion into local tissues and the development of metastases.

Substances that may act as carcinogens or initiators include chemical, physical, and biologic agents (Cheung 2005). Exposure to chemicals may occur by virtue of occupational and environmental means, as well as lifestyle habits. The association of aniline dye exposure and bladder cancer is one such example. Benzene is known to cause leukemia.

Some drugs and hormones used for therapeutic purposes are also classified as carcinogenic chemicals like anabolic steroids, coalters etc. Physical agents that act as carcinogens include ionizing radiation and ultraviolet light. These types of radiation induce mutations by forming free radicals that damage DNA and other cellular components. Viruses are biologic agents that are associated with certain cancers. The Epstein-Barr virus is believed to be an important factor in the initiation of Burkitt’s lymphoma. Likewise, infection with human papilloma virus is known to be a major cause of cervical cancer. All the previously mentioned carcinogens, as well as age, gender, diet, growth factors, and chronic irritation, are among the factors considered to be promoters of carcinogenesis.
Pathology of Cancer:

Tumor Origin

Tumors may arise from any of four basic tissue types: epithelial tissue, connective tissue (i.e., muscle, bone, and cartilage), lymphoid tissue, and nerve tissue. Although some malignant cells are atypical of their cells of origin, the involved cells usually retain enough of their parent’s traits to identify their origin. Benign tumors are named by adding the suffix -oma to the name of the cell type. Hence, adenomas are benign growths of glandular origin, or growths that exhibit a glandular pattern.

Some cancers are preceded by cellular changes that are abnormal, but not yet malignant. Correction of these early changes could potentially prevent the occurrence of a cancer. Precancerous lesions may be described as consisting of either hyperplastic or dysplastic cells. Hyperplasia is an increase in the number of cells in a particular tissue or organ, which results in an increased size of the organ. It should not be confused with hypertrophy, which is an increase in the size of the individual cells. Hyperplasia occurs in response to a stimulus and reverses when the stimulus is removed. Dysplasia is defined as an abnormal change in the size, shape, or organization of cells or tissues. Hyperplasia and dysplasia may precede the appearance of a cancer by several months or years.

Malignant cells are divided into those of epithelial origin or the other tissue types. Carcinomas are malignant growths arising from epithelial cells. Malignant growths of muscle or connective tissue are called sarcomas. An adenocarcinoma is a malignant tumor arising from glandular tissue. Another term used frequently in the description of malignancy is carcinoma in situ. In this instance, the cancer is limited to the epithelial cells of origin; it has not yet invaded the basement membrane. Carcinoma in situ is a preinvasive stage of malignancy, and most tumors have progressed well beyond this stage at diagnosis. Like all classification systems, there are exceptions to these rules. Malignancies of hematologic origin, such as leukemias and lymphomas, are classified separately.
**Tumor Characteristics**

Tumors may be either benign or malignant. Benign tumors are non-cancerous growths that are often encapsulated, localized, and indolent. Cells of benign tumors resemble the cells from which they developed. These masses seldom metastasize, and once removed they rarely recur. In contrast, malignant tumors invade and destroy the surrounding tissue. The cells of malignant tumors are genetically unstable, and loss of normal cell architecture results in cells that are atypical of their tissue or cell of origin. These cells lose the ability to perform their usual functions. This loss of structure and function is defined as anaplasia. In contrast to benign tumors, malignant tumors tend to metastasize, and consequently, recurrences are common after removal or destruction of the primary tumor.

**Invasion and Metastasis**

Metastasis is the spread of neoplastic cells from the primary tumor site to distant sites (Cheung 2005, Liebisch 2006). Despite advances in diagnostic techniques and screening for cancer, many patients have detectable metastatic disease at diagnosis. Once clinically evident distant metastases are present, cancers are seldom curable. Newly diagnosed cancer patients may also have microscopic cancer metastases. Although clinically undetectable, these small clusters of diseased cells must be present, because many patients subsequently relapse at distant sites despite removal of the primary tumor. Some patients with micrometastatic disease may be cured with systemic chemotherapy.

The two primary pathways of metastasis are hematogenous and lymphatic. Other less-common modes of disease spread include dissemination via cerebrospinal fluid and transabdominal spread within the peritoneal cavity. Tumors are constantly shedding neoplastic cells into the systemic circulation or surrounding lymphatics. This process may begin early in the life of the tumor and often increases with time. The time course for metastasis depends largely on the biology of the tumor. Breast cancer, for example, tends to metastasize very early.
Not all of the shed cancer cells or “seeds” result in a metastatic lesion. The “seed” must first find the appropriate “soil” or an environment suitable for growth (Liebisch 2006). This process is illustrated in the diverse patterns of metastasis that are characteristic of individual types of cancer. An example is prostate cancer, which commonly metastasizes to bone, but rarely to the brain.

The process of invasion and metastasis involves several essential steps. After neoplastic transformation, the malignant cells and surrounding host tissue secrete substances that stimulate the formation of new blood vessels to provide oxygen and nutrients. This process is known as angiogenesis or neovascularization (Tosi 2006). Tumor cells must then detach from the primary mass and invade surrounding blood and lymph vessels. The tumor cells or cell aggregates detach and embolize through these vessels, but most do not survive circulation. The disseminated cells must then attach to the vascular endothelium. The cells may proliferate within the lumen of the vessel, but most commonly extravasate into the surrounding tissue. The local microenvironment may provide growth factors that can serve as “fertilizer” to potentiate the proliferation of the metastasis. At every step of the way, the potential metastatic cell must fight the host immune system. Last, the metastasis must again initiate angiogenesis to ensure continued growth and proliferation. Because angiogenesis has been recognized as a critical element in primary tumor growth as well as metastasis, it has become a target for development of new anticancer agents.
Cancer Treatment:

Cancer is treated using a number of techniques that include surgery, chemotherapy, radiation, immunotherapy, gene therapy, hormone therapy etc. that come in the category of oncology.

Chemotherapy is a term associated with the cancer treatment, by using chemicals and antineoplastic drug. Usually cancer cells grow and divide very fast. Chemotherapy works by attacking the cancer cells and stopping them from growing further. Most of the people suffering from cancer are treated with chemotherapy. It provides a healthy treatment of the disease and helps the patient to lead a normal life. Chemotherapy is the process, where the cancer cells get killed and their further growth is restricted. It is either injected into the veins of the patients or given in the form of pills. There is a variation among every drug provided to the patients. This is because growth of some cancer cells are quick while some are slower and the drug requirement is different in both the cases. Though the results are positive in most of the cases, however, while applying it to the cancer cells, some of the normal growing cells also get affected, which in turn causes damage of blood cells and hair loss. The plan designed for cancer treatment, by the doctors for every individual patient depends on the stage of advancement of the disease. Different patient reacts in different ways to the treatment. Applying chemotherapy can be interrupted due to low count of white blood cells. Hence, a proper schedule is maintained by the doctors for treating the same. Chemotherapy requires the patients to be capable of undergoing the treatment.

The long term effect of chemotherapy is that the patient’s immune system becomes very weak and they stops responding to nutrition. People are often found afraid of chemotherapy, due to its side effects, like low count of red and white blood cells, hair loss, vomiting, nausea and fatigue. However, side effect management is possible this days and chemotherapy can be considered as a process with the best outcome.
Clinical Pharmacology of Chemotherapy:
Agents used in cancer chemotherapy are commonly categorized by their mechanism of action or by their origin. The alkylating agents exert their effects on DNA and protein synthesis by binding to DNA and preventing the unwinding of the DNA molecule. The antimetabolites resemble naturally occurring nuclear structural components (“metabolites”), such as the nucleotide bases, or inhibit enzymes involved in the synthesis of DNA and proteins. Antitumor antibiotics derive their name from their source; they are fermentation products of *Streptomyces* species.
3.3 SIGNIFICANCE OF WHEAT GRASS IN HEALTH AND DISEASE WITH RESPECT TO CLINICAL STUDIES

The most important light-absorbing pigments, in the thylakoid membrane of a plant leaf, are the chlorophylls, green pigments with polycyclic, planar structures resembling the protoporphyrin of hemoglobin, except that \( \text{Mg}^{2+} \), not \( \text{Fe}^{2+} \), occupies the central position. The four inward oriented nitrogen atoms of chlorophyll are coordinated with the \( \text{Mg}^{2+} \). The heterocyclic five-ring system that surrounds the \( \text{Mg}^{2+} \) has an extended polyene structure, with alternating single and double bonds. Such polyenes, characteristically show, strong absorption in the visible region of spectrum; the chlorophylls exhibit high molar absorption coefficients and are therefore well suited for absorbing visible light during photosynthesis.

Chloroplasts of higher plants always contain two types of chlorophyll. One is invariably chlorophyll a, and the second in many species is chlorophyll b, which has an aldehyde group instead of a methyl group attached to ring II. Although both are green, their absorption spectra are slightly different, allowing the two pigments to complement each other’s range of light absorption in visible region. Higher plants contain about twice as much chlorophyll a as chlorophyll b. Chlorophyll itself is not a single molecule but a family of related molecules, designated chlorophyll a, b, c, and d. Chlorophyll a is the molecule found in all plant cells and therefore its concentration is what is reported during chlorophyll analysis. Chlorophyll d is found only in marine red algae, but chlorophylls b and c are common in fresh water. Chlorophyll a and chlorophyll b can be separated by shaking a light petroleum solution of chlorophyll with aqueous methanol: chlorophyll-a remains in the light petroleum but chlorophyll b is transferred into the aqueous methanol. Chlorophyll is a bluish-green solid and chlorophyll b is a dark green solid, both giving a green solution in organic solutions. In natural chlorophyll there is a ratio of 3 to 1 (of a to b) of the two components. In addition to chlorophylls, the thylakoid membranes secondary light absorbing pigments, together called the accessory pigments, the carotenoids and phycobilins.
Carotenoids may be yellow, red or purple. The most important are beta-carotene, a red-orange isoprenoid compound that is the precursor of vitamin A in animals, and the yellow carotenoid, xanthophyll. The carotenoid pigments absorb light at wavelengths other than those absorbed by chlorophylls and thus are supplementary light receptors. Phycobilins are linear tetrapyrroles that have the extended polyenes system found in chlorophylls, but not their cyclic structure or central Mg$^{2+}$. Examples are phycoerythrin and phycocyanin.

All the above-mentioned pigments allow plants to absorb energy from visible light. In addition to this, chlorophyll has been reported to be useful in several clinical conditions. Possibly, the presence of magnesium offers wide range of therapeutically useful effects.

Degradation of chlorophyll following ingestion by humans produces several chlorophyll derivatives, of which, pheophytin, pyro-pheophytin, and pheophorbide have been under study for their potential medical benefits (Chernomorsky 1999). Magnesium is rather easily displaced from the molecule of chlorophyll, when it is heated in the presence of organic acids. Treatment of chlorophyll-a, with acid removes the magnesium ion replacing it with two hydrogen atoms giving an olive-brown solid, pheophytin-a. Pheophytins are much more stable than naturally occurring chlorophyll. Hydrolysis of pheophytin-a splits off phytol and gives pheophorbide-a. Similar compounds are obtained if chlorophyll b is used. Removal of the phytol group from the molecule of chlorophyll is catalyzed by the enzyme chlorophyllase, found in some vegetables. Hydrolysis of the ester linkage yields a compound, known as a chlorophyllide, which is water-soluble. Further degradation of chlorophyllide produces pheophorbide. Thus, when a chlorophyll molecule breaks down, a number of distinct pheophytins, chlorophyllides, and pheophorbides will be produced, depending on the parent molecule. Chlorophyll can also be reacted with a base, which yields a series of phyllins, magnesium porphyrin compounds. Treatment of phyllins with acid gives porphyrins. Numerous studies have shown that
chlorophyll and Cu-chlorophyllin, a water-soluble chlorophyll derivative, possess antimutagenic and antigenotoxic activities. Little information about the absorption and metabolism of these compounds by humans is available. In vitro digestion has been used to access the relative bioavailability of numerous minerals and nutrients from different food matrices. The general consensus among researchers was that chlorophyll molecules were too large to be absorbed by the body. But in recent studies, chlorophylls potential as a health promoter has taken a leap forward (Chernomorsky 1999). In the study, artificial digestion system, known as the Coupled In Vitro Digestion and Caco-2 Human Cell Model, showed that absorptive cells lining the small intestine actually do uptake pheophytins chlorophyll derivatives such as pheophytins, pyropheophytins and chlorophyllins formed during digestion of spinach puree.

Ulcerative colitis is a common and sometimes serious disorder of the large intestine that can cause abdominal pain, diarrhea, and bleeding. Ben-Ayre et al., (2002) reported that people taking wheat grass juice experience a significant improvement of their ulcerative colitis symptoms on a scale that measured overall disease activity, compared with people taking a placebo. Wheat grass juice also significantly reduced the severity of rectal bleeding and abdominal pain. The initial dose of wheat grass juice, 20 mL (two-thirds of an ounce) per day, was increased over a period of several days to a maximum of 100 mL (3.5 ounces) per day. In addition to the positive results mentioned above, an examination of the colon (sigmoidoscopy) showed improvement in 78% of the people receiving wheat grass juice, compared with only 30% of those receiving placebo. No serious side effects were seen. Although nausea was reported by 33% of the participants receiving wheat grass juice, 41% noted an increase in vitality while taking the supplement.

A clinical pilot study was carried out by Marwaha et al., (2004) at the Advanced Pediatric Centre, Postgraduate Institute of Medical Education and Research, Chandigarh, India. It was reported that during period of wheat grass juice
ingestion: all participants experienced lower blood transfusion requirements, 50% had at least 25% reduction in transfusion requirements, the mean interval between transfusions increased 29.5%, overall, hemoglobin levels were not compromised by reduced transfusion volumes.

There is scientific evidence that chlorophyll and the nutrients found in green foods offer protection against toxic chemicals and radiation. In 1980, Dr. Chiu Nan Lai at the University of Texas Medical Center reported that extracts of wheat grass and other green vegetables inhibit the cancer-causing effects of two mutagens (benzopyrene and methylicholanthrene) (Lai et al, 1980). More chlorophyll in the vegetable, greater the protection from carcinogen. Several laboratories have verified that chlorophyll can reduce the ability of carcinogens to cause gene mutations in the last decade. Chlorophyll-rich plant extracts, as well as water solutions of a chlorophyll derivative (chlorophyllin), dramatically inhibit the carcinogenic effects of common dietary and environmental chemicals (Kimm et al, 1982, Ong et al, 1986). Ames testing shows that chlorophyllin neutralizes the cancer-causing action of mixtures of coal dust, tobacco, fried beef, red wine, and other compounds. In this capacity, chlorophyllin is more effective than vitamin A, vitamin C, or vitamin E against mutations induced by the same mixtures (Ong et al, 1986, Ong et al, 1989). Chlorophyll and its related chemicals are being investigated for potential protection against carcinogens, including the dietary carcinogens found in cooked muscle meats (heterocyclic amines), smoked or barbecued foods (polycyclic hydrocarbons), and peanut mold (aflatoxin) (Zitzler 1995). Epidemiological evidence has suggested that diets containing yellow-green vegetables are associated with protection against carcinogenic effects and related mutagenic, clastogenic, and genotoxic activities (Sarkar 1994, Sarkar 1996, Waladkhani 1998). Unlike other chemoprotective phytochemicals, which are present naturally in small quantities in plants, chlorophyll is especially abundant in green leafy plants. For example, spinach can contain up to 5.7% chlorophyll on a dry-weight basis (Gentile 1991, Harttig 1998). Chlorophyll, ubiquitous to all green plants, and having been shown to have medical value, has
received tremendous interest as a nutritional supplement. However, some evidence suggests that the observed medical effects of chlorophyll in plants are actually the results of the total effect of the interaction between different components of the whole plant, in addition to the sole effects of chlorophyll (Waladkhani 1998, Lea 1999). Other phytochemicals that may work in therapeutic combination with chlorophylls include carotenoids, flavonoids, indole, isothiocyanate, polyphenolic compounds, protease inhibitors, sulfides, and terpenes (Waladkhani 1998). Epidemiological surveys have demonstrated that the frequent consumption of fresh vegetables and fruit is associated with reduced risk of some cancers, including gastrointestinal cancer in humans (Sarkar 1994, Nakamura 1996, Sarkar 1996). Chlorophylls and chlorophyll-related products, both natural and man-made, have been shown to support normal cellular function, as demonstrated experimentally through in vitro, in vivo, and animal studies (Higashi Okai 1998). Studies demonstrate that chlorophyll and chlorophyll-related products support normal cellular health through anti-mutagenic activity. Studies have found that chlorophyll binds carcinogens and reduces their uptake (Nakamura 1996, Harttig 1998). Chlorophylls have shown a dose-related protective effect against carcinogens found in the human diet. Chlorophylls form complexes with carcinogens while they are still in the digestive tract, thereby limiting their bioavailability. Chlorophylls also reduce the binding of carcinogens to DNA in the liver (Zitgler 1995). Terwell and van der Hoeven have shown through in vivo studies that chlorophyll and chlorophyllin are inhibitors for the mutagenicity of cigarette smoke condensate (Negishi 1989). Chlorophyllin has been used as a dietary supplement or to diminish the intensity of the discomforting side effects of anticancer therapy. The increase in micronuclei in bone marrow polychromatic erythrocytes in response to anticancer therapy was reduced by concomitant chlorophyllin treatment. We conclude that chlorophyllin may have beneficial effects when used in combination with anticancer therapy (Te et al., 1997).
Chlorophyll and its derivatives are also being studied in relation to photodynamic therapy, a procedure that harnesses the energy of light to treat certain diseases. In photodynamic therapy, chlorophyll and its derivatives are used as photosensitizers that kill cancer cells and act as antiviral agents (Lee 1990). Chlorophyll-related compounds have been effective in photodynamic treatment of pancreatic cancer cells, gross leukemia virus and malignant melanoma, in various *in vitro*, *in vivo* and animal studies (Fiedor 1993, Rosenbach and Belkin 1996, Lee 1999).

Researchers have studied the use of wheat grass in cancer, summary of research presented as below;

- Fresh extract of wheat grass juice appeared effective and safe as a single or adjuvant treatment of active Distal Ulcerative Colitis (Ben-Arye Apr 2002).
- Consumption of wheat grass juice was found to have beneficial effect on the transfusion requirements in 50% of patients in this pilot study (Marwaha 2004).
- Inhibition of *in vitro* metabolic activation of carcinogens by wheat sprout extracts (Lai 1978, Lai 1979).
- A randomized, double-blind, placebo trial was undertaken with 23 patients in Israel. Treatment with wheat grass (100 cc a day for one month) reduced the overall disease activity and severity of rectal bleeding in patients with active distal ulcerative colitis. No serious side effects were found. The authors concluded that ‘wheat grass juice appeared effective and safe as a single or adjuvant treatment of active distal ulcerative colitis’ (Ben-Ayre. 2002).
- In human breast cell studies, chlorophyllin was one of the most effective compounds protecting against DNA adduct formation. Chlorophyllin inhibited adduct formation 65% at 30 micromolar concentrations, and it was also a very effective inhibitor at 15 micromoles, a level obtainable in vivo in the tissues of humans (http://www.lef.org/protocols/prtcls-txt/t-prtcl-149.shtml).
In the prospective matched control study, 60 patients with breast carcinoma on chemotherapy were enrolled and assigned to an intervention or control arm. Those in the intervention arm (A) were given 60 cc of wheat grass juice orally daily during the first three cycles of chemotherapy, while those in the control arm (B) received only regular supportive therapy.” It was found that Wheat Grass Juice taken during chemotherapy may reduce myelotoxicity, dose reductions, and need for granulocyte-colony stimulating factor support, without diminishing efficacy of chemotherapy (Bar-Sela Gil 2007).

During a period from January 2003 to December 2005, 400 solid organ cancer patients were selected in the palliative care unit of Netaji Subhash Chandra Bose Cancer Research Institute (India) to see the effect of wheat grass haemoglobin levels, serum proteins & performance status. It was concluded that wheat grass juice is an effective alternative (natural) for blood transfusion. Its use in terminally ill cancer patients should be encouraged (Dey S 2006).

In 1979, Dr. Chiu Nan Lai, suggested that wheat grass may have cancer-preventive properties. Using the standard Ames test, she showed that an extract of wheat grass, when applied to known chemical mutagens (which cause cells to become cancerous), decreased their cancer-causing ability by up to 99 percent (Lai 1979).

Dr. Otto Warburg (1931) concluded that Cancer can be prevented by blood supply of oxygen to all cells (www.homeopathyone.com/wheat grass.html).

Dr. Wigmore (1985) reported that Wheat grass contains abscisic acid and laetrile which may have anti-cancer activity (Wigmore 1985).

Soma Mukhopadhyay et al. (2008) concluded that wheat grass juice is an effective alternative of blood transfusion. It’s use in terminally ill cancer patients should be encouraged (Soma Mukhopadhyay 2008).
Peryt B et al. (1992) demonstrated that wheat sprout extract shown to be antimutagenic towards benzo[a]pyrene (BP), reduced formation of BP metabolites by hepatic microsomes of either benzo[a]pyrene- or phenobarbital-treated rats as analyzed in high-pressure liquid chromatography (HPLC) (Peryt 1992).

Noorjahan Banu Alitheen et al. conducted study, which effectively demonstrated that wheat grass and fibers food supplements have a good anti-leukemia potential with less or no toxic effects towards healthy immune system. In general, wheat grass has the potential to help in fight against disease, increase immunity and improving the life style of cancer patients (Noorjahan Banu Alitheen 2011).

Dr. M. Badamchian of the George Washington University Medical Centre showed that barley grass extract directly inhibited growth of three different human prostate cancer cells. The extract also killed human breast and melanoma cancer cells both in vitro and in vivo. Human prostate and melanoma cancers were later grafted on to mice that grew into tumours. These were “significantly reduced” by the barley extract. (http://www.acadianawheatgrass.com).

Another possible anti-cancer property of wheat grass juice, first brought to my’ attention in a lecture given by the well known biochemist and researcher, Dr. Enst Krebs, Jr. is Vitamin B17 (laetrile). In his research, Dr. Krebs extracted laetrile from apricot pits, but it is also found in whole foods and especially wheat grass. This vitamin has shown the ability to selectively destroy cancer cells, while leaving non-cancerous alone (http://bionutraceuticals.eu/brochure/clinical_studies_on_wheat_grass.pdf).

At the Linus Pauling Institute of Science and medicine, Dr. Arthur Robinson studied the various effects of live foods, wheat grass and synthetic vitamin C on cancer in laboratory mice. (http://bionutraceuticals.eu/brochure/clinical_studies_on_wheat_grass.pdf).
Review of Literature

- Wheat grass has a high enzyme content, which boosts the system by enriching and cleansing the blood, removing wastes, attacking viruses and enhancing digestion. 1946, Nobel prize winner Dr. James B. Sumner, claims that the tired run-down ‘middle age’ feeling is due to diminishes enzymes. (Survival into the 21st century) Chlorophyll improves varicose veins and many skin disorders. According to the American Journal of Surgery (1940), 1200 cases, ranging from deep internal infections such as brain ulcer and peritonitis to skin disorders had been treated using chlorophyll and were discharged as cured (http://www.acadianawheatgrass.com/wheatgrass%20and%20cancer%20prevention.pdf).

- Oya Sena AYDOS concluded that Wheat grass extract has an antioxidant activity, inhibits proliferation of leukemia cells, and induces apoptosis; thus, this finding may represent a novel therapeutic approach for the treatment of CML (Oya Sena AYDOS 2011).

- Karadag et al. (2007) study results showed that the wheat grass extract inhibited growth of 32Dp210 cells in a dose dependent manner compared to the control cell line (32D). At 6.5% (w/v) and 13% (w/v) concentrations of wheat grass extracts induced apoptosis at 72, 24 hours respectively. In this study, it has been calculated that the death risk of 32Dp210 was found 6.2 times higher than 32D. It is concluded that wheat grass extract inhibits proliferation of 32Dp210 cells through the induction of apoptosis (Karadag 2007).

- Laboratory experiments at National Institute of Health and Medical Research, Marseille, France have proved that fermented wheat germ extract has reduced chemotherapy-induced febrile neutropenia in pediatric cancer patients. Wheat grass juice supplementation to cancer patients has also proved to have a positive impact (http://www.avinuty.ac.in/fsn_ugc_project.pdf).

- Cade (2006) reported the beneficial effect of 20 gm of wheat bran supplementation on colon cancer patients. As 20 gm of wheat bran provides 8.56 gm of dietary fiber and also has been well tolerated by the colon rectal cancer subjects (Cade 2006).
- Shirude Anup Ashok studied the antioxidant activity of the wheat grass juice with the standard drug ascorbic acid and it shows that wheat grass juice is having significant antioxidant activity that is comparable to the standard drug ascorbic acid (Shirude Anup Ashok 2011).
- As per András Telekes and Márta Hegedüs article, the experimental studies have shown many effects including immunologic, cytotoxic, metabolic, and signal transduction. These effects lead to the inhibition of tumor cell proliferation and increase survival of tumor-bearing animals. Avemar alters the glucose metabolism of cancer cells in several ways such as reducing glucose uptake, shifting the use of glucose derived carbon atoms from ribose synthesis to pentose cycle and fatty acid synthesis, and inhibiting key enzymes of glucose metabolism. All of these lead to reduced nucleic-acid synthesis in tumor cells. Avemar also exerts beneficial effects on the immune system. (András Telekes 2009).
- As per Boros et al. Avemar significantly enhanced the antimetastatic activity of DTIC and 5-FU in the treatment of B16 melanoma and C38 colorectal carcinoma, respectively (Boros 2005).
- Borowicki A et al. (2010) studied that fermented wheat aleurone is able to act as a secondary chemopreventive agent by modulating parameters of cell growth and survival, whereas cells of an early transformation stage are more sensitive (Borowicki 2010).
- In a randomized, pilot, phase II clinical trial, the efficacy of dacarbazine (DTIC)-based adjuvant chemotherapy on survival parameters of melanoma patients was compared to that of the same treatment supplemented with a 1-year long administration of fermented wheat germ extract (FWGE). At the end of an additional 7-year-long follow-up period, log-rank analyses (Kaplan-Meier estimates) showed significant differences in both progression-free (PFS) and overall survival (OS) in favor of the FWGE group. The inclusion of Avemar into the adjuvant protocols of high-risk skin melanoma patients is highly recommended (Demidov 2008).
Wheat sprouts contain a very high level of organic phosphates and a powerful cocktail of different molecules such as enzymes, reducing glycosides and polyphenols. The antioxidant properties of wheat sprouts have been widely documented and it has been shown that they are able to protect DNA against free-radicals mediated oxidative damage (Amici 2008).

Hepatocytes are an important physiological model for evaluation of metabolic and biological effects of xenobiotics. They do not proliferate in culture and are extremely sensitive to damage during freezing and thawing, even after the addition of classical cryoprotectants. Thus improved cryopreservation techniques are needed to reduce cell injury and functional impairment. Here, we describe a new and efficient cryopreservation method, which permits long-term storage and recovery of large quantities of healthy cells that maintain high hepatospecific functions. In culture, the morphology of hepatocytes cryopreserved with wheat protein extracts (WPE) was similar to that of fresh cells. Furthermore, hepatospecific functions such as albumin secretion and biotransformation of ammonium to urea were well maintained during 4 days in culture. Inductions of CYP1A1 and CYP2B in hepatocytes cryopreserved with WPEs were similar to those in fresh hepatocytes. These findings clearly show that WPEs are an excellent cryopreservant for primary hepatocytes. The extract was also found to cryopreserve other human and animal cell types such as lung carcinoma, colorectal adenocarcinoma, Chinese hamster ovary transfected with TGF-b1 cDNA, cervical cancer taken from Henrietta Lacks, intestinal epithelium, and T cell leukemia. WPEs have potential as a universal cryopreservant agent of mammalian cells. It is an economic, efficient and non-toxic agent (Hamel 2006).
The antiproliferative and antitumor effect of wheat leaf ribonuclease was tested in vitro on the human ML-2 cell line and in vivo on athymic nude mice bearing human melanoma tumors. Interestingly, immunosuppressive effect of wheat leaf ribonuclease tested on mixed lymphocyte culture-stimulated human lymphocytes reached the same level as that of bovine seminal RNase. The antibodies against wheat leaf ribonuclease produced in the injected mice did not inactivate the biological effect of this plant RNase in vivo. This is probably the first paper in which plant ribonuclease was used as antiproliferative and antitumor drug against animal and human normal and tumor cells and tissues in comparison with animal ribonucleases (Skvor 2006).

As per Farkas E., article, Supportive application of fermented wheat germ extract in colorectal cancer is highly recommended (Farkas 2005).

Whole grain consumption has been associated with reduced risk of chronic diseases, such as cardiovascular diseases and cancer. These beneficial effects have been attributed to the unique phytochemicals of grains that complement those found in fruits and vegetables. Wheat is one of the major grains in the human diet; however, little is known about the inherent varietal differences in phytochemical profiles, total phenolic and carotenoid contents, or total antioxidant activities of different wheat varieties, which ultimately influence the associated nutritional and health benefits of wheat and wheat products. The objectives of this study were to determine the phytochemical profiles and total antioxidant activity for 11 diverse wheat varieties and experimental lines. The profiles included free, soluble-conjugated, and insoluble-bound forms of total phenolics, flavonoids, and ferulic acids and carotenoid content including lutein, zeaxanthin, and beta-cryptoxanthin. The results showed that total phenolic content (709.8-860.0 micromol of gallic acid equiv/100 gm of wheat), total antioxidant activity (37.6-46.4 micromol of vitamin C/gm), and total flavonoid content (105.8-141.8 micromol of catechin equiv/100 gm of wheat) did not vary greatly among the 11 wheat lines. However, significant differences in total ferulic acid content (p < 0.05) and carotenoid content (p <
0.05) among the varieties were observed, with carotenoid content exhibiting the greatest range of values. Carotenoid content among the 11 wheat varieties exhibited 5-fold, 3-fold, and 12-fold differences in lutein, zeaxanthin, and beta-cryptoxanthin, respectively. A synthetic wheat experimental line, W7985, gave the lowest carotenoid concentrations of any of the genotypes in this study. Such large genotypic differences in carotenoid content may open up new opportunities for breeding wheat varieties with higher nutritional value (Adom 2003).

- Cell growth and proliferation were measured by microculture tetrazolium (MTT) assay, cell colony-forming assay and the synthesis of DNA by 3H-thymidine incorporation. Flavonoid extract of wheat germ resulted to a dose-dependent, time-dependent growth inhibition, reduction of colony and 3H-thymidine incorporation in DNA of human breast cancer cell BCap-37. These findings indicated that the flavonoid extract of wheat germ can inhibit tumor cell growth and proliferation by blocking DNA synthesis in vitro (Ferguson 1998, Wei Sheng Yan Jiu 1999, Ferguson 1999).