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Original Article

Antitumor and antioxidant activity of *Triticum Aestivum* against CACO-2 cell line induced colon cancer

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ABSTRACT

Plant-based diet supplements help the prevention and therapy of several kinds of cancer because they contain micronutrients, which exhibit chemopreventive and chemotherapeutic activities. In present study invitro cytotoxic effect of wheat grass was evaluated on Caco2 cell line by MTT assay. Further antitumor and antioxidant activity of wheatgrass was evaluated against the Caco2 cell line induced colon cancer in mice. After 24 h of tumor inoculation intraperitoneally, wheatgrass was administered daily for 30 days. After administration of last dose followed by 18 h fasting, mice were sacrificed for observation of antitumor activity. The change in body weight, abdominal circumference of tumor bearing hosts and simultaneous alterations in hematological profile, serum (SGPT, LDH, GGT, ALP and glucose) and liver biochemical parameters (lipid peroxidation, GSH and antioxidant enzymes-CAT, GPx) were estimated. The changes in serum carcinoembryonic antigen and ferritin levels were estimated. The wheatgrass exhibited apoptosis of cell lines and inhibited cell growth (invivo). The wheatgrass maintained the abdominal circumference and body weight of tumor bearing mice. Hematological profile reverted towards normal levels in wheatgrass treated mice. Treatment with wheatgrass restored serum biochemical parameters towards normal levels and decreased levels of lipid peroxidation and increased levels of reduced glutathione and other antioxidant enzymes (CAT and GPx). The wheatgrass treatment restored Carcino embryonic antigen and ferritin levels in tumor induced mice. The wheatgrass exhibited antitumor effect by modulating hematological parameters, lipid peroxidation and augmenting antioxidant defense system in tumor bearing mice.

Key words: Wheatgrass extract, Caco2 cell line, antitumor effect, apoptosis, antioxidant activity

INTRODUCTION

Wheat (Triticum aestivum L.) a cereal grass of the graminae (Poaceae) family, is an important component of the human diet, particularly in developing countries. Epidemiological studies have shown that the consumption of whole grain and whole-grain products are protective against chronic diseases such as cardiovascular disease, diabetes, and cancer [1-4]. Wheat germinated over a period of 6-10 days is generally called wheatgrass [5]. During germination, vitamins, minerals, and phenolic compounds including flavonoids are synthesized in wheat sprouts, and wheat sprouts reach the maximum antioxidant potential [5]. Wheatgrass contains minerals and trace elements including calcium, iodine, magnesium, selenium, zinc, chromium, antioxidants like vitamin C, vitamin E, β-carotene, vitamin B_1, antianemic factors like vitamin B_{12}, iron, folic acid, pyridoxine, abscissic acid, ferulic acid, and vanilic acid the concentrations of which increase with the germination period [6], and wheatgrass also contains Chlorophyll, which was found to be responsible for inhibiting the metabolic activation of Carcinogens [7, 8]. Wheat grass extract is known to contain antioxidant enzymes superoxide dismutase (SOD) and cytochrome oxidase that have the potential to convert reactive oxygen species (ROS) to a hydrogen peroxide and an oxygen molecule. There are reports on the antimutagenic effect of oxidative DNA damage towards benzopyrene induced mutagenicity [9]. Falcioni et al. demonstrated the inhibition effect of wheatgrass on oxidative DNA damage [10]. It has been shown that wheatgrass extracts contain significant amounts of phenolic compounds including flavonoids [11]. Phenolic compounds of

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plant products are mainly responsible for the antioxidant activity to reverse the effect of ROS mechanism by various pathways, and they have a potent effect to reduce incidence of cancer [12]. Reactive oxygen species (ROS) are produced as a by-product of various metabolic processes, mainly during respiration, in living organisms. Normal physiological concentrations of ROS usually have a role of regulation of cell activities, whereas higher concentrations cause oxidative damage. Wheat grass extract has been shown to possess anti-cancer activity [13], anti-ulcer activity [14], antioxidant activity [15], anti-arthritic activity [16], and blood building activity in Thalassemia Major [17].

Although there are some reports on inhibition of in vitro metabolic activation of carcinogens by wheat sprout extracts [7, 8] and the antimutagenic effect of oxidative DNA damage towards benzopyrene induced mutagenicity [9], there are no reports on whether wheatgrass extract has an effect on human cancer-breast cancer and colon cancer cell lines. Considering the rich antioxidant and vitamin contents of wheatgrass, this study investigated possible antitumor effects and antioxidant status of ethanol extract of wheatgrass on the caco2 cell line induced colon cancer in mice.

MATERIALS AND METHODS

Growing of Wheat grass extract The grass of *T. aestivum* used in this study was grown under indoor conditions. Over-night soaked *T. aestivum* seeds were used to cultivate. Little quantities of water were sprinkled evenly over soil and 3-4 hours indirect sunlight was allowed daily for growth of grass. On the Seventh day, grass is harvested and used for further studies.

Preparation of plant extract The harvested wheatgrass (*T. aestivum) is* washed dried at room temperature. The dried grass was subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. This powder was packed into soxhlet apparatus and made to extract using ethanol. The extract was then filtered and concentrated under reduced pressure using a rotator evaporator at 40°C until the solvent completely dried. The yield of the ethanolic extract was 30%. The extract obtained was then dissolved in 2% Gum acacia for the pharmacological studies.

Preliminary phytochemical screening: The ethanol extract of *T. aestivum* was screened for the presence of various phytoconstituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, amino acids, saponins, terpenoids, tannins and phenolic compounds using the standard procedures.

Determination of LD_{50} of extract of wheatgrass: Acute toxicity study of wheatgrass extract was carried out for determination of LD_{50} by adopting fixed dose method of CPCSEA, OECD guideline no.423. A group of albino mice was used for this study. Acute toxicity studies were conducted and no mortality was observed till the dose of 2000mg/kg. Hence 1/5 of the dose 2000mg/kg i.e. 400mg/kg has been fixed for the study.

Cell lines: Caco2 colon cancer cell line. The cell line was obtained from National Institute of Nutrition, Hyderabad. These cells were maintained in bovine serum albumin medium at 37 °C in a humidified atmosphere of 5% CO2 in air.

Animals: Male Wistar Albino rats weighing 150gms-250gms were obtained from National Institute of Nutrition, Hyderabad. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3° C and 35-60% humidity). Standard pelletized feed and tap water were provided *ad libitum*. All the pharmacological experimental protocols were approved by the Institutional Animal Ethics Committee (Reg no: MRCP/CPCSEA/IAEC/2012-13/MPCOL/08).

MTT assay for cell viability: The extent of the cell proliferation and cell viability was determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Cell proliferation and viability were determined by MTT (Cell proliferation Kit I, Roche, Germany) assay after wheatgrass extract treatments according to the manufacturer's protocol. A Thoma slide was used to count the cells in the culture flask. In brief, 1 × 105 cells were incubated in 96-well plates. Cells were treated with 10 µL/well of MTT reagent for 4 h at 37 °C and then treated with 100 µL/well of solubilization solution at 37 °C. After this incubation period, a water-insoluble formazan dye was formed. Following the solubilization, the formazan dye was quantitated by measuring absorbances of OD550-OD690 with a SOFT max Pro 3.12 program. The blank control contained cell culture medium only. At least 3 independent experiments were performed.

Anti-tumor activity of wheatgrass extract against Caco2 cell line induced colorectal cancer in Swiss albino mice [18, 19]: Twenty four male Swiss Albino mice weighing 20-30g were divided into four groups of six animals each.
Group 1: Male Control group (1% gum acacia 1ml)
Group 2: Caco2 cell line 2×106 cells/mouse i.p.
Group 3: Caco2 cell line 2×106 cells/mouse i.p.+ 5-Flourouracil 20mg/kg body weight I.P.

Group 4: Caco2 cell line 2×106 cells/mouse i.p. + Wheatgrass extract 400 mg/kg body weight p.o.

Treatment schedule: Animals were grouped into 4 groups as explained above. The control group animals were given 1% gum acacia 1ml for 30 days. Group 2 animals were given Caco2 cell line 2×10^6 cells/mouse i.p. Group 3 animals were given Caco2 cell line 2×106 cells/mouse i.p. and 5-Flourouracil 20mg/kg body weight i.p. until 30th day respectively. Group 4 animals were given Caco2 cell line 2×10^6 cells/mouse i.p. and Wheatgrass 400 mg/kg body weight p.o until 30th day respectively.

Blood sample preparation: The animals were sacrificed on 30th day using ether anesthesia, blood was collected by carotid bleeding and transferred to anticoagulant EDTA tubes for the estimation of hematological parameters like Hb, RBC and WBC.

Serum sample preparation: The animals were sacrificed on 30th day using ether anesthesia; blood was collected by carotid bleeding and was centrifuged using Remi cool centrifuge at 4000 rpm for 15 minutes. Serum was separated for the estimation of various biochemical parameters like serum SGPT, alkaline phosphatase, Ferritin, Carcino embryonic antigen, LDH, GGT and glucose.

Tissue sample preparation: At the end of the experiment, animals were sacrificed with light ether anesthesia. Liver tissue was separated and washed with phosphate buffer saline (0.05M, P^H7.4). The liver was taken later and minced into small pieces and homogenized in ice cold phosphate buffer saline (0.05M, P^H7.4) using tissue homogenizer to obtain 1:9 (w/v) (10%) whole homogenate. A part of the liver homogenate was taken and mixed with equal volume of 10% Trichloro acetic acid (TCA) for the estimation of malondialdehyde. Homogenate was centrifuged using Remi cool centrifuge at 8000 rpm for 30 min. The supernatant was separated and used for estimation of anti-oxidant levels of different enzymes i.e. Catalase and reduced glutathione, malondialdehyde and glutathione peroxidase.

Histopathological studies: At the end of the experimental period, the mice were sacrificed and colon was removed. The tissue sample from each group was selected and stored in 10% buffered formalin solution and further embedded in paraffin with wax. The blocks were processed for sectioning; the sections were then stained with haematoxylin and eosin as nuclear and cytoplasmic stains, respectively to assess the activity. Pathological changes, if any, were viewed under light microscope and recorded.

Statistical Analysis: The experimental results were expressed as the Mean ± SEM with six rats in each group. Statistical significance of difference between groups was determined by Student's *t*-test.

RESULTS

Effect of wheatgrass extract on viability of Caco-2 colon cancer cell line: The effect of wheatgrass extract was tested on Caco-2 cell line. Data show that control cells were of high density with well-defined morphological characteristics of certain cultures. 24 h after addition of Wheatgrass extract noticeable changes were visible in the morphology and density of treated cells. Almost all treated cells became rounded and their number was reduced in comparison with the control cell culture. In order to quantify the toxicity of Wheatgrass extract a MTT assay was performed. In the higher concentration (0.1mg/ml) Wheatgrass extract exhibited statistically significant cytotoxic activity against Caco-2 tumor cells (~ 95 %). The cytotoxic activity was dose dependent and it was up to 10% for lower dose (0.01 mg/ml) and up to 95% for higher dose (0.1 mg/ml) of Wheatgrass extract against tumor cell line (Figure 1).

Effect of Wheatgrass extract on body weight and abdominal circumference of colon cancer induced mice: There was a significant ($P<0.011$) increase in the body weight and abdominal circumference of colon cancer induced mice from second week onwards during a growth period of 30 days when compared to normal group. Treatment with5-Fluorouracil and Wheatgrass extract significantly ($P<0.001$) maintained the body weight and abdominal circumference of tumor induced mice (Figure 2).

Effect of Wheatgrass extract on Hematological parameters of colon cancer induced mice: Hemoglobin content and RBC count were significantly ($P<0.001$) decreased and total WBC count was significantly ($P<0.001$) decreased in the colon cancer group as compared to the Normal group. Treatment with 5-Fluorouracil and Wheatgrass extract significantly ($P<0.001$) restored the RBC and hemoglobin levels towards the normal (Figure 3).

Effect of Wheatgrass extract on serum biochemical enzymes of colon cancer induced mice: There was a significant ($P<0.01$) decrease in serum glucose, significant ($P<0.01$) increase in serum SGPT, LDH, GGT and ALP activity of colon cancer group when compared to normal group and treatment with 5-Fluorouracil and Wheatgrass extract significantly ($P<0.001$) increased the glucose level, significantly ($P<0.001$)

decreased the enzyme activity as compared to colon cancer group and restored to normal levels (Table 1).

Effect of Wheatgrass extract on Catalase, MDA, GSH and GPx in colon cancer induced mice: Catalase, GSH, and GPx were significantly (P<0.001) decreased and MDA levels were significantly (P<0.001) increased in the colon cancer group when compared to the normal group. Treatment with 5 fluorouracil and Wheatgrass extract significantly (P<0.001) decreased the MDA levels and increased the Catalase, GSH, and GPx levels towards the normal (Table 2).

Effect of Wheatgrass extract on Ferritin and Carcino embryonic antigen levels in colon cancer induced mice: Ferritin levels were significantly (P<0.001) decreased in the colon cancer induced group compared to the normal control group. Treatment with 5 fluorouracil and wheatgrass extract significantly (P<0.001) restored the levels of Ferritin towards the normal. The levels of Carcino embryonic antigen were significantly increased in the colon cancer induced group compared to the normal control group. Treatment with 5 fluorouracil and wheatgrass extract significantly (P<0.001) restored the levels of Carcino embryonic antigen towards the normal (Figure 4, 5).

DISCUSSION

In recent years, it has increasingly been recognized that malignancy may not exclusively result from enhanced cell proliferation but also from decreased physiological cell death, i.e. apoptosis. Apoptotic induction has been a new target for innovative mechanism-based drug discovery. Chemoprevention, a relatively new strategy to prevent cancer, depends on the use of nontoxic chemical substances, to block, reverse, or retard the process of carcinogenesis. Plant-based diet is regarded one of the potential chemo preventive agents. If a plant-derived extract induces apoptosis and has anti-proliferative and antioxidant effects, it might protect normal cells from the damage caused by ROS while inducing apoptosis and inhibiting proliferation in tumor cells. In this study, we investigated in vitro effects of wheatgrass extract on the human Caco-2 cell lines and further investigated the effect of wheatgrass extract in mice. Our results showed that Wheatgrass extract inhibited the growth of colon cancer cells in a concentration dependent manner, compared to the controls. Wheatgrass extract at a concentration of 0.1mg/ml exhibited a maximum of 95% inhibition of growth of colon cancer (Caco-2) cells and a minimum of 10% inhibition at a concentration of 0.01mg/ml. From the graph plotted between the concentrations (X-axis) of wheatgrass extract and cell viability (Y-axis) it was found that the IC50 concentrations of wheatgrass extract were found to be 0.403mg/ml on colon cancer cells.

With their antioxidant potential, wheatgrass extract can be used as a dietary supplement in some diseases as well as in cancer. In other previous studies, it was found that wheatgrass extract induced apoptosis in 32Dp210 cells 6.2 times higher than in32D cells and CAT and SOD activities were increased in the wheatgrass extract added groups; it seemed that this compensatory change could not prevent cell death. Thus, the mechanism of the apoptosis might be based on some reasons other than oxidative stress. Abscisic acid is one of the major constituent in the wheatgrass. ABA may represent a potential cancer treatment due to its ability to modulate calcium signaling. More specifically, modulates pathways in plants and animals involving cADPR, which control the increase in [Ca2+]. In plants, ABA depolarizes plasma membranes, which activates potassium ion channels and thereby extrudes K+ outside the cells. Ion channel depolarization is dependent on calcium, which is an ABA second messenger. As calcium signaling is a key regulator of apoptosis, changes in calcium distribution in the cell activate cellular cascades which lead to cell death. In conclusion, to the best of our knowledge, this is the first study to demonstrate that wheatgrass extracts exert significant anti-proliferative and apoptotic effects against Caco-2 cells in a concentration dependent manner. Thus, it can be concluded that wheatgrass extract might have therapeutic value against colon cancer. However, further investigations on a cellular or molecular level are necessary to describe possible mechanism(s) that cause these effects of wheatgrass.

There is increasing evidence suggesting that certain antioxidants compounds act as preventive or protective factors. The present study is preliminary to measure the antioxidant levels and Hematological parameters in colon cancer induced mice and in treated groups.

The decrease in Hb (hemoglobin) concentration and RBC (red blood cells) value indicates the presence of anemia in all cancer induced mice. This anemia may be caused by GSH depletion in cancer induced mice which is important as cellular antioxidant so its depletion lead to red blood cell destruction which lead to decrease Hb value and the other cause may be the bone marrow failure which is caused by replacement of its normal elements by cancer cells in varying degrees [20] .

These levels were restored to normal in wheatgrass treated groups; chlorophyll is one of the major constituent in the wheatgrass which is structurally similar to the hemoglobin except the central element Iron which is magnesium in chlorophyll. As the chlorophyll is similar to Hb the levels of RBC and hemoglobin may have been restored to normal in wheatgrass treated groups.

A significant difference in WBC count between cancer induced mice and control was noticed in this study. The WBC count in colon cancer induced mice was lower as compared to their controls. The reduced number of WBC count may be due to the loss of blood in stools and may be due to the replacement of normal bone marrow cells by the cancer cells. The levels of WBC were restored to normal in wheatgrass treated and 5-fluorouracil treated groups [21].

Our study showed depletion of reduced glutathione concentration in colon cancer induced mice as shown these results were in agreement with other studies. There can be two reasons for GSH depletion in cancer. Firstly; elevated glutathione peroxidase will use more GSH in an attempt to cope with the excessive production of oxy radicals as revealed by elevated lipid peroxidation. Secondly, if little replenishment of GSH occurred, the level of GSH would become lower.

Oxidative stress plays an important role in the pathogenesis of chronic diseases, such as cancer and atherosclerosis. In these pathological states, the increased production or ineffective scavenging of oxidants may play a crucial role in determining tissue injury. Prime targets of reactive oxygen species are the polyunsaturated fatty acids in cell membranes and their interaction results in lipid peroxidation. Enhanced lipid peroxidation and impairment in antioxidant defense mechanisms were demonstrated in patients with lung and breast cancers [22]. Serum MDA levels were significantly elevated in colon cancer induced groups when compared to controls.

Increased lipid peroxidation in serum and tissues has been reported in colon cancer induced mice. The lipid peroxidation products such as MDA can structurally alter DNA, proteins and other biomolecules. Our findings are in agreement with most of the earlier studies suggesting that colon cancer induced mice might be at risk from oxidative cell damage. Oxidative stress arises when there is an imbalance between oxygen-free radical (OFR) formation and scavenging by antioxidants. Excess generation of free radical can cause oxidative damage to biomolecules resulting in lipid peroxidation. OFR-induced lipid peroxidation has been implicated in neoplastic transformation [23]. The increase in the rate of lipid peroxidation causes the increased production of MDA that leaks into the blood stream, consequently causing increased levels of MDA in mice induced with colon cancer. Wheatgrass extract significantly ($p<0.01$) reduced the levels of MDA in colon cancer treated groups; this may be due to the Super oxide dismutase present in the wheatgrass that converts oxygen free radicals in to hydrogen peroxide and water.

Gamma GT is an oncofoetal protein, a glycoprotein whose levels have been shown to be altered during development and carcinogenesis. In most of the liver diseases, both malignant and non-malignant, GGT estimation has been reported to be a sensitive but nonspecific indicator of the disease [24, 25]. Some studies have shown that GGT levels are also elevated in malignant tumors of the other tissues. This increase in serum GGT activity in cancer induced mice is due to rapid turnover of malignant cells, which release the enzymes in to blood streams. The levels of GGT were restored to normal in wheatgrass treated groups [26, 27, 28].

Lactate dehydrogenase is an oxidation reduction enzyme which reversibly catalyses the reaction between pyruvic acid to lactic acid. It is distributed widely in body tissues and is raised in variety of physiological and pathological status. LDH is thus derived from the tissues and its high levels in serum indicate the destruction of the cells [29]. There was significant rise ($p < 0.001$) in LDH levels in colon cancer induced mice than in control group mice. When serum LDH levels was compared in different stages of cancer, the enzyme levels raised significantly with increase in severity (stage) of the disease [30, 31, 32]. It has been reported that tumor cells excrete large amounts of lactic acid, because glucose is metabolized via the anaerobic glycolytic pathway to lactic acid when compared to normal cell. And serum levels of glycolytic enzymes were found to be increased in mice with colon and breast carcinoma. Raised levels of LDH are seen in malignancies because of high rate of glycolysis, increased production of enzyme by tumor cells, change in the permeability of cells, allowing leakage of soluble enzyme into circulation and because of tumor blockade of the duct system through which enzyme passes. Induction of LDH synthesis in the normal tissues of the host by the tumor also contributes to raise LDH levels. Post treatment serum enzyme studies on the 30th day in the cancer group showed that, the enzyme levels tend to decline but do not touch the base line. Thus LDH levels are a good adjunct in the diagnosis, are an indicator of the stage of the disease, response to treatment and prognosis of mice.

The levels of SGPT, ALP and Glucose were significantly (P<0.001) changed in colon cancer induced groups compared to their respective controls and these levels were restored in the wheatgrass treated groups.

Carcino embryonic antigen is normally produced during fetal development, but the production of CEA stops before birth. Therefore, it is not usually present in the blood of healthy adults, although levels are raised in heavy smokers. It was found that serum from individuals with colorectal, gastric, pancreatic, lung and cervical carcinomas had higher levels of CEA than healthy individuals. CEA testing is of significant value in the monitoring of patients with diagnosed malignancies in whom changing concentrations of CEA are observed. CEA is encoded by the CEA gene which is a member of the immunoglobulin super family. The human CEA gene family is clustered on chromosome 19q. In humans, the Carcino embryonic antigen family consists of 29 genes; of these, 18 are expressed, with 7 belonging to the CEA subgroup and 11 to the pregnancy-specific glycoprotein subgroup CEA measurement is mainly used to identify recurrences after surgical resection. Elevated CEA levels should return to normal after surgical resection, as elevation of CEA during follow up is an indicator of recurrence of tumor. CEA is a substance normally found in a fetus which, when found at elevated levels in the blood of adults, may indicate the presence of colorectal cancer or other types of cancer. CEA is therefore called a tumor marker. CEA levels can also be an indication of the effectiveness of treatment. Tests for its presence in the serum of the cancer patients aid in screening, in evaluating recurrent or disseminated disease, and in gauging the success of surgical removal of malignant tumors. CEA is most frequently tested in blood. It can also be tested in body fluids and in biopsy tissue. The best use of CEA is as a tumor marker, especially for cancers of the gastrointestinal tract and cervical cancers. In our study the levels of CEA were significantly (p<0.001) increased in cancer induced mice compared to normal controls, whose levels were significantly (p<0.001) decreased/restored to normal in wheatgrass treated groups [33].

Ferritin, a 450 KDa protein consisting of 24 subunits is present in every cell type. In vertebrates, these subunits are both the light (L) and the heavy (H) type with an apparent molecular weight of 19 KDa or 21 KDa respectively. In plants and bacteria the complex only consists of the H-chain type. Inside the Ferritin shell, iron ions form crystallites together with phosphate and hydroxide ions [34]. The resulting particle is similar to the mineral ferrihydrite. Each Ferritin complex can store about 4500 iron (Fe3+) ions. Decreased Ferritin levels are reported in advanced cancers of breast, ovaries, lungs, colon and esophagus. In our study the levels of Ferritin were decreased significantly (P<0.001) in cancer induced groups compared to the normal control groups and the levels were restored to normal levels in the wheatgrass treated groups.

Appearance of HCG in tumor cells, induced or not by a specific agent as bacterium or it occurring in placenta or membranes of sperm cells, is a natural mechanism for protecting foreign cells against the immune system of a host organism. HCG is a sialoglycoprotein hormone produced by the human placenta having by function maintenance of the steroid hormone secretions of the corpus luteum and protecting the embryo and fetus against the immune system of the mother. A medicine against cancer must have a fundamental property of counteracting HCG to facilitate viability of tumor destruction by the immune system. Abscisic acid is one of the major constituent in the wheatgrass that neutralizes the negative groups present on the HCG there by facilitating the body immune system to act against the cancer cells [35].

Wheatgrass has 60% chlorophyll also known as wheatgrass chlorophyll which is the basis of plant life. Both chlorophyll and hemoglobin share a similar atom structure to create their respective molecules. Human blood and hemoglobin consist of iron, while in chlorophyll the metallic atom is magnesium. Magnesium found in the proton of chlorophyll is essential and beneficial for about 30 enzymes of our body.

Water-based (i.e. wheatgrass juice) and alcohol-based extracts of wheatgrass showed antioxidant levels in phenolic and flavonoid compounds. The Wheat grass juice may prevent myelotoxicity when applied with chemotherapy. The Wheat grass juice is an effective iron chelator, and it is used in reducing serum ferritin, myelodysplastic syndrome, and other diseases [35]. These phyoconstituents may be responsible for antitumor activity of wheat grass.

From the histopathology study, mucosal degeneration of intestinal epithelial cells in the mucosa was observed in colon cancer induced group. Mucosal cell hyperplasia was observed in the mucosal layer in the wheatgrass extract and 5-FU treated groups. Sub mucosa and muscular region appeared normal in wheatgrass and 5-FU treated groups which is similar to normal control group. In conclusion, to the best of our knowledge, this is the first study to demonstrate that wheatgrass

extracts exert significant anti-proliferative and antioxidant against Caco2 cells in a concentration- and time-dependent manner. Thus, it can be concluded that wheatgrass extract might have therapeutic value against colon cancer. However, further investigations on a cellular or molecular level are necessary to describe possible mechanism(s) that cause these effects of wheatgrass.

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Table 1: Effect of Wheatgrass on SGPT, LDH, GGT, ALP and Glucose in Caco-2 cell line induced mice.

Groups	SGPT(IU/L)	LDH(IU/L)	GGT(IU/L)	ALP(IU/L)	Glucose (mg %)
Group I Normal control	20.9±0.98	45.32±0.83	22.24±1.26	9.46±0.24	110.12±3.19
Group II Cell line induced	39.67±1.4^^^	57.32±0.61***	28.29±1.26^^	20.44±1.19^^^	92.14±1.27^^^
Group III 5- FU	24.23±1.32***	38.83±1.24^^^	18.14±0.86***	15.36±0.51**	147.12±1.86***
Group IV Wheat grass	28.33±0.88**	48.26±1.36***	18.85±0.34**	12.6±0.43***	129.2±6.2***

Values are expressed as mean ± SEM., Data was analyzed by unpaired t test. ^^^p<0.001, ^^p<0.01 and ^p<0.05as compared with the normal control; ***p<0.001**p<0.01and *p<0.05 as compared with cell line induced group. 5-FU= 5 Fluorouracil.

Table 2: Effect of Wheatgrass on Catalase, MDA, GSH and GPx in Caco-2 cell line induced mice.

Groups	Catalase (µm/min/mg pt)	MDA (m/L)	GSH (mg/g tissue)	GPx (µm/min/mg pt)
Group I Normal control	0.41±0.07	50.25±2.4	14.67±0.77	7.87±0.35
Group II Cell line induced	0.24±0.01^	97.30±2.8^^	7.57±0.13^^	4.23±0.32^^
Group III 5- FU	0.34±0.09**	74.58±0.8**	12.68±0.93	6.24±1.6
Group IV Wheat grass	0.36±0.02**	68.42±2.62***	11.80±0.8**	5.84±0.44*

Values are expressed as mean ± SEM., Data was analyzed by unpaired t test. ^^^p<0.001, ^^p<0.01 and ^p<0.05as compared with the normal control; ***p<0.001**p<0.01and *p<0.05 as compared with cell line induced group. 5-FU= 5 Fluorouracil, MDA=malondialdehyde, GSH=reduced glutathione, GPx=glutathione peroxidase

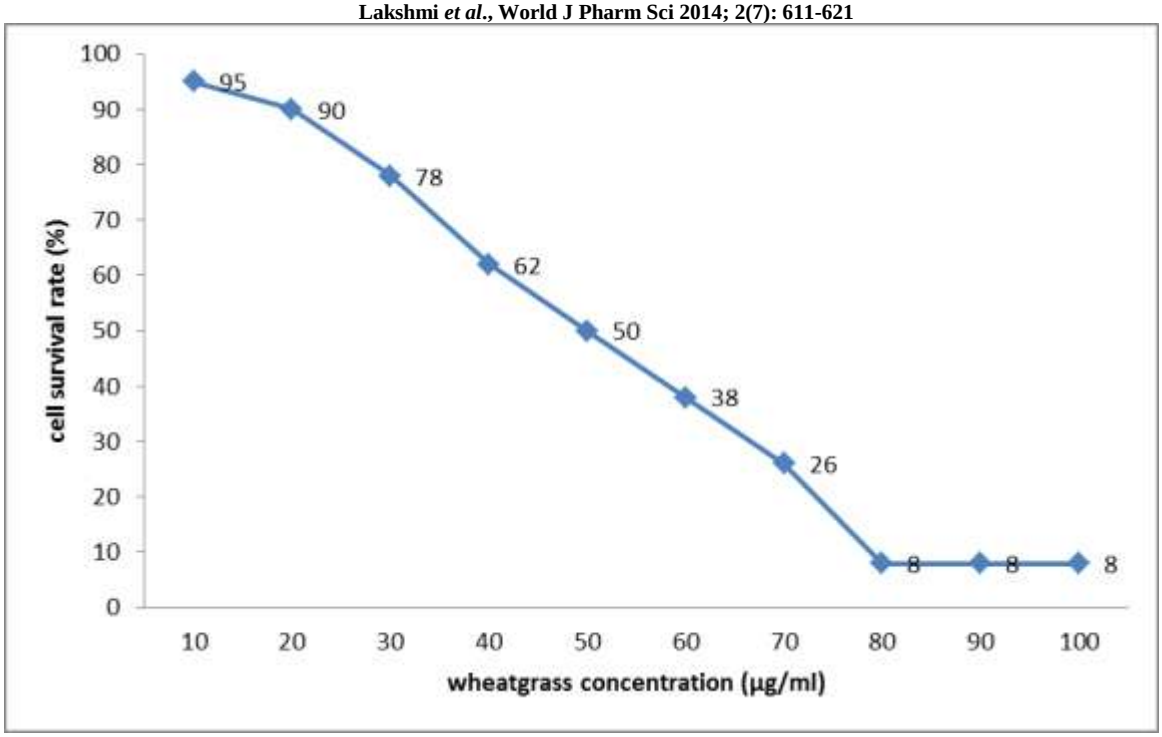

Figure 1: Effect of wheatgrass extract on survival rate of Caco-2 colon cancer cells

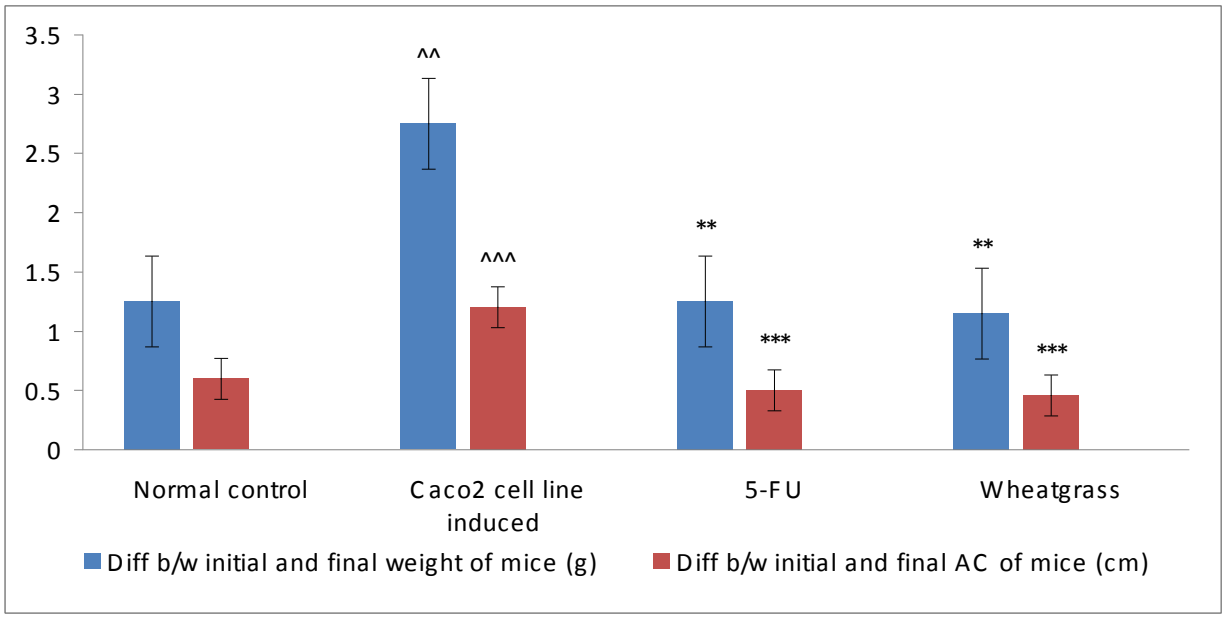

Figure 2: Effect of Wheatgrass on weight and abdominal circumference in Caco-2 cell line induced mice. Values are expressed as mean ± SEM., Data was analyzed by unpaired t test. ^^^p<0.001, ^^p<0.01 and ^p<0.05as compared with the normal control; ***p<0.001**p<0.01and *p<0.05 as compared with cell line induced group. 5-FU= 5 Fluorouracil, AC= abdominal circumference

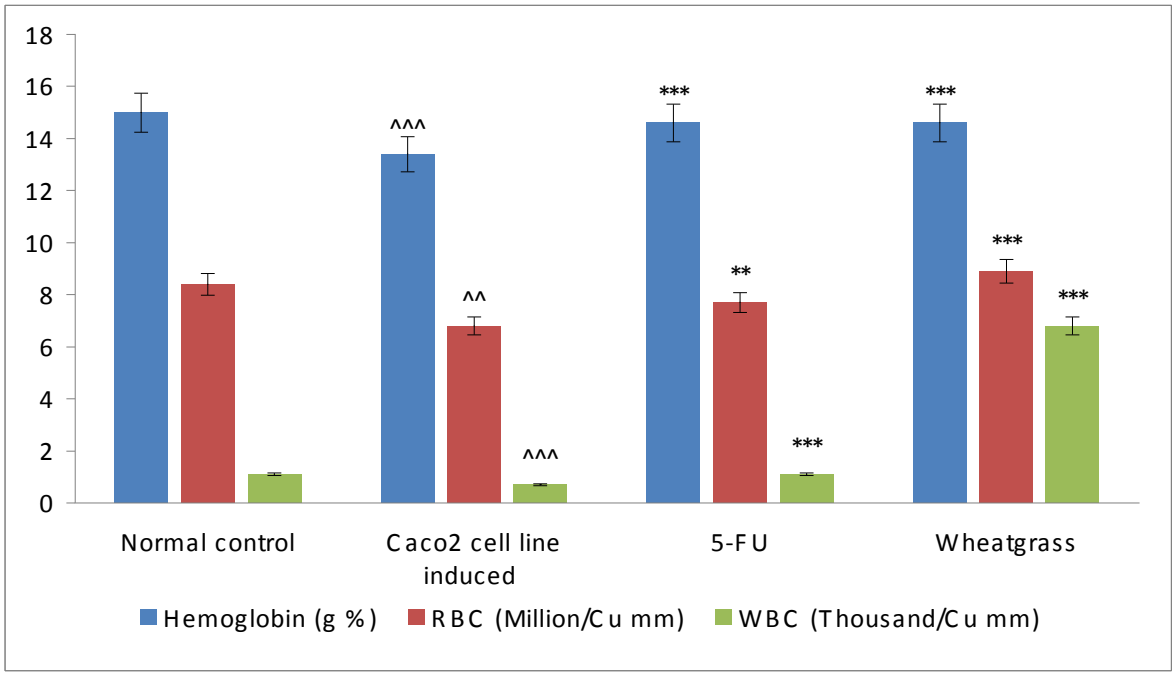

Figure 3: Effect of Wheatgrass on Hemoglobin, RBC, and WBC in Caco-2 cell line induced mice. Values are expressed as mean ± SEM., Data was analyzed by unpaired t test. $^{\wedge\wedge\wedge}p<0.001$, $^{\wedge\wedge}p<0.01$ and $^{\wedge}p<0.05$as compared with the normal control; ***p<0.001**p<0.01and *p<0.05 as compared with cell line induced group. 5-FU= 5 Fluorouracil.

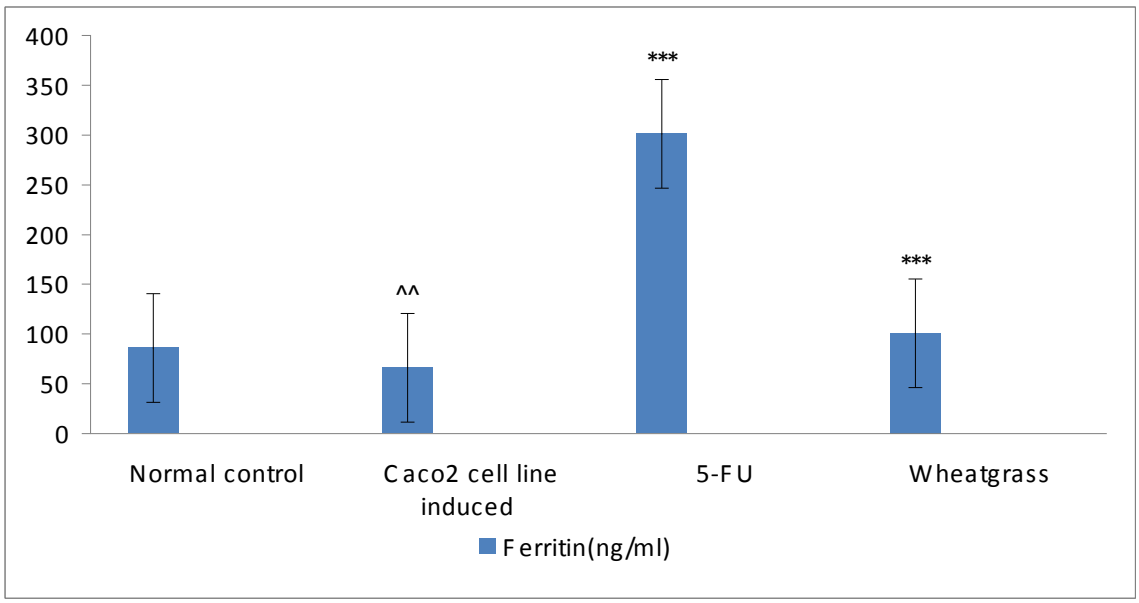

Figure 4: Effect of Wheatgrass on Ferritin in Caco-2 cell line induced mice. Values are expressed as mean ± SEM., Data was analyzed by unpaired t test. $^{\wedge\wedge\wedge}p<0.001$, $^{\wedge\wedge}p<0.01$ and $^{\wedge}p<0.05$as compared with the normal control; ***p<0.001**p<0.01and *p<0.05 as compared with cell line induced group. 5-FU= 5 Fluorouracil.

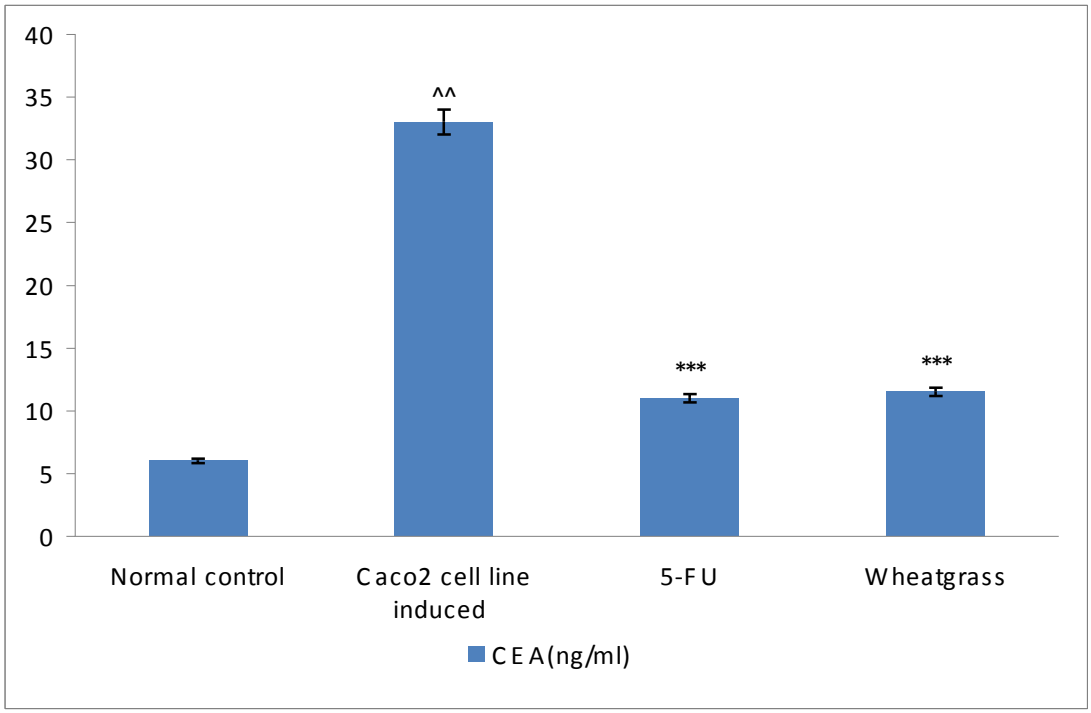

Figure 5: Effect of Wheatgrass on Carcino embryonic antigen in Caco-2 cell line induced mice. Values are expressed as mean ± SEM., Data was analyzed by unpaired t test. $^{\wedge\wedge\wedge}p<0.001$, $^{\wedge\wedge}p<0.01$ and $^{\wedge}p<0.05$as compared with the normal control; ***p<0.001**p<0.01and *p<0.05 as compared with cell line induced group. 5-FU= 5 Fluorouracil, CEA=Carcino embryonic antigen.

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