Research Article **ISSN:2321-2624**

International Journal of Medicine and Pharmaceutical Research
www.pharmaresearchlibrary.com/ijmpr

Antitumor Activity of Triticum Aestivum against MCF-7 Cell Line Induced Breast Cancer and $CaCo_2$ Cell Line Induced Colon Cancers

[1]Kranthi Ponugoti[*], [1]Rohitha Kesireddy, [2]Kartheek.S, [3]K.Satish

[1]Malla Reddy College of Pharmacy, Hyderabad, India
[2]Annamalai University, Tamilnadu, India
[3]Vikascollege of Pharmaceutical Sciences, Suryapet, India
Received: 28 July 2014, Accepted: 29 August 2014, Published Online: 10 October 2014

Abstract

Antitumor activity of wheatgrass (400 mg/kg) was evaluated against the MCF-7 and Caco2 cell lines and these cell lines induced breast and colon cancer in mice. Materials and Methods in used Invitro cytotoxicity of wheatgrass was observed by MTT assay. After 24 hr of tumor inoculation, the wheatgrass was administered daily for 30 days. After administration of the last dose followed by 18 hr fasting, mice were sacrificed for observation of antitumor activity. The effect of wheatgrass on cell proliferation (invitro) was estimated. The change in body weight, abdominal circumference of tumor bearing hosts and simultaneous alterations in the hematological profile, serum (SGPT, LDH, ALP and glucose) and liver biochemical parameters (lipid peroxidation, GSH and antioxidant enzymes) were estimated. The changes in Carcinoembryonic antigen and ferritin levels were estimated. The wheatgrass inhibited the cell growth (invivo) and exhibited apoptosis of cell lines. 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 the serum biochemical parameters towards normal levels and decreased the levels of lipid peroxidation and increased the levels of reduced glutathione and other antioxidant enzymes (CAT and GPx). The wheatgrass treatment restored the Carcino embryonic antigen and ferritin levels in tumor induced mice. The wheatgrass exhibited antitumor effect by modulating the hematological parameters, lipid peroxidation and augmenting antioxidant defense system in tumor bearing mice.

Keywords: Cancer Tumor, Invitro & Invivo Studies, Cell line, Plant Extracts, Animals (Mice).

Contents

***Corresponding author**
Kranthi Ponugoti
Malla Reddy College of Pharmacy, Hyderabad, India
Manuscript ID: IJMPR2258

PAPER-QR CODE

1. Introduction

Herbal medicine (or "herbalism") is the study and use of medicinal properties of plants. Herbalism is a traditional medicinal practice based on the use of plants and plant extracts. Herbalism is also known as botanical medicine, medical herbalism, herbal medicine, herbology and chemical compounds that act upon the body and are used to prevent or treat disease or promote health and well-being. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. The use of herbs to treat disease is almost universal phytotherapy. Herb plants produce and contain a variety of among non-industrialized societies. With over 80% of the world's population relying on herbs for health[1, 2]. Cancer is a leading cause of death worldwide. In 2005 7.6 million people died of cancer (13% of all deaths worldwide), the range between 55 and 75 years being the age where most cancer mortalities occur. The cancer types with the highest mortality rates are lung, stomach, liver, and colon and breast cancer.

A tumor is made up of two components, the parenchyma and the supporting, non-neoplastic cells. The latter part is made up of connective tissue and blood vessels. It supplies the parenchyma with nutrients via the blood stream and support the growth of the parenchyma cells. The parenchyma is made up of neoplastic cells. This part determines the tumors biological behavior.

Cancer tumors arise from one single cell, and originate in genetic damage. This genetic damage can be initiated by environmental agents, such as chemicals, radiation or viruses. This mutation can result in activation of growth-promoting oncogenes, alterations of genes that regulate apoptosis or inactivation of cancer suppressor genes. Some of the changes in cell physiology that occurs in cancer tumors compared to normal tissue are self-sufficiency in growth signals, the cells do not respond to growth-inhibitory signals, respond to programmed cell death, sustained angiogenesis and gain the ability to invade and metastasize. Tumors can spread through seeding within body cavities, through the lymphatic system or the blood circulation. [3, 4]

Cancer cell Proliferation is the growth or cell division of cells. The proliferation of cancer cells is often more rapidly and less controlled than normal cells. Stimulation of a normal cell into proliferation depends upon the external signals of growth factors. This eventually leads to cell division. Tumor cells are not dependent on external growth factors in the same way as normal cells. They are able to proliferate when the concentration of growth factors are much lower than those required by normal cells. When normal dividing cells reach a certain density they stop proliferating. Cancer cells continue to replicate too much higher densities than normal cells. [3]

An important mechanism that regulates proliferation is the cell cycle. After a growth stimulus, cells may enter the cell cycle in G1 phase, pass through S phase, in which DNA synthesis is stimulated, and progress to G2 phase ready for mitosis (M phase). CDK-inhibitors stops cell progression through the cell cycle. The relative levels of cyclins, CDKs and CDK-inhibitors change throughout the cell cycle. Flow cytometry is a method used to measure the proportion of cells in each phase of the cell cycle. The DNA of the cells is stained with a fluorescent dye before they are sent in a fluid stream through a laser beam. It is cells size, granularity, internal complexion and fluorescence intensity that are measured. [3]

Angiogenesis is induced by the tumor cells own production of angiogenic factors. Some of the most important angiogenic factors are vascular endothelial growth factor (VEGF) and fibroblast growth factor. When a tumor is subject to hypoxia, hypoxia-inducible factor-1 (HIF-1) is released. HIF-1 controls transcription of VEGF. Thus, hypoxia can induce apoptosis. [3]

Cell line Cell culture refers to the removal of cells from an animal or plant and their subsequent growth in a favorable artificial environment. Primary culture refers to the stage of the culture after the cells are isolated from the tissue and proliferated under the appropriate conditions until they occupy all of the available substrate. Cell Line After the first subculture, the primary culture becomes known as a cell line or sub clone.

2. Materials and Method

Plant: Wheatgrass was purchased from the normal distributor.

Cell lines: MCF-7 Breast cancer cell lines and Caco2 colon cancer cell lines. The cell lines were obtained from National Institute of Nutrition.

Animal selection:

Twenty four male Swiss Albino mice weighing 20gms-30gms and twenty four Female Swiss Albino mice weighing 20gms-30gms were obtained from National Institute of Nutrition, Hyderabad. The mice were housed in polypropylene cages and maintained under standard conditions (12 hr 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/12).

Table 1: Equipments

Name Of Instrument	Manufacturer
Tissue Homogenizer	Remi
U.V- Visible spectrophotometer	Elico
Cool centrifuge	Remi, Mumbai
Digital colorimeter	Lab med
Deep refrigerator	Remi

Table 2: Chemicals

ALP kit, LDH kit, SGPT kit	Span cogent
Acetic acid, Hydrochloric acid, Phosphoric acid	Srl
Catalase, Glutathione reductase.	Sigma
CDNB	Arva synthesis
DTNB	Bio chemicaka fluka
H_2O_2, Methanol	Finar
K_2HPO_4, Na_2HPO_4, NaOH, TBA, Sodium Citrate	Hi-media
Glucose kit	Erba
SLS	Finar
GGT kit	Crest biosystems

Methodology:

Preliminary phytochemical screening:

Preliminary phytochemical screening was done on Wheatgrass for the presence of carbohydrates, amino acids, proteins, saponins, alkaloids, flavonoids, glycosides, sterols, tannins, tri-terpenoids and phenolic compounds according to the procedures described in "Text book of Practical Pharmacognosy" by C.K. Kokate.

Determination of LD_{50} of Extract of Wheatgrass:

Acute toxicity study of wheatgrass 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 at the dose of 2000mg/kg. Hence 1/5 of the dose 2000mg/kg i.e. 400mg/kg has been fixed for the study.

Determination of Invitro cytotoxicity of wheat grass against MCF-7 breast cancer and Caco2 colon cancer cell lines.

MTT Assay for Experimental Samples:

a. Plate cells at the concentration determined using the procedure on pages 3 - 4. Plate triplicate wells at 100 µL/well for each variable. Be sure to plate enough wells to include cell-based controls, and include three wells of cell culture medium alone.
b. Incubate the cells to allow them to recover and reattach (if adherent) and treat according to your established experimental protocol.
c. Add 10 µL of MTT reagent to each well. If more than 100 L of cell culture medium was used per well, increase the amount of MTT added proportionately.
d. Incubate the plate for approximately 2 - 4 hours at 37° C. View the cells periodically for the appearance of punctate, intracellular precipitate using an inverted microscope. Longer incubation times (up to 24 hours) may be required, depending on the cell type and experimental conditions.
e. When purple precipitate is clearly visible under the microscope, add 100 µL of Detergent Reagent to all wells, including control wells. Do not shake. Leave plate covered in the dark at 18 - 24° C for at least 2 hours to overnight. Samples may be read after 2 hours, but if the readings are low and there are crystals remaining, return the plate to the dark and incubate for a longer period. Room temperature (18 - 24° C) incubation is sufficient, but incubation at 37° C may help to shorten the solubilization time.
f. Remove the plate cover and measure the absorbance of the wells, including the blanks, at 570 nm with a reference wavelength of 650 nm. If a 570 nm filter is not available, absorbance may be read with any filter in the wavelength range of550 - 600 nm. The blanks should give values of 0 0.1 O.D. units.
g. Determine the average values from triplicate readings and subtract the average value for the blank. Plot the absorbance on y-axis and treatment on x-axis.

Determination of Anti-Tumor Activity of Wheatgrass Against Cell Line Induced Breast And Colorectal Cancer In Swiss Albino Mice:

Experimental Design:

Twenty four male Swiss Albino mice weighing 20gms-30gms and twenty four Female Swiss Albino mice weighing 20gms-30gmswere divided into eight groups of six animals each.

For breast cancer:

Group 1: Female Control group (1% gum acacia 1ml)
Group 2: MCF-7 cell line 2×106 cells/mouse I.P
Group 3: MCF-7 cell line 2×106 cells/mouse I.P. + 5-Flourouracil 20mg/kg body weight I.P.
Group 4: MCF-7 cell line 2×106 cells/mouse I.P. + Wheatgrass extract 400 mg/kg body weight p.o.

For colon cancer:

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.

Experimental Protocol:

Treatment schedule:

Animals were grouped into 8 groups as explained above. The two control group animals were given 1% gum acacia 1ml for 30 days. Group 2 animals were given and MCF-7 cell line 2×10^6 cells/mouse I.P Caco2 cell line 2×10^6 cells/mouse I.P. Group 3 animals were given MCF-7 cell line 2×10^6 cells/mouse I.P and 5-Flourouracil 20mg/kg body weight I.P until 30th day and Caco2 cell line 2×106 cells/mouse I.P and 5-Flourouracil 20mg/kg body weight I.P until 30th day. Group 4 animals were given MCF-7 cell line 2×106 cells/mouse I.P and Wheatgrass 400 mg/kg body weight p.o until 30th day and Caco2 cell line 2×106 cells/mouse I.P and Wheatgrass 400 mg/kg body weight p.o until 30th day.

Blood sample preparation:

The animals were sacrificed on 30^{th} day using ether anesthesia, blood was collected by carotid puncture method. Blood was collected 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 30^{th} day using ether anesthesia, blood was collected by carotid puncture method. Blood 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 supernant was separated and used for estimation of anti-oxidant levels of different enzymes i.e. Catalase and reduced glutathione, malondialdehyde and glutathione peroxidase.

3. Results and Discussion

Results

Acute Toxicity Study of Wheatgrass:

No signs of toxicity were found up to the dose of 2000 mg/kg body weight.
Hence $1/5^{th}$ dose i.e. 400 mg/kg has been fixed as ED_{50} for present study.

Preliminary Phytochemical Screening of Wheatgrass:

The main chemical constituents that are found in the extract of Wheatgrass are given in the

Table 3: Preliminary Phytochemical Screening of Wheatgrass

Chemical Constituent	Result
Carbohydrates,Proteins, Aminoacids, Saponins, Alkaloids,Tannins and phenolic compounds Triterpenoids	+
Glycosides, Fats/oils	-

'+' represents presence of compounds
'-' represents absence of compounds

Effect of wheatgrass on cell lines:

Wheatgrass concentration (µg/ml)	Cell survival rate (%)
100	96
200	82
300	69
400	46
500	31
600	16
700	10
800	10
900	10
1000	10

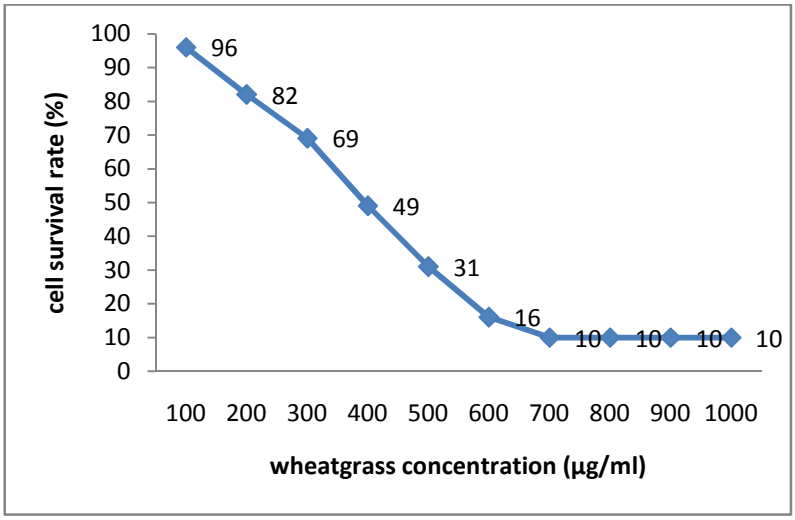


Figure 1: Effect of wheatgrass on survival rate of MCF-7breast cancer cells

Wheatgrass concentration (µg/ml)	Cell survival rate (%)
10	95
20	90
30	78
40	62
50	50
60	38
70	26
80	8
90	8
100	8

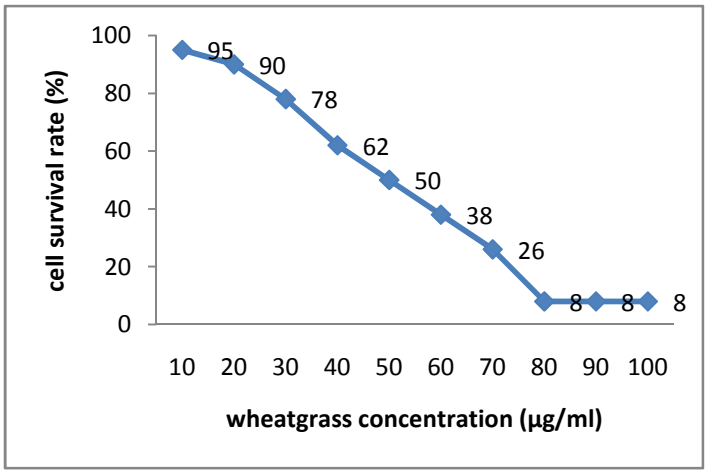


Figure 2: Effect of wheatgrass on survival rate of Caco-2 colon cancer cells

Invivo Results:

Groups	Diff b/w initial and final weight (g)	Diff b/w initial and final AC (cm)
Group I Normal control	2.25±0.48	0.70±0.05
Group II Cell line induced	5.50±0.65^^	1.10±0.058^^^
Group III 5- FU	1.25±0.25***	0.53±0.03***
Group IV WHEATGRASS	1.50±0.29***	0.43±0.03***

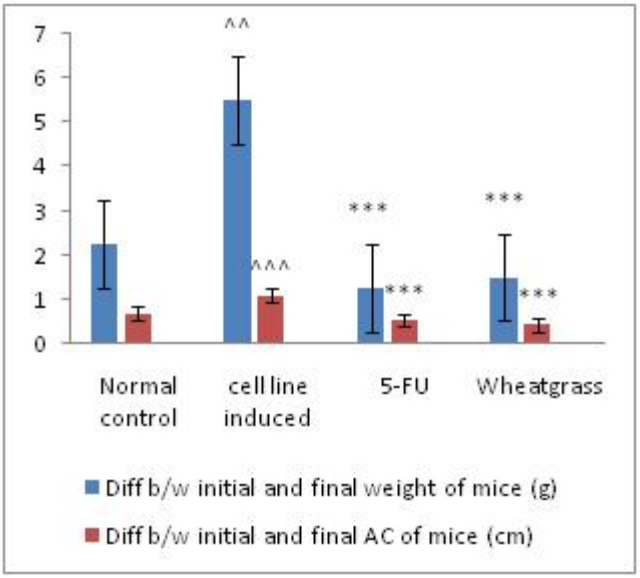


Figure 3: Effect of Wheatgrass on weight and abdominal circumference in MCF-7 cell line induced mice

Groups	Hemoglobin (g %)	RBC (Million/Cu mm)	WBC (Thousand/Cu mm)
Group I Normal control	15.15±0.25	8.7±0.15	0.95±0.05
Group II Cell line induced	13.57±0.13^^^	6.60±0.20^^^	1.55±0.07^^^
Group III 5- FU	14.3±0.1**	7.40±0.10**	1.10±0.04***
Group IV WHEATGRASS	14.7±0.1***	7.60±0.25**	0.30±0.06***

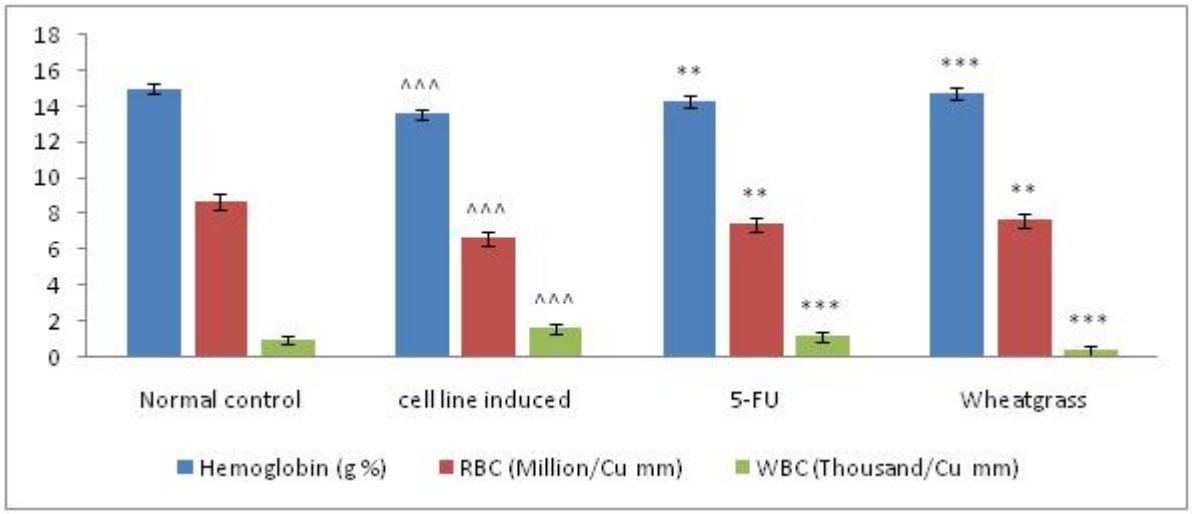


Figure 4: Effect of Wheatgrass on Hemoglobin, RBC, and WBC in MCF-7 cell line induced mice

Groups	SGPT(IU/L)	LDH(IU/L)	GGT(IU/L)	ALP(IU/L)	Glucose (mg %)
Group I Normal control	18.2±2.32	34.52±0.93	16.24±0.66	8.81±0.78	120.14±5.32
Group II Cell line induced	42.07±0.38^^^	52.78±1.39***	19.24±1.36	13.36±0.64^^	95.14±1.6^^
Group III 5- FU	20.04±0.70***	35.84±0.55^^^	16.46±0.85	11.45±0.60	115.3±4.53**
Group IV WHEATGRASS	27.67±0.79***	42.68±1.16***	15.5±0.29*	10.10±0.41**	144±6.8***

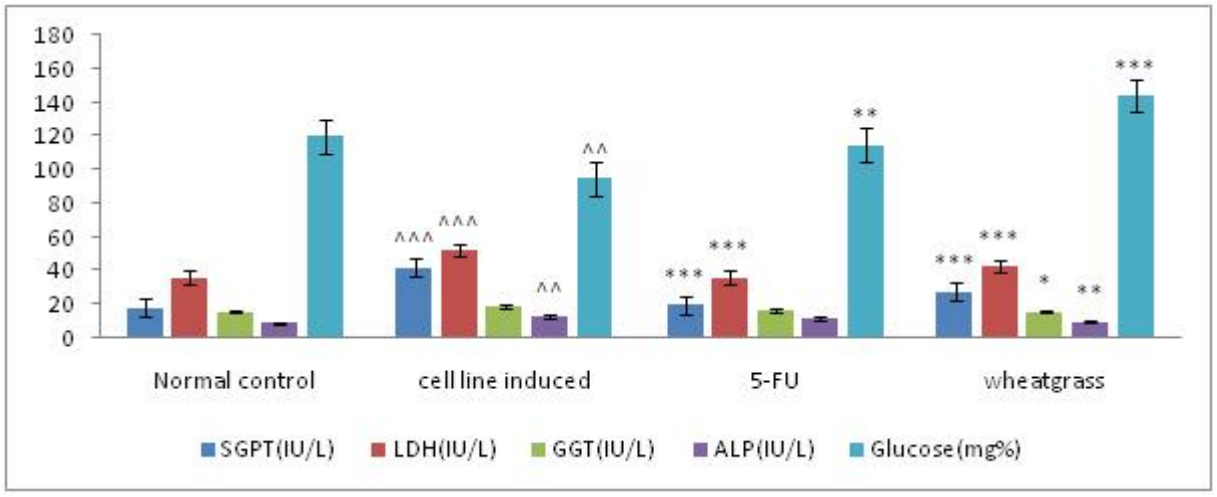


Figure 5: Effect of Wheatgrass on SGPT, LDH, GGT, ALP and Glucose in MCF-7 cell line induced mice

Groups	Catalase (µm/min/mg pt)	MDA (m/L)	GSH (mg/g tissue)	GPX (µm/min/mg pt)
Group I Normal control	0.38±0.01	71.02±0.8	12.68±0.77	8.67±0.68
Group II Cell line induced	0.26±0.06^^	110.84±2.48^^	7.12±0.16^^	5.23±0.86^
Group III 5- FU	0.32±0.07**	83.20±1.3**	10.4±1.6	7.12±0.93
Group IV WHEATGRASS	0.34±0.042*	84.60±2.34***	11.22±1.10**	7.80±0.46*

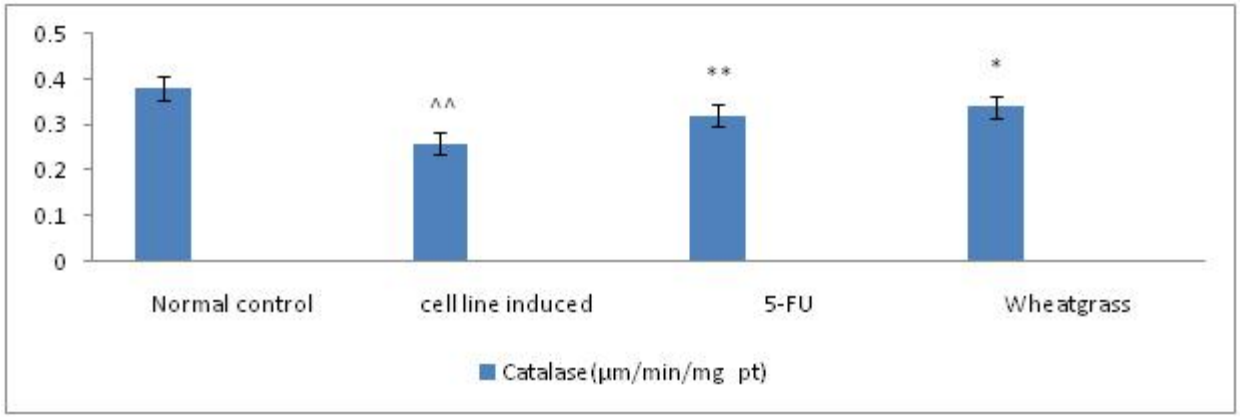
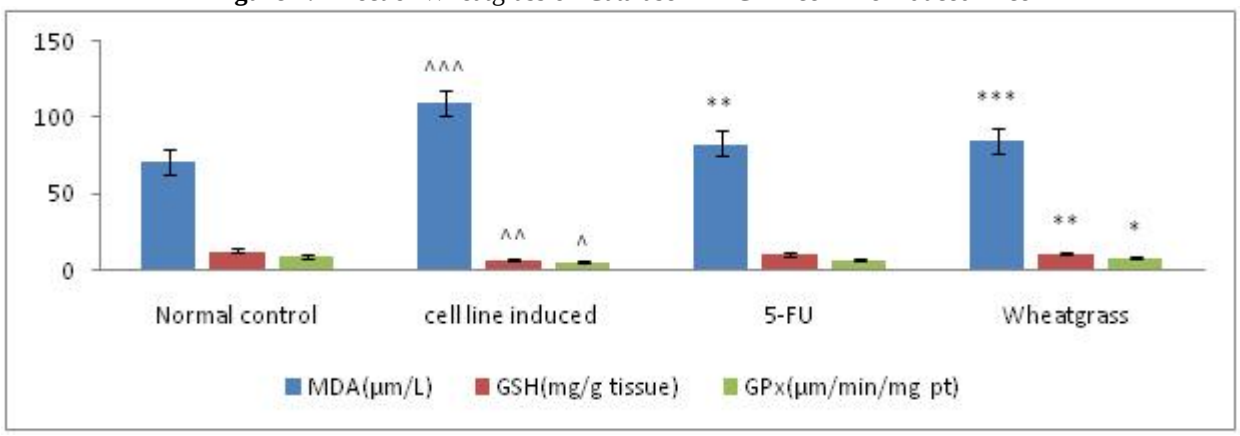


Figure 6: Effect of Wheatgrass on Catalase, MDA, GSH and GPx in MCF-7 cell line induced mice

Figure 7: Effect of Wheatgrass on Catalase in MCF-7 cell line induced mice



Effect of Wheatgrass on MDA, GSH and GPx in MCF-7 cell line induced mice.

Groups	Ferritin (nag/ml)	CEA (nag/ml)
Group I Normal control	180.60±6.44	6.10±0.34
Group II Cell line induced	140.20±5.60^^^	24.80±0.82^^^
Group III 5- FU	204.60±6.25***	11.80±0.36***
Group IV WHEAT GRASS	199.80±6.40***	12.40±0.42***

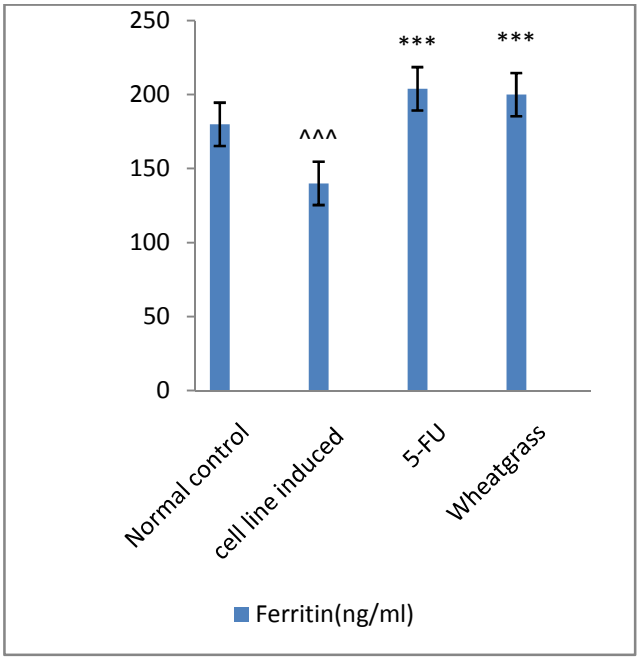


Figure 8: Effect of Wheatgrass on Ferritin and Carcino embryonic antigen (CEA) in MCF-7 cell line induced mice

Effect of Wheatgrass on Ferritin in MCF-7 cell line induced mice

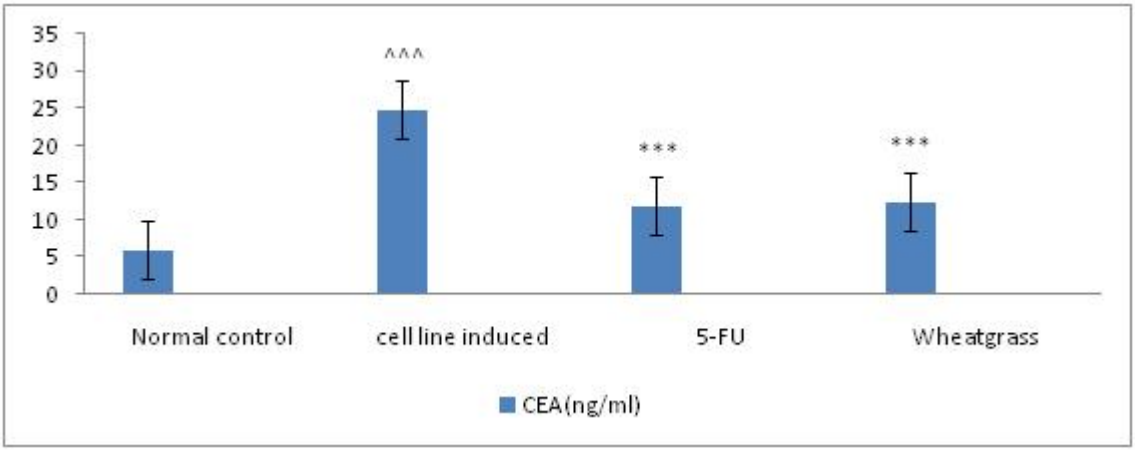


Figure 9: Effect of Wheatgrass on Carcino embryonic antigen in MCF-7 cell line induced mice

Discussion:

From the histopathology study it was observed that there is a proliferation and inflammation of cells in the breast cancer induced tissue and we observed the hyperplasia of the epithelium in the cancer induced group. The breast tissue was normal in wheatgrass treated group which is similar to the normal control group and 5-FU treated group. 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 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.

4. Conclusion

The wheatgrass exhibited antitumor effect by modulating the hematological parameters, lipid peroxidation and augmenting antioxidant defense system in tumor bearing mice. The wheatgrass also restored the hepatic lipid peroxidation and free radical scavenging enzyme GSH as well as other antioxidant enzymes such as CAT, GGT and GPx in tumor bearing mice to near normal levels.Thus, it can be concluded that wheatgrass might have therapeutic value against colon and breast cancer. However, further investigations on a cellular or molecular level are necessary to describe possible mechanism(s) that cause these effects of wheatgrass.

5. References

1. Memory Elvin Lewis. Should we be concerned about herbal remedies; *Journal of Ethno pharmacology*. **2001**, 75(2): 141–164.
2. Duke James A. Triticum aestivum L. Handbook of Energy Crops, **1983**.
3. Schneider, K. **2001**. Counseling about cancer: Strategies for genetic counseling. 2d ed. New York: John Wiley & Sons.
4. Lichtenstein et al. Environmental and heritable factors in the causation of cancer: analyses of cohorts of twins from Sweden, Denmark, and Finland. *N Engl J Med*, **2000**, 343(2): 78-85.
5. Avis N, Crawford S, Manuel J, et al. Quality of life among younger women with breast cancer. *J Clin Oncol*. **2005,** 23: 3322–3330.
6. Azim HA Jr, et al. Safety of pregnancy following breast cancer diagnosis: a meta-analysis of 14 studies. *EurJCancer*. **2011** Jan, 47(1):74-83.
7. Compton C, et al. Colon cancer. In: *Abeloff's Clinical Oncology*. 4th ed. Philadelphia, Pa: Elsevier; **2008**:1477–1534.
8. Johns LE, Houlston RS. A systematic review and meta-analysis of familial colorectal cancerrisk. *Am J Gastroenterol*. **2001** Oct, 96(10): 2992-3003.
9. Adachi A, et al. Productive, persistent infection of human colorectal cell lines with human immunodeficiency virus. *J. Virol*. **1987**, 61: 209-213, *Pub Med*: 3640832
10. OyaSena, et al, Anti proliferative, apoptotic and antioxidant activities of wheatgrass (Triticum aestivum L.) extract on CML (K562) cell line. *Turk J Med Sci.*, **2011**, 41(4): 657-663.
11. Sean M. Wheatgrass, healthy for the body and the bank account. *Australia's National Rural Affairs Weekly- Landline*. 13-10-**2002**, Retrieved 2006-1006.

12. Shirude et al, Phytochemical and Pharmacological Screening of Wheatgrass Juice Volume 9, Issue 1, July – August **2011**, Article-029.
13. Dey S et al, Effect of Wheat grass Juice in supportive care of terminally ill cancer patients- A tertiary cancer centre Experience from India. Journal of Clinical Oncology, **2006**.
14. Ben-Arye E, Wheat Grass Juice in the Treatment of Active Distal Ulcerative Colitis: A Randomized Double-blind Placebo-controlled Trial. **2002**, 37(4): 444-449.
15. Hawk ET, Levin B. Colorectal cancer prevention. *J Clin Oncol*. **2005**, 23: 378–388.
16. Kulkarni et al, Determination of elemental concentration profiles in tender wheat grass (Triticum aestivum L.) using instrumental neutron activation analysis. Food Chemistry, **2006**, 4: 699-70.
17. Swati P, et al, Multitude potential of Wheat grass Juice (Green Blood): An overview. Chronicles of Young Scientist, **2010**, 1(2): 23-28.
18. Bar-sella P. Rejuvenation- Health According to Dr. Ann Wigmore's Teachings. Kiryat Ono. Israel: Shachar Ltd, (in Hebrew), **1998**: 142-152.