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RESEARCH ARTICLE

**ANTICANCER AND CYTOTOXIC POTENTIAL OF AQUEOUS EXTRACT OF
TRITICUM AESTIVUM ON HELA CELL LINE****Dr. Patel Janki Bhulabhai ***

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ABSTRACT

The objective of the study was to analyze the anticancer property of the leaves of *Triticum aestivum* on HeLa cells. The Indian medicinal plant *Triticum aestivum* that is used in traditional medicine for cancer and non cancerous diseases was collected. The crude aqueous extract was prepared by using standard protocols. The antiproliferative effect of the aqueous extract was evaluated *in vitro* by employing MTT assay. The potency of each plant extract concentration was calculated in terms of percent cell inhibition of VERO and HeLa cells. The extract showed dose dependent anticancer activity on the cancer cell line i.e HeLa cell line while the extract did not show any cell toxic potential to the normal cell line i.e. Vero cell line. . The MTT assay showed an anti proliferative activity (IC₅₀) for the HeLa cell line at 133.6 µg/ml of crude extract.

Keywords: *Triticum aestivum*, HeLa cells, VERO Cell line, MTT assay, cytotoxic, aqueous extract

INTRODUCTION

In India, cervical cancer is a leading cancer among women with annual incidence of about 130,000 cases and 70-75,000 deaths¹. Thus, India shares about one fourth of the global cervical cancer burden. A large number of risk factors are known to contribute to high incidence of this disease but most important of them are early age of marriage (<18 years), multiple sexual partners, multiple pregnancies, poor genital hygiene, smoking, use of oral contraceptives, religion, ethnicity, etc.² Natural products have been used as traditional medicines in many parts of the world like Egypt, China, Greece, and India from ancient times. It is from these medicinal plants which have immense medicinal values that the modern drugs been developed.³

Triticum aestivum

Wheat, (*Triticum* species) a cereal grass of the *Gramineae* (*Poaceae*) family, is the world's largest edible grain cereal-grass crop. It is commonly 60-150 cm. in height, but may be as short as 30 cm. Stem is tufted, erect or semi-erect to prostrate, generally hollow with thin walls, in stem nodes are present generally 5-7 at 3-4 cm. Leaves are long and narrow having glabrous or hairy on one or both surface.^{4,5}

Scientific reports on nutritional analysis of wheatgrass have been published frequently in various journals^{6,7,8}. These reports and the chemical analyses undertaken reveal that wheatgrass is rich in chlorophyll, minerals

and trace elements including calcium, iodine, magnesium, selenium, zinc, chromium, antioxidants like betacarotene (pro-vitamin A), vitamin B1, vitamin E, vitamin C, antianemic factors like vitamin B12, iron, folic acid, pyridoxine and many other minerals, amino acids and enzymes, which have significant nutritious and medicinal value⁴.

Wheatgrass 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 molecule⁹. Chlorophyll, one of the primary components in the wheatgrass, was found to augment blood formation and strengthen the immune system through inhibition of metabolic activation of carcinogens¹⁰⁻¹¹. It also possesses the ability to inhibit oxidative DNA damage¹².

Dr. Ann Wigmore, founder director of the Hippocrates Health Institute, Boston, U.S.A., she claimed that wheatgrass is a safe and effective treatment for ailments such as high blood pressure, some cancers, obesity, diabetes, gastritis, ulcers, anemia, asthma and eczema¹³. Few clinical trials have been accomplished that have shown on consumption of wheatgrass juice, the number of transfusions in patients with thalassemia major is decreased¹⁴. Reduction in the overall disease activity index and the severity of rectal bleeding in

cases of distal ulcerative colitis on consumption of wheatgrass juice has also been observed¹⁵.

VERO Cell line

The Vero lineage was isolated from kidney epithelial cells extracted from an African green monkey. The lineage was developed on 27 March 1962, by Yasumura and Kawakita at the Chiba University in Chiba, Japan¹⁶. The original cell line was named "Vero" after an abbreviation of "Verda Reno", which means "green kidney" in esperanto, while "Vero" itself means "truth" also in Esperanto¹⁷. Vero cells are one of the most common mammalian continuous cell lines used in research¹⁸.

HeLa cell line

A HeLa cell is an immortal cell line used in medical research. The cell line was derived from cervical cancer cells taken from Henrietta Lacks, who died from her cancer in 1951. Initially, the cell line was said to be named after a "Helen Lane" in order to preserve Lacks's anonymity¹⁹.

MATERIALS AND METHODS

Plant Material

Certified sample of *Triticum aestivum* (Wheatgrass), was acquired from Anand Agricultural University, Gujarat. The authenticity of this certified sample was also confirmed by comparing its morphological characters with the description mentioned in different standard texts and floras²⁰. Voucher specimen of the plant has been deposited at Department of Pharmacognosy, B. Pharmacy College, Rampura, Kakanpura, Godhra, Dist. Panchmahal, Gujarat, India for future reference. This wheat variety was grown in plastic tray as per the standard procedure described below¹³.

Procedure for growing wheatgrass

- Adequate quantities of unpolished wheat grain were soaked overnight in water in a container.
- On the next day, the soaked wheat-grain were spread on the surface of the soil filled in plastic trays. Care was taken so that the grains did not touch one another.
- A thin layer of soil was sprinkled on the wheat grains and then tray was covered with a newspaper to provide darkness, which helps the sprouting.
- The tray was kept in a covered balcony. Next day the tray was uncovered to spray on some water and was covered again with the newspaper.
- Previous step was repeated every day until sprouting took place, after which the tray was left uncovered and watered everyday for 8 days.
- On 9th day the wheatgrass was harvested by cutting it with a clean pair of scissors about 1/2" above the surface of the soil.

Preparation of aqueous extract

For preparation of aqueous extract, 100 g of fresh wheatgrass was crushed thoroughly, using mortar and pestle. The crushed wheatgrass was completely exhausted by adding small quantities of water several times followed by filtration, to yield final volume of 1 liter. The extract was filtered and concentrated to dryness under reduced pressure and controlled temperature (40 °C to 50 °C) in a rotary evaporator. The dried extract was dissolved in Dimethyl sulphoxide (DMSO) to prepare the stock solution 100 mg/ml.

In-vitro evaluation of anticancer activity by MTT assay

Cell culture

The human cervical cancer cell line (HeLa) & Vero (normal kidney cells) was provided by National Centre for Cell Science (NCCS), Pune. Stock cells of these cell lines were cultured in DMEM (high glucose) or Eagle's Minimum Essential Medium (EMEM) with 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 5% CO₂, 95% air. Cells were used in experiments during the linear phase of growth.

Preparation of working herbal extracts

0.5ml of stock (100 mg/ml) herbal extract was dissolved in 4.5 ml of DMSO giving a concentration of 10mg/ml. 10 µl of 10 mg conc. of test compound was added in to 900 µl of complete media and as a result 100 µg conc. of test sample was obtained. Than 1:3 dilution of test sample was done as shown in Table 1. It was done by mixing 50 µl of test compound with 100 µl of complete media. For this, initially 100 µl of complete media was added in to well no. 1 – 9. Well 10 contained 150 µl test substance only, from that 50 µl was pipetted out and added into well no. 9 which already contain 100 µl of complete media, which lead to 1:3 dilution of test sample. Same procedure was repeated 9 times in order to get final conc. of test Sample up to 0.005 µm. (Table 1).

Cytotoxicity assay

Principle

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, purple colored formazan product which is measured spectrophotometrically²¹⁻²³. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity²⁴⁻²⁵.

Table 1: (1:3) dilution of test compound used in the assay

well no:1-9 contain complete media 100 µl + Test Compound 30 µl										
Well no:	1	2	3	4	5	6	7	8	9	10
Compound dilution	50 µl T.C from well 2	50 µl T.C from well 3	50 µl T.C from well 4	50 µl T.C from well 5	50µl T.C from well6	50µl T.C from well 7	50µl T.C from well 8	50µl T.C from well 9	50µl T.C from well 10	150µl T.C
Final conc. (µm)	0.05 µg	0.15 µg	0.46 µg	1.37 µg	4.12 µg	12.35 µg	37.04 µg	111.1 µg	333.3 µg	1000 µg

Where, T.C= Test Compound; C.M= Culture Media

MTT assay^{20,23,26,28}

Protocol:

- Cells were preincubated at a concentration of 1×10^6 cells / ml in culture medium for 3 hrs at 37°C and 6.5 % CO₂, 75 % Relative Humidity.
- Cells were seeded at a concentration of 5×10^4 cells / well in 100 µl culture medium and various amounts of compound (final concentration, e.g. 1000 µg/ml – 0.05µg/ml) were added into microplates (tissue culture grade, 96 wells, flat bottom).
- Cell cultures were incubated for 24 hrs at 37 °C and 6.5% CO₂.
- 10 µl MTT labeling mixture was added and incubate for 4 hrs at 37 °C and 6.5 % CO₂, 75 % Relative Humidity.
- 100 µl of solubilization solution was added to each well and incubate for overnight.
- Absorbance of the samples was measured using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product is between 540 and 600 nm according to the filters available for the ELISA reader, used. (The reference wavelength should be more than 650 nm).

DATA INTERPRETATION:

Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell death; evidence of cell death may be inferred from morphological changes.

After 24 hrs, the cytotoxicity data were evaluated by determining absorbance and calculating the correspondent chemical concentrations. Linear regression analysis with 95 % confidence limit and R^2 were used to define dose-response curves and to compute the concentration of chemical agents needed to reduce absorbance of the formazan by 50 % (IC₅₀).

Percentage cell growth inhibition or percentage cytotoxicity was calculated by following formula:

$$\% \text{ viability} = (A_T - A_B) / (A_C - A_B) \times 100 \dots (1)$$

Where,

A_T = Absorbance of treated cells (drug)

A_B = Absorbance of blank (only media)

A_C = Absorbance of control (untreated)

There by,

$$\% \text{ cytotoxicity} = 100 - \% \text{ cell survival} \dots (2)$$

$$\text{Cell viability \%} = \text{Mean OD of wells receiving each plant extract dilution} / \text{Mean OD of control wells} \times 100 \dots (3)$$

$$\text{Cell death \%} = 1 - (\text{OD of sample} / \text{OD of control}) \times 100 \dots (4)$$

DETERMINATION OF IC₅₀ VALUE:

According to the FDA, IC₅₀ represents the concentration of a drug that is required for 50 % inhibition *in-vitro*. In the present study, IC₅₀ is a concentration of drug at which 50 % of cell population die.

For primary screening, a threshold of 50 % cell growth inhibition as a cut off for compound toxicity against cell lines. IC₅₀ determined from plot of Dose Response curve between log of compound concentration and percentage growth inhibition. IC₅₀ values have been derived using curve fitting methods with *GraphPad Prism* as statistical software (Ver. 5.02).

IC₅₀ values were calculated using the nonlinear regression program Origin The average of two (duplicates manner) were taken in determination.

Graph was plotted by keeping log concentration of drug on X axis and % cell growth inhibition or % cytotoxicity Y axis. IC₅₀ was estimated as a concentration of the drug at 50 % position on the Y axis.

The relationship should be sigmoidal, log drug concentration on the X axis and 'response /

measurement' of the Y axis. The prism web site has some good guides for this.

RESULTS AND DISCUSSION

In order to evaluate the cytotoxic effect of aqueous extract of *Triticum aestivum*, a MTT assay with HeLa (human cervical cancer) & Vero (normal kidney cells) cell line was performed. The extract was screened for its cytotoxicity at different concentrations to determine the IC₅₀ (50% growth inhibition) value.

A chart was plotted using the % cell inhibition in Y-axis and concentration of the plant extract in X-axis.

The results are tabulated in (Table 2) and graphically represented (Fig. 1).

When HeLa and VERO cells were treated with the aqueous extract of the leaves of *Triticum aestivum*, there was a concentration dependent cytotoxic effect. As the concentration increased from 0.05 – 10,000 µg/ml, percentage of inhibition increases from -93.85% to 37.49 for VERO cell line and -6.66% to 63.2% for HeLa cell line. The IC₅₀ value was found to be 1000 µg/ml for VERO cell line and 133.6 µg/ml for HeLa cell line from the graph and R² values 0.9783 for VERO cell line and 0.9071 for HeLa cell line (Table 3).

Table 2: % Cell inhibition on VERO & HeLa cell lines by aqueous extract of leaves of *Triticum aestivum*

Sr.No	Concentration (µg/ml)	Log conc	VERO	HeLa
1	0.05	-1.29	-93.85	-6.66
2	0.15	-0.82	-116.4	-11.75
3	0.46	-0.34	-115	-13.57
4	1.37	0.14	-115.6	-11.38
5	4.12	0.61	-115.4	-14.74
6	12.35	1.09	-114	-16.58
7	37.04	1.57	-91.66	-15.43
8	111.11	2.05	-60.82	42.79
9	333.33	2.52	1.28	63.06
10	1000	3	37.49	63.2

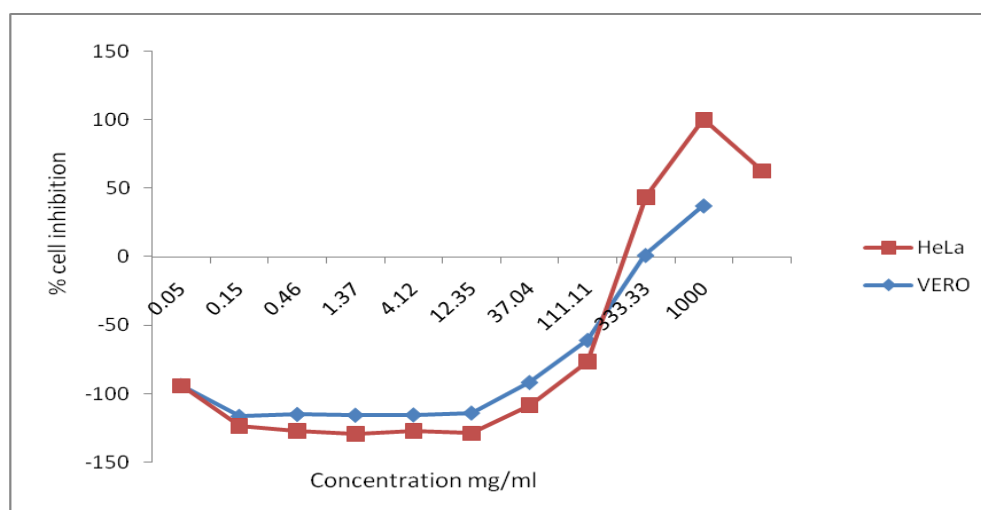


Figure 1: Dose response curve of aqueous extract of leaves of *Triticum aestivum* on HeLa and VERO-cell-lines.

Table 3: IC₅₀ and R² values of aqueous extract of leaves of *Triticum aestivum*

Parameters	VERO	HeLa
IC ₅₀ Value	1000	133.6
R ²	0.9783	0.9071

Traditionally many medicinal plants, which possess the ability to prevent and even to stall the progress of cancer, were in use. Plants possess certain chemicals, which have the ability to modify the physiological function of cells and hence act as anti-cancer drugs to arrest the proliferation of cancer cells. The mode of action of the drugs is unknown but successfully integrating our documented knowledge of plant properties and modern technological tools, effective anti-cancer drugs can be derived from plant sources and their mechanism can be elucidated²⁹⁻³¹.

The present need is to develop drugs that can potentially target cancer cells by means of their inherent difference to normal cells. The development of such drugs with differential action will be very valuable in cancer chemotherapy without the observed side effects. The methodology involves use of cancer cell lines to test the efficacy of the plant extracts *in vitro*.

The potential use of *Triticum aestivum* as therapeutic agent holds great promise as the isolation of one or more cytotoxic chemicals from crude extract and the judicious use of such chemicals can control the progression of cancer and also can prevent the formation of tumour in individuals who are highly susceptible to developing a tumour.

CONCLUSION

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The results obtained from the *in-vitro* studies performed using the HeLa cell lines reveals that the aqueous extract of *Triticum aestivum* has a moderate anticancer activity. The extract did not show any cell toxic potential to the normal cell line i.e. Vero cell line. Even though there was increase in the cell growth inhibition when concentration of sample was increased, the IC50 value was 133.6 µg/ml for the HeLa cell line as shown by the MTT assay method. This holds great promise for future research in human beings. The anticancer property of *Triticum aestivum* will provide useful information in the possible application in the prevention and treatment of cancer.

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