**INTRODUCTION**

In India, cervical cancer is a leading cancer among women with annual incidence of about 130,000 cases and 70-75,000 deaths.\(^1\) 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 value.\(^9\)

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 molecule. 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 carcinogens.\(^10\)\(^11\) It also possesses the ability to inhibit oxidative DNA damage.\(^12\)

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 eczema.\(^13\) Few clinical trials have been accomplished that have shown on consumption of wheatgrass juice, the number of transfusions in patients with thalassemia major is decreased.\(^14\) 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 observed.\(^15\)

**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 anonymity.\(^16\)

**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 floras.\(^17\) 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 below.\(^13\)

**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 methanol extract**

For preparation of methanol extract, 100 g of fresh wheatgrass was crushed thoroughly, using mortar and pestle. The crushed wheatgrass was completely exhausted by adding small quantities of methanol several times followed by filtration, to yield final volume of 1 liter. The extract was filtered and concentrated to dryness by adding small quantities of methanol 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. 100 mg of the extract was dissolved in 1 ml of 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) was grown in Eagles Minimum Essential Medium (HIMEDIA Laboratories Pvt. Ltd.) which contained 10% fetal bovine serum (HIMEDIA Laboratories Pvt. Ltd.) 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. Using the 10mg/ml concentration herbal extract nine serial doubling dilutions of the extract of 500 μl each was prepared in DMSO to get the concentration of the extract from 0.0195-10mg/ml as indicated in Table 1 and the diluted extracts transferred to 96- well culture plate. 500 μl of culture of HeLa cells at a concentration of 10^5 cells/ml was added to each well. 8 wells receive only cell suspension without extract and they serve as control. The plate incubated in a humidified CO2 incubator at 37° C for 72 hrs. The plate was microscopically examined for confluent monolayer of cells, turbidity and toxicity.

**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 spectrophotometrically. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity.

**MTT assay**

After 72hrs of incubation, the medium from the wells aspirated carefully and discarded. 50 μl of MTT solution was added, and the plates were gently shaken to solubilize the formed formazan. The suspension transferred to a spectrophotometer cuvette and absorbance values read at 570nm using DMSO as blank. The % cell viability and % cell death were calculated with the following formulae:

\[
\text{Cell viability } \% = \frac{\text{Mean OD of wells receiving each plant extract dilution}}{\text{Mean OD of control wells}} \times 100
\]

\[
\text{Cell death } \% = 1 - \left( \frac{\text{OD of sample}}{\text{OD of control}} \right) \times 100
\]

**Table 1: Cytotoxicity on HeLa cells by methanol extract of Triticum aestivum leaves (MTT Assay)**

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Concentration (mg/ml)</th>
<th>Absorbance at 570nm</th>
<th>% Cell Viability</th>
<th>% Cell Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.11</td>
<td>27.7</td>
<td>72.3</td>
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<tr>
<td>2</td>
<td>5</td>
<td>0.12</td>
<td>30.2</td>
<td>69.8</td>
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<td>0.133</td>
<td>33.5</td>
<td>66.5</td>
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<td>62.3</td>
</tr>
<tr>
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<td>0.625</td>
<td>0.162</td>
<td>40.8</td>
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<td>0.197</td>
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<tr>
<td>8</td>
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<td>0.256</td>
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<tr>
<td>9</td>
<td>0.039</td>
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<td>10</td>
<td>0.0195</td>
<td>0.35</td>
<td>88.1</td>
<td>11.9</td>
</tr>
<tr>
<td>11</td>
<td>cell control</td>
<td>0.397</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

**Fig. 1: Effect of methanol extract of Triticum aestivum leaves on HeLa cell viability**

**Fig. 2: Effect of methanol extract of Triticum aestivum leaves on HeLa cell death**
RESULTS AND DISCUSSION

In order to evaluate the cytotoxic effect of methanolic extract of *Triticum aestivum*, a MTT assay with HeLa (human cervical cancer) 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 viability in Y-axis and concentration of the plant extract in X-axis. Another chart was prepared using the % cell death in Y-axis and concentration of plant extract in X-axis. Cell control was included in each assay to compare the full cell viability in cytotoxicity and antitumor activity assessments. The results are tabulated in Table-1 and graphically represented in Fig. 1 and Fig. 2.

When HeLa cells were treated with the methanolic extract of the leaves of *Triticum aestivum*, there was a concentration dependent cytotoxic effect. As the concentration increased from 19.9 – 10,000 μg/ml, percentage of inhibition increases from 11.9% - 72.3 % . The IC50 value was found to be 156μg/ml from the graph.

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 elucidated.

The potential new 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

The results obtained from the in-vitro studies performed using the HeLa cell lines reveals that the methanolic leaf extract of *Triticum aestivum* has a moderate anticancer activity. Even though there was increase in the cell growth inhibition when concentration of sample was increased, the IC50 value was 156μg/ml for the cell line studies 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 a useful information in the possible application in the prevention and treatment of cancer.

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REFERENCES

4. The wealth of India, Council of Scientific & Industrial research, New Delhi, 10: 312-323.