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Genotoxicity Potential of Triticum Aestivum (Wheatgrass) On Oral Cancer Cell Lines by DNA Fragmentation

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Abstract

Aim:
To determine the genotoxicity analysis of *Triticum aestivum (wheatgrass)* on oral cancer cell lines by DNA fragmentation method.

Objective:
This study was done to evaluate the anti-cancer activity of *Triticum aestivum (wheatgrass)* extract.

Background:
Triticum aestivum (wheatgrass) richest sources of proteins, vitamins and minerals grown throughout temperate regions of North America and Europe. used as an herbal medicine , non-toxic herb , evades Food and Drug Administration screening and clearance. It has been traditionally used as a herbal medicine in a number of serious diseases like believed to strengthen the immune system and increase the life span of cancer patients by regressing the spread of cancer cells.

Reason:
This study may help in the formulation of economical and new anticancer agents derived from *Triticum aestivum (wheatgrass).*

Result:
The *Triticum aestivum (wheatgrass)* extract showed effective anticancer activity against oral cancer cell lines.

INTRODUCTION

Oral cancer is among the 10 most common cancers in the world. Squamous cell carcinoma is the most prevalent malignant neoplasm in the oral cavity. The risk factors are tobacco and heavy alcohol consumption[(1)]. Almost eighty three percent of the oral cancer patients use tobacco and seventeen percent did not. The mean age of tobacco users is 51 years and of non-users was 52 years[(2)].

So, it mostly occurs in middle aged and older individual. Women have been more equally exposed to known oral carcinogens such as tobacco and alcohol[(3)].

Nature serves to be a vital spring of medicinal herbs. Plants and macrofungi are considered to be a fundamental source of medicines and medicinal agents. Wheatgrass is grown from common wheatgrass (Triticum aestivum), a subspecies of the family Poaceae[4] is a gift of nature given to mankind. The systematic viewpoint was engrossed by a food scientist who established about the health effects of wheatgrass. A number of scientific research on *Thinopyrum intermedium* (wheatgrass) establishes it's anticancer[5] potential. Wheat grass is one of the richest sources of proteins, vitamins and minerals. It is widely grown throughout temperate regions of North America and Europe. Indigenously, wheatgrass has been used as an herbal medicine since ages[6]. It's been manufactured and marketed by various consumer-based product companies. Being a non-toxic herb it also evades Food and Drug Administration screening and clearance. It has been traditionally used as a herbal medicine in a number of serious diseases. In addition, it has also been believed to strengthen the immune system and increase the life span of cancer patients by regressing the spread of cancer cells[7]. This study is done to analyse the genotoxic activity of wheatgrass against oral cancer cell line.

Plant derived compounds have been an important source of several clinically useful anticancer agents[(8)]. India is sitting on a gold mine of well recorded and traditionally well practiced knowledge of herbal medicine. Although, more than 1500 anticancer drugs are in active development with over 500 of the drugs under clinical trials, there is an urgent need to develop much effective and less toxic drugs[(9)]. Some studies suggest that herbs could increase the anti-cancer activity in several cancer cell lines including KB.

The several components of the herb follow diverse anticancer pathways. An interesting phenomenon suggests that some herbs might be targeted for cancer cells instead of normal cells[10]. The pharmacological mechanisms of most natural anticancer compounds remaining elusive, have become one of the major obstacles in developing novel effective anticancer agents[11]. Most of the anticancerous compounds used in medicine are natural products. Of the 140 anti-cancer agents approved since 1940 and available for use, over 60% can be traced to a natural product. Of the 126 small molecules among them, 67% are natural in origin[12].

MATERIALS AND METHODS

The chemicals used in the assay are procured from Himedia.

Preparation of wheatgrass extract

The wheatgrass was collected from the herb. Only wheatgrass of uniform size and shape, without injuries was selected. The plants were washed, wiped and cut into small pieces. They were homogenized with a clean pestle and mortar using distilled water and ethanol (10% w/v). The extracts were centrifuged at 15,000 rpm for 20 min at 4 °C and the supernatants were stored at −20 °C until further use [13].

Maintenance of cell line

The vial containing KB cell lines acquired from ATCC, was removed from liquid nitrogen freezer and the vial was thawed for 2 minutes by mild agitation in a 37°C water bath. Then it was centrifuged for 10 minutes at 150 to 200g, room temperature. Supernatant was disposed and cells were cleaned with Eagle's minimum essential medium to remove residual DMSO. The cell pellet was re-suspended in 3ml of DMEM with 10% FBS. It was then incubated in a CO_2 incubator (eg: phenol red) in a medium which changed color as an indicator. The culture was then kept in a growth medium with 10% fetal bovine serum, until cell line were re-established.The cell lines was incubated with varying concentration of wheat grass extract and assessed for its genotoxicity.

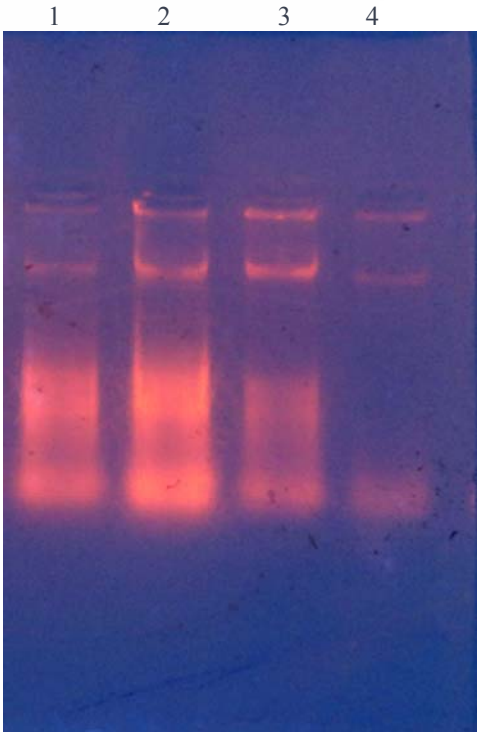
Isolation of DNA

$1*10^6$ cells were incubated with 100µl of cell lysis buffer at room temperature for one hour. This was centrifuged for 15 min at 3000rpm at 4°C to sediment the cell debris. To the supernatant equal volume of phenol: chloroform: isoamylalcohol mixture was added and mixed well. This was centrifuged at 5000rpm for 15 min. The supernatant was transferred to new tube. And the centrifugation was repeated again. To the final aqueous phase 40 µl of 3.5M ammonium acetate was added, to this ice cold isopropanol was added to precipitate the DNA. This was incubated at -20degree C for 1 hour, followed by the centrifugation at 10000 rpm for 15min. The pellet was retained and washed with 70% ethanol and stored in 20-50 µl of TE buffer. The samples were analyzed in agarose gel stained with Ethidium bromide.

Genotoxicity Analysis by Agarose Gel Electrophoresis

The agarose gel has to be prepared with 1X TAE buffer and stained with 2µl of ethidium bromide. The % of agarose depends upon the molecule to be separated. DNA Samples isolated were loaded with loading dye (2 µl of loading dye is used). Electrophoresis of DNA fragments at 50 volts. Visualization of DNA fragments in the UV trans-illuminator was done.

RESULTS AND DISCUSSION



Lane 4 – 1 kb ladder
Lane 3 – DNA from KB cells treated with 100µg sample
Lane 1 – DNA from KB cells treated with 200µg sample
Lane 2 – DNA from KB cells treated with 300µg sample

The gel picture debicts the DNA fragmentation of the KB cells incubated in the wheatgrass extract ,thus showing the genotoxicity effect of wheatgrass extract. As the concentration of the wheatgrass extract increases, the DNA fragmentation also increased. Wheatgrass inhibits HeLa cell proliferation and accumulation of ascites. Induction of apoptosis on KB cells by Wheatgrass extract was validated by DNA fragmentation analysis using Agarose gel electrophoresis technique.

In diseases such as cancer, induction of apoptosis has been a new target for mechanism-based drug discovery. Cancer cells evolve to avoid apoptosis-inducing signaling pathway in order to survive. Understanding the mechanistic machinery of apoptosis is vital because programmed cell death is a component of both health and disease, being initiated by various physiologic and pathologic stimuli. Thus, induction of apoptosis in cancer cells can be a promising treatment method in cancer therapy. Natural-derived products, extracts or isolated active compounds, had drawn growing attention as agent in

cancer therapy, due to their ability to modulate apoptosis. Apoptosis involves characteristic morphological and biochemical events ultimately leading to cell demise[14,15].

CONCLUSION

The chemotherapeutic agents used in oncologic treatment produce deleterious side effects that augment the mortality and morbidity caused by cancer. Safer treatments are thus desperately needed. The anticancer activity of the medicinal herbs selectively targets KB cells without affecting the normal cells and inhibits their growth. From the present study, it was concluded that the extract of Wheatgrass acts against oral cancer (KB) cells which may be due to the syngerstic effect of the secondary metabolites such as flavonoids present in the extract. Thus, the anticancer activity of Wheatgrass may be useful in the treatment of patients with oral carcinoma[16].

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