



Genotoxicity of *Hibiscus rosa sinensis* on Oral Cancer Cell Line

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ABSTRACT

Aim of the study is to analyse the genotoxicity of hibiscus extract on oral cancer cell lines. The objective of the study is to update the knowledge and to observe the genotoxic activity of hibiscus on oral cancer cell lines. Cancer cell lines are genetic representatives of tumours hence serves as useful tools to understand the molecular changes associated with oral cancer. Working on these cancer cell lines provides a basis for any drug formulation and on the effect on cancer as a whole. Cancer is a growing concern in the Indian population and the use of a common substance such as hibiscus extract would help in the same. Hibiscus is widely known for various pains relieving property. Therefore hibiscus has an effect on oral cancer. Cancer is a growing concern, and is very much prevalent in the Indian population. The usage of common flowers such as hibiscus helps to provide cure among the population. Hence assessing the genotoxic activity will help in the analysis. From the result, it is found that, DNA fragmentation has occurred. Hence this proves that Hibiscus oil extract exhibits genotoxicity and anti-cancerous properties.

Keywords: Genotoxicity; Oral Cancer; Hibiscus extract; anti-cancerous.

INTRODUCTION

Oral cancer includes cancers of the lip tongue, cheeks, floor of the mouth, hard and soft palate. Cancer is a multi-step disease incorporating physical, environmental, metabolic, chemical and genetic factors, which play a direct and/or indirect role in the induction and deterioration of cancers and is one of the most life-threatening diseases¹. A substances that has the property of genotoxicity is known as a genotoxin. Genotoxins are mutagens. They include both radiation and chemical genotoxins². In genetics; genotoxicity describes the property of chemical agents that damages the genetic information and nucleic acid within a cell causing mutations, which may lead to cancer. These changes can be observed using DNA fragmentation.

To assess genotoxicity, different endpoints must be taken into considerations: beside point mutations induction, a compound can induce changes in chromosomal number (polyploidy or aneuploidy) or in chromosome structure (breaks, deletions, rearrangements)³.

The most common symptoms of oral cancer include swellings and thickenings, lumps or bumps, rough spots or eroded areas on the lips, gums, or other areas inside the mouth. The development of velvety white, red, or speckled (white and red) patches and unexplained bleeding may also occur in the mouth⁴. Cigarette smoke (CS) is the main inducer of oral cancer, increasing the prevalence by 4-7 times⁵. Cancer cell lines are powerful and robust experimental tools used for understanding how genetic alterations lead to tumour initiation and progression⁶. A primary cell culture is the initial culture set up directly from a body tissue. Primary cancer cultures

can be initiated and derived from a variety of tissue types such as solid tumour fragments (primary or metastatic) or cell suspensions. Cell suspensions can be particularly convenient for developing cell lines as they are already growing as single cells or clusters, avoiding the need for mechanical or enzymatic dispersion. Cancer cells differ from most normal cell types in their ability to grow in suspension, for example, in agar, but generally cultures are initiated by allowing cells to adhere to a substrate before proliferating⁷. A number of strategies have been developed to help disperse fragments of tissue and these include mechanical and enzymatic methods. Many human tumours induce an immune response in the host⁸.

The genus *Hibiscus* thrives in a variety of climates and produces a diversity of natural compounds with bioactive properties⁹. The authors reported that the hypoglycaemic activity of this extract is not mediated through insulin release and this increase the potential use of this species for human health purposes. Moreover, there is very important evidence of the anticancer action of *H. rosa sinensis* extract against the tumour promotion stage of cancer development, in mouse skin with ultraviolet radiation.

MATERIALS AND METHODS

Chemicals used were procured from Cyrus Enterprises. Cell line was purchased from ATCC.

Maintenance of Cell Lines

The oral cancer cell lines i.e., KB (ATCC CCL-17) were acquired from ATCC. Oral cancer cells were seeded in 24 well plate and kept in CO₂ incubator.



Treatment of cell lines with different concentrations of hibiscus extract

Cells were treated with the Hibiscus oil extract in 2 different concentrations (75 µg, 125µg) for 24 hrs. Treated cells were subjected to DNA fragmentation assay according to Alexei G.¹⁰

Isolation of Genomic DNA

1*10⁶ 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: isoamyl alcohol mixture was added to the supernatant and mixed well. This was centrifuged at 5000 rpm for 15min. The supernatant was transferred to new tube. The 3rd step was repeated once. The pellet was retained and washed with 70% ethanol and stored in 20-50µl of TE buffer. The samples were analyzed in 2% agarose gel stained with Ethidium bromide.

Analysis of DNA fragmentation by Agarose gel electrophoresis:

The extracted DNA is loaded to Agarose gel with the loading dye, DNA fragments was visualised under UV transilluminator¹¹

RESULTS AND DISCUSSIONS

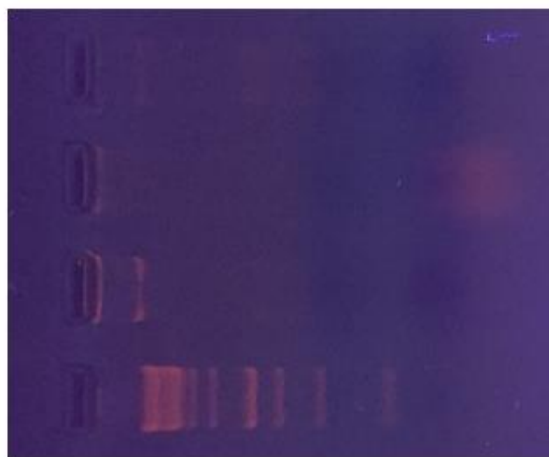


Figure 1

Lane 1 – 1 kb ladder

Lane 2 – DNA from untreated cells

Lane 3 – DNA from cells treated with 75 µg sample

Lane 4 – DNA from cells treated with 125 µg sample

Results show that *Hibiscus rosa sinensis* oil extract exhibits genotoxicity as the existing DNA got fragmented. All the four concentrations have shown DNA fragmentation.

From the results obtained in the image above (figure 1), we can see that DNA fragmentation has occurred. Hence this proves that Hibiscus oil extract exhibits genotoxicity and anti-cancerous properties.

CONCLUSION

From the above experiment and research it's evident that Hibiscus oil has all the vitality to treat oral cancer¹². The results showed that fragmentation of DNA occurred in both the concentrations that were tested. Treatment with hibiscus extract inhibited the growth and proliferation of cancer cells¹³. Though research is still going on various parts of the world to make use of this plant extract to treat cancer, oral-cancer in specific, there is less awareness among the masses¹⁴.

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