Research Article

Genotoxicity Analysis of Pineapple Extract on Oral Cancer Cell Line by DNA Fragmentation

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ABSTRACT

The aim of the study is to assess the genotoxicity of pineapple extract on oral cancer cell line by DNA fragmentation. The objective of the study is to observe the effect of genotoxicity on oral cancer cells by using the method of DNA fragmentation. Genotoxicity describes the property of chemical agents that damages the genetic information within a cell causing mutations, which may lead to cancer. It is a property possessed by some substances that makes them harmful to the genetic information contained in organisms. A substance that has the property of genotoxicity is called a genotoxin. Bromelain is a complex mixture of substances that can be extracted from the stem and core fruit of the pineapple. Among dozens of components known to exist in this crude extract, the best studied components are a group of protein-digesting enzymes called cysteine proteinases. Vitamin C is also a major component of the extract. The genotoxic substance invades the nucleus and causes damage to the nucleic acid. These changes can be observed using DNA fragmentation. The oral cancer cell lines are maintained and Bromelain is added to it. This is followed by DNA isolation and DNA fragmentation in a step-wise manner. Agarose gel electrophoresis is done and the DNA is viewed using ethidium bromide under the UV light. DNA fragmentation occurred in all the three concentrations. Bromelain was proved to contain anti-cancerous properties.

Keywords: Agarose gel electrophoresis, Anti-cancerous, Bromelain, DNA fragmentation, Oral cancer cell lines.

INTRODUCTION

Genotoxicity, in genetics is defined as a destructive effect on the cell's genetic material affecting it's integrity.[1] It is the property of chemical agents that damages the genetic information within a cell causing mutations, which may lead to cancer. A substance that has the property of genotoxicity is known as a genotoxin. Genotoxins are mutagens. They include both radiation and chemical genotoxins.[1] The genotoxic substance invades the nucleus and causes damage to the nucleic acid. These changes can be observed using DNA fragmentation. Genotoxicity is a property possessed by some substances that makes them harmful to the genetic information contained in organisms. Agarose gel electrophoresis is a method that is very suitable for clinical routine analyses of proteins in plasma and other body fluids since a good resolution is obtained with patterns which are easy to interpret. It is also useful for lipoprotein analyses in a slightly modified form.[2]

Bromelain, a proteolytic enzyme is derived from pineapple which is botanically known as *Ananas comosus*. Bromelain has been used as medicine for centuries by the indigenous inhabitants to treat a range of ailments[3] including edema, thrombophlebitis, sinusitis, inflammation, rheumatic arthritis, and as adjuvant cancer treatment.[4] It comprises of sulphydryl-containing proteolytic enzymes, peroxidase, acid phosphatase, glucosidases, cellulases, several protease inhibitors, glycoproteins and organically bound calcium.[3] Bromelain seems to cause the body to produce substances that fight pain and swelling (inflammation). It also contains chemicals that interfere with the growth of tumor cells and slow blood clotting. Some people use a product (Phlogenzym) for arthritis that combines bromelain with trypsin and rutin (a substance found in buckwheat). Bromelain used in this way seems to reduce pain and improve knee function in people with arthritis.

Although poorly understood, the pleiotropic effects of bromelain are considered to be due to the complex mixture of these compounds such as closely related cysteine proteinases, proteinase inhibitors, phosphatases, glucosidases, peroxidases, and other undefined compounds.[5,6] In addition, bromelain has shown both anti-proliferative and anti-metastatic effects in tumor models in vitro and in vivo.[7-11] Medicinal qualities of bromelain include anti-inflammatory, anti-thrombotic, fibrinolytic and anti-cancer functions.[12] Bromelain proteases are usually unstable and sensitive under stress conditions for example elevated temperature, acidity and gastric proteases in stomach juice, organic solvents and chemicals.[13]

Oral cancer appears as a growth or sore in the mouth which includes cancers of the lips, tongue, cheeks, floor of the mouth, hard and soft palate, sinuses, and pharynx (throat). It can be life threatening if not diagnosed and treated early. 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 mouth.

Cigarette smoke (CS) is the main inducer of oral cancer, increasing the prevalence by 4-7 times.[14] Cancer starts when the structure of the DNA (deoxyribonucleic acid) alters and a genetic mutation occurs. When there is a genetic mutation cells grow in an uncontrollable manner, eventually producing a lump (tumor).[15] Cancer cell lines are powerful and robust experimental tools used for understanding how genetic alterations lead to tumor initiation and progression.[16]

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 tumor 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 proliferating.[17] 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 host.[18]

MATERIALS AND METHODS

Maintenance of cell lines

The vial containing KB cell lines acquired from ATCC (CCL-17) was removed from liquid nitrogen freezer and immediately placed in a 37 degree Celsius water bath. It was agitated continuously until the medium thawed. Then it was centrifuged for 10 min at 150 to 200 x g, room temperature. Supernatant was discarded and cells were washed with fresh medium to remove residual DMSO. The cell pellet was re-suspended in 3ml of DMEM with 10% FBS. The cells were seeded in 24 well plate and kept in CO_2 incubator. Cells were treated with the pineapple extracts (bromelain) in three different concentrations (100µg, 200 µg, 300µg) for 24 h. Treated cells were subjected to DNA fragmentation assay.

Induction of DNA fragmentation by Bromelain

Cells were treated with the pineapple extracts in three different concentrations (100 µg, 200 µg, 300µg) for 24h. Treated cells were subjected to DNA fragmentation assay.

Isolationof DNA by cell lysis method

$1*10^6$ cells were incubated with 100µl of celllysis buffer at room temperature for one hour. This was then 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 mixture was then centrifuged at5000 rpm for 15min.The supernatant was transferred to new tube. To the final aqueous phase, 40µl of 3.5M ammonium acetate was added and ice cold isopropanol was added to precipitate the DNA. This was then incubated at -20°C for 1hour, followed by the centrifugation at 10000 rpm for 15min.The pellet obtained was retained and washed with 70% ethanol and stored in 20-50µl of TE buffer. The samples were then analyzed in 2% agarose gel stained with Ethidium bromide.

Analysis of DNA fragmentation by Agarose gel electrophoresis:

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

RESULTS AND DISCUSSION

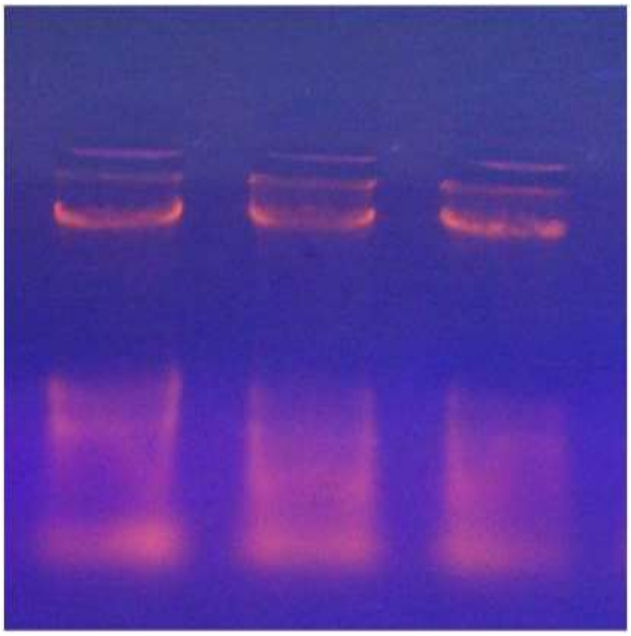

Figure 1

Lane 1 – DNA from KB cells treated with 100µg sample

Lane 2 – DNA from KB cells treated with 200 µg sample

Lane 3 – DNA from KB cells treated with 300 µg sample

Results show that pineapple extract exhibits genotoxicity as the existing DNA got fragmented. All the three concentrations have shown DNA fragmentation.

Apoptosis has been characterized biochemically by the activation of a nuclear endonuclease that cleaves the DNA into multimers of 180-200 base pairs and can be visualized as an 'oligosome ladder' by standard agarose gel electrophoresis.[19] From the results obtained in the image above, we can see that DNA fragmentation has occurred. Hence this proves that pineapple extract exhibits genotoxicity and anti-cancerous properties.

CONCLUSION

Pineapple extract is a mixture of cysteine proteases and non-proteases components, extracted from pineapple plant. This proteolytic enzymes used as a phytomedical

compound demonstrates anti-inflammatory, anti-edematous, absorption of antibiotic drugs, anti-thrombotic, inhibition of tumor cells proliferation and exhibition of strong immunogenicity.[13] Existing evidence derived from clinical observations as well as from mouse and cell-based models suggests that pineapple extract acts systemically, affecting multiple cellular and molecular targets.[12] In recent years, studies have shown that pineapple extract has the capacity to modulate key pathways that support malignancy.[12] In the present research conducted, the oral cancer cells lines were subjected to DNA fragmentation by pineapple extract. The results showed that fragmentation of DNA occurred in all the three concentrations that were tested. Treatment with pineapple extract inhibited the growth and proliferation of cancer cells.[3] Hence it has been proved that pineapple extract contains genotoxic properties.

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