Research Article

Apoptotic Induction Potentials of Pineapple Extract in Oral Cell Line Cancer

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ABSTRACT

The aim of the study is to study the apoptotic induction potentials of pineapple extract in oral cancer cell line. Pineapples contain high quantities of manganese and vitamin C. Apart from these, pineapples also contain high quantities of thiamin, a B vitamin that are involved simultaneously in energy production. Quantitative analysis of caspian enzymes to study apoptotic induction potentials of pineapple extract in oral cancer cell line. Cancer has always had the reputation as a deadly disease. Taken as a whole, about half of the patients suffering from invasive cancer receiving treatment, die from its treatment or cancer itself. A requirement for finding of new ways of treating cancer would be essential to be able to save the lives of those with cancer rather than just extending their lives by a few weeks. Apoptotic induction potentials of pineapple extract along oral cancer cell line was studied.

Keywords: oral cancer, apoptosis, pineapple, caspase enzyme, KB cell maintenance.

INTRODUCTION

One of the most important commercial fruit crops in the world is the pineapple [Ananas comosus (L.) Merr. Family: Bromeliaceae[1]. Due to its excellent flavour and taste, it is known as the queen of fruits[2]. Pineapple peel is a potential source for the extraction of bioactive compounds which can be beneficial due to the huge amount of waste after processing[3]. Pineapple is cultivated for fresh or canned fruit and juice mainly. It is also the only source of bromelain. Bromelain is a complex proteolytic enzyme which is used in the pharmaceutical market and is also used as a meat-tenderizing agent[4]. Pineapple is rich in various nutrients. Minerals such as Chlorine, Calcium, Phosphorus, Potassium and Sodium are found in fresh pineapple[5].

Pineapple has several health benefits if consumed. It decreases the risk of obesity and diabetes, overall mortality, heart diseases and promotes a healthy hair and complexion, increased energy, overall lowers weight. It also decreases risk of and progression of macular degeneration related to age, prevents asthma due to presence of beta-carotene and lowers blood pressure. One of the main health benefits is due to its high content of strong antioxidant vitamin C. The action of vitamin C is that it combats the formation of free radicals which cause cancer.

Cancer, also known malignancy, is an abnormal growth of cells. Proliferation of the tumor has prognostic value in resected early-stage non-small cell lung cancer (NSCLC)[6]. More than 100 types of cancer, including breast cancer, skin cancer, lung cancer, colon cancer, prostate cancer, and lymphoma are present. Symptoms vary depending on the type of cancer. The capability of a tumor to grow in number and proliferate is dependent on a small group of cells within a tumor, called cancer stem cells[7]. There are more than 20 different cancer-associated HPV types, but very little knowledge has been discovered about their geographic variation[8].

Apoptosis is considered a vital component of various processes including normal cell turnover, proper functioning and development of the immune system, hormone-dependent atrophy, embryonic development and chemical-induced cell death[9]. A great number of apoptosis-regulating genes including bc1-2, c-myc, and p53 have recently been discovered. Apoptosis plays an important role in regulation of tumor proliferation and tumor response to various forms of cancer therapy, including chemotherapy and radiotherapy. This has been suggested by recent experimental evidences[10]. Along with the other group members like Apo-2L , Fas/Apo-1 ligand and TNF may serve as an extracellular signal that activates programmed cell death[11]. The decision to die cannot be taken lightly, and the activity of many genes influence the likelihood of a cell activating its self-destruction programme[12]. Ultrastructural examination confirms the occurrence of apoptotic bodies[13]. Extensive lymphocyte apoptosis is caused by caspase-3-mediated apoptosis[14].

Caspases, also known as cysteinyl aspartate-specific proteases, are a family of important molecules involved in signaling with various tasks depending on the subdivision and organ involved. The activation of caspases also is a marker for damage along cellular level in diseases such as stroke and myocardial infarction. Although the precise role in the initiation and progression of apoptosis is not known in case of all caspases, their involvement as an indicator alone and as a potential leverage point for research of drug makes them largely researched molecules.

This protease has been implicated as an "effector" caspase which is associated with the "death cascade" initiation and is therefore an important marker of the cell's entrance point into the pathway of apoptotic signaling. Caspase-3 is activated by the upstream caspase-8 and caspase-9, and since it serves as a point of convergence for different signaling pathways, it is well suited as a read-out in an apoptosis assay. Active caspase-3 detection can be used in different cell lines or primary cells, does not require the transfection techniques usage, and can be multiplexed with other probes to get a deep understanding of signaling events with cell-by-cell resolution. The induction of apoptosis leads to an activation of caspases. These enzymes are cysteine proteases that are produced by the cell as inactive pro-enzymes. After activation the pro–caspase is hydrolyzed to its active form, caspase. Caspases belong to a family of proteases which are responsible for the cell death induction. The activation of the caspase induces the cell fragmentation and thus the development of "apoptopic bodies"[15].

MATERIALS AND METHODS

Preparation of Pineapple Extract

The stumps of mature pineapple plants were stripped of leaves and then pared to remove the roots and the epidermal layer. The cleaned stumps were then quartered and fed into a three roll experimental sugar mill press. The pressed residue was rerun through the mill an additional three times. Water was added to the pulp to increase the efficiency of the extraction process.

Maintenance of KB cell line

Cell culture flasks were selected by the method of confluency. They were observed under inverted microscope. In order to maintain cell line, the technique of subculturing was performed. This is to facilitate cell growth by extraction of cells from the existing medium and placing them into a fresh new medium. For cell maintenance, enzymatic methods using TPVG were prevalently used. Growth medium is then extracted completely from the flasks and the cell were further subjected to incubation at 37°C after the addition of the enzyme. This, initially, detach the cells from the surface. The cells were then suspended in 5ml of the medium. This suspension was repeated a few times to break the cell clumps. The date of seeding, cell line and the passage number were then marked on the bottom of the T-flask. The cell suspension in 5ml of medium was then transferred into a fresh new T-flask.

Determination of Caspase activity

Activities of caspases were determined by colorimetric assays using caspase-3 and Caspase-9 activation kits according to the protocol of the manufacturer. After treated with designated concentrations of extract (50-150 µg/ml, 0µg/ml (control)), cell lysates were prepared by incubating 2×106cells/ml in cell lysis buffer for 10 min on ice. Lysates were centrifuged for 1 min at 10,000×g.

The supernatants (cytosolic extract) were collected and concentration of protein was determined by the Bradford's method using BSA as a standard to test against. 100–200 µg protein (cellular extracts) was diluted for each assay in 50 µl cell lysis buffer. Cellular extracts were then incubated in 96-well microtiter plates with 5 µl of the 4mM pnitroanilide (pNA) substrates, DEVD-ala-pNA (caspase-3 activity) and Ac-LEHD-pNA (CASPASE -9 activity) at 37°C for 2 hours.

Caspase activity was measured by cleavage of the above substrates to free pNA. Free pNA (cleaved substrates) was measured by absorbance in a microtiter plate reader at 405nm. Relative caspase-3 activity was calculated as a ratio of the absorbance of treated cells to untreated cells.

RESULTS

Table 1: Caspase-3 activity of Pineapple extracts using Oral cancer cell lines (KB).

	OD Value			% of caspase activation	
Concentration	Control	Caspase 3	Caspase 9	% of Caspase 3 activation	% of Caspase 9 activation
50 µg	0.5116	0.5921	0.5521	115.7349492	107.9163409
100 µg	0.5116	0.7023	0.6221	137.275215	121.5989054
150 µg	0.5116	0.8034	0.7056	157.0367475	137.9202502

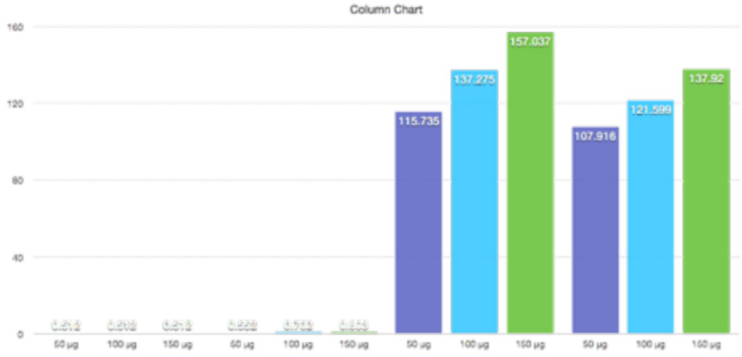

Figure 2: Graph indicating the control and activity of the enzymes

DISCUSSION

The conventional anticancer drugs which are being used, acts on both normal cells and tumor cells, causing brutal side effects and tumor resistance. Anticancer activity by apoptotic induction by herbs doesn't show any side effects.

One of the most extremely conserved biochemical features of apoptotic cell death is the activation of caspases. Once activated, these destructive proteases proceed to systematically deconstruct the cell to ensure its effective removal without damage to surrounding cells and tissue. In this study, apoptotic induction potential of various concentrations of pineapple extract (50 , 100, 150 µg) on KB cells was studied by monitoring the activation of caspase3 and caspase9. Percentage activation of caspase3 was more compared to caspase9 [Table 1 Figure 1].

CONCLUSION

The mechanism that can activate caspases may therefore represent a possibly feasible approach for effective tumor treatment which has several advantages over both conventional therapies and the more current "designer" approaches.

The secondary metabolites from herbs are always promising with antioxidant and anticancer activity. The ability of apoptotic induction by pineapple extract can be utilized in anticancer formulation.

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