Case Series

Comparison of Apoptotic Effect of Withania Coagulans in Hela1, Viro and BHK Cancer Cell Lines

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Objectives: Cancer is the second leading cause of death and has a complex etiology. Efforts are being made to target different components for its treatment. Developments in the field of molecular biology have led the researchers to target apoptotic pathway. This study has been designed to assess the apoptotic effect of the extract of withaniacoagulans on different cancer cell lines.

Methodology: This study was designed to check the anticancer activity of withaniacoagulans and to compare it in different cancer cell lines.

Results: The extract of withaniacoagulans proved to be equally effective in hela, vero and bhk cell lines even at concentration of 10µg/ml.

Conclusions: The extract has a potential to act as apoptotic compound. Study can be elaborated by isolating different components and assessing their role as apoptotic molecules on individual basis.

Key words: Withaniacoagulans, cancer cell lines & apoptosis

Introduction

XCancer a disease of complex etiology is one of the leading causes of death in the world and is still difficult to treat.[1,2] The programmed cell death serves as a natural barrier to cancer development.[3] Cell death can proceed by different mechanisms apoptosis being the typical route of cell demise under physiological conditions.[4-6] Imperfections in the cell death mechanisms not only result in cancer but also ensure the pathological cell growth and proliferation which results in progression of cancer. Several mechanisms by which cancer cells escape endogenous cell death have been identified, hence identifying how cancer cells achieve selective advantage of survival. Molecules that create the barriers to cell death within tumors have been identified as suitable targets for drug discovery. The main idea is either to restore the integrity of natural pathways for cell turnover or promoting cell death or to induce the activation of the activators of endogenous cell death which are sometimes silenced in cancer cells.[7,8] Resistance of cancers to conventional therapies has stimulated the researchers to search for novel stratagems for the cancer cell demise.[3,4]

The goal of any therapeutic strategy should be to treat the cancer cells with limited harmful effect to the normal cell function. In existing years, the naturally occurring compounds and their synthetic analogs have attained great attention of the researchers in the field of cancer research as they have proved to be the promising anti-cancer agents because of their non-toxic or less toxic effects and compelling anti-cancer properties. In addition to that different epidemiological studies have revealed that in individuals whose main component of food is from plant sources are a lower risk of cancer.[9] Therefore, identifying anti-cancer compounds in plant extracts has become the major strategy to treat cancers. Nowadays almost 80% of the world's population is using plant derived medicine for the maintenance of health and treatment of diseases because of it's very less side effects.[10]

Pakistan is very rich in plant resources specially the medicinal ones. Almost 1,000 species of medicinal plants have been reported in the Peshawar region only and 500 species of them are being used for health care practices. Moreover the medicinal plants have mammoth potential but unluckily very little is known about the actual size of production, their capabilities, their conservation status etc. and very little research is carried out in this field so far in Pakistan.[11]

Withania Coagulans is usually found in Afghanistan, India and southern Pakistan. The common name of Withania Coagulans is panir or vegetable rennet and it belongs to Solanaceae family. Withaniacoagulans is an important medicinal herb and a number of phytochemicals have been isolated from it, which are used in different herbal pharmaceutical products. Phytochemical analysis of the hydro alcoholic

fraction of withania showed the presence of steroids, alkaloids, phenolic compounds, tannins, saponins, carbohydrates, proteins, amino acids and organic acids. The chloroform fraction showed the presence of steroids and alkaloids as main components. Pharmacological evaluation has shown the association of activities with the specific steroidal lactones known as Withanolides present in Withania. Major Withanolides present in Withaniacoagulans are Withaferin A and Withanolide A and Withanone.[11-13] Withanolides have proved to be potent suppressors of NF-KB (nuclear factor kappa-light-chain-enhancer of activated B cells). This suppression is mediated through inhibition of IKK (IκB kinase). This mechanism may account for the ability of Withanolides to suppress the expression of gene products that regulate apoptosis, proliferation, angiogenesis and invasion and hence may prove to be important anticancer agent.[10,14,15]

Methodology

This study was designed to check the anticancer activity of withaniacoagulans and to compare it in different cancer cell lines.

Identification of plant:

Identification of the plant was done by Prof. Dr. Tahira Mughal (Prof. of Botany at Lahore College for Women University).

Extraction procedure:

The plant was dried in the shade after collection and identification. The plant material was ground to powder. Then the powder was placed in the thimble of Soxhelt and methanolic extract was collected at 60oC. The methanol was evaporated under vacuum by rotatory evaporator to get crude methanolic extract.

Stock solution:

1 gram of extract was dissolved in 1 ml of DMSO (Dimethyl sulfoxide) to prepare stock solution of 1000µg/µl then serial dilutions were made (250µg/µl, 100µg/µl, 50µg/µl, 25µg/µl, 10µg/µl).

Cell Lines: Three cell lines HeLa (developed from cervical cancer cells), Viro (developed from kidney epithelial cells) and BHK (derived from baby hamster kidney fibroblasts) were obtained from School of biological Sciences University of The Punjab and were cultured according to the standard procedure.

Reagents & apparatus: All reagents used were of analytical quality and were obtained from GIBCO Invitrogen USA. Methyltetrazolium salt (MTT salt) and DMSO were obtained from MB cell (Korea). Haemocytometerused to count cells was by Marienfeld Germany and all plastic ware required for the experiment was procured from Oxygen life sciences, California.

HeLa, Viro and BHK cell lines were cultured at 370C with 5% CO2 in Dulbaco's Modified Eagle's Medium (DMEM) containing 10% heat inactivated fetal bovine serum (FBS), 2mM L- glutamate, 100 U/ml penicillin and 100 U/ml of streptomycin and cultures were split 1:3 in 25cm culture flask as follows:

1. Wash the cells twice with about 1ml PBS (phosphate buffer saline)
2. Then about 1-2 ml trypsin was added to the flask and placed at 37 C for 5-10 minutes.
3. After the cells were detached from the surface, about 1ml culture medium was added and the cells transferred to a 15ml falcon tube. Cells were transferred into the new culture flasks with fresh culture medium for future use.

Preparation of 96 wells culture plate for MTT assay:

The stock solutions were taken from the primary cultures. Three 96 wells culture plates were prepared for the performance of MTT assay to evaluate anticancer effect of WithaniaCoagulans in Hela, Viro and BHK cell lines. Cells were counted with the help of Haemocytometer. After calculation of the total number of cells, cell suspensions of desired dilutions were prepared to obtain 5×103 cells per well.

MTT Assay:

Short 96 well assay: Assay was performed in duplicates.

Preparation of Solutions:

1. MTT 5mg/ml of Phosphate Buffer Saline (PBS)
2. Extraction Buffer (SDS 20%; DMSO 50%)

Procedure:

1. 5×103 cells per well in 96 cavity plates were taken in 0.2ml of the medium (DMEM).
2. Different concentrations of withaniacoagulans extract solution were added and incubated at 370 C in the presence of 5% CO2 for 24 hrs.
3. 0.025 ml of MTT reagent was added & was incubated for two hrs at 370 C.
4. Then the media was aspirated and the plates were dried while patting slowly on a tissue paper.
5. 1 ml of extraction buffer was added & was incubated again overnight
6. Optical density was read at 570nm in Elisa Reader.
7. Cell viability was calculated as follows

$$\text{Relative cell viability} = \frac{\text{OD at 570nm of the sample} \times 100}{\text{OD at 570 nm of Blank}}$$

Results

With aniacoagulansmethanolic extract was used in

Vero and Bhk cell lines. Extract induced apoptosis even at concentration of 10µg/µl (minimum concentration used) (see Fig:1). Fig:2 shows that the apoptotic effect of withaniacoagulans extract is comparable and same extent of apoptosis occurs in three cell lines even at very low concentration.

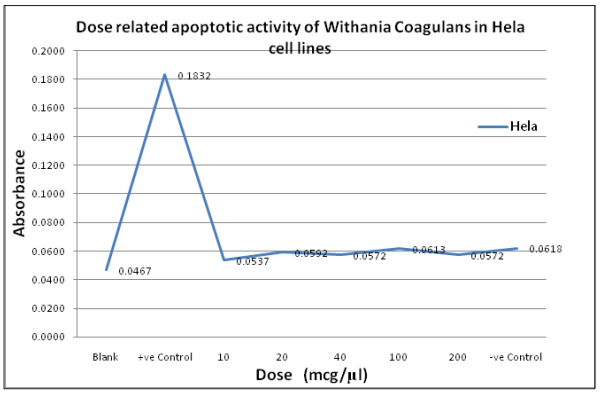

Fig-1: showing the apoptotic effect of Withania Coagulans in Hela cell lines.

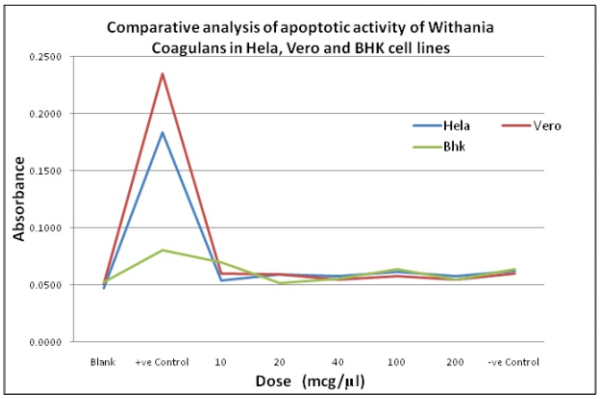

Fig-2: Showing comparison of apoptotic effect of Withania Coagulans in Hela, Vero and BHK cell lines

Discussion

Our study has proved that the active compounds in WithaniaCoagulans have apoptotic activity in human cancer cell lines even at a dose of 10µgm/µl. When a comparison was made for the apoptotic activity of WithaniaCoagulans amongst the three cell lines namely Hela, Vero and BHK, it showed that all the cell lines almost behaved the same way (see fig: 2). With positive controls they exhibited variable absorbance but when they were treated with WithaniaCoagulans extract the absorbance was markedly decreased becoming zero in Hela cell lines at a concentration of 10µgm/µl, in Vero cell lines at a concentration of 40µgm/µl and in BHK cell lines at a concentration of 20µgm/µl. Our results are comparable with studies conducted with the extracts of WithaniaSomnifera in human and with WithaniaCoagulans in animals.[10,11,16]

Conclusion

It will prove to be very effective for the treatment of different types of cancers and can be used to design new therapy regimes targeting cell death mechanisms in cancer cells.

Limitations and Future Scope

The components of the extract should be separated out to find out the effective anti cancer compound. Moreover a study tracing the intracellular pathway to which the specific compounds of the extract target will be more conclusive.

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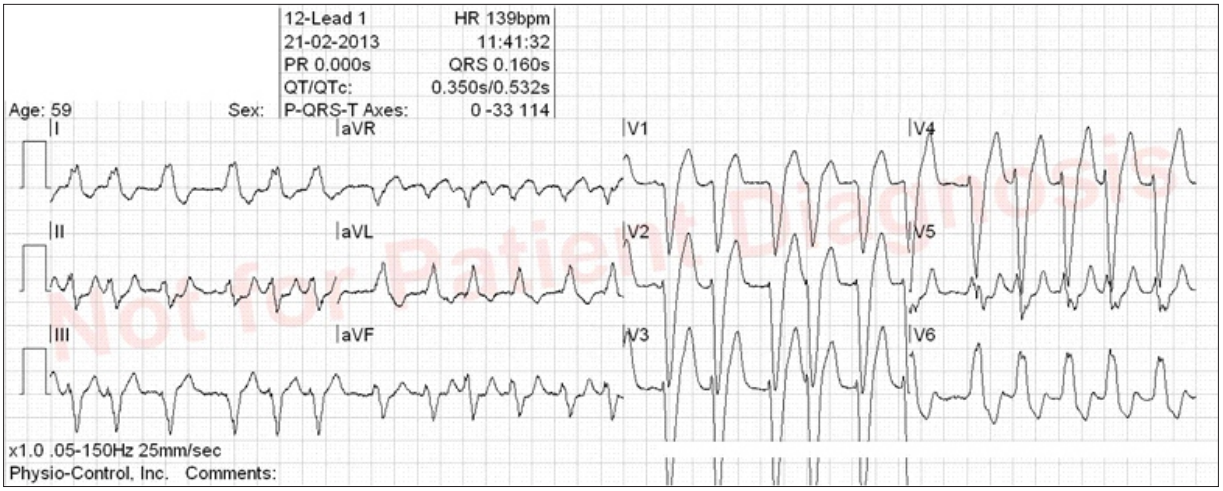
Picture Quiz

This patient woke up 4 hours ago with shortness of breath, presyncope.
What is diagnosis?



See answer on page # 31