Original Article

Cytotoxicity study of plant *Aloe vera* (Linn)

Abstract

Background: The objective of this study has been to evaluate the *in-vitro* antitumor activity of *Aloe vera* extract of in cultured B16F10 melanoma cell line by measuring cell viability using "Trypan blue exclusion assay" method. **Aim:** To find out such kind of anticancer drug which is a cheap, safe, less toxic, and more potent drug compared to chemotherapy drug. **Materials and Methods:** *In-vitro* antitumor activity cell culture1, drug treatment (standard and test extract) and Trypan blue exclusion assay growth and viability test 1 were used. Treatment of *Aloe vera* extract against B16F10 melanoma cell line, in all concentration range, showed decrease in percent cell viability, as compared to that of negative when examined by "Trypan blue exclusion assay". **Results:** In overall variation of test samples, *Aloe vera* extract showed its best activity in the concentration of 300 μg/ml, which was approximately equal to the activity of standard drug doxorubicin. Evaluation of *in-vitro* antitumor activity revealed that *Aloe vera* extract exhibits good cytotoxic activity. The best cytotoxic activity by *Aloe vera* was shown at 200 μg/ml concentration. **Conclusion:** The study of cytoprotection against normal cells by micronucleus assay has shown that the herbal extracts have less toxic effects to the normal blood lymphocytes, as compared to that of standard anticancer drug.

Key words:
Aloe extract, B16F10 melanoma cell, cytotoxicity, trypan blue exclusion assay

Introduction

Plant-based systems continue to play an essential role in the primary health care of 80% of the world's population.[1] An increasing number of chemotherapeutic agents are discovered as a result of chemical studies directed toward the isolation of the active substances from plants used in traditional medicine.[2] Our study analyzed the cytotoxic potential of AE, a hydroxyanthraquinon, naturally present in the leaves of *Aloe vera*.[3,4] The presence of free radicals in the body induces cell and tissue damages. This sort of damage is known as oxidative damage.[5] Cancer is a disease characterized by uncontrolled multiplication and spread of abnormal forms of the body's own cells. It is one of the major causes of death in the developed nations. At first sight, incidence figures for the past 100 years or so give the impression that the disease is increasing in developed countries, but cancer is largely a disease of later life, and with advances in public health and medical science many more people now live to an age where they are more liable to contract cancer.[6] Aloes have been used therapeutically, certainly since Roman times and perhaps long before (Morton, 1961; Crosswhite and Crosswhite, 1984), different properties being ascribed to the inner, colorless, leaf gel and to the exudate from the outer layers. During the 12 years since the last major review of *Aloe vera* (L.) Burm. F gel (Grindlay and Reynolds, 1986) popular interest and use of the gel have increased dramatically. It has larger demands and is traded in medicinal drug markets of the world for flavoring liquid and as a source of 'Aloin' (4.5 to 25%). This study is mainly a cytotoxic study on tumor cell using crude *Aloe Vera* leaves extract.

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Materials and Methods

Plant material

Specimen of *Aloe vera (L.)* was harvested from Tindal, Erode

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district, Tamil Nadu and collected plant has been identified by Botanical survey of India at Coimbatore. Fresh leaves of this cultivated plant were used in this study.[6]

Extraction

Aloe Vera leaves (six big and healthy leaves) were weighed, washed and cut in the middle, the gel was separated by scratching with a spoon and pulp was cut in to small pieces (514 g) and homogenized with phosphate buffered saline solution (PBS; pH 7; 600 ml) by means of a blender. The extract was kept at 4°C overnight, and then filtered through muslin cloth and the filtrate was centrifuged at 20000 rpm for 30 min at 2°C in a refrigerated centrifuge. The green pallet was discarded and the clear yellow supernatant was taken and lyophilized, thus 10 g of *Aloe vera* leaves pulp extract was obtained. The extract used in the experiment was (7.5%) prepared by dissolving the powder in PBS and mixing it thoroughly via a magnetic stirrer.[3]

Experimental protocols

In-vitro antitumor activity

Cell culture

The B16F10 melanoma cell line was kindly provided by A.R. Lab Salem, (T.N.). Cells were cultured in Eagle's minimum essential medium (EMEM), supplemented with 10% fetal calf serum (FCS) and streptomycin plus penicillin (100 µg/ml and 100 IU/ml, respectively). Cells were cultured in a 5% CO_2 humidified atmosphere at 37°C until near confluence. All the processes were carried out in a vertical laminar flow chamber.[7]

Drug treatment

Saline extracts of *Aloe vera* were prepared in increasing final concentrations, ranging from 25 to 300 µg/ml. The drug extracts were treated with plates containing confluent monolayer of B16F10 melanoma cell lines. Negative control group was B16F10 cell line only, and the positive control group was treated with doxorubicin (0.032 µg/ml). After incubating for 24 h at 37°C, the cells were trypsinized (0.25% in PBS) and then centrifuged at 1000 rpm for 5 min, washed twice with fresh medium, and re-suspended with fresh medium. Cell viability was counted for each concentrations of crude extract as well as for control.

Trypan blue exclusion assay: Growth and viability test

To evaluate the growth and viability of the treated and untreated cells, the percentage of viable and non-viable cells was determined, using Trypan blue exclusivity stain.[1] Cell growth and viability were measured by adding 0.4% Trypan blue in 0.9% saline to a 50% dilution, and the cells were counted using a hemocytometer. Briefly, 0.5 ml of the Trypan blue solution was transferred to a test tube and 0.3 ml of PBS plus 0.2 ml of the trypsinized cell suspension (dilution factor of 4) were added. The final solution was thoroughly and gently mixed and allowed to stand for 5 min. Then a drop of this dye-cell suspension was loaded onto both chambers of the hemocytometer. Cells were examined and counted in duplicates under light microscope at 100 x (Olympus, Japan). Percentage cell viability was calculated by the formula:

Cell viability = No of viable cells (unstained cells)×l00

Total no of cells (stained and unstained)

Summary and Conclusion

This piece of work is mechanized to prove the potentiality of herbal extracts of *Aloe vera to* kill the cancerous cells and to protect the normal cells from the cytotoxic effect of anticancer drugs. Here the culture is being performed for cancer cell, the molecular involvement and potency of chemotherapeutic drug in cancer cell is well presented. Evaluation of *in-vitro* antitumor activity revealed that *Aloe vera* exhibits good antitumor activity. The best antitumor activity by *Aloe vera* was shown at 300 µg/ml concentration. In future, this piece of work can be mechanized for purification and identification of active constituents present in *Aloe vera by* using various analytical techniques. Also, this work can be extended for *in-vivo* study of antitumor activity by measuring tumor volume and also

Table 1: Effect of different concentration of *Aloe vera* on cell viability

Drug conc. (µg/ml)	Total cells	Viable cells	Nonviable cells
Control	100	95±1.33	5±1.33
25	100	62±1.34	38±1.34
50	100	57±1.76	43±1.76
100	100	48±1.98	52±1.98
150	100	43±1.51	57±1.51
200	100	38±1.34	62±1.34
250	100	34±1.21	66±1.21
300	100	24±1.81	76±1.81
Doxorubicin (0.032)	100±1.24	20±1.22	80±1.113

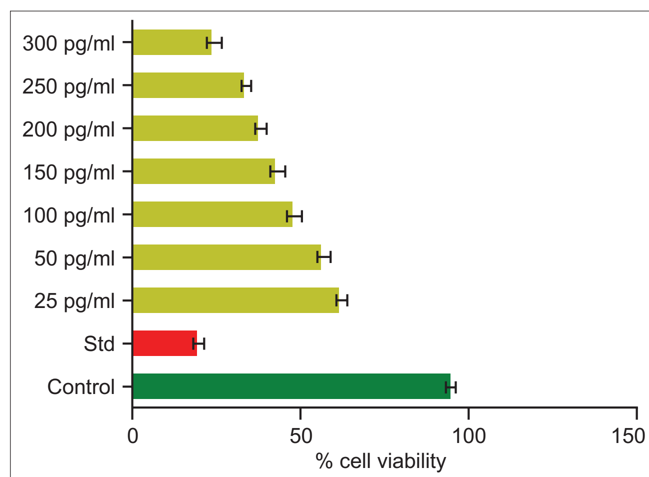

Figure 1: Effect of different conc. of *Aloe vera* on cell viability (concentration vs percent viability)

for *in-vivo* study of cytoprotection activity and immune-modulatory activity.

The statistical correlation [Table 1 and Figure 1] can be established on the above-based information, so that this wonderful plant of *Aloe vera*, which is well known for its various activities and used as long as "gripe water, can be used as a drug of choice during cancer chemotherapy. Its low cost makes it a drug of choice for poor people. In near future, such products will be a boon to mankind and cancer patients.

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