Journal of Advanced Pharmaceutical Technology & Research

IN VITRO CYTOTOXICITY STUDY OF *AGAVE AMERICANA, STRYCHNOS NUX-VOMICA* AND *ARECA CATECHU* EXTRACTS USING MCF-7 CELL LINE

Anajwala Chetan C.[*1], Patel Rajesh M.[2], Dakhara Sanjay L.[1], Jariwala Jitesh K.[3]

1. Department of Pharmaceutics, Bhagwan Mahavir College of Pharmacy, Bharthana-Vesu, Surat, Gujarat, (India)
2. Department of Pharmaceutical Biotechnology, S. K. Patel College of Pharmaceutical Education and Research, Kherva, Dist. Mehsana, Ganpat Vidyanagar, Gujarat, (India)
3. Department of Pharmacognosy, Babaria Institute of Pharmacy, Vadodara, Gujarat, (India)

Corresponding Author's E-mail: - anajwalachetan@yahoo.co.in

Received: 15th April 2010 Revised: 02nd June 2010 Accepted: 08th June 2010

ABSTRACT

Research is focusing on the search for new types of natural chemotherapeutic agent that is plant based medicines which are proving to be excellent sources of new compounds. In present research study, an attempt was made to prove cytotoxicity activity of various parts of medicinal plants such as *Agave americana, Strychnos nux-vomica* and *Areca catechu* using MCF-7 and Vero cell line. Various parts of the medicinal plants were extracted by soxhlet apparatus using solvents likes methanol and water. By trypan blue dye exclusion method, Viability of MCF-7 and Vero cell lines were 85.50 and 81.13%, respectively. IC_{50} value of methanol extract of *Agave americana* leaves and aqueous extract of *Areca catechu* fruits were found to be 545.9 & 826.1 µg/ml by SRB assay and 775.1 & 1461µg/ml by MTT assay, respectively, against MCF-7 cell line. From cytotoxicity study data by SRB and MTT assay, it revealed that methanol extract of *Agave americana* and aqueous extract of *Areca catechu* are potent cytotoxic.

Keywords: Cytotoxicity, *Agave americana, Strychnos nux-vomica, Areca catechu*, MCF-7.

INTRODUCTION

A recent survey lists over 1400 genera of herbs that have a history of use in cancer treatments [1]. Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives each year [2]. On a yearly basis in US, 0.5% of the population is diagnosed with cancer [3]. *Agave americana* belongs to family of Agavaceae. It is traditionally used in Africa to treat high blood pressure. Concentrated agave sap has antibacterial activity, and extracts from

tropical agave plants have anti-inflammatory effects [4]. *Strychnos nuxvomica* belongs to family of *Loganiaceae*. It is an energetic poison, exerting its influence chiefly upon the cerebro-spinal system. It is a favorite medicine for paralysis and nervous debility generally. *Areca catechu* belongs to family of Arecaceae. In ayurvedic medicine, the nut is used in the treatment of headaches, fever and rheumatism. It induces a cardio-acceleratory response in humans; this leads to heightened alertness [5, 6]. The MCF-7 breast cancer cell line originated from a 69-year-old Caucasian woman who previously underwent two mastectomies in a five-year span. The tissue removed at the first mastectomy was benign, but the second operation found a malignant adenocarcinoma at Michigan Cancer Foundation (MCF). The MCF-7 cell line was derived from this pleural effusion in 1970 [7]. Vero cells are lineages of cells used in cell cultures. The *Vero* lineage was isolated from kidney epithelial cells extracted from African green monkey (Cercopithecus aethiops). The lineage was developed on 27 Mar 1962, by Yasumura and Kawakita at the Chiba University in Chiba, Japan [8].

MATERIALS AND METHODS

Plant material collection

The leaves of *Agave americana* were collected in winter from Bilimora Nursery & Farm, Surat, Gujarat, India. The fruits of *Areca catechu* and seeds of *Strychnos nux-vomica* were collected in winter from Hakeem Chichi Sons, Hakeem Chichi Street, Rani Talao, Surat, Gujarat, India. All parts of plants were identified by Dr. Minoobhai Parabia at Department of Biological Sciences; Veer Narmad South Gujarat University, Surat and by Ritesh Vaidh at Department of Botany, School of Bioscience, Ganpat university, Kherva, India.

Preparation of Extracts

The leaves of *Agave americana* (300 g) were air-dried, powdered and exhaustively methanol extracted by soxhlet apparatus. The fruits of *Areca catechu* (100 g) and seeds of *Strychnos nux-vomica* (100 g) were powdered and exhaustively extracted by soxhlet apparatus using water and methanol, respectively. Each extract was cooled at room temperature and evaporated to complete dryness by vacuum distillation. All dried powder was stored in air-tight container.

Cells

Human MCF-7 breast carcinoma cells and Vero cells were obtained from National Centre for Cancer Science (NCCS), pune and grown as a monolayer in DMEM (Dulbecco's Modified Eagles Medium; US Biological, Lot No: 7020976) supplemented with 10% FBS (Fetal Bovine Serum; Bioclot, Lot No:07310) at

37°C in an atmosphere of 5% CO_2. Cells were grown up to 60% confluence and then treated with different extracts at a final concentration ranging from 0.0196-10 mg/ml for indicated periods of time. Stock solutions were prepared in DMSO (Dimethyl Sulfoxide; MP Biomedicals) and stored at 20°C until used.

Determination of the percentage viability

Proliferation of both cells and their sensitivity to increasing concentrations of extracts were determined by the Trypan Blue Dye (Hyclone, Lot no: JRH27098) Exclusion Assay [9]. As described recently in more detail, Trypan Blue Dye Exclusion Assay is based on quantification of the cellular ATP levels. Tests were performed at least in quadruplicate to count cells using inverted fluorescence microscope.

SRB assay

Cell were grown in micro titer plates in a final volume of 100 µl culture medium containing 10% FBS per well. Cells were incubated for 24 h at 37^{0}C and 5% CO_2. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed once and 100µl of different test compound concentrations were added to the cells in microtitre plates. The plates were then incubated at 37^{O}C for 72 hours in 5% CO_2 incubator and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, 25µl of 50% TCA (trichloroacetic acid) was added to the wells gently such that it forms a thin layer over the test compounds to form an overall concentration 10%. The air-dried plates were stained with 100 µl SRB (Sulphorhodamine-B; MP Biomedicals) and kept for 30 minutes at room temperature. The unbound dye was removed by rapidly washing four times with 1% acetic acid. 100 µl of 10mM Tris base was then added to the wells to solubilize the dye. The absorbance was measured using microplate reader at a wavelength of 540nm [10, 11].

MTT assay

Cell were grown in micro titer plates in a final volume of 100 µl culture medium per well. Cells were incubated for 24 h at 37^{0}C & 5% CO_2. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed once and different test compound concentrations were added into micro plates (96 wells, flat bottom). After incubation period, 10 µl of the MTT (3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide; Roche applied sciences, Cat. No. 11 465 007 001) labeling mixture were added to each well. Solubilization solution (100 µl) was added into each well and incubated the microtiter plate for 24 hrs in incubator. Results were measured on a Microplate

reader using a wavelength of 490 nm [12].

RESULT

Viability and Characterization of Cell Lines

Cell lines were checked for sterility, viability and free from any kind of bacterial and fungal contamination. Viability and characterization of both cell lines (MCF-7 and Vero) are shown in below table no. 1.

Table 1: Percentage Cell Viability and Characterization of Cell Line

Cell line	Live cell count	Total cell count	pH	% Viability
VERO	1.72 x10^5	2.12 x10^5	7.5	81.13
MCF7	1.69 x10^5	2.0x10^5	7.5	85.50

Cytotoxicity study

The cytotoxicity assays were carried out for plant extracts of *Agave americana*, *Strychnos nux-vomica* and *Areca catechu*. These extracts were screened for its cytotoxicity activity against MCF-7 and Vero cell lines at different concentrations to determine the IC_{50} (50% inhibition concentration) by SRB and MTT assay and plotted DRC (Dose Response Curve) using Graph Pad Prism Software V-5.

Determination of Total Cell protein content by Sulphorhodamine B (SRB) assay

The percentage growth inhibition was found to be increasing with increasing concentration range from 0.0196 to 10 mg/ml of test plant extract. The methanol extract of *Agave americana* and aqueous extract of *Areca catechu* were found potent cytotoxic effect against MCF-7 cancer cell line and IC_{50} values were found to be 545.9 and 775.1 µg/ml, respectively. IC_{50} of methanol extract of *Strychnos nux-vomica* was 668.7µg/ml. But from DRC, it did not show identical cytotoxic activity. Against Vero cell line, IC_{50} of methanol extract of *Agave americana* was found to be 1854 µg/ml whereas aqueous extract of *Areca catechu* and methanol extract of *Strychnos nux-vomica* were not showed any significant cytotoxicity activity. All data are tabulated in table no. 2 and graphically represented in figure no. 1, 2 and 3. These data suggests that methanol extract of *Agave americana* and aqueous extract of *Areca catechu* were shown higher cytotoxicity on MCF-7 cell line and have good proportionality for % Inhibition vs. log [dose].

Determination of Cytotoxicity by MTT assay

Determination of Cytotoxicity by MTT assay is based on the formation of formazan blue colour due to reduction of MTT by mitochondrial Succinate dehydrogenase that was directly proportional to the number of viable cells and the absorbance was read out

by 490 nm. The methanol extracts of *Agave americana* and aqueous extract of *Areca catechu* were tested for cytotoxic activity against MCF-7 cancer cell line and its IC_{50} values were found to be 826.1 and 1461 µg/ml respectively. IC_{50} of methanol extract of *Strychnos nux-vomica* was 18134µg/ml. From dose response curve (DRC) and all data, it did not any significant cytotoxic activity. Against Vero cell line, IC_{50} of methanol extract of *Agave americana* was 8.455µg/ml. The aqueous extract of *Areca catechu* and methanol extract of *Strychnos nux-vomica* were not found IC_{50} values. These data were not represented cytotoxic activity for Vero cell line. All data are tabulated in table no. 3 and graphically represented in figure no. 4, 5 and 6. Finally, the methanol extract of *Agave americana* and aqueous extract of *Areca catechu* were shown higher cytotoxicity on MCF-7 cell line and have good proportionality for % cell inhibition vs. log [dose].

Table 2: Determination of Cytotoxicity by SRB assay

Plant Extract	Cell line	IC_{50} (µg/ml)	Cell line	IC_{50} (µg/ml)
Agave americana	MCF-7 (Human Breast Cancer cell line)	545.9	Vero (African green monkey kidney cell line)	1854
Strychnos nux-vomica		668.7		-
Areca catechu		775.1		-

Table 3: Determination of Cytotoxicity by MTT assay

Plant Extract	Cell line	IC_{50} (µg/ml)	Cell line	IC_{50} (µg/ml)
Agave americana	MCF-7 (Human Breast Cancer cell line)	826.1	Vero (African green monkey kidney cell line)	8.455
Strychnos nux-vomica		18134		-
Areca catechu		1461		-

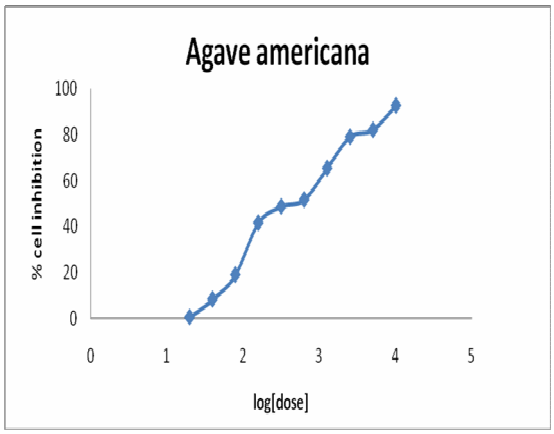

Fig. 1 DRC of methanol extract of *Agave americana* for MCF-7 cell line by SRB assay

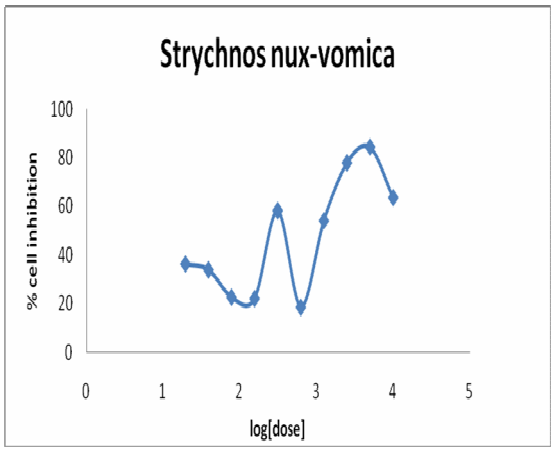

Fig. 2 DRC of methanol extract of *Strychnos nux-vomica* for MCF-7 cell line by SRB assay

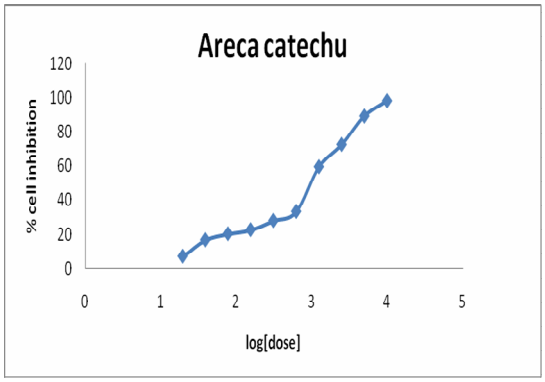

Fig. 3 DRC of Aqueous extract of *Areca catechu* for MCF-7 cell line by SRB assay

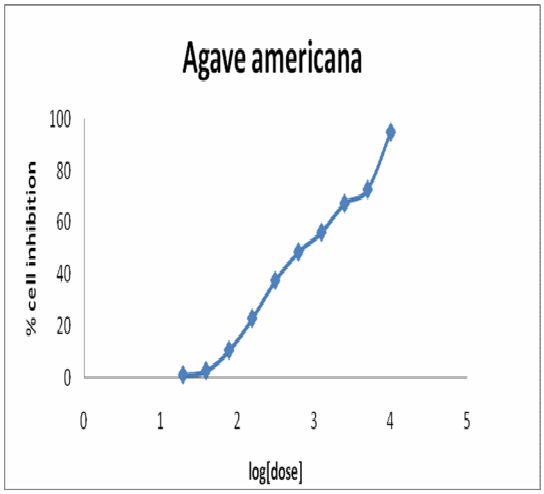

Fig. 4 DRC of methanol extract of *Agave americana* for MCF-7 cell line by MTT assay

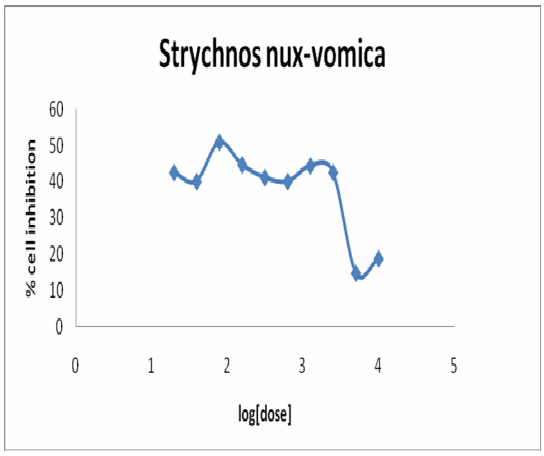

Fig. 5 DRC of methanol extract of *Strychnos nux-vomica* for MCF-7 cell line by MTT assay

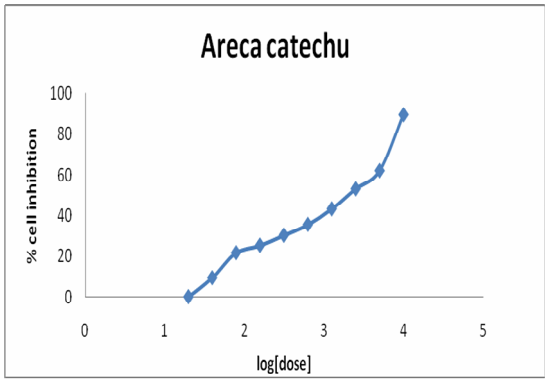

Fig. 6 DRC of Aqueous extract of *Areca catechu* for MCF-7 cell line by MTT assay

DISCUSSION

Human MCF-7 breast cancer cells are relatively resistant to chemotherapy. MCF-7 cells have a low invasive capability *in vitro*. *In vivo*, the MCF-7 cells are capable of forming tumors in nude mice, but grow substantially better when the mouse is treated with 17 b estradiol [13]. Vero cells are used as host cells which is continuous and aneuploid. A continuous cell lineage can be replicated through many cycles of division and not become senescent. Aneuploidy is the characteristic of having an abnormal number of chromosomes [8]. Recently, we showed that extract of *Areca catechu* and *Agave americana* arrested human MCF- 7 breast cancer cells. On the other hand extract of *Strychnos nux-vomica* showed no potential activity to arrest human MCF- 7 breast cancer cells. In vitro studies suggested that water and ethanol extracts have ACE-inhibitor activity. In vivo studies indicate anti-inflammatory, molluscucidal, uterine

stimulant, and larvicidal properties. Other studies indicate antibacterial, antifungal, tumor-enhancing, and spermaticidal activities. Crude extracts of *Agave americana* contain two compounds that are similar to the neurotransmitter, acetylcholine [4]. Arecoline resembles Pilocarpine in its effects. It has parasympathomimetic properties acting on both the muscarinic and nicotinic receptors. Arecoline, an active alkaloid of *Areca catechu L.*, and sodium nitrite, a food additive, are highly cytotoxic and cytostatic on the Hep 2 cell line when administered in an acidic environment (pH 4.2) [14, 15]. *Strychnos nux-vomica* showed anti-inflammation and analgesic effects. Isostrychnine and isobrucine showed most potent cytotoxicity to tumor cell lines of K562, HeLa and HEP-2. [15] As shown in the present study, *Agave americana* and *Areca catechu* are strongly affected the distribution of MCF-7 cells. These results are consistent with previous observations that *Agave americana* and *Areca catechu* are able to induce a cell cycle arrest in MCF-7 cells. Interestingly, *Agave americana* and *Areca catechu* seems to exert an effect on the cellular levels of its downstream target. Furthermore, it leads to apoptosis in a dose-dependent manner.

CONCLUSION

The percentage viability of both cell lines, MCF-7-cancer cell line and Vero-normal cell line, were found 85.5 and 81.13, respectively, by trypan blue dye exclusion assay. From cytotoxicity study data by SRB and MTT assay, it revealed that methanol extract of *Agave americana* and aqueous extract of *Areca catechu* are potent cytotoxic. Methanol extract of *Strychnos nux-vomica* shows no effective cytotoxicity by calculating its 50% cell growth inhibition Concentration (IC_{50}) and graphically representation against MCF-7 cell line.

REFERENCES

1. Hartwell J.L. Plants used against Cancer. A survey. Lloydia 1971; 32, 204-255.
2. Abdullaev FI, Rivera Luna R, Roitenburd Belacortu V, Espinosa Aguirre J. Pattern of childhood cancer mortality in Mexico. Arch Med Res 2000; 31(5):526–531.
3. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, et al. Cancer statistics 2005. CA Cancer J Clin 2005; 55 (1): 10–30.
4. Hackman DA, Giese N, Markowitz JS. Agave (*Agave americana*): an evidence-based systematic review by the Natural Standard Research Collaboration. J Herbal Pharmacother. 2006; 6(2): 101-122.
5. Chitra, S., Ashok, L., Anand, L., Srinivasan, V., and Jayanthi, V. Risk factors for esophageal cancer in Coimbatore, southern India: a hospital-based case-control study.

Indian J Gastroenterol. 2004; 23(1):19-21.

6. Huang Z, Xiao B, Wang X, Li Y. and Deng H. Betel nut indulgence as a cause of epilepsy, Seizure. 2003; 12(6):406-408.
7. Dickson R.B., Bates S.E., Mc Manaway M.E., Lippman M.E. Characterization of estrogen responsive transforming activity in human breast cancer cell lines, Cancer Res. 1986; 46: 1707-1713.
8. Yasumura Y, Kawakita M. The research for the SV40 by means of tissue culture technique. Nippon Rinsh 1963; 21 (6): 1201–1219.
9. K. L. Phetteplace. Trypan blue assay SOP. New Hampshire Community Technical College, 320 Corporate Drive, Portsmouth, NH 03801, doc. No. 2.5.
10. Knick V.C., Eberwein D.J. and Miller C.G. Vinorelbine tartrate and paclitaxel combinations: enhanced activity against in vivo P388 murine leukemia cells. J. Natl. Cancer Inst. 1995; 87:1071.
11. P. Skehan. R. Storeng, D Scudiero, A Monks, J Mc mahon, D Vistica, JT Warren et al. New colorimetric cytotoxicity assay for anti cancer drug screening. J. Natl. Cancer Inst. 2003; 82:1107-1112.
12. Roche applied science, cell proliferation kit I (MTT) manual, Colorimetric assay (MTT based) for the non-radioactive quantification of cell proliferation and viability, Cat. No. 11 465 007 001.
13. Shafie S.M., Liotta L.A. Formation of metastasis by human breast carcinoma cells (MCF-7) in nude mice. Cancer Lett. 1980; 11: 81-87.
14. K. K. Wary. Cytotoxic and cytostatic effects of arecoline and sodium nitrite on human cells *in vitro*. International Journal of Cancer. 2006; 47(3): 396 – 400.
15. P. C. Sharma, M. B. Yelne, T. J. Dennis. Database on medicinal plants used in ayurveda, central council for research in ayurveda and siddha. 2002; 5:141.

