



In-Vitro Cytotoxicity Activity of Some Selected Ethanomedicinal Plants Against Vero Cell Line

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ABSTRACT

The study was aimed to evaluate the cytotoxic activity of selected ethanomedicinal plants on Vero (African green monkey kidney normal cell) cell line. Nine different ethanomedicinal plants were selected. Alcoholic and aqueous extracts were prepared of selected ethanomedicinal plants. These extracts were tested for its inhibitory effect on Vero Cell Line at different ranges of concentration. The percentage viability of the cell line was carried out by using Trypan blue dye exclusion method. The cytotoxicity of plant extracts on Vero cells was evaluated by MTT assay. All the plant extracts show very less cytotoxic effect on Vero cells. So these all selected ethanomedicinal plants can be taken for further studies.

Keywords: Cytotoxicity Activity, MTT Assay, ethanomedicinal plants, Vero Cell Line.

INTRODUCTION

Since last many years, plants have beneficial activity in different type of diseases producing in human beings. As per WHO calculation about 80% of the world's inhabitants problem should be treated by medicinal herbal drug for their primary health care¹⁻².

Plants have long history used in the treatment of cancer.

Active constituents of *Catharanthus roseus*, *Angelica Gigas*, *Podophyllum peltatum*, *Taxus brevifolia*, *Podophyllum emodii*, *Ocrosia elliptica*, and *Campototheca acuminata* have been used in the treatment of advanced stages of various malignancies³.

There were numbers of studies dealing with cytotoxicity screening of plant extracts⁴⁻⁶ but mostly tested in cancerous cells rather than in normal cell cultures.

Cytotoxicity studies with normal cell culture systems of local plant extracts or folk medicinal plant extracts has not been studied extensively and this is vital for the safety evaluation or any herbal preparations⁷.

Thus, the objective of this study was to evaluate the potential cytotoxicity activity of some selected ethanomedicinal plants against Vero cell line.

MATERIALS AND METHODS

Materials

Collection and authentication of selected ethanomedicinal plant material

All the selected ethanomedicinal plants were collected at the different geographical sources when it fully grown with flowering.

Plants raw material was authenticated by taxonomist. Plants having Herbarium specimen number PH/015/001

to PH/015/009 as deposited at Pharmacognosy Department, K.B.I.P.E.R., Gandhinagar, Gujarat, India for future reference.

The raw material were dried under shade and reduced mechanically to moderate coarse powder.

Cell lines used for Cytotoxicity Screening

African green monkey kidney Normal cell line (Vero), Details of cell line as mentioned below:

Cell line	Morphology	Origin	Species	supplier
Vero	Epithelial	Kidney	Monkey	NCCS,PUNE

Reagents

Trypan blue, Sodium bicarbonate, EDTA, DPBS (Dulbecco's phosphate buffer saline), Trypsin, MTT Salt

Media

DMEM (Dulbecco's Modified Eagles medium, high glucose), FBS (Fetal Bovine Serum)

Glasswares and plastic wares

96-well micro titer plate, Tissue culture flasks, Falcon tubes, Reagent bottles

Equipments: Inverted microscope, Biosafety cabinet class II, CO₂ incubator, Deep freezer, ELISA plate reader, Micropipettes, RO water system

Methods

Selection of plants

Plants were selected on the basis of their ethanomedicinal used in tribal areas. Following nine plants were selected to check its cytotoxicity effect on Vero cell line.



Selected ethanomedicinal plants for cytotoxicity study on Vero cell line

Plant name	Botanical source	Family	Part used
Amla ⁸	<i>Emblica officinalis</i>	<i>Euphorbiaceae</i>	Fruit
Baheda ⁹	<i>Terminalia bellerica</i>	<i>Combretaceae</i>	Fruit
Harde ¹⁰	<i>Terminalia chebulla</i>	<i>Combretaceae</i>	Fruit
Ashwagandha ¹¹	<i>Withania somnifera</i>	<i>Solanaceae</i>	Roots
Nagarmoth ¹²	<i>Cyperus rotundus</i>	<i>Cyperaceae</i>	Rhizomes
Rasna ¹³	<i>Alpinia galanga</i>	<i>Zingiberaceae</i>	Rizomes
Tulsi ¹⁴	<i>Oscimum sanctum</i>	<i>Liliaceae</i>	Leaf
Vasaka ¹⁵	<i>Adhatoda vasica</i>	<i>Acanthaceae</i>	Leaf
Long pepper ¹⁶	<i>Piper longum</i>	<i>Piperaceae</i>	Fruit

Preparation of plant extracts

20 gm of powder of selected plants were taken to prepare its different extracts. Aqueous and alcoholic extracts were prepared by maceration of powder material for 48 hours. Solvents were removed by rota evaporator. Percentage yield were calculated.

Cytotoxicity Assay**Trypan blue dye exclusion technique**

Trypan Blue is a blue acid dye that has two azo chromophore groups. Trypan blue will not enter into the cell wall of plant cells grown in culture. Trypan Blue is an essential dye, use in estimating the number of viable cells present in a population.

Make a cell suspension in a fixed volume of cells (e.g. 1ml). Although an aseptic technique is not essential in all stages of this procedure, it is good laboratory practice to maintain sterility throughout the procedure¹⁷. Take 50uL of cell suspension and mix it with an equal volume of trypan blue. Mix solution well using a pipette. Transfer to a hemocytometer and count the live cell as clear form and dead cell as blue cells. After staining with trypan blue solution counting should commence in less than 5minutes as after that time the cells will begin to take up the dye. Using a pipette place some of the cell suspension: trypan blue mixture into the hemocytometer and overlay with a cover slip.

The cell suspension will pass under the cover slip by capillary action unless there is an air bubble.

Make sure the wells are not overfilled and that the cover slip is not moved once it is placed on the grid and the cell solution is added. Place the hemocytometer on the stage of an inverted microscope.

Adjust focus and power until a single counting square fills the field. Calculate the number of cells per ml, and the total number of cells, using the following formula¹⁸.

Calculate percent viability by using formula:

$$\% \text{ viability} = (\text{live cell count} / \text{total cell count}) * 100$$

Microculture tetrazolium (MTT) assay

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically^{19,20}.

Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

Procedure: The monolayer cell culture was trypsinized and the cell count was adjusted to 3-lakh cells/ml using medium containing 10% fetal bovine serum.

Cells were seeded in a flat-bottomed 96-well plate and incubated for 24 hour at 37°C and in 5% CO₂. Vero cell line was treated with different plant extracts at various concentrations (1000µg/ml, 500µg/ml and 100µg/ml) for 48 hours. Isoniazid +Rifampicin were used as a Positive Standard and The DMSO treated cells served as control.

Cells were then treated with MTT reagent (0.5 mg/ml as final concentration, i.e. 20µl/well of stock) for 4 h at 37°C. All the media and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; thiazolyl blue) reagent was removed from the wells and DMSO (200 µl) was added to each well to dissolve the formazan crystals.

The optical density (OD) was recorded at 570 nm in a Microplate (ELISA) reader.²¹

Percentage of cell viability was determined as (Avg. OD of treated cells/Avg. OD of control cells) ×100.

RESULTS AND DISCUSSION

In-Vitro assay of selected ethanomedicinal plants were carried out for their confirmation of cytotoxic effect on vero cell line. Percentage of viable cell can be obtained by performing trypan blue dye exclusion technique.

The cytotoxicity activity is carried out by using MTT assay. Cell lines derived from NCCS, Pune were free from any kind of bacterial and fungal contamination.



Table 1: Percentage cell viability and characterization of cell line

Cell line	% viability	Live Cell count	Total cell count
Vero	81.13%	1.72×10^5	2.12×10^5

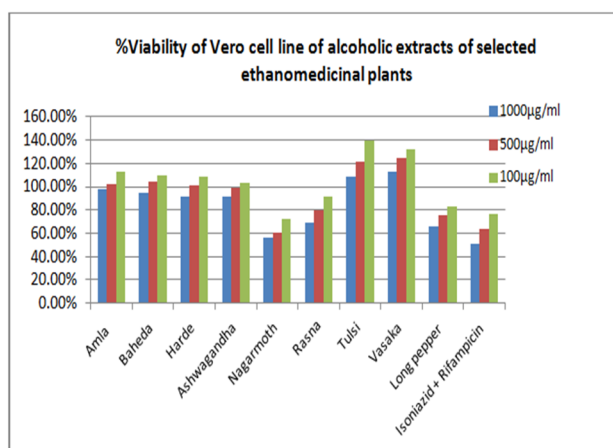
Percentage cell viability of cell line was carried out by using trypan blue dye exclusion technique. From the Table 1, it shows that the % viability of Vero cell line is 81.13%, which is most suitable to perform cytotoxicity study.

The cytotoxicity study was carried out for plant extracts. These extracts were screened for its cytotoxicity against Vero cell line at different concentrations to determine the IC_{50} (50% growth inhibition) by MTT assay.

Results of % Viability of vero cell line of alcoholic and aqueous extracts of selected ethanomedicinal plants are tabulated in Table 2 and 3 and graphically represented in Fig. 1 and 2. The percentage viability was found to be increasing with decreasing concentration of test compounds.

Table 2: % Viability of vero cell line of alcoholic extracts of selected ethanomedicinal plants

Name of plant extract	1000 μ g/ml	500 μ g/ml	100 μ g/ml
Amla	98.23%	102.56%	112.39%
Baheda	94.63%	104.87%	109.98%
Harde	91.25%	100.64%	108.31%
Ashwagandha	91.26%	98.99%	103.74%
Nagarmoth	56.32%	60.00%	72.31%
Rasna	69.45%	79.55%	91.36%
Tulsi	108.17%	121.28%	139.36%
Vasaka	112.38%	124.68%	132.38%
Long pepper	65.87%	75.00%	82.48%
Isoniazid + Rifampicin	50.63%	63.87%	76.25%

**Figure 1:** %Viability of Vero cell line of alcoholic extracts of selected ethanomedicinal plants**Table 3:** %Viability of vero cell line of aqueous extracts of selected ethanomedicinal plants

Name of plant extract	1000 μ g/ml	500 μ g/ml	100 μ g/ml
Amla	98.23%	100.65%	103.42%
Baheda	96.74%	101.32%	108.36%
Harde	86.00%	97.55%	104.96%
Ashwagandha	82.34%	94.83%	102.35%
Nagarmoth	61.36%	68.21%	76.00%
rasna	52.36%	70.41%	83.23%
Tulsi	101.86%	113.69%	136.85%
Vasaka	100.58%	112.39%	142.71%
Long pepper	59.19%	68.72%	76.31%
Isoniazid+Rifampicin	40.69%	56.37%	69.56%

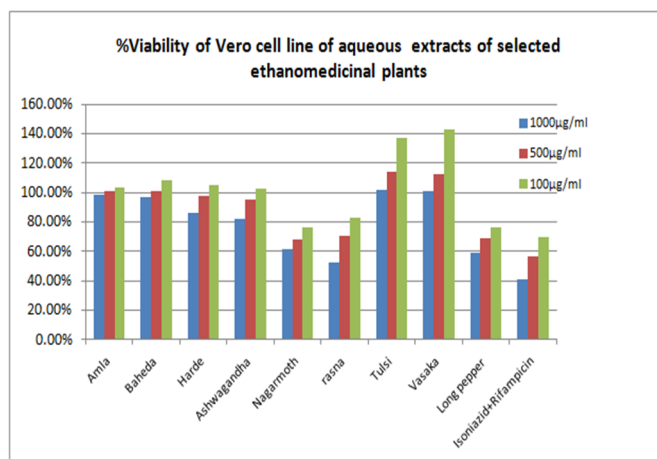


Figure 2: %Viability of Vero cell line of aqueous extracts of selected ethnomedicinal plants

CONCLUSION

The above study shows that selected plants do not have any significant cytotoxicity on the normal vero cell line. The study suggests that, the extracts might have increased the proliferation of the kidney cells, which can be further studied by cell proliferation assay. Thus, the toxicity of the plant has to be studied for further exploration of various biological activities.

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