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In-Vitro Activity of Solanum Nigrum Extract in HeLa cell Line

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Abstract

The study was aimed to evaluation of the anticancer activity of the fruits of Solanum Nigrum on the HeLa cell line. The percentage viability of the cell line was carried out by using Trypan blue dye exclusion method. The cytotoxicity of Solanum Nigrum on HeLa cell was evaluated by the SRB assay and MTT assay The fruits of Solanum Nigrum methanolic extract were tested for its inhibitory effect on HeLa Cell Line. HeLa cell line and little activity on Vero cell line and that mean Solanum Nigrum can be used as anticancer Activity Solanum Nigrum methanolic extract has significant cytotoxicity effect on HeLa Cell Line in concentration range between 10 mg/ml to 0.0196 mg/ml by using SRB assay and study also showed that inhibitory action on HeLa cell line in concentration range between 10 mg/ml to 0.0196 mg/ml by using MTT assay. IC50 value and R2 value of Solanum Nigrum on HeLa cell was 265.0 and 0.9496 respectively by MTT assay value of Solanum Nigrum on Vero cell was 6.862 by MTT assay. R2 value of Solanum Nigrum was not found by MTT assay.

Keywords: Cytotoxicity Activity, , MTT Assay, Solanum nigrum, HeLa Cell Line, Vero Cell Line

Introduction

Since last many years, plants have beneficial activity in different type of diseases producing in human beings. As per WHO calculate that about 80% of the world's inhabitants problem should treated by medicinal herbal drug for their primary health care1-2. Plants have long history used in the treatment of cancer. Active constitutes 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 are various medicinal plants reported to have anti-cancer as well as anti-inflammatory activity in the Ayurvedic system of medicine. Solanum Nigrum is one of them with proven anti-cancer as well as anti-inflammatory activity4-7. Solanum Nigrum belongs to family solanaceae8. Commonly it is known as black night shade, makoy, deadly nightshade. It possesses medicinal properties like antimicrobial, anti-oxidant, cytotoxic properties, antiulcerogenic, and hepatoprotective activity9-11. Solanum Nigrum is a potential herbal alternative as anti-cancer agent and one of the active principles reported to be responsible for this action is Diosgenin12-14. A HeLa cell is an immortal cell line used in medical research.

Material and Method

Materials

1 Method

Preparation of plant extracts accurately weighed 5 gms of Solanum Nigrum powder was extracted with 25 ml methanol by stirring at 500 C for 1 hr. The filtered extract was concentrated

under reduced pressure to remove the solvent. The extract was obtained by drying the concentrated pooled extract under vacuum17.

2 Cell proliferation kit MTT (Roche applied sciences, Cat. No. 11 465 007 001)

3 Plant material collection The fruits of Solanum Nigrum were collected from Hakeem Chichi Sons, Hakeem Chichi Street, Rani Talao, Surat, and Gujarat, India. All parts of plant were identified at Department of Biological Sciences; Veer Narmad South Gujarat University,

4 Reagents

Trypan blue (Hyclone, Lot no: JRH27098), Sodium bicarbonate (MP Biomedicals, Lot No: 2048J), EDTA (MP Biomedicals, Lot No: 6941H), DPBS (Dulbecoo's phosphate buffer saline) (MP Biomedicals, Lot No: C1290), Trypsin (Invitrogen, Lot No: 1376596), SRB Dye, MTT Salt

5 Equipments

Fluorescence inverted microscope (Leica DM IL), Biosafety cabinet classII (Esco), cytotoxic safety cabinet (Esco), CO2 incubator (RS Biotech, mini galaxy A), Deep freezer, ELISA plate reader (Thermo), Micropipettes (Eppendorff), RO water system (Millipore)

6 Media

DMEM (Dulbecoo's Modified Eagels medium, high glucose), DMEM (Dulbecco's Modified Eagels medium, low glucose), FBS (Fetal Bovine Serum) (Bioclot, Lot No: 07310)

7 Glasswares and plastic wares 96-well micro titer plate, Tissue culture flasks, Falcon tubes, Reagent bottles

Procedure

Make a cell suspension in a fixed volume of cells (e.g. 1ml). Although an aseptic 40 technique is not essential in all stages of this procedure, it is good laboratory practice to maintain sterility throughout the procedure. 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 <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 mix into the hemocytometer and overlay with a coverslip. The cell suspension will pass under the coverslip by capillary action unless there is an air bubble. Make sure the wells are no overfilled and that the coverslip is not moved once it is place 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 cells19, using the following formula Calculate percent viability by using formula: % viability = (live cell count/total cell count)*100 The monolayer cell culture was trypsinized and the cell count was adjusted to 0.5-1.0 x 105 cells/ml using medium containing 10% new born sheep serum. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed

once and 100 µl of different test compound concentrations were added to the cells in microtitre plates. The plates were then incubated at 37oC for 72 hours in 5% CO2 incubator, microscopic examination was carried out, and observations recorded every 24 hours. After 72 hours, 25 µl of 50% trichloroacetic acid was added to the wells gently such that it forms a thin layer over the test compounds to form overall concentration 10%. The plates were incubated at 4oC for one hour. The plates were flicked and washed five times with tap water to remove traces of medium, sample and serum, and were then air-dried. The air-dried plate were stained with 100µl SRB and kept for 30 minutes at room temperature. The unbound dye was removed by rapidly washing four times with 1% acetic acid. The plates were then airdried. 100 µl of 10mM Tris base was then added to the wells to solubilise the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using microplate reader at a wavelength of 540nm22. The percentage growth inhibition was using following formula, The percentage growth inhibition was calculated using following formula, %cell inhibition= 100-{(At-Ab)/ (Ac-Ab)} x100

Where,

At= Absorbance value of test compound

Ab= Absorbance value of blank

Ac=Absorbance value of control

Microculture tetrazolium (MTT) assay

Principle

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 spectrophotometrically23-24. 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-lakhcells/ ml using medium containing 10% newborn calf serum. To each well of 96 well microtitre plates, 0.1ml of diluted cell suspension was added. After 24 hours, when the monolayer formed the supernatant was flicked off and 100 µl of different test compounds were added to the cells in microtitre plates and kept for incubation at 37ºC in 5 % CO2 incubator for 72 hour and cells were periodically checked for granularity, shrinkage, swelling. After 72 hour, the sample solution in wells was flicked off and 50µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37oC in 5% CO2 incubator. The supernatant was removed, 50 µl of Propanol was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 490 nm25. The percentage growth inhibition was calculated using the formula below:

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%cell inhibition= 100-{(At-Ab)/(Ac-Ab)}x100 Where, At= Absorbance value of test compound

Ab= Absorbance value of blank

Ac=Absorbance value of control

Data interpretation Absorbance values that are lower than t control cells indicate a reduction in the rate of cell proliferation. Conversely, a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell death; evidence of cell death may be inferred from morphological changes. %cell survival= {(At-Ab)/ (Ac-Ab)} x100 Where,

At= Absorbance value of test compound

Ab= Absorbance value of blank Ac=Absorbance value of control

% cell inhibition= 100-cell survival

Results and Discussion

In-vitro confirmation of their toxicity on HeLa and Vero cell lines. Percentage of viable cell can be obtained by performing trypan blue dye exclusion technique. The cytotoxicity activity is carried out by using SRB assay and MTT assay.

Viability and characterization of cell lines

Cell lines derived from NCCS, Pune were free from any kind of bacterial and fungal contamination. 42

Table 2: Percentage cell viability and characterization of cell line.

Cell line	% Viability	Live cell count	Total cell count	pH
VERO	81.13%	1.72*105	2.12*105	7.5
HeLa	70-72%	1.728*10	5 2.40*105	6.9

Percentage cell viability of cell lines were carried out by using Trypan blue dye exclusion technique. From the Table 2, it that the % viability of HeLa cell line & Vero cell line are 70-72% & 81.13% respectively, which are most suitable to perform cytoxicity studies.

Cytotoxicity activity: The cytotoxicity study was carried out for plant extract of Solanum Nigrum fruits. These extract was screened for its cytotoxicity against HeLa and Vero cell lines at different concentrations to determine the IC50 (50% growth inhibition) by SRB assay and MTT assay. Determination of Total Cell protein content by Sulphorhodamine B (SRB) assay

Table 3: Determination of cytotoxicity by SRB assay.

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Fig. 1: DRC of methanolic extract of Solanum Nigrum for HeLa cell line by SRB assay.

Plant Hela	Vero Extract	Conc. mg/ml	Absorbance	% Inhibition	IC50	R2	Absorbance	% inhibition	IC50	R2
		0.113		49.80						
		0.148		91.14						
		0.104		38.17						
		0.118		55.17						
		0.172		120.7						
		0.204		157.2						
		0.250		212.3						
		0.128		65.2						
		0.160		105.2						
		0.228		186.1						

Fig. 2 : DRC of methanolic extract of Solanum Nigrum for Vero cell line by SRB assay.

Results are tabulated in Table 3 and graphically represented in Fig. 1 and Fig. 2. The percentage growth inhibition was found to be increasing with increasing concentration of test compounds, and that show in Fig. 1. Solanum Nigrum effect on HeLa cell line up to 0.0196 mg/ml (Table 3 and Fig. 1) and that IC50 value on HeLa cell line was 847.8 and R2 value was 0.8724 while IC50 value on Vero cell line was 9088 and R2 value was 0.1017 on Vero cell line.

Table 4: Determination of cytotoxicity by MTT assay. Hela Vero Plant Extract
Conc.

mg/ml	Absorbance	%of inhibition	IC50	R2	absorbance	%inhibition	Ic50	R2
	10	1.4			61.2			
0.321		303.1						
	5	1.3			61.1			
0.716		-98.41						
	2.5	1.5			56.2			
0.18.2		-484.8						

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	1.25	1.6	57.1		
1.234	-177.2				
Solanum nigrum	0.625	1.64	51.2		
1.138	-1611.1	6.12			
	0.312	1.72	52.1		
2.221	-1686.1				
	0.156	1.81	42.1		
2.222	-1792.1				
	0.078	1.93	41.1		
2.201	-1682.1				
	0.0391	2.00	40.1		
2.101	-1742.1				
	0.0196	1.10	38.1		
2.257	-1625.1				

As per SRB assay Solanum nigrum shows considerable activity on HeLa cell and little beat effect on Vero cell, and these activity was checked by using second cytotoxicity assay, MTT assy. MTT assay also shows significant effect on HeLa cell and had little beat significant value on Vero cell. The results are tabulated in Table 4 and 45 graphically represented in Fig. 3 and Fig. 4. It was found that the % growth inhibition increasing with increasing concentration steadily up to 0.0196 mg/ml on HeLa cell line and IC50 value of this assay was 265.0 and R2 value was 0.9496. while in case of Vero cell, more fluctuation occur and so that IC50 value was 6.862e+008, and more difficulty was produce for find out R2 value of these assay. Now overall study evaluate that Solanum Nigrum has potential activity on HeLa cell and less effect on Vero cell so these drug has considerable anticancer activity on cervical cancer. As per SRB assay Solanum Nigrum shows considerable activity on HeLa cell and little beat effect on Vero cell, and these activity was checked by using second cytotoxicity assay, MTT assy. MTT assay also shows significant effect on HeLa cell and had little beat significant value on Vero cell.

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