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Research Article

In Vitro Cytotoxicity of Methanolic Extract of *Solanum nigrum* Using MCF-7 Cell Line

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ABSTRACT

The discovery of the increased complexity of cancer has led us to consider it as a group of diverse diseases more than an etiopathology. Cancer can be treated by surgery, chemotherapy, radiation therapy, immunotherapy, monoclonal antibody therapy or other methods. *Solanum nigrum* strongly promote perspiration. Numerous types of bioactive compounds have been isolated from plant sources. *Solanum nigrum* can be poisonous and the juice or an ointment prepared from the *solanum nigrum* can be used for skin problems and tumors. Methanolic extract of the *solanum nigrum* was studied for its anticancer activity in the form of anti tumor activity against MCF-7 cell line and VERO Cell line (African green monkey kidney cell line). From this cytotoxicity screening data by MTT and SRB assay, IC50 value methanolic extract of *solanum nigrum* 148.70 and 279.00, so it demonstrate that Methanolic extract of *Solanum Nigrum* was potentially cytotoxic effective against MCF-7 cells.

Key Words: Antitumor, *Solanum nigrum*, Cytotoxicity

INTRODUCTION

Cancer is one of the leading causes of death in the prosperous countries of the world, where one person in five die because of this disease. The discovery of the increased complexity of cancer has led us to consider it as a group of relatively heterogeneous of diseases more than distinctive etiopathology. Cancer may affect people at all ages, even fetuses, but risk for the more common varieties tends to increase with age. Cancer causes about 13% of all deaths. Nearly all cancers are caused by abnormalities in the genetic material of the transformed cells. These abnormalities may be due to the effects of carcinogens, such as tobacco smoke, radiation, chemicals, or infectious agents.

Cancer can be treated by surgery, chemotherapy, radiation therapy, immunotherapy, monoclonal antibody therapy or other methods. Numerous types of bioactive compounds have been isolated from plant sources. Natural origin is defined as natural products, derivatives of natural products or synthetic pharmaceuticals based on natural product [1]. *Solanum Nigrum* has also been used as an agricultural insecticide, chronic hepatitis, Infective hepatitis [2]. Evidence for the utility of *invitro* cytotoxicity tests has led many pharmaceutical companies to screen compound libraries to remove potentially toxic compounds early in the drug discovery process [3, 4].

The MCF-7 breast cancer cell line produced from a Caucasian woman who already underwent two mastectomies during five years. Vero cells are lineages of cells used in cell cultures [5]. The Vero lineage was isolated from kidney epithelial cells extracted from African green monkey [6].

MATERIAL AND METHODS

The fruits of *Solanum nigrum* were collected from Hakeem Chichi Sons, Hakeem Chichi Street, Rani Talao, Surat. *Solanum nigrum* was authentified at Department of Biological Sciences; Veer Narmad South Gujarat University, Surat by Dr. Minoobhai Parabia.

Plant material extraction

The fruits of *Solanum Nigrum* were powdered and exhaustively 100 gm of powder extracted by percolation using methanol with continuously flushed with fresh solvent. The extraction is continued until sufficient compound is extracted. If necessary, the same material can be re-extracted with a second solvent. Extract was concentrated and evaporated to air dryness [7].

Anticancer Activity by in vitro Methods

MCF-7 and VERO cell lines were obtained from NCCS, Pune. The cell line was maintained in monolayer cultures in supplemented Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated fetal bovine serum (FBS), glutamine, streptomycin and incubated at 37°C in humidified 5% CO_2/ 95% air. Cell viability had been maintained with in complete media with trypan blue assay method and inhibitory effect of the extract was identified by MTT and SRB assay. A cytotoxicity assay has been recently introduced that is sensitive and that offers increased efficiency in terms of sample handling and quantification [8].

Identification of cytotoxicity by 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, coloured formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

Cells were grown in micro titer plates in culture medium per well. Cells were incubated for 24h at 37° C & 5% CO_2. Different Concentrations of test samples were added into micro plates (96 wells, flat bottom). After incubation period MTT labeling mixture were added. Incubate micro titer plate for 4 to 24 hrs in incubator Read plate on a Microplate reader using a wavelength between 450 nm [9].

Identification of total cell protein content by sulphorhodamine B (SRB) assay

SRB is a bright pink aminoxanthine dye with two sulfonic groups. Under mild acidic conditions, SRB binds to basic amino acid residues in TCA(Trichloro acetic acid) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of visible at least two order of magnitude. The developed colour can be measured over a broad range of visible wavelength in either a spectrophotometer or a 96 well plate reader.

The monolayer cell culture was trypsinized different test concentrations were used to the cells in microtitre plates and incubated at 37° C for 72 hours in 5% CO_2 incubator and microscopic examination was carried out and observations recorded. 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 plates were stained with 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 air-dried. Tris base was then added to the wells to solubilize the dye with continuous shaking. The absorbance was measured using micro plate reader at a wavelength of 540nm [10, 11].

The percentage growth inhibition was calculated using Formula:

$$\% \text{ Cell inhibition} = 100 - \left\{\frac{(A_t - A_b)}{(A_c - A_b)}\right\} \times 100$$

Where, A_t= Absorbance value of test compound
A_b= Absorbance value of blank
A_c=Absorbance value of control

RESULT & DISCUSSION

Solanum Nigrum can be used for treatment of skin problems, tumors, as an agricultural insecticide, chronic hepatitis, Infective hepatitis. The methanolic extract of *Solanum nigrum* was prepared by percolation method to identify cytotoxicity activity against MCF-7 cell line by identifying the 50% inhibition concentration. The MCF-7 breast cancer cell line produced from a Caucasian woman who already underwent two mastectomies during five years.

Table 1 Identification of cytotoxicity by MTT assay method

Plant Extract	MCF-7	IC50 (µg/ml)	Vero	IC50 (µg/ml)
Solanum nigrum		148.7		4.473

Characterization of cell line

MCF-7 cell line is continuous linage of the cancer cells multiplied by cell divisions. Cell viability had been maintained 80.10% for MCF-7 cell line and 82.50% for VERO cell line in complete media with trypan blue assay method and inhibitory effect of the extract was identified by MTT and SRB assay.

Identification of Cytotoxicity by MTT assay

The methanolic extract of *Solanum Nigrum* were shown cytotoxic effect against MCF-7 cancer cell line and its IC_{50} values obtained 148.7 µg/ml. Against Vero cell line, IC_{50} of methanolic extract of *Solanum nigrum* was 4.473 µg/ml **(Table 1).** IC_{50} of methanolic extract of *solanum nigrum* shown higher cytotoxicity on MCF-7 cell line and good proportionality for % cell inhibition Vs log [dose] **(Figure 1).**

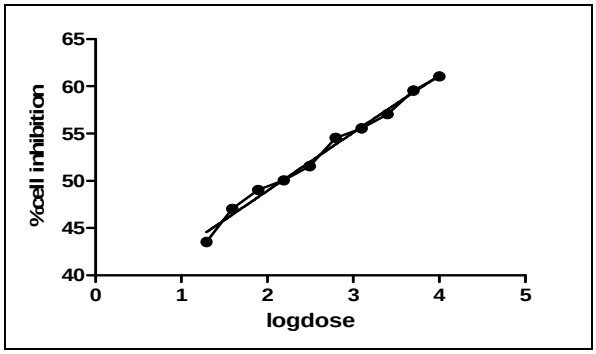

Figure 1 Graphical Representation of DRC of *Solanum Nigrum* on MCF-7 cell by MTT assay

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

The percentage cell growth inhibition was found to be increasing with increasing concentration of test compounds. The methanolic extract of *solanum nigrum* was found potent cytotoxic effect against MCF-7 cancer cell line and IC_{50} value was 279.0 µg/ml. From dose response curve (DRC), IC_{50} of methanolic extract of *solanum nigrum* was 2.651 µg/ml against VERO cell line **(Table 2)**. These data suggests that methanolic extract of *solanum nigrum* was shown good cytotoxicity on MCF-7 cell line **(Figure 2)**. As shown in study, *solanum nigrum* are significantly affecting the growth of MCF-7 cells.

Table 2 Identification of cytotoxicity by SRB assay method

Plant Extract	MCF-7	IC50	Vero	IC50
Solanum nigrum		279.0		2.651

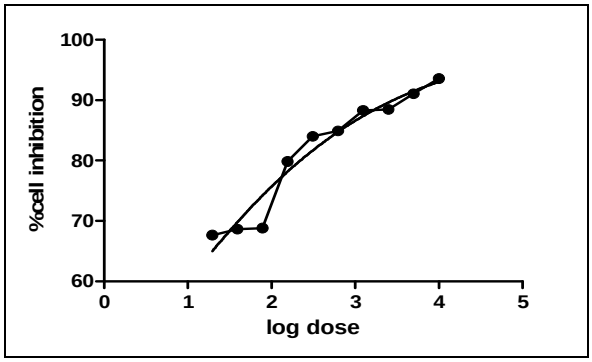

Figure 2 Graphical Representation of DRC of *Solanum nigrum* on MCF-7 cell by SRB assay

CONCLUSION

From the above study, it concluded that methanolic extract of *Solanum nigrum* showed potent cytotoxic activity against MCF-7 cell line. Graphically representation of dose response curve gives the idea about the % inhibition concentration 50(% IC50) in dose dependent manner. From cytotoxicity screening data by MTT and SRB assay, Methanolic extract of *Solanum nigrum* was potentially cytotoxic effective may be due to impede the cell division in MCF-7 cells by causing the apoptosis.

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