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In-Vitro Cytotoxicity Study of Some Indigenous Medicinal Plants on Vero Cell Line

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ABSTRACT

The ethno botany and ubiquitous plants as a source of medicine has been an important component of the health care system providing a rich resource for natural drug research and development. Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads, because of unmatched availability of chemical diversity. In the present study, six commonly used plants, Amla, Baheda, Harde, Ashwagandha, Turmeric and Kali Musli are selected. The *in-vitro* cytotoxicity study has been done using MTT assay on the Vero cell line using cisplatin as a standard drug for toxicity. The hydroalcoholic extracts of the above drugs have been tested for their cell viability at different concentrations, ranging from 100 µg/ml to1000 µg/ml. From the performed assay, almost all the plants did not show any significant cytotoxic effect on the Vero cells, promoting their use as potential nephro-protective agents. The possible mechanism of action can further be validated by identifying the molecules from the plant extracts and subjecting them to various studies like docking studies, and their binding affinity with the targets.

1. Introduction

Plants are an important component of the health care system in India. They have been used as a source of medicine since ancient times. The sound ethnobotanical knowledge of plants provides a rich resource for natural drug research and development. There is an unmatched availability of chemical diversity of natural products from medicinal plants and hence they are either used as pure compounds or as standardized extracts providing unlimited opportunities for new drug leads**[1]**. More than 80% of the world's population relies on traditional system of medicine to meet their primary healthcare needs as per World Health Organization. Plants used in the traditional medicines contain a large number of substances that can be used to treat chronic, as well as infectious diseases**[2]**. The main drawback of the chemically synthesized drugs is development of adverse effects and microbial resistance and hence, recent medicinal researches are focusing towards the use of phyto-chemicals from plants as safe alternatives with lesser adverse effects**[3]**.

The major cause of morbidity and mortality, especially in the developed countries is cancer. Chemotherapy is one of the potential treatments for prolonging the patient's life. Almost 60% of anticancer drugs are of natural origin, such as plants (*i.e.*, vincristine, irinotecan, camptothecines) and microorganisms (*i.e.*, doxorubicin, dactinomicines, mitomycin and bleomycin)**[4]**. But the major side effect of these anticancer drugs is that they are also cytotoxic to normal cells of the body. In the present study we have aimed to study the cytotoxic effect of the six commonly used indigenous medicinal plants (Table 1) on the Vero cell line, which are also reported to have its use in cancer namely Kali musli, Ashwagandha, Amla, Baheda, Harde and Turmeric.

2. Materials and Methods

Procurement of plant material and extraction procedure

The plant materials were collected from the fields of Gandhinagar district, Gujarat and from the local supplier of herbal drugs, Lallu Vrajlal Gandhi, Ahmedabad. The voucher specimen was submitted to Department of Pharmacognosy, KBIPER, Gandhinagar. The 70% alcoholic extract was prepared by heating for 1hour and occasional shaking over a water bath. Then it was filtered and concentrated. The concentrate was evaporated to dryness and stored in an airtight container for further use.

Cell lines and maintenance

Vero (African Green Monkey-kidney) cell line was procured from National Centre for Cell Science (Pune, India). Vero was maintained in Minimum essential medium (MEM) (Eagle) with Non-essential amino acids, with 10% fetal bovine serum in a humidified atmosphere at 37 ºC with 5% CO_2. The cell line was maintained in their growing phase at 70% confluency with regular passaging.

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Table No. 1: Information about the selected Indian Medicinal Plants[5, 6-10]

Name of the plant	Family	Common name	Parts Used	Uses
Curculigo orchioides	*Hypoxidaceae*	Kali Musli	Rhizomes	Diuretic, anti-cancer, aphrodisiac
Curcuma longa	*Zingiberaceae*	Turmeric	Rhizomes	Anti-inflammatory, antioxidant, anti-cancer
Emblica officinalis	*Euphorbiaceae*	Amla	Fruits	Antioxidant, anti-carcinogenic, anti-inflammatory
Teriminalia bellerica	*Combretaceae*	Baheda	Fruits	Blood purifier, Anti-cancer, Throat diseases
Teriminalia chebula	*Combretaceae*	Harde	Fruits	Fever, cough,astringent, Anti-cancer
Withania somnifera	*Solanaceae*	Ashvagandha	Leaves	Anti-inflammatory, anti-tumour, anti-stress,anti-oxidant

Cytotoxicity assessment: MTT assay[11,12]

The prepared Extracts were tested for its cytotoxicity by MTT-assay. Vero cells were seeded in their respective culture medium (200 µl, 1 x 10^4cells/well) in a 96-well plate and incubated at 37 ºC for 24 h with 5% CO_2 supply. After incubation, the control wells were replenished with fresh medium and the test wells were treated with 100, 500 and 1000 µg/ml of extracts. The cells were further incubated for 48 h maintaining the same conditions. After the treatment incubation period, medium in each well was replenished with 200µl of fresh medium plus 20µl of MTT (0.5 mg/ml). The plate was then incubated for 4 h in the same conditions after which the absorbance was measured at 570 nm using ELISA reader.

Percentage cell viability was calculated by the following formula:

Avg. OD of treated cells/Avg. OD of control cells×100.
(OD = Optical density)

3. Results

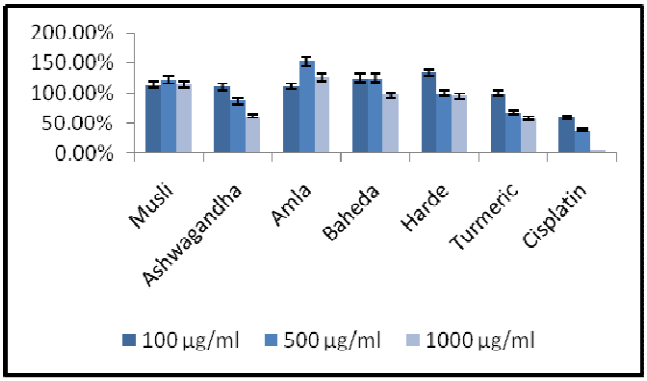

Figure No. 1: MTT Assay: Percentage Cell Viability of the Vero cells (70% Alcoholic Extracts)

4. Discussion

The focus on the pharmacological effects of bioactive compounds on cancer treatments and prevention has increased recently. It has been shown to possess numerous anti-cancer activities in various cancer cells through different forms of cytotoxic effects without exhibiting considerable damage to normal cells. The prepared extracts were studied for their *in-vitro* cytotoxic activity using MTT assay. The MTT Assay is a sensitive, quantitative and reliable colorimetric assay that measures viability, proliferation and activation of cells. The assay is based on the capacity of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble substrate 3-(4,5- dimethylthiazol-2-y1)-2,5-diphenyl tetrazolium bromide (MTT) into a dark blue formazan product that is insoluble in water. Viable cells are able to reduce the yellow MTT under tetrazolium ring cleavage to a water-insoluble purple-blue formation which precipitates in the cellular cytosol and can be dissolved after cell lysis, whereas cells being dead following a toxic damage, cannot transform MTT. This formation production is proportionate to the viable cell number and inversely proportional to the degree of cytotoxicity. The reaction is mediated by dehydrogenases enzymes associated with the endoplasmatic reticulum and the mitochondria[11, 12]. The positive standard used is cisplatin, because Vero cells are normal kidney cells and cisplatin is known for its nephro-toxic potential. The present data suggests that amongst all the plant extracts only Turmeric shows moderate cytotoxicity on the Vero cells and all the other cells did not show cytotoxicity on the Vero cells. At the dose of 100µg/ml the cells show the percentage viability above 100 percent, which suggests that the extracts might have increased the cell proliferation, which can be further studied by cell proliferation assays. In order to validate, the possible mechanism of action, the plant extracts can be subjected to various cell based studies.

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