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Research Article

EVALUATION OF ANTICANCER ACTIVITY OF *VETIVERIA ZIZANIOIDES* AGAINST HUMAN BREAST CANCER CELL LINE

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ABSTRACT

Objective: In the present study, the aqueous extract of *Vetiveria zizanioides* root was tested for cytotoxic effect against human cancer cell line (MCF-7 human breast cancer cell line)

Methods: The aqueous extract of the root of*Vetiveria zizanioides*were evaluated through MTT (3-(4,5-dimethyl thiazolyl-2 - yl)-2, 5-diphenyl tetrazolium bromide) and assessment of cell morphology by usingAcridine Orange & Ethidium Bromide staining.

Results:The aqueous extract of root exhibited cytotoxicity towards the cancer cell line. IC_{50} concentration of 5µg/ml to 50µg/ml against human breast adenocarcinoma MCF-7 cell line was recorded. The maximum inhibition of concentration showed in 31µg/ml to 37µg/ml.

Conclusion: The results indicated us the feasible anticancer nature of root aqueous crude extract.

Keywords: Cytotoxic, MCF-7 cell line, MTT assay, *Vetiveria zizanioides*

INTRODUCTION

Medicinal plants have been used as remedies for human diseases for centuries. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value. Plant materials have served as medicines across cultures and throughout time. Knowledge about plants that were found to be most effective against particular ailments was passed down to the succeeding generations. Medicinal plants can be a promising source of novel chemotherapeutic agents including cancer. Cancer is the most common and fatal disease responsible for 2-3% of deaths recorded worldwide annually. While in women breast cancer is most wide spread **[7]** and its incidence in Pakistan is reported highest among South Central Asian countries. About 60% anticancer drugs used nowadays are obtained from natural resources **[10]**.

Vetiveria zizanioides (Nash), belonging to the family Poaceae. Commonly known as Khash-Khas, Khas or khus grass in India. Historically Vetiver grass was well known in tropical countries for its aromatic and medicinal properties **[9]**. It is a perennial grass with thick fibrous adventitious roots which are aromatic and highly valued. India is inhabited by a wide variety of tribal populations who dwell in forested areas and depend on surrounding resources for their livelihood **[12]**. Among the several hundreds of plants are gathered by tribal populations, khas grass, particularly in North Indian plains, takes a leading role **[6]**. Various tribes are the different parts of the grass for many of their ailments such as mouth ulcer, fever, boil, epilepsy, burn, snakebite, scorpion sting, rheumatism, headache etc.**[2,11]**. Present investigation was aimed to assess the cytotoxic potential of aqueous crude extract of *Vetiveria zizanioides* root against MCF-7 human breast cancer cell.

MATERIALS AND METHODS

Plant collection

Vetiveria zizanioides (Nash) roots were collected in Nehru Herbal Gardens from Nehru Arts and Science College, Coimbatore, Tamil Nadu, India. The identification was confirmed with Botanical Survey of India, Coimbatore, Tamil Nadu, India (Ref. No: BSI/SRC/5/23/2011-12/Tech-1673). The roots were collected; shade dried for a week and powdered using pulveriser. A fine powder obtained was stored in air tight polythene bags kept at room temperature and used for preparation of extract.

Preparation of extract

An aqueous extraction has done with 25g of the powdered root material were taken in a Soxhlet apparatus and kept on round bottom flask containing 250ml of water. The extraction was done for 16 h and finally the extract was kept in the water bath at 100ºC to remove the water content from the extract. The final greasy crude extract was collected, air dried and stored at room temperature until use.

In vitro cytotoxic assay

Following were the chemicals used in the present study, (3-(4,5-dimethyl thiazolyl-2 - yl)-2, 5-diphenyl tetrazolium bromide (MTT), Fetal Bovine Serum (FBS), Phosphate Buffered Saline (PBS), RPMI 1640 medium, and antibiotics from Sigma Aldrich and Himedia, Mumbai.

Cell Culture

Human breast adenocarcinoma MCF-7 cell were procured from National Centre for Cell Science at Pune was maintained in RPMI-1640 supplemented with 10% FBS, antibiotic 2% (Penicillin or Streptomycin) in a humidified atmosphere of 5% CO_2 at 37ºC until confluent. The stock cultures were grow in 25cm^2 culture flask and the experiments were carried out in 96 micro titer plate.

Dilution of extract

The crude extract was separately dissolved in DMSO (Dimethylsulfoxide) at different concentrations from 5µg/ml to 50µg/ml under sterile conditions.

MTT Cytotoxic Assay

Standard MTT assay was used to evaluate cell line viability in the presence of extracts **[5]**. In 96 well plate, 100µl medium (RPMI 1640) was poured in each well and selected with 5000-10,000 human breast adenocarcinoma MCF-7 cells/well. Cells were allowed to attach overnight and then various concentration of the crude extract were added to respective wells. After 24 h incubation at 37ºC, 5% CO_2 and relative humidity 20µl of MTT (5mg/ml) was added to each cell. After further 4 h incubation at 37ºC, 100µl of DMSO solutions was added to each well to solubilize MTT crystals. The plates were again incubated overnight at conditions mentioned above. The plates were read for optical density at 570nm as test wave length and 630nm as the reference using a plate reader. Percentage inhibition was calculated by the following formula.

$$\text{Percentage of cytotoxicity} = \frac{\text{Control} - \text{Test Sample}}{\text{Control}} \times 100$$

Assessment of cell morphology

Materials

Acridine Orange & Ethidium Bromide staining one part of 100µg/ml acridine orange in PBS and one part of 100µg/ml ethidium bromide in PBS.

Working solution

5 µl of the stock solution was taken and the volume was made up to 500µl using distilled water.

Method

Cells were grown in 6 well plates (5x10^{3}cells/well) for 24 hr. The cells were then incubated with the IC50 dose of drug for 24 and 48 hr. The medium was discarded and the cells were washed with PBS. The cells were then trypsinised and placed on a glass slide and stained with acridine orange & ethidium bromide, or Hoechst stain. The cells were then viewed in an epifluorescent microscope (Olympus, Japan)

RESULTS

Effect of extract on the proliferation of MCF-7 adenocarcinoma cell line

To determine the cytotoxicity of extract to human breast adenocarcinoma MCF-7 cells, it was treated with increasing concentrations of plant extract and viable cells detected with MTT assay. The results depicted in Figure 1 summarize the cytotoxic effects of the extract on MCF-7 breast cancer cell lines. The extract showed cytotoxic activity on the breast adenocarcinoma (MCF-7) cell line in a concentration-dependent manner. The extract on MCF-7 cell line produced a 50% of net killing (IC_{50}) at the doses 37µg and 31µg/ml at 24 and 48 hrs respectively.

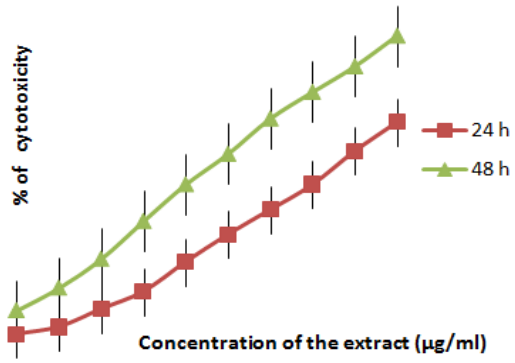
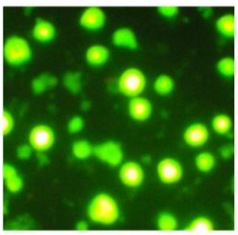
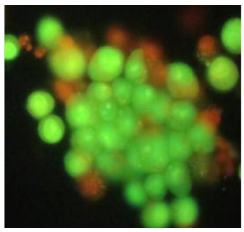


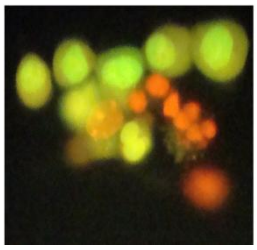
Fig. 1: Survival of MCF-7 cells after exposure to various concentrations of extract for 24 and 48 h.



Control cell showing normal morphology (green fluorescence)



Cells treated with extract for 24h showing, cell membrane blebbing and vacuolation (green and red fluorescence)



Cells treated with extract for 48h showing, nuclear fragmentation and chromatin condensation (red fluorescence).

Fig. 2: Acridine orange / Ethidium bromide staining

DISCUSSION

The search for anticancer agents from natural sources has been successful worldwide. Active constituents that have been isolated nowadays are used to treat human diseases. The Ethnopharmacological knowledge is helpful to lead the search for plants with potential cytotoxic activity **[4]**. In the present study the results reveal that the maximum inhibiton of concentration (IC_{50}) was in between 31µg/ml-37µg/ml. Similar results have been estabilished by **[13]** that dose dependent activity was found in crude extract of *Morinda lucida*. Some of the findings revealed the facts by **(1, 3, 8, 9)**. Crude extract of *Aerulus indica* proved active showing inhibition ranging from 34.2% at 10µg/ml to 94% at 500µg/ml. The extract showed activity in a dose dependent manner from lowest to highest concentration**[13]**. The plant species of *Vetiveria* roots have been reported to be utilised as remedies against fever, mouth ulcer, epilepsy, snake bite, scorpion sting, rheumatism, headache, etc. No such studies on crude root extract of *Vetiveria zizanioides* have been reported earlier so the present study explore that the *Vetiveria* crude root extract has growth inhibitory and cytotoxic effects on human breast adenocarcinoma (MCF-7) cell. This preliminary results could be helpul to find out the major components present in roots of *Vetiveria zizanioides* against the cancer activities.

CONCLUSION

Aqueous crude root extract of *Vetiveria zizanioides* can be suitable for isolation of active components against MCF-7 cell line. This extract should be tried on different cancer cell lines in order to measure the potential difference in cytotoxicity which could make their findings even more valuable.

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