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Cytotoxicity of *Amaranthus viridis* and *Vetiveria zizanioides* against human embryonic kidney cells

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ABSTRACT

Extracts from plants with minimal to no toxicity to the host are vital for the successful formulation development of a pharmaceutical drug. This study is an effort to determine the cytotoxic effect of two medicinal plants, *Amaranthus viridis* and *Vetiveria zizanioides* against human embryonic kidney cells. Cytotoxic effect of methanolic extracts of *Amaranthus viridis* and *Vetiveria zizanioides* on HEK 239 cell lines were determined by MTT assay. The results of this study revealed that both the plant samples tested were non-cytotoxic to the kidney cells, thereby justifies their usage in traditional medicines to treat urinary infections.

Keywords: *Amaranthus viridis*, *Vetiveria zizanioides*, Cell Line, HEK 239, MTT assay, Cytotoxicity.

INTRODUCTION

Medicinal plants continue to play a major role in preserving human health with new therapies. Although medicinal plants are abundant with structurally diverse valuable chemical compounds having different therapeutic effects on biological system, certain compounds might be lethal causing certain allergic reactions, destruction of red blood cells, irritation to the gastrointestinal tract, carcinogenicity and damage to organs like liver, heart and kidney in humans. Hence it is necessary to investigate these medicinal plants for their efficacy, quality, safety and toxicity [1,2].

An initial step to determine the potential toxicity of a test substance is the cytotoxicity studies. For the successful formulation development of a pharmaceutical drug, it is very essential that the plant extracts or plant derived compounds should exhibit least or no toxic effect to the host. The safety of a potential therapeutic agent against host cells must be ascertained by evaluating the cytotoxicity level of the plant from which the agent is developed. One of the most commonly used cytotoxicity assays is the MTT assay [1,3,4].

The MTT assay is a simple, rapid test used to assess cellular metabolic activity that measures cell viability, proliferation and cytotoxicity. The working principle of MTT assay is that the reduction of the water-soluble yellow tetrazolium salt (MTT) by NAD(P)H-dependent oxidoreductase enzymes of metabolically active cells into water insoluble purple formazan crystals and then the concentration of formazan crystals solubilized by DMSO is quantified using spectrophotometer [4,5].

In this study, the cytotoxic effect of methanolic extracts of two medicinal plants viz. *Amaranthus viridis* and *Vetiveria zizanioides* were determined against human embryonic kidney (HEK) cells.

MATERIAL AND METHODS

Chemicals and reagents

Culture media- Dulbecco's Modified Eagles Medium (DMEM- HiMedia), Fetal bovine serum (FBS), antibiotics (Penicillin (100 U/ml), Streptomycin (100 µg/ml) and Amphotericin B (2.5 µg/ml)), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) dye, phosphate buffer saline and Dimethyl sulfoxide (DMSO).

Collection and Preparation of Plant Extracts

Fresh, healthy leaves of *Amaranthus viridis* and roots of *Vetiveria zizanioides* were identified and collected with the help of traditional drug sellers of Kaliyakkavilai, Kanya Kumari district, Tamil Nadu. The collected plant parts were washed thoroughly using running water and distilled water, then shade dried at 28±2ºC. The dried plant parts were ground to fine powder and stored in labeled airtight

containers. About 100 ml of methanol was added to 10 gms of the finely powdered plant parts taken in labeled conical flasks separately. The flasks were covered using aluminium foils and stored under dark condition at room temperature. The contents were filtered using Whatman No.1 filter paper, after 3 days. After evaporation, the filtrates were collected and used for further study [6,7].

Test Sample Used

The methanolic extract of leaves of *Amaranthus viridis* and roots of *Vetiveria zizanioides*.

Cell Lines

HEK 239 cell line procured from the National Centre for Cell Sciences (NCCS), Pune, India.

Cytotoxicity Assay

The test samples were subjected to MTT assay for evaluating cytotoxic effect with slight modifications [5,8]. HEK cells (2500 cells/well) seeded on 96 well microtiter plates were allowed to acclimatize to the culture conditions (37°C and 5% CO_2 environment in the incubator for 24 hrs). The test samples prepared and diluted with DMEM media (100 mg/ml) were added to the wells with cultured cells of various concentrations (6.25, 12.5, 25, 50 and 100 µg/ml) and the plates were further incubated for 24 hrs. Each of the concentrations was tested thrice and mean values were calculated keeping untreated wells as control. The media was aspirated after incubation, then 100 µl of 0.5 mg/ml MTT solution in PBS was added to the wells and kept for incubation for 2 hrs. After removing the supernatant, 100 µl DMSO (100%) were added to each of the wells and the microtiter plates were read for cell viability at 570 nm using a microplate reader by the formula:

$$\text{Cell viability (\%)} = \frac{\text{Average absorbance of treated}}{\text{Average absorbance of control}} \times 100$$

RESULTS

The methanolic extracts of the leaves of *Amaranthus viridis* and the roots of *Vetiveria zizanioides* were evaluated for its cytotoxic effect on human embryonic kidney (HEK 239) cells by MTT assay and the observations were recorded in table 1 and table 2.

Table 1: Cytotoxic effect of *Amaranthus viridis* on HEK Cells

Concentration µg/ml	Triplicate 1	Triplicate 2	Triplicate 3	Mean	Percentage of Viability
Control	0.768	0.779	0.758	0.768	-
6.25	0.764	0.772	0.753	0.763	99.30
12.5	0.752	0.748	0.744	0.748	97.35
25	0.741	0.732	0.724	0.732	95.31
50	0.699	0.692	0.679	0.69	89.80
100	0.656	0.647	0.642	0.648	84.38

The results of cytotoxic effect of methanolic extract of leaves of *Amaranthus viridis* at varying concentrations ranging from 6.25-100 µg/ml against human embryonic kidney cells was shown in figure 1.

The methanolic leaf extract of *Amaranthus viridis* cause no significant reduction in cell viability indicating that it is non-cytotoxic to the kidney cells.

The cytotoxic effect of methanolic extract of roots of *Vetiveria zizanioides* at varying concentrations ranging from 6.25-100 µg/ml against human embryonic kidney cells was shown in figure 2.

The methanolic root extracts of *Vetiveria zizanioides* cause no significant reduction in cell viability indicating that it is non-cytotoxic to the kidney cells.

Table 2: Cytotoxic effect of Vetiveria zizanioides on HEK Cell

Concentration µg/ml	Triplicate 1	Triplicate 2	Triplicate 3	Mean	Percentage of Viability
Control	0.768	0.779	0.758	0.768	-
6.25	0.751	0.766	0.747	0.754	98.22
12.5	0.738	0.741	0.733	0.737	95.96
25	0.722	0.727	0.711	0.72	93.70
50	0.686	0.681	0.672	0.679	88.45
100	0.647	0.638	0.633	0.639	83.21

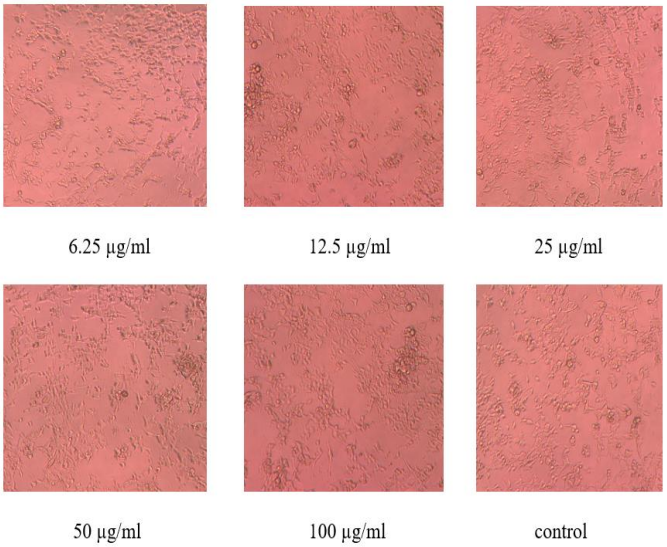

Figure 1: Cytotoxic effect of *Amaranthus viridis* on HEK cells

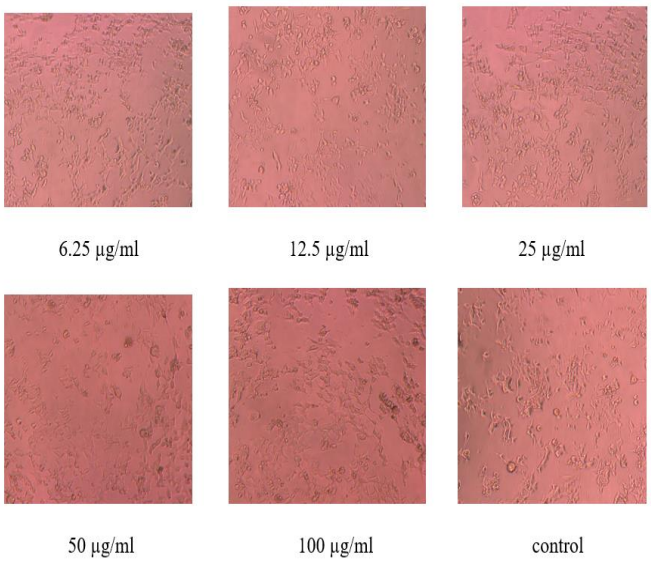

Figure 2: Cytotoxic effect of *Vetiveria zizanioides* on HEK cells

DISCUSSION

The risks associated with the use of medicinal plants could be revealed by toxicity testing. Toxicity can be determined by the ability of a plant extract to inhibit cellular growth and viability [9]. The cytotoxicity testing was greatly aided by MTT assay, a calorimetric method that measures the absorbance of pink colour formazan crystals formed from reduction of MTT that correlates the number of viable cells [10].

The present study was aimed to investigate the cytotoxic effect of the methanolic extracts of the leaves of *Amaranthus viridis* and roots of *Vetiveria zizanioides*, at varying concentrations ranging from 6.25-100 µg/ml against human embryonic kidney cells (HEK 239 cells) using MTT assay. The two plants used in this study were *Amaranthus viridis* belonging to *Amaranthaceae* family, a leafy vegetable having pharmacological properties like antiallergic, antidiabetic, antihepatotoxic, antihyperlipidemic, anti-inflammatory, antioxidant, antiulcer, antimicrobial actions [11, 12] and *Vetiveria zizanioides* belonging to *Poaceae family*, a perennial grass with aromatic roots having pharmacological properties like amenorrhoea, cooling, diaphoretic, expectorant, antioxidant and antifungal activities [13]. However, the cytotoxic potential of *Amaranthus viridis* and *Vetiveria zizanioides* were not much explored. Although some studies have reported the cytotoxicity effects of both plants on other cell lines, as far as our knowledge this is the first study to report the cytotoxicity effect of both the plants on Human Embryonic Kidney (HEK 239) cells.

Larbie et al., 2015 evaluated the anti-proliferative effects of ethanolic extracts of leaves (AVL) and stem (AVS) of *Amaranthus viridis* against three human leukemic cell lines (JURKAT, CEM and L-60). From the MTT assay, the sample AVL was found to exhibit better anti-proliferative activity than the sample AVS, showing decreasing cell viability with increasing concentration of *Amaranthus viridis* extracts [14]. Similarly, our study reports shows that the methanolic leaf extracts of *Amaranthus viridis* on HEK 239 cell lines exhibited no significant reduction in cell viability indicating the extracts were non-cytotoxic to kidney cells.

Chitra et al., 2014 determined the cytotoxic effect of the aqueous root extracts of *Vetiveria zizanioides* against human cancer cell line (MCF-7 human breast cancer cell line) using MTT assay and found that the aqueous extract of root of *Vetiveria zizanioides* exhibit cytotoxicity towards the cancer cell line indicating the feasible anticancer nature of aqueous crude root extract [15]. The results of our study also showed the cytotoxicity exhibited by root extracts of *Vetiveria zizanioides* against the HEK cells causing no significant reduction in cell viability thus, indicating the root extracts were non-cytotoxic to kidney cells.

The study thus derived the point that both were plant extracts cause no damage to kidney cells, justifying its usage in traditional medicines. However more studies should be conducted using higher concentrations of the extracts to determine the time-dependent activity involving various other cytotoxicity assays too.

CONCLUSION

The current study served as scientific evidence for the use of *Amaranthus viridis* and *Vetiveria zizanioides* in traditional medicine by people of Kanyakumari district, Tamil Nadu for the treatment of urinary tract infections. This study concluded stating that the leaves of *Amaranthus viridis* and roots of *Vetiveria zizanioides* were non-cytotoxic to human embryonic kidney cells in this model of cytotoxicity assay and could be used as a potent candidate to develop therapeutic agents to combat urinary tract infections. However, further studies using different toxicity models are essential to confirm the use of both the plants in traditional medicine.

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Conflict of interest

The authors declared no conflict of interest.

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