Anticancer activity of Triphala extract against oral cancer cell lines

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INTRODUCTION

Herbal products contain a wide variety of chemical compounds with potent biological effects, including anticancer activity. Identification of the active components in herbal products and their mechanism of action is of growing interest in the field of pharmaceutical medication for clinical use development.

Triphala (Sanskrit; tri = three and phala = fruits) is a well-recognized and revered polyherbal medicine consisting of dried fruits of the three plant Emblica officinalis (Family Euphorbiaceae), Terminalia bellerica (Family Combretaceae), and Terminalia chebula (Family Combretaceae) that are native to the Indian subcontinent.

Triphala is one of the highly potential herbal medicines in cancer treatment and prevention because all three compositions of Triphala have been found to possess notable anticancer properties. It is an important medicine of the “Rasayana” group and is believed to promote general health, immunity, and longevity.

Triphala extract, rich in antioxidants, is a frequently used Ayurvedic medicine to treat many diseases such as anemia, jaundice, constipation, asthma, fever, and chronic ulcers. Most people practicing Ayurvedic medicine consume Triphala as a “health tonic.” Recent studies have reported anticlastogenic and antitumor properties of E. officinalis and antiproliferative effect of T. chebula. However, the anticancer property of TPL has not been reported to the best of our knowledge.

Oral cancer, a type of head and neck cancer and any cancerous tissue growth located in the oral cavity. There are several types of oral cancers, but around 90% are SCCs, originating in the tissues that line the oral cavity. Oral cancer mostly involves the tongue. It may also occur on the floor of the mouth, the cheek lining, the gingiva (gums), the lips, or the palate (roof of the mouth). Most oral cancers look very similar under the microscope and are called squamous cell carcinoma, but less commonly other types of oral cancer occur, such as Kaposi’s sarcoma. In 2013 oral cancer resulted in 135,000 deaths up from 84,000 deaths in 1990. 5-years survival rates in the United States are 63%.

The utility of cell lines procured from tumors allows the investigation of tumor cells in a simplified and controlled environment. There are advantages and disadvantages to exploit cancer cell lines over animal models. These dictate the nature of the experiment that can be organized. First, the cost involved in sustaining them is significantly less than maintaining animal
subjects. They are promptly available and research studies can be implemented relatively quickly.[17,18]

Cytotoxicity is the degree to which an agent has a specific destructive action on certain cells. It is the possession of this destructive action, particularly in reference to lysis of cells by immune phenomena. Cell proliferation rates or viability levels are very good indicators of cell health. Proliferation or viability analysis which is important for cell growth and differentiation studies and is often combined with metabolism analysis. Assessing compound cytotoxicity is also an important step in pharmaceutical development. These assays in oncological settings are also used to evaluate both compound toxicity and inhibition of tumor cell growth during drug development.

Cancer is the single largest cause of death, in both men and women equally. Recently, resistance to anticancer drugs has been observed.[19,20] Therefore, the need of the hour is to develop treatment modalities using plant derivatives which obscure potent side effects and act as effective therapeutic agents.

MATERIALS AND METHODS

Triphala Extract

For preparing the extract, 10 g of the Triphala powdered sample was extracted with ethanol using Soxhlet extraction method. The filtrate was condensed using rotary vacuum evaporator at 45°C, and the extract was stored at 4°C till used. The dried extract was dissolved in dimethyl sulfoxide solution (0.1% DMSO) (Sigma) at a concentration of 100 mg/ml and kept as a stock sample.

Cell Culture

KB cells were procured, placed in 25 cm² culture flasks and cultured in RPMI 1640 culture medium, with 10% fetal bovine serum, L–glutamine, 1% penicillin (100 U/ml), and streptomycin (100 µg/ml) at 37°C in a humidified CO₂ (5%) chamber and 95% air. The cells were detached using 0.25% EDTA Trypsin. Neutralization of the Trypsin was achieved using DMEM containing 10% FBS and PSGF, and cells were mechanically separated using a pipette. There were 96-well plastic culture plates filled with 200 µl of medium containing in each well. The plates were then incubated at 37°C in a humidified atmosphere containing 5% CO₂ and 95% air for 24 h to permit attachment of the cells to the plates.

Cytotoxicity Assay

The MTT assay has been widely used to assess cell viability.[21] MTT test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid) was performed in three independent experiments. During the exponential growth phase of KB cells, the cultures were seeded in 96-well plates in 100 µl of complete α-MEM (minimal essential medium). KB cells were seeded with a cell suspension of 5 × 104 cells/cm². After 24 h of incubation, the Triphala extract (50–800 µg/ml) and standard 5-flurouracil were diluted in fresh complete α-MEM, just before replacing the initial culture medium with 100 µl of treatment medium. From each concentration, 100 µl was added to the wells. Subsequently, 24 h later, 200 µl of the MTT solution were added to each well and incubated for 3–4 h in standard conditions. Then, the culture medium was removed and 100 µl of DMSO was added to each well. The plates were agitated for 5 min before being introduced in a microplate reader. The absorbance of the extract was read at a wavelength of 570 nm. The average absorbance values of controls were taken as 100% cell viability. IC50 values were measured as the concentration of test sample which decreased the absorbance of the treated cells up to 50% of that of the control cells (DMSO treated).

Percentage of viable cell concentration was calculated thus:

Viability (%) = [Test sample OD/Control OD] × 100

Statistical Analysis

Results were expressed as mean ± SD. Statistical significance was determined by one-way analysis of variance and post hoc least-significant difference test. P < 0.05 was considered significant.

RESULTS AND DISCUSSION

This graph depicts the cell viability changes after 24 h. Results were expressed as Mean ± SD (n = 3). *P < 0.05 significantly different as compared with KB control.

Cell proliferation rates or viability levels are very good indicators of cell health. Assessing compound cytotoxicity is also an important step in pharmaceutical development. Triphala extract is an ayurvedic drug made of herbal constituents. When tested for the cytotoxic potential of the extract against KB cell lines [Graph 1] Triphala extract exhibited a consistent cytotoxicity in a dose dependent manner. With a concentration of 400 microgram/ml of Triphala

Graph 1: Cell viability assay
extract there was 50% cell viability as compared with the standard drug 5 fluoro uracil.

**CONCLUSION**

Natural products are widely used, nowadays, to avoid the various side effects caused by carcinogenic drugs. Based on the above results, the herb exhibits anticancer activity against oral cancer cells.

Thus, the potential to develop Triphala as an anticancer drug is a thrust area for future research in the drug designing industry.

**REFERENCES**