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In vitro Studies on anticancer activity of fungal taxol against human breast cancer cell line MCF-7 cells

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ABSTRACT

Objective: To prove the anticancer activity of fungal taxol obtained from *Pestalotiopsis pauciseta* VM1 endophytic fungus of *Tabebuia pentaphylla* on human breast cancer cell line MCF-7 by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. record ethnobotanical information from a hill-dwelling aboriginal tribe of Odisha. **Methods:** Different concentrations of fungal taxol ranging from 100 µg to 700 µg were tested against the MCF-7 breast cancer cell line showed significant decrease in the concentration of 350 µg. **Results:** This cell viability of control cells was consistently 85–90%, The cell shrinkage increased progressively. **Conclusions:** Thus, the fungal taxol isolated from *Pestalotiopsis pauciseta* VM1, exhibited a very high degree of *in vitro* cytotoxic activity against MCF-7 breast cancer cell line.

1. Introduction

The most important member of the clinically useful natural anticancer agent is Paclitaxel (Taxol®), which was first extracted in the bark of western yew *Taxus brevifolia* (Wani et al., 1971). This compound is the world's first billion-dollar anticancer drug, and it is used to treat a number of other human tissue-proliferating diseases as well. The projected increase in the use of taxol for basic research and cancer chemotherapy warrants effort to improve existing production processes for this important natural product. The amount of taxol found in yew tree is relatively small, 0.01–0.03% dry weight and this has been a major factor contributing to its high price.

However over the past few years, the renewable sources for taxol such as the needles of *Taxus* spp. or cell cultures and other alternative chemical biosynthetic methodologies have been utilized for the production of taxol are found to be too expensive for commercialization. A new possibility

to produce taxol in a cheaper way is industrial fermentation has come from the discovery that some endophytic fungi belonging to different genera such as *Taxomyces*, *Pestalotiopsis*, *Alternaria* and *Periconia* (Stierle et al., 1993; Li et al., 1998a, b).

Thus, in this study taxol producing endophytic fungi *Pestalotiopsis pauciseta* VM1 (Vennila and Muthumary, 2010) have been undertaken to investigate the anticancer activity against the human breast cancer cell line MCF-7 by MTT assay (David Pineiro et al., 2007).

2. Materials and methods

2.1 Cell line

MCF-7 cell line was obtained from National Centre for Cell Science (NCCS), Pune, India.

2.2 Cell culture reagents

10 g of Dulbecco's modified eagle medium (DMEM) was dissolved in 990 mL of sterilized double distilled water. To

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this solution, 1.5 g of sodium bicarbonate, 110 mg of sodium pyruvate and 10 mL of penicillin–streptomycin cocktail (100–units/mL penicillin and 10 μ L/mL streptomycin) were added and mixed thoroughly. The pH was adjusted to 7.4 using 1 N HCl or 1 N NaOH. Then the medium was filter sterilized using (0.22 μ M) membrane filter and dispensed into sterilized container and stored at 4°C.

Trypsin –EDTA solution (0.25% per 0.02%) in PBS was used. This was aliquoted and stored frozen until use. (Note: Freeze/thaw process does not affect the enzyme activity. Thawing was done at room temperature).

0.63 g of Sodium dihydrogen phosphate (NaH_2PO_4), 170 mg of Disodium hydrogen phosphate (Na_2HPO_4) and 4.5 g of sodium chloride were dissolved in 500 mL of sterile distilled water. The pH was adjusted to 7.4 using 1 N HCl or 1 N NaOH, filtered and then stored in a sterile container.

2.3 Cell Viability Test

Cell viability was assessed by MTT (3–4, 5–dimethylthiazolyl–2–2, 5–diphenyltetrazolium bromide) method. 5 mg of MTT was dissolved in 10 mL of serum free DMEM medium. 5 mL of DMF was made up to 10 mL with distilled water and 2 g of SDS were added and mixed well. Approximately 5000 cells were plated in 96 well plates with DMEM medium containing 10% Foetal Bovine Serum (FBS). The cells were incubated for 24 hrs under 5% CO_2 , 95% O_2 at 37°C. The serum medium was removed, washed with Phosphate Buffered Saline (PBS) and then serum free medium was added and kept for 1 h in the incubator. Then, serum free medium (SFM) was removed and the control plates received SFM and treatment plates received 100 μ g to 700 μ g of fungal taxol containing medium. The treatment protocol is represented in the experimental protocol.

Control cultures were treated with DMSO. The maximum concentration of dimethyl sulfoxide (DMSO) added to the medium in this study was 0.01%. 96 well plates were divided into five sections, with one section being treated by control culture media, the other treated with one of the following 100 μ L each: culture media containing 100 μ g to 700 μ g of fungal taxol. The cultures were again incubated

as above. After 24, 48 and 72 h 100 μ L of 0.5 mg/mL MTT solution was added to each well and the cultures were further incubated for 4 h and then 100 μ L of 20% SDS in 50% dimethylformamide (DMF) was added and the formed crystals were dissolved gently by pipetting 2 to 3 times. A micro plate reader was used to measure absorbance at 650 nm for each well. Growth inhibition rate was calculated as follows;

$$\% \text{ Growth inhibition} = \frac{A_{650/\text{nm of treated cells}}}{A_{650/\text{nm of control cells}}} \times 100$$

2.4 In vitro morphology

MCF–7 cells were grown in 24–well plates and, at 50% confluency, were treated with either DMSO alone or with concentrations of 350 μ g of fungal taxol. After 24 and 48 h the cells were observed with an inverted phase contrast microscope. Photographs were taken with a Nikon FM 10 camera.

3. Results

The cytotoxic effects of fungal taxol was tested by the MTT assay, which showed that the effect of fungal taxol on the cell viability in MCF–7 cell line for 24, 48 and 72 hours. It was observed that cell viability of control cells was consistently 85–90%. A significant decrease was seen in the cell viability for the concentration ranging 100 to 700 μ g of fungal taxol. In 350 μ g of fungal taxol treatment, only 50% of cells were viable in 48 h treatment. The IC_{50} value of fungal taxol was calculated as 350 μ g/ml (Fig. 1).

Light Microscope observations showed modifications in the morphology of MCF–7 cells after treatment with fungal taxol at 24 and 48 h, DMSO treated control MCF–7 cells grew as irregular confluent aggregates with rounded and polygonal cell morphology. At 24 h and 48 h of treatment, the fungal taxol induced the appearance of polygonal cells that began to shrink and spherical in shape. The cell shrinkage increased progressively (Fig. 2).

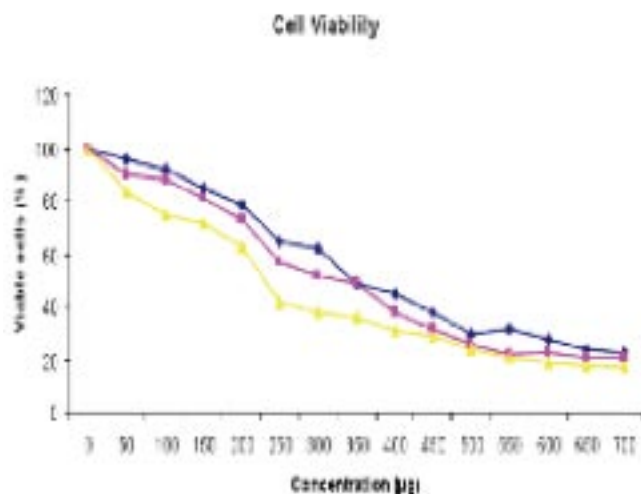


Figure 1. Cell viability of MCF–7 human breast cancer cell line.

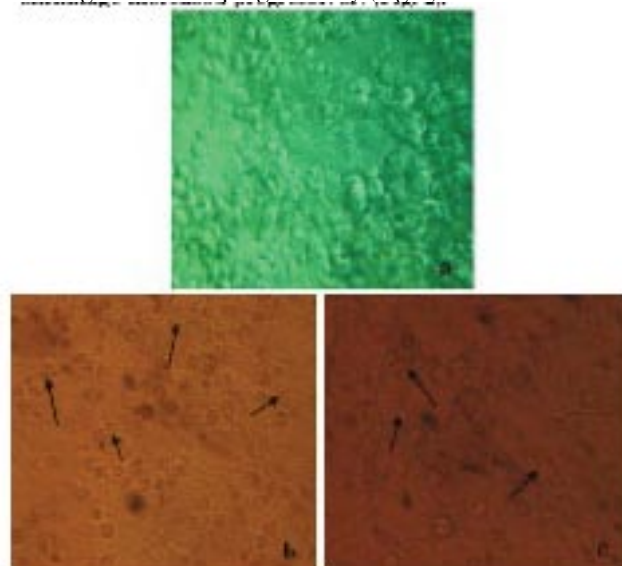


Figure 2. Morphology of MCF–7 cells after treatment with fungal taxol at 24 and 48 h. a: control; b: 24 h; c: 48 h.

4. Discussion

In the present study, we have investigated the effects of fungal taxol on MCF-7 breast cancer cell proliferation and apoptosis. The cytotoxic effects of fungal taxol was tested by the MTT assay, which showed that the effect of fungal taxol on the cell viability in MCF-7 cell line for 24, 48 and 72 hours. It was observed that cell viability of control cells was consistently 85–90%. A significant decrease was seen in the cell viability for the concentration ranging 100 to 700µg of fungal taxol. In 350µg of fungal taxol treatment, only 50% of cells were viable in 48 hours treatment. The IC₅₀ value of fungal taxol was calculated as 350 µg/ml. Treatment of cells with taxol interferes with the normal reorganization of the microtubule network, and inhibits the formation of normal spindle at metaphase required for mitosis and cell proliferation. These effects lead to the arrest of the cells in the G2/M phase of the cell cycle and eventually to apoptotic cell death (Woods et al., 1995; Rowinsky and Donehower, 1995; Eastman and Rigas 1999; Shi et al., 2008; Samadi et al., 2009). Thus, the fungal taxol isolated from *Pestalotiopsis pauciseta* VM1, exhibited a very high degree of in vitro cytotoxic activity against MCF-7 breast cancer cell line.

Conflict of interest statement

We declare that we have no conflict of interest.

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