ABSTRACT

The study of the influence of aqueous and hydro alcoholic extracts of Tinospora cordifolia on human mammary tumor cell line MCF-7 is a common way of discovering potential therapeutics for treating people suffering from hormone-dependent problems and diseases. In the present study, the cells were treated with the extracts at three different doses viz., 200 µg/ml, 400 µg/ml and 600 µg/ml. Both the extracts produced degenerative changes in the cell in dose-dependent manner with maximum effect being noticed at the dose level of 600 µg/ml. Thus, these findings suggest that the extracts from Tinospora cordifolia have potential for acting as an antiproliferative agent in mammary tumour.

Key words: Tinospora cordifolia, MCF-7 cell line, Degenerative changes, Doses

Cancer is the second leading cause of death in most parts of the world. Among cancers, cancer in mammary gland is of malignant type and chance of developing has increased to many folds. Variations in circulating levels of hormones or growth factors show significant association with cancer risk (Ponder, 2001). Kute et al. (1985) viewed that the growth patterns of mammary tissues are related to the regulatory factors like steroid hormones which alter cell kinetic activity. Pardhasaradhi et al. (2005) explored the antitumour activity of organic and aqueous extracts from the defatted seeds of Annona squamosa (custard apple) on different human tumour cell lines MCF-7 and K-562 resulting in nuclear condensation, DNA fragmentation, induction of reactive oxygen species (ROS) generation and reduced intracellular glutathione levels. Jun et al. (2006) observed that Siegesbeckia glabrescens induced apoptosis in MCF-7 and MDA-MB-231 cells which was determined by loss of cell viability, nuclear shrinkage and apoptotic body formation. Singh et al. (2005) reported that an alcoholic extract of Tinospora cordifolia had enhanced the differentiation of tumour associated macrophages to dentritic cells in response to granulocyte / macrophage –colony stimulating factor, interleukin-4 and tumour necrosis factor.

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Taking into consideration, the use of *T. cordifolia* in the treatment of cancer, the present investigation was undertaken to evaluate its effect on MCF-7 cell morphology.

Aqueous and hydro alcoholic extracts of stem part of *T. cordifolia* were obtained from M/s. Natural Remedies Pvt. Ltd., Bangalore. Human breast carcinoma cell line MDA-MB-231 was obtained from the National Centre for Cell Sciences (Pune, India) and doxorubicin from M/s Dabur Pharma Ltd. (Himachal Pradesh, India). Cells were grown in Minimum Essential Medium (MEM; Gibco) with 10% foetal bovine serum (Gibco) and 1% penicillin-streptomycin (Gibco BRL) at standard culture conditions.

**Treatment**: Six-well culture plates were incubated at 37°C in a humidified atmosphere containing 5% CO₂. Two days after seeding, doxorubicin (positive control; PC-2 ìg/ml), medium (NC) and plant extracts (alcoholic; T AE and hydro alcoholic; T HAE) at different concentrations (200, 400 and 600 ìg/ml) were added to the medium for a period of up to 48 hours and the effect of compounds with six replications for each dose were conducted. The drugs were dissolved in medium to give the desired drug concentration, just before use. After treatment, the adherent cells were visualized under a microscope at 40X object.

**Effect on cell morphology**: The effect of aqueous and hydro-alcoholic extracts of *Tinospora cordifolia* on MCF-7 cells is depicted in Fig:1. Negative control MCF-7 cells displayed flat cell bodies and a monolayer with a sheet like spread over the substratum. The cells treated with the aqueous extract of *T. cordifolia* showed degenerative changes, like cell swelling and loss of cell sheet. There was a dose dependent increase in cells getting swollen and bursting out. All these changes were more intense in the T AE 600 ìg/ml group.

After treatment with the hydro alcoholic extract of *T. cordifolia*, the monolayer cells began to show increased degeneration and clumping in a dose-dependent manner. The majority of cells was floating and had irregular cell walls. There was some cell debris in the medium. The cells also became rounded and detached from the substratum. These changes were high in the T HAE 600 ìg/ml group and maximum in the doxorubicin treated group. A dose-dependent inhibition of cell growth observed in our study was similar to the findings of Hostanska *et al.* (2004) using *Cimicifuga racemosa* extracts on MCF-7 and MDA-MB-231 cells to inhibit cell growth. Both extracts of *T. cordifolia* have exhibited significant antiproliferative effect as is evident in the degenerative changes after treatment with these extracts in a dose-dependent manner. Banerjee *et al.* (2002) treated MCF-7 cells with two fold serial dilutions of resveratrol for 72 h and reported that resveratrol inhibited the growth of cells dose-dependently, with almost 60% suppression of cell viability at 100ìm concentration. These results were similar to our observations, where there was a maximum reduction in viability (50% inhibition) by both the extracts at 600 ìg/ml.

It was concluded that both aqueous and hydro alcoholic extracts of *Tinospora cordifolia* were found to induce degenerative changes in
Effect of tinospora cordifolia on MCF-7 cell morphology

The MCF-7 tumour cell culture in dose-dependent manner as confirmed by cell morphological changes effect at 600 µg/ml dose.

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Fig. 1. : The effect of aqueous and hydro alcoholic extracts of *T. cordifolia* on MCF-7 cells (object in 40X). Morphological changes of cells after treatment with different concentrations of extracts and positive control. Vehicle – 48 h subculture (a), Doxorubicin - 2 µg/ml (b), T_{HAE} 200 µg/ml (c), T_{HAE} 400 µg/ml (d), T_{HAE} 600 µg/ml (e), T_{AE} 200 µg/ml (f), T_{AE} 400 µg/ml (g) and T_{AE} 600 µg/ml (h). AE - Aqueous extracts, HAE – Hydro alcoholic extracts.
REFERENCES


