Research Article

International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com

ANTI-PROLIFERATIVE ACTIVITY OF *TINOSPORA CORDIFOLIA* DETERMINED BY CELL COUNT AND TRYPAN BLUE DYE EXCLUSION METHOD IN MCF-7 CELLS

Sakthi Priya M[1]*, KV Venkateswaran[1], LN Mathuram[2], M ParthibanT[3] and Vijayanand[4]

[1]Department of Veterinary Pharmacology and Toxicology, Madras Veterinary College, Chennai-600 007, India
[2]Education cell, Madras Veterinary College, Chennai-600 007, India
[3]Department of Animal Biotechnology, Madras Veterinary College, Chennai-600 007, India
[4]Viral Vaccine Division, Bharat Biotech International Limited, Hyderabad, India

Received for publication: December 19, 2012; **Revised:** January 12, 2013; **Accepted:** February 21, 2013

Abstract: An *in-vitro* study was performed in mammary tumor cell line MCF-7 to find out the antiproliferative activity of aqueous and hydro-alcoholic extracts of Guduchi *Tinospora cordifolia*, each at three different doses viz., 200µg/ml, 400µg/ml and 600µg/ml. Their effects on the proliferation of cells were analyzed by cell count assay and cell viability was detected by using trypan blue dye exclusion method. Both of the extracts produced significant decrease in cell count and cell viability, with maximum effect being noticed at the dose level of 600µg/ml. This result suggest that aqueous and hydro-alcoholic extracts of *Tinospora cordifolia* could reduce cell count and cell viability in MCF-7 cell line and act as effective anti-proliferative agent in mammary tumor.

Keywords: *Tinospora cordifolia*, Anti-proliferative activity, MCF-7cell line, Cell count, Trypan blue.

INTRODUCTION

Cancer is an umbrella term covering a plethora of conditions characterized by unscheduled and uncontrolled cellular proliferation. It is a pathogenic disturbance of growth characterized by an excessive and unceasing proliferation of cells and is one of the major causes of death in man and animals in 21st century. As the average age of man rises, so do cancer related deaths (Pulverer, 2001)[1]. Pherson *et al.* (2000)[2] indicated that breast cancer is the most common malignancy in women accounting for 18% of all cancers. Emerging, although controversial, evidence suggested that exposure to various forms of stress may influence vulnerability to breast cancer and affect length of survival once a particular tumor had developed (Clarke *et al.*, 1994)[3]. Human breast cancer cell lines are widely used for screening and identification of new anticancer drugs. In recent years, breast cancers have aroused much concern. Chow *et al.* (2004)[4] emphasized that the growing incidence of breast cancer all over the world had highlighted the importance of developing a new chemotherapeutic drug in combating it. In the present era, a notion has evolved among the drug designers that natural products frequently exert a valuable role in broadening the scope of disease intervention strategies (Sharma *et al.*, 2004)[5].

Indian Tinospora (*Tinospora cordifolia*) commonly called 'guduchi' in Asia is a deciduous climbing shrub from the tropical Indian subcontinent. Categorized as "Rasayana" in Ayurveda, it is used for its general adaptogenic and pro-host immuno-modulatory activity in fighting infections. (Sharma and Khosa, 1993)[6]. The preliminary studies on the stem extracts of *Tinospora cordifolia* have shown promising responses in cultured HeLa cells, in a dose-dependent manner (Jagetia *et al.*, 1998)[7]. A polysaccharide present in *Tinospora cordifolia* inhibited metastases in the lungs of syngeneic C57BL/6 mice, when the drug was administered simultaneously with tumor challenge (Leyon and Kuttan, 2004_a)[8]. The antineoplastic activity of dichloromethane extract of *Tinospora cordifolia* on mice transplanted with Ehrlich ascites carcinoma had shown that the cytotoxic effect on tumor cells was exerted by reducing the glutathione concentrations and increasing lipid peroxidation simultaneously (Jagetia and Rao, 2006)[9].

Singh *et al.* (2006)[10] reported a significant influence of alcoholic extract of *Tinospora cordifolia* on the proliferation and myeloid differentiation of bone marrow progenitor cells and the recruitment of macrophages in response to tumor growth *in situ*. Therefore, it was desired to explore the anti-proliferative activity of *Tinospora cordifolia* on MCF-7 cell line by estimating count and viability and to compare its effect with known therapeutic anticancer drug used.

MATERIALS AND METHODS

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 MCF-7 was obtained from the

***Corresponding Author:**
Dr. M. Sakthi Priya
Assistant Professor,
Krishi Vigyan Kendra, VC7RI campus,
Namakkal, Chennai-600 007, India

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% pencillin-streptomycin (Gibco BRL) at standard culture conditions. Other standard chemicals with analytical grade were used throughout the study.

Treatment:

Six-well culture plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Two days after seeding, doxorubicin (positive control; PC-2 µg/ml), medium (NC) and plant extracts (alcoholic; T_{AE} and hydroalcoholic; T_{HAE}) at different concentrations (200, 400 and 600 µg/ml) were added to the medium for a period of upto 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 harvested by trypsinization, centrifuged at 10,000 rpm for 10 mins and re-suspended in 0.5 ml of 1X PBS to yield cell suspension.

Cell proliferation:

Cell number was estimated by microscopic cell counting using a haemocytometer, as suggested by Freshney (2000)[11]. After the treatment period, 20µl of the cell suspension was taken from each group respectively and transferred to the improved Neubauer haemocytometer chamber and cells in four 1 mm^2 squares were counted. The average was taken to represent the cell number. The cell concentration was calculated using the formula:

$$\text{Cell Concentration} = \frac{Cell\ number\ counted}{4}\ x\ 10^5$$

Cell viability by Trypan blue dye exclusion method:

Cell viability was assessed by trypan blue dye exclusion test as reported by Chakraborty *et al.* (2004)[12]. 200 mg of trypan blue powder was dissolved in 100 ml of triple distilled water and filtered, to get 0.2% solution for this study.

After the treatment period, 0.2 ml of the cell suspension was taken in a small tube and 0.2 ml of 0.2% trypan blue solution was added and mixed well. From this, 20µl was charged into the haemocytometer chamber and examined immediately. Live cells excluded the dye whereas the dye entered and stained the dead cells blue in color. Both stained and unstained cells were counted, and cell viability was calculated using the formula:

$$\text{Cell Viability (\%)} = \frac{\text{Total cells unstained}}{\text{Total cells stained} + \text{Total cells unstained}}\ x\ 100$$

RESULTS

The effect of aqueous and hydro-alcoholic extracts of *Tinospora cordifolia*, each at three different dose levels on the proliferation and viability of MCF-7 cells is presented in Tables 1 and in Figures 1 and 2.

Table.1: Effect of *Tinospora cordifolia* on cell proliferation and viability by trypan blue method in MCF-7 cells (Mean±S.E)

Treatment groups		Cell count (10^5cells/ml)	Cell viability (%)
Negative control		$13.19^{e} \pm 0.66$	$94.19^{f} \pm 0.02$
Doxorubicin 2µg/ml		$3.5^{a} \pm 0.47$	$48.86^{a} \pm 0.08$
T. cordifolia (T_{AE})	200 µg/ml	$9.45^{cd} \pm 0.40$	$84.87^{ef} \pm 0.04$
	400 µg/ml	$8.44^{bc} \pm 0.33$	$74.65^{cd} \pm 0.02$
	600 µg/ml	$7.44^{b} \pm 0.26$	$63.30^{b} \pm 0.02$
T. cordifolia (T_{HAE})	200 µg/ml	$11.12^{d} \pm 0.50$	$87.79^{f} \pm 0.06$
	400 µg/ml	$9.86^{cd} \pm 0.41$	$79.29^{de} \pm 0.08$
	600 µg/ml	$8.63^{bc} \pm 0.26$	$66.36^{bc} \pm 0.03$

*Means with different superscripts between rows within extracts differ significantly (P < 0.01)
µg – microgram; ml – milliliter; (n = 6)

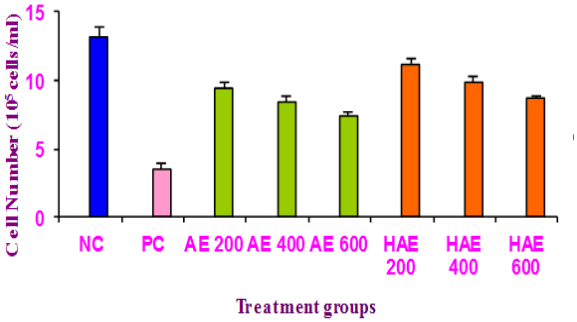

Figure.1: Effect of *Tinospora cordifolia* on cell proliferation in MCF-7 cells

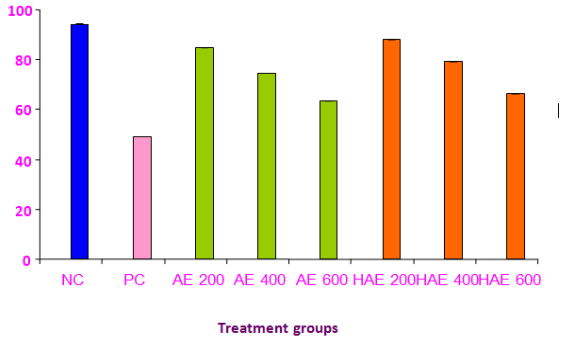

Figure.2: Effect of *Tinospora cordifolia* on cell viability by trypan blue method in MCF-7 cells
NC- Negative control; PC- Positive control; AE- Aqueous extract; HAE- Hydro-alcoholic extract.

Cell Proliferation:

There was a significant (P<0.01) decrease in cell counts of the treatment groups T_{AE} 200, T_{AE} 400 and T_{AE} 600 (9.45 ± 0.40, 8.44 ± 0.33, 7.44 ± 0.26 × 10^5cells/ml) when compared to the negative control.

There was no significant difference between the treatment group T_{AE} 200 and T_{AE} 400, and also between T_{AE} 400 and T_{AE} 600. Maximum inhibition in cell proliferation was noticed in the positive control group (3.5 ± 0.47 × 10^5cells/ml), which was significantly different from all the other treatment groups. Also, a maximum cell count was noticed in the negative control group (13.19 ± 0.66 × 10^5cells/ml), which was significantly different from the other treatment groups. In case of hydro-alcoholic extract of *T. cordifolia*, there was a significant (P<0.01) reduction in cell proliferation in all of the treatment groups T_{HAE} 200, T_{HAE} 400 and T_{HAE} 600 (11.12 ± 0.50, 9.86 ± 0.41 and 8.63 ± 0.26 × 10^5cells/ml) and positive control group (3.5 ± 0.47 × 10^5cells/ml) respectively when compared to the negative control (13.19 ± 0.66 × 10^5cells/ml). There was no significant difference between T_{HAE} 200 and T_{HAE} 400 and also between T_{HAE} 400 and T_{HAE} 600.

Cell viability:

There was a significant (P<0.01) decrease in cell viability in the treatment groups T_{AE} 400 and T_{AE} 600 (74.65 ± 0.02 and 63.30 ± 0.02%) and the positive control group (48.86 ± 0.08%) when compared to the negative control (94.19 ± 0.02%). There was no significant difference between T_{AE} 200 (84.87 ± 0.04%) and the negative control. There was a significant difference between T_{AE} 600 and the positive control group.

In case of hydro-alcoholic extract, there was a significant decrease (P<0.01) of cell viability in T_{HAE} 400 and T_{HAE} 600 (79.29 ± 0.08 and 66.36 ± 0.03 %) and the positive control group (48.86 ± 0.08%) when compared to the negative control (94.19 ± 0.02%). There was no significant difference between T_{HAE} 200 (87.79 ± 0.06%) and the negative control. Marked reduction in cell viability was noticed in the T_{HAE} 600 group.

DISCUSSION

A dose-dependent inhibition of cell growth observed in our study was similar to the findings of Yang *et al*. (2006)[13] who reported a decline in the proliferation rate with respect to inhibition of breast cancer cells by *Antrodia camphorata*. Our observations on the cell proliferation are in accordance with these findings in which the aqueous and hydro-alcoholic extracts of *Tinospora cordifolia* have exhibited significant anti-proliferative effect as is evident in the decreased cell proliferation after treatment, in a dose-dependent manner. Banerjee *et al*. (2002)[14] treated MCF-7 cells with two fold serial dilutions of resveratrol for 72 hours and reported that resveratrol inhibited the growth of cells in a dose-dependent manner, In the present study, both the extracts of *Tinospora cordifolia* were able to produce a significant decline in cell viability when compared to the negative control in MCF-7 cells.

CONCLUSION

It was concluded that both aqueous and hydro-alcoholic extracts of *Tinospora cordifolia* were found to possess anti-proliferative effect and this was dose-dependent as confirmed by detection of cell count and viability by Trypan blue dye exclusion method with the maximum effect at 600µg/ml dose.

ACKNOWLEDGEMENT

The authors are highly thankful to the Dean, Madras Veterinary College for having provided necessary infrastructure facilities to carry out the research project.

REFERENCES

1. Pulverer B, Nature insight: cancer. *Nature,* 411 (2001) 335.
2. Pherson MCK, Steel CM and Dixon JM, ABC of the breast diseases, breast cancer epidemiology, risk factors and genetics. *B. M. Journal,* 321 (2000) 624.
3. Clarke HL, Rowland J, Clarke R and Lippman M E, Psychosocial factors in the development and progression of breast cancer. *Breast Cancer Res. Treat*, 29 (1994) 141.
4. Chow SKY, Chan JWY and Fung KP, Suppression of cell proliferation and regulation of estrogen receptor and signaling pathway by arsenic trioxide on human breast cancer MCF-7 cells. *J. Endocrinol*, 182 (2004) 325.
5. Sharma GA, K Tyagi, RP Singh, DC Chan and R Agarwal, 2004. Synergistic anti-cancer effects of a grape seed extract and conventional cytotoxic agent doxorubicin against human breast carcinoma cells. *Breast Cancer Res. Treat.*, 85: 1-12
6. Sharma DNK, and Khosa RH, Chemistry and pharmacology of *Tinospora cordifolia* Miers. *Indian drugs*, 30 (1993) 549.
7. Jagetia GC, Nayak V and Vidyasagar MS, Evaluation of the antineoplastic activity of guduchi (*Tinospora cordifolia*) in cultured Hela cells. *Cancer Lett*, 127 (1998) 71.
8. Leyon PV, and Kuttan G, Inhibitory effect of a polysaccharide from *Tinospora cordifolia* on experimental metastasis, 2004. *J. Ethnopharmacol,* 90 (2004_a) 233.
9. Jagetia GC, and Rao SK, Evaluation of the antineoplastic activity of Gudichi (*Tinospora cordifolia*) in Ehrlich Ascites carcinoma bearing mice. *Biol. Pharm. bull*, 29 (2006) 460.

10. Singh SM, N Singh and P Shrivastava, 2006. Effect of alcoholic extract of Ayurvedic herb *Tinospora cordifolia* on the proliferation and myeloid differentiation of bone marrow processor cells in a tumor-bearing host. *Fitoterapia*, 77: 1-11.

11. Freshney RI, Quantitation and Experimental Design, *In Culture of animal cells. A manual of basic technique.* Fourth edition (Wiley-Liss, Inc, New York) 2000,267.

12. Chakraborty S, Roy M, Taraphdar A K and Bhattacharya R K, Cytotoxic effect of root extract of *Tiliacora racemosa* and oil of *Semecarpus anacardium* nut in human tumor cells. *Phytother. Res*, 18 (2004) 595.

13. Yang HL, CS Chen WH Chang FJ Lu, YC Hai, CC Chen, THH Sell, C T Kuo and YC Hseu, 2006. Growth inhibition and induction of apoptosis in MCF-7 breast cancer cells by *Antrodia camphorata. Cancer Lett.*, 231: 215-227.

14. Banerjee S, Ramos CB and Aggarwal BB, Suppression of 7, 12 – Dimethyl benz (a) anthracene induced mammary carcinogenesis in rats by resveratrol, *Cancer Res,* 62 (2002) 4945.

Source of support: Nil
Conflict of interest: None Declared