Antiproliferative effect of Tinospora cordifolia on MDA-MB-231 cell line

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Abstract

The present study was taken up to explore the antiproliferative activity of aqueous and hydro-alcoholic extracts of Tinospora cordifolia in MDA-MB-231 human breast cancer cells each at three different doses viz. 200, 400 and 600 µg/ml. Their influence on the proliferation of cells were analysed by estimation of cell count, cell viability and cytotoxicity using trypan blue dye exclusion method and MTT assay, detection of apoptosis by flow cytometry and expression of apoptosis related genes. The expression of telomerase was determined by using RT-PCR. Both the extracts produced significant inhibition of cell count and cell viability, with maximum effect being noticed at the dose level of 600 µg/ml. Induction of apoptosis was shown by changes in nuclear morphology and formation of apoptotic bodies, though there was no DNA laddering in agarose gel electrophoresis. There was an expression of the apoptosis related genes in the treated groups. Flow cytometric analysis revealed an increase in the percentage of apoptotic cells dose-dependently, after treatment with both the extracts of the plant. Telomerase RNA and hTERT mRNA was expressed in the control but not in the treated groups by RT-PCR analysis. These results suggest that aqueous and hydro-alcoholic extracts of Tinospora cordifolia could suppress cell proliferation and induce apoptosis in MDA-MB-231 cell line and act as effective natural anticancer agents in mammary tumour.

Keywords: Tinospora cordifolia, antiproliferative activity, MDA-MB-231 cell line, apoptosis, MTT assay

Received: 09th December; Revised: 29th December; Accepted: 10th January; © IJCS New Liberty Group 2012

Introduction

Mammary cancer is one of the most common malignancies diagnosed in women and the frequency of mammary neoplasia in different species varies tremendously. It is extremely difficult to treat due to several distinct classes of tumours that exhibit different treatment response (Otin and Diamandis, 1998). 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). Tinospora cordifolia (Guduchi) has been used in the Indian system of medicine since ancient times. It is a glabrous climbing succulent shrub, commonly found in hedges. It is native to India and thrives easily on tropical region (Prince et al., 1999). T. cordifolia has been extensively screened for many pharmacological activities. It had been shown to possess hypolipidaemic (Prince et al., 1999), antioxidantive and hepatoprotective (Bishayi et al., 2002), immunostimulant (Nair et al., 2004), antiangiogenic (Leyon and Kuttan, 2004), antiallergic (Badar et al., 2005), anti-inflammatory and analgesic activity (Thejomoorthy and Raju, 2007) and causes retardation in the tumour development (Jagetia and Rao, 2006). In accordance with above different pharmacological activities, the present investigations were carried out to elicit the antiproliferative potential of aqueous and hydro-alcoholic extracts of T. cordifolia on MDA-MB-231 mammary tumour cell line by evaluating the possible mechanisms of cell death.

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 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% pencillin-streptomycin (Gibco BRL) at standard culture conditions.
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% CO₂. Two days after seeding, doxorubicin (positive control; PC-2 μg/ml), medium (NC) and plant extracts (alcoholic; TAE and hydroalcoholic; THAE) at different concentrations (200, 400 and 600 µg/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 harvested by trypsinization, centrifuged at 10,000 rpm for 10 mins and resuspended in 0.5 ml of 1X PBS to yield cell suspension.

**Cell proliferation assays**

20 µl of the cell suspension was transferred to the improved Neubauer haemocytometer chamber and cells in four 1 mm² squares were counted and the average was taken to represent the cell number. Cell number was estimated by microscopic cell counting using a haemocytometer, as suggested by Freshney (2000). The cell concentration was calculated using the formula as,

\[
\text{Cell Concentration} = \frac{\text{cell number counted}}{4} \times 10^5.
\]

**Cell viability and cytotoxicity**

The viability of cells was determined by the trypan blue dye exclusion method and cytotoxicity by the MTT assay (Chakraborty et al., 2004). A 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, 20 µl 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 colour. Both stained and unstained cells were counted and cell viability was calculated using the formula,

\[
\text{Cell viability} (%) = \frac{1 - \text{Absorbance of the experiment well}}{\text{Absorbance of the negative control well}} \times 100
\]

For MTT assay, 20 µl of MTT solution (5 mg/ml) was added to each well after treatment period, and the microplates were further incubated at 37°C for 4 hours. The unreactive supernatants in the wells were discarded and 100 µl of DMSO was added to each well and mixed thoroughly to dissolve the dark blue crystals of formazan. The absorbance values of each well were determined with an ELISA reader equipped with a 570 nm filter. The results were presented as the percentage viability which was determined as,

\[
\text{Cell viability} (%) = \frac{\text{Total cell unstained} - \text{Total cell stained}}{\text{Total cell unstained}} \times 100
\]

**Detection of apoptosis**

**Acridine orange/Ethidium bromide (AO/EB) staining**

To visualize and quantify the number of viable cells (green nuclei), apoptotic cells (nucleus condensed and coloured in orange) and necrotic cells (red nuclei), 2 µl of the working solution was added to 20 µl of the cell suspension and immediately examined with a 40X oil immersion objective using a fluorescence microscope. Several fields randomly chosen, were digitalized and minimum 600-800 nuclei for each sample were counted and scored. Results were expressed as the relative percentages of viable, apoptotic and necrotic cells to the total number of cells scored (Fromigue et al., 2000).

**DNA fragmentation analysis**

The percentages of internucleosomal DNA cleavage in MDA-MB-231 cells were investigated using the modified method of Wan et al. (2006). After treatment, both detached cells and adherent cells (harvested by trypsinization) were pelleted by centrifugation (2500 rpm, 5 min) at 4°C. Cells were lysed in ice-cold lysis buffer, incubated on ice for 20 min and centrifuged at 12000 rpm for 20 minutes at 4°C to separate the low molecular weight DNA from the intact chromatin. The supernatant was extracted by phenol/chloroform and DNA was precipitated by ethanol. The DNA pellet was dissolved in 300 µl of TE buffer and 5 ml of DNase-free RNase-A was added. After incubation at 37°C for 30 min, phenol/chloroform extraction and ethanol precipitation were repeated and DNA was dissolved in 15 µl TE buffer and then separated by electrophoresis on a 2 % agarose gel containing 0.5 mg/ml ethidium bromide. DNA bands were visualized by a UV transilluminator image system.

**Flow cytometric analysis**

Flow cytometric analysis was performed to identify and quantify the apoptotic cells by using the Vybrant® Apoptosis assay Kit # 2 (Invitrogen Detection Technologies, USA). After treatment period, both floating and adherent cells were harvested and subjected to centrifugation and washed in cold PBS solution. The supernatants were discarded and the cells were then
resuspended in 1X annexin binding buffer. Cell density was determined and diluted in 1X annexin binding buffer to approximately 1 x 10^6 cells/ml to have 100 μl per assay. 5 μl of Alexa Flour® 488 annexinV and 1 μl of the 100 μg/ml propidium iodide working solution was added to each 100 μl of the cell suspension and incubated at room temperature for 15 minutes. After the incubation period, 400 μl of 1X annexin binding buffer was added, mixed gently and kept on ice. As quickly as possible, the stained cells were analysed by flow cytometer (Becton, Dickinson), measuring the fluorescence emission at 530 nm and greater than 575 nm.

Detection of apoptosis related genes and of tumour marker enzyme by RT-PCR

The mRNA expression levels of widely established apoptosis-related genes (Bcl-2, Bax and Fas) and Tumour marker enzyme (Telomerase activity) were carried out using RT-PCR as described by Kahta et al. (2006) and Kosciolek et al. (2003), respectively. The cells were cultured in T-25 flasks and treated with the plant extracts and incubated for 2 hours. After incubation, the total cellular RNA was isolated using Tri reagent (MRC, Ohio) according to the manufacturer’s protocol. Subsequently, 10 μl was reverse transcribed into cDNA by using the cDNA synthesis kit (First strand cDNA synthesis kit- Fermentas, USA) using the random hexamer primer. PCR was carried out in a final concentration of 25 μl containing 12.5 μl of Mastermix (Fermentas, USA), 2 μl of cDNA, 1 μl of each forward and reverse primer (10 pmol) and 8.5 μl DEPC treated water. The primers used were as follows;

β-actin: forward 5'- ggcatcctcaccctgaagta-3', reverse 5'-ggggttgtaaggtctcaaa-3',

Bcl-2: forward 5'-gagaaggtgagaagccctga-3', reverse 5'-gcccagggatgaataaacct-3',

Bax: forward 5'-tgtgcctaggtttccatcc-3', reverse 5'gcatctctgatgattcatcc-3',

Fas: forward 5'-tgtgcctaggtttccatcc-3', reverse 5'-gcatctctgatgattcatcc-3',

Telomerase RNA: forward 5'-ctgggggggttggtcttcattt-3', reverse 5'-cgcaggggccagcagcatc-3',

hTERT mRNA: forward 5'-gccagaagttgccgagaga-3', reverse 5'-aatcatccacaaagcaggag-3' and GAPDH: forward 5'-cagccgagccacatcg-3', reverse 5'-tgaggttggttgtcacatccc-3'.

The PCR protocol consisted of initial cycles of denaturation at 94°C for 2 minutes, 94°C for 30 seconds, annealing (at 60°C with 30 seconds for apoptosis-related genes, at 50°C with 45 seconds for telomerase, at 48°C with 45 seconds for hTERT and 58°C with 30 seconds), 72°C for 1 min and extension at 72°C for 7 min. The PCR cycle conditions include 28 (β-actin), 33 (Fas), 38 (Bax and Bcl-2), 25 (telomerase), 30 (hTERT) and 25 (GAPDH). The PCR products were electrophoresed on a 2 % agarose gel and visualized with ethidium bromide staining. The expected sizes of the PCR products for β-actin, Bcl-2, Bax, Fas, telomerase, hTERT and GAPDH were 203, 179, 342, 269, 179, 649 and 460 base pairs (bp), respectively.

Nucleotide sequencing

The PCR products of the hTERT gene were purified using Purelink (PCR purification kit, Invitrogen, USA) and the purified PCR products were subjected to automatic sequencer (ABI, Switzerland). The nucleotide sequence data was then used for blast analysis.

Statistical analysis

Mean data values are presented with their deviation (mean±SE). The data were analyzed using SPSS (Version 10). Statistical significance was defined as P<0.01 for all tests.

Results and discussion

Cancer is the second leading cause of death in most parts of the world, specifically cancer in mammary gland is of malignant type and chances of developing have increased to many folds. Chemotherapy, radiation therapy and immunotherapy all rely heavily on apoptotic mechanisms to kill breast cancer cells. Many tumours initially respond to therapy. But cells can subsequently survive and gain resistance to these treatments. Resistance to available chemotherapeutic agents has forced us to think of novel approaches to cancer therapy. A great promise has been obtained from plant products for the cure of cancer. Therefore, novel therapeutic approaches to the treatment of breast cancer were proposed utilizing plant extracts, and in the present study, an attempt was made to focus on the in vitro antiproliferative effect of T. cordifolia on MDA-MB-231 cells.
Effect of T. cordifolia on cell morphology, viability and proliferation

There was a dose dependent increase in cells getting swollen and bursting out in the aqueous and hydro-alcoholic extract treated groups. All these changes were more intense in the T$_{AE}$ 600 group. Similarly in the hydro-alcoholic extract treated groups, the monolayer cells began to show increased degeneration and clumping and maximum effect being noticed in T$_{HAE}$ 600 groups which was comparable to positive control (doxorubicin treated groups) (Figure 1). 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. In continuance with the above findings, both extracts of T. cordifolia have exhibited significant antiproliferative effect as is evident in the decreased cell proliferation 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 hrs and reported that resveratrol inhibited the growth of cells dose-dependently, with almost 60% suppression of cell viability at 100µm concentration. These results were similar to our observations, where there was a maximum reduction in viability (50 % inhibition) by both the extracts at 600 µg/ml. Our observations on the cell proliferation are in accordance with these findings indicating a possible beneficial effect in humans.

Induction of apoptosis by T. cordifolia

Apoptosis or programmed cell death is a series of genetically controlled events and is a highly organized physiological phenomenon. Induction of apoptosis is a highly desirable mode as a chemotherapeutic as well as a chemopreventive strategy for cancer control. In our study, double staining with a mixture of acridine orange and ethidium bromide was used and we could visualize and quantify the number of viable (large green nuclei), necrotic (red nuclei) and apoptotic cells (nuclear condensation) (Fromigue et al., 2000). Evans and Dive (1993) based on their findings on results of staining technique, reported that JB1 hepatoma cells underwent apoptosis following cisplatin treatment as evidenced by 62% of detached cells undergoing apoptotic changes whereas only one to four per cent of attached cells were apoptotic (Table 1 and Fig. 2). In continuation with these staining results, flow cytometry results also revealed a significant (P<0.01) decrease in the proportion of live cells and an increase in the proportion of apoptotic and necrotic cells in a dose dependent manner in all the treatment groups in comparison to negative control (Fig. 3). Morphological features of apoptosis such as cell shrinkage, chromatin condensation and nuclear fragmentation evident in our study were similar to the findings reported by Chakraborty et al. (2004) in human tumour cells using the root extract of Tiliacora racemosa and oil of Semecarpus anacardium. Presence of a significant number of apoptotic cells in our study suggests that T. cordifolia extracts would have induced the changes in membrane permeability which precede nuclear changes that occur during apoptosis. Journe et al. (2006) demonstrated that high ibandronate concentrations induced apoptotic cell death, as documented by the detection of annexin - positive and propidium iodide-negative MCF-7 cells. This finding was similar to the results obtained in our study, where there was a dose-dependent increase in the percentages of annexin - positive cells in the treated groups indicate that the inhibition of cell growth by T. cordifolia in this study could be attributed to the induction of apoptotic mechanisms.

Agarose gel electrophoresis of the DNA, isolated from the treatment groups of both the extracts of T. cordifolia on MDA-MB-231 cells revealed smearing of DNA in all the treatment groups when compared to the negative control (Figure 4). In support with our findings, evodiamine produced typical DNA fragmentation in HeLa cells and smearing like DNA degradation in A375-S2 cells which was due to apoptosis at the early stages and necrosis at later stages (Zhang et al., 2004). DNA ladder formation is characteristic of apoptosis, as has been demonstrated in HL-60 and K-562 (Chakraborty et al., 2004) and MCF-7 human cancer cell line (Yang et al., 2006). On the contrary, Janicke et al., (1998) did not observe DNA fragmentation in MCF-7 cells treated with various apoptotic stimuli and cells undergoing death. They attributed the absence of DNA fragmentation to the low levels of caspase-3 in cancer cells. In the present study too, DNA laddering was not observable in any one.
Table 1. Effect of *Tinospora cordifolia* on cell proliferation and viability, induction of apoptosis in MDA-MB-231 cells. Viable cell number was measured with MTT assay. Data were expressed as mean ± SE of 6 replicates. AE - Aqueous extracts, HAE - Hydro-alcoholic extracts.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Cell count (10^3 cells/ml)</th>
<th>Cell viability (%)</th>
<th>Apoptosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trypan blue</td>
<td>MTT assay</td>
<td>Live cell</td>
</tr>
<tr>
<td>Negative control</td>
<td>13.04±0.61</td>
<td>95.63±0.07</td>
<td>92.23±0.08</td>
</tr>
<tr>
<td>Doxorubicin 2 µg/ml</td>
<td>5.67±0.26</td>
<td>64.04±0.02</td>
<td>41.07±0.05</td>
</tr>
<tr>
<td><em>T. cordifolia</em></td>
<td>200 µg/ml</td>
<td>8.90±0.12</td>
<td>93.68±0.07</td>
</tr>
<tr>
<td></td>
<td>400 µg/ml</td>
<td>7.94±0.10</td>
<td>86.71±0.03</td>
</tr>
<tr>
<td></td>
<td>600 µg/ml</td>
<td>7.05±0.17</td>
<td>71.76±0.01</td>
</tr>
<tr>
<td><em>T. cordifolia</em></td>
<td>200 µg/ml</td>
<td>9.29±0.08</td>
<td>94.98±0.05</td>
</tr>
<tr>
<td></td>
<td>400 µg/ml</td>
<td>8.29±0.06</td>
<td>88.48±0.03</td>
</tr>
<tr>
<td></td>
<td>600 µg/ml</td>
<td>7.14±0.18</td>
<td>73.67±0.01</td>
</tr>
</tbody>
</table>

Means with different superscripts between rows within extracts differ significantly (P < 0.01)

Fig. 1. The effect of aqueous and hydro-alcoholic extracts of *T. cordifolia* on MDA-MB-231 cells (object in 40X). Morphological changes of cells after treatment with different concentrations of extracts and positive control. Vehicle – 48 hr subculture (a), Doxorubicin - 2 µg/ml (b), *T. AE* 200 µg/ml (c), *T. AE* 400 µg/ml (d), *T. AE* 600 µg/ml (e), *T. HAE* 200 µg/ml (f), *T. HAE* 400 µg/ml (g) and *T. HAE* 600 µg/ml (h). AE - Aqueous extracts, HAE - Hydro-alcoholic extracts.

Fig. 2. The effect of *T. cordifolia* extracts on induction of apoptosis in MDA-MB-231 cells were analysed by AO/EB staining. The cells were treated with vehicle (a), Doxorubicin - 2 µg/ml (b), *T. AE* 200 µg/ml (c), *T. AE* 400 µg/ml (d), *T. AE* 600 µg/ml (e), *T. HAE* 200 µg/ml (f), *T. HAE* 400 µg/ml (g) and *T. HAE* 600 µg/ml (h). Arrows show dark stained nuclei which indicate DNA fragmentation and nuclear condensation. AE - Aqueous extracts, HAE - Hydro-alcoholic extracts.
Fig. 3. The effect of the aqueous and hydro-alcoholic extracts of *T. cordifolia* in three different doses on MDA-MB-231 cells. Apoptosis was examined by flow cytometry after Annexin V-PI double staining. Necrotic cells lose membrane integrity, permitting PI entry. Viable cells exhibit Annexin V (-)/PI (-); early apoptotic cells exhibit Annexin (+)/PI (-); late apoptotic cells or necrotic cells exhibit Annexin V (+)/PI (+). The cells were treated with vehicle (a), Doxorubicin - 2 µg/ml (b), TAE 200 µg/ml (c), TAE 400 µg/ml (d), TAE 600 µg/ml (e), T_HAE 200 µg/ml (f), T_HAE 400 µg/ml (g) and T_HAE 600 µg/ml (h). AE - Aqueous extracts, HAE - Hydro-alcoholic extracts, L- Live cells, A- Apoptotic cells, N- Necrotic cells.

Fig. 4. DNA fragmentation analysis of *Tinospora cordifolia* treated MDA-MB-231 cells. Agarose gel electrophoresis of the DNA isolated from the eight groups of culture of MDA-MB-231 cells. Each lane represented as: 1, cells were treated with vehicle; 2, Doxorubicin - 2 µg/ml; 3, TAE 200 µg/ml; 4, TAE 400 µg/ml; 5, TAE 600 µg/ml; 6, T_HAE 200 µg/ml; 7, T_HAE 400 µg/ml and 8, T_HAE 600 µg/ml. AE - Aqueous extracts, HAE - Hydro-alcoholic extracts.

Fig. 5. The effect of aqueous and hydro-alcoholic extracts of *T. cordifolia* in high doses on the expression level of apoptosis related genes Bcl-2, Bax, Fas, keeping β-actin as an internal control on MDA-MB-231 cells. A) DNA extracted from vehicle (lane 2 – 5) and doxorubicin (lane 6 – 9) treated cells. B) DNA extracted from T_AE 600 µg/ml treated cells. C) DNA extracted from T_HAE 600 µg/ml treated cells. Each lane represented as: 1, Marker; 2 & 6, β actin (203 bp); 3 & 7, Bcl2 (179 bp); 4 & 8, Fas (269 bp) and 5 & 9, Bax (342 bp). AE - Aqueous extracts, HAE - Hydro-alcoholic extracts.
of the treatment groups. Yang et al. (2006) reported that internucleosomal DNA fragmentation is not essential for apoptotic cell death whereas some necrotic cell death may be accompanied by internucleosomal fragmentation. It has been reported that if DNA cleavage during apoptosis does not proceed to internucleosomal sections but stops at 50 to 300 kbp size fragments, bigger fragments are not detectable by conventional agarose gel electrophoresis (Squier and Cohen, 2001). This could explain the lack of DNA fragmentation in this study. Therefore, the lack of DNA fragmentation in our study may not indicate the absence of apoptosis.

Continuance with DNA fragment analysis, expression of apoptotic genes were noticed in all the treatment groups when compared to the negative control where there was expression of antiapoptotic gene (Figure 5). Bcl-2, Bax and Fas play an important role in apoptosis. Bcl-2 has an antiapoptotic effect whereas Bax and Fas are apoptotic in nature (Lai and Thomas, 1999). The increased ratio of Bax/Bcl-2 might contribute to the induction of apoptosis in resveratrol (Kim et al., 2004) and Antrodia camphorata (Yang et al., 2006) treated MCF-7 cells. Treatment with Siegesbeckia glabrescens decreased the level of Bcl-2 mRNA expression and increased the level of Bax expression in MDA-MB-231 cells (Jun et al., 2006). Our studies also showed the similar expression of Bcl-2 gene in the negative control and Bax and Fas in the treated groups.

Effect of T. cordifolia on tumour marker enzyme (Telomerase)

Increase in telomerase activity and hTERT mRNA expression are features of the early stages of the development of squamous cell carcinoma of the lung, with strong telomerase activity and hTERT mRNA expression being prominent during the later stages (Shibuya et al., 2001). In the present findings, negative control showed the expression of GAPDH, telomerase RNA and hTERT mRNA (214 bp), whereas treatment with the aqueous and hydro-alcoholic extracts of T. cordifolia and the positive control showed only the expression of GAPDH (Fig. 6). Some of the proven anticancer compounds like adriamycin and 5-flourouracil reduced telomerase activity and human telomerase mRNA expression in MCF-7 cells (Ishikawa et al., 1999). In our study too, decrease in telomerase activity is found as is evident by the absence of hTERT amplification. However, in the negative control, the gene amplified corresponds to a much smaller size (214 bp for MDA-MB-231 cells). This may be attributed to the following reasons. (i) Alternative spliced variants encoding different isoforms (Yokoyama et al., 2001). (ii) The amplified gene was further sequenced and the blast analysis of the sequenced product revealed to be similar to a U5 snRNP (small nuclear Ribonucleo protein) which is responsible for maintaining cell viability in Saccharomyces cerevisiae (Dix et al., 1998). It is possible that the plant extract could induce cell death through a different pathway by involving U5 snRNP. The blast analysis also revealed that hTERT primers did not show homology with the U5 snRNP. Hence the mechanism by which U5 snRNP was amplified is uncertain. However the results of our study point that telomerase activity could be a useful marker in in vitro assessment of tumour cell chemosensitivity. Hence, to confirm identity of the expressed products, it was subjected to nucleotide sequencing. Blast analysis of the sequenced product was found to be homologous to U5 snRNP present in Homo

Fig. 6. The effect of aqueous and hydro-alcoholic extracts of T. cordifolia in high doses, on the expression of telomerase RNA (lane 6 – 9), hTERT mRNA (lane 10 – 13) keeping GAPDH as an internal control (lane 2 – 5) on cDNA extracted from MDA-MB-231 treated cells. Each lane represented as: 1, Marker; Lane 2,6,10, vehicle; Lane 3,7,11 , Doxorubicin - 2 µg/ml; Lane 4,8,12, TAE 600 µg/ml; Lane 5,9,13,THAE 600 µg/ml. AE - Aqueous extracts, HAE - Hydro-alcoholic extracts.
sapiens chromosome with the accession number NT 010718. However the blast analysis of hTERT primers with the U5 snRNP did not show homology.

In conclusion, our findings demonstrates that, Tinospora cordifolia functions as an inhibitor of breast cancer cells, MCF-7 in vitro mediated through modulation of cell proliferation and apoptosis. These findings illustrate that Tinospora cordifolia could be a promising anti-cancer agent for human breast cancer and contribute to clarify the complex chemopreventive and antiproliferative properties and thus additional studies are warranted to determine this phytochemical compound as an inhibitor of cancer.

References


