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Evaluation of Antimetastatic Activity of *Tinospora Cordifolia* on MCF-7 breast cancer cell line

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ABSTRACT

Tinosporacordifolia, (TC) sometimes referred to as *Guduchi* in technical terms, is a key component of Indian medicine. Since ancient times, it has been utilized as a medicine and is a member of the *Menispermaceae* family. Other names for it are *Giloy*, *Amrita*, and Indian bitter. It can be found all over India in deep, dry woods, and at higher altitudes, it develops into small trees and shrubs. TC leaves, root, and stem are all medicinally useful. TC is described as a treatment for a variety of diseases in the classic textbooks of ancient Ayurveda, such as *Charaka Samhita*, *Sushruta Samhita*, and other literature, under a variety of names. *Tinospora cordifolia* are medicinal plants that have long been used in Ayurveda for a variety of purposes, including *Rasayana*, *Ayushprada*, *Vayah-Sthapana*, *Cakshuya*, *Varnya*, *Keshya*, *Vatapitajit*, *Raktaprasadana*, *Varnahar*, *Shothhar*, *Vishghan*, and *Chhardighan*. However limited informations are available regarding *Tinospora cordifolia* cytotoxicity potential. The objective of this study was to determine the antiproliferative activity of Ethanolic extract of *Tinospora cordifolia*(TCE) on MCF-7 breast cancer cell line. Viability of cell was measured using the MTT assay while cell morphology was captured using phase contrast microscope. Results obtained showed that the cell viability decreased in a dose dependent manner TCE similar to the result seen when tamoxifen, an established human anticancer drug is being used. It has been observed IC₅₀ values for ethanol 5.0 ug/ml. Furthermore, cell treated with *Tinospora cordifolia* extracts detected to be shrunken and detached compared to the untreated cell after 72 hours incubation period. As conclusion, TC has a dose dependent antiproliferative activity against MCF-7 breast cancer cells and the lowest IC₅₀ was seen in ethanol extract. Further studies are needed to elucidate the mechanism of cell death induced by TC.

Key words: *Tinospora cordifolia* (Guduchi), antiproliferative activity, breast cancer, MCF-7 cell lines etc

INTRODUCTION

In India, TC is a well-known traditional medicine plant. To date, there are numerous studies on this plant emphasizing its antioxidant, antidiabetic, antimalarial and cosmetic effects. A comprehensive range of pharmacological benefits including: antioxidant, immunomodulatory, brain-strengthening and memory-enhancing, gastro protective, anti-inflammatory, antiulcer, and wound-healing and antibacterial characteristics, have been well demonstrated by modern research. On the other hand, cancer and associated chemo/radiation therapy side effects have escalated into a significant global public health issue, including in India.

Since ancient times, the Indian shrub TC, often known as "*Guduchi*," a member of the *Menispermaceae* family, has been a key component of traditional Indian medicine. TC is a 300 m tall, spiky deciduous shrub that can be found in China and the tropical Indian subcontinent [1]. Particularly noteworthy is how it is used in various sections of the nation in traditional or tribal medicine. In ancient Ayurvedic literature, TC is referred to as "heavenly nectar" and "nectar of immortality" and has been used to heal a number of ailments [2]. "*Amrita*" is another name for it, due to its immunomodulatory and adaptogenic qualities [1]. TC has been used traditionally for millennia to treat a variety of conditions, including psoriasis, gout, asthma, diabetes, anemia, dysentery, and diarrhea [3–4]. The plant contains alkaloids such as palmatin, berberine, and magnoflorin as well as glycosides like tinocorcid, tinocardifolioside, cordioside, and palmitoside, steroids like -sitosterol, and ecdysterone. The active components of 11-hydroxymystacon, a few diterpene lactones, and aliphatic chemicals are what give its therapeutic qualities. Various TC preparations are said to have antioxidant, antidiabetic, anxiolytic, antidepressive, and chemopreventive properties [5]. *Curcuma Longa* and *T. cordifolia* extract work incredibly well together to avoid hepatotoxicity [6]. Chloroform glycosides, which were separated from the chloroform extract of *T. cordifolia*, have been shown in several recent researches to have beneficial nutritional and anticancer properties [7–8].

A 50% ethanolic extract of *T. cordifolia* (TCE) has recently been shown to have anticancer and differentiation-inducing properties in the treatment of nerve cancer [9–10]. TCE was purified utilizing increasing polarity solvents, including butanol, hexane, chloroform, and ethyl acetate. The goal of the current investigation was to find out whether TCE in the solvents chloroform, hexane, ethyl acetate, and butanol have any anticarcinogenic properties. The human neuroblastoma cell lines employed as a model system in this study, IMR-32 and U87MG, stage IV human glioblastoma multiforme, have a long history of usage in vitro studies of brain malignancies. Only Chl-TCE and Hex-TCE, out of the four fractions, exhibited antiproliferative activity, according to the initial MTT screen. Then, these two portions were applied to thorough analyses of molecular markers. Expression of the glial fibrous acid protein (GFAP) is indicative of glioblastoma differentiation, whereas expression of the microtubule-associated protein (MAP-2) is indicative of neuroblastoma differentiation [11–12]. Studies on GFAP expression are extremely helpful for understanding neurological diseases as well as brain physiology [13]. The dendrites of terminally differentiated postmitotic neurons have been found to express MAP-2, and this in vitro expression also causes microtubule bundle stabilization [14]. The ability to accelerate aging was also tested by putting the heat shock proteins Lethal and HSP70 to the test. Mortalin, an age marker, is involved in the regulation of cell proliferation and the suppression of apoptosis, whereas HSP70, a stress response protein, is crucial for cell differentiation and proliferation [15–16]. By monitoring the expression of the neural cell adhesion molecule (NCAM), which is also crucial for preventing the growth of malignant tumors by controlling neurite outgrowth and matrix membership, the antimigratory potential was, examined [17–19].

Primary astrocytes and hippocampus neurons from the brains of 0–2 day old rat pups were also treated to these extracts in order to determine whether they specifically destroy tumor cells or are hazardous to normal cells.

MATERIAL AND METHOD

Preparation of a Ethanolic Extract of *T. Cordifolia*(TCE)

Stem of *Tinospora cordifolia* (TC) collected from fields in the month of January 2023 from Local area of Hyderabad India, and the collection procedure was in compliance with the national and international guidelines and legislation. The stem of TC was dried in shade at room temperature for 07-08 days and ground using electric grinder. First, the dried sample was extracted with a solvent of ethanol (2500 mL) at 40°C for 28 h in a Soxhlet apparatus. The residue was dried at room temperature on a rotary vacuum evaporator and stored the Ethanolic extract *Tinospora Cordifolia*(TCE) using a 4°C temperature.

Cancer cell cultures and In vitro Cytotoxicity Assay

MCF-7 cells were purchased from the National Center for Cell Science (NCCS) in Pune, India. The cell culture medium used in this study was DMEM/F12 with 50 units/ml penicillin, 50 µg/ml streptomycin and 10% bovine serum (FBS). The antitumor potential of the fractions was assessed using the MTT test with minor modifications (Gomathi AC et al. 2020). Briefly, 1 x10⁶ cells per well were placed in a 96-well plate and then incubated for 24 hours. The culture medium was discarded and the cells were treated for 24 hours at different concentrations (200- 2000 µg/ml) in a humidified incubator at 37°C with a 5% CO₂ chamber. After 24 hours of incubation, the MTT test was performed. Briefly, the culture medium was discarded again and fresh FBS-free culture medium (200 µl) containing 20 µl MTT (5 mg/ml) was added to each well and incubated at 37°C for 4 hours to generate a formazan crystal. The crystals were dissolved by adding 150 µl DMSO and incubated for 15 minutes. The absorbance was recorded at 570 nm with an ELISA microplate reader. Cell viability values were determined using the following formula:

Cell viability (%) = (experimental absorbance/control absorbance) x 100%. Using Invitrogen

EVOS fluorescence microscopies (Life Technologies), morphological changes in cells were examined and bright field images of control and treated cells were taken. At least three independent experiments were performed to obtain accurate data.

RESULT AND DISCUSSION

The antitumor potential of the fractions was assessed with minor modifications using the MTT test (Gomathi et al. 2020, Pandey et al. 2020). The result of the MTT tests shows that the TCE has a greater cytotoxic effect by reducing cell viability over the MCF-7 cell line. The fraction showed remarkable inhibition of MCF-7 cell lines with an IC₅₀ value 750 µg/ml. To examine the nature of the morphological changes, bright field bioimaging of cells treated with the control group was performed. Morphologic bioimaging reveals cytotoxic and anti-proliferative effects showing that the morphology and growth of MCF-7 cells exhibit typical apoptotic morphologic changes including separation from surrounding cells, cell shrinkage and rounding more common in treated cells. The fractions showed antitumor potential and reduced cell growth in a dose- dependent manner.

In some previous studies, namely Siripong et al. (1992) reported the cytotoxic effect of a crude methanolic extract of dried and coarsely powdered *Tinospora cordifolia* against P388 cells (lymphoid leukemia) and KB cells (human squamous cell carcinoma), while Matsuda et al. (1994) reported the antitumor potential of aerial part of *Tinospora cordifolia* extract and some of its compounds isolated on M1 cells (mouse myeloid leukemia). These reports are consistent with our results obtained with MCF-7 cells.

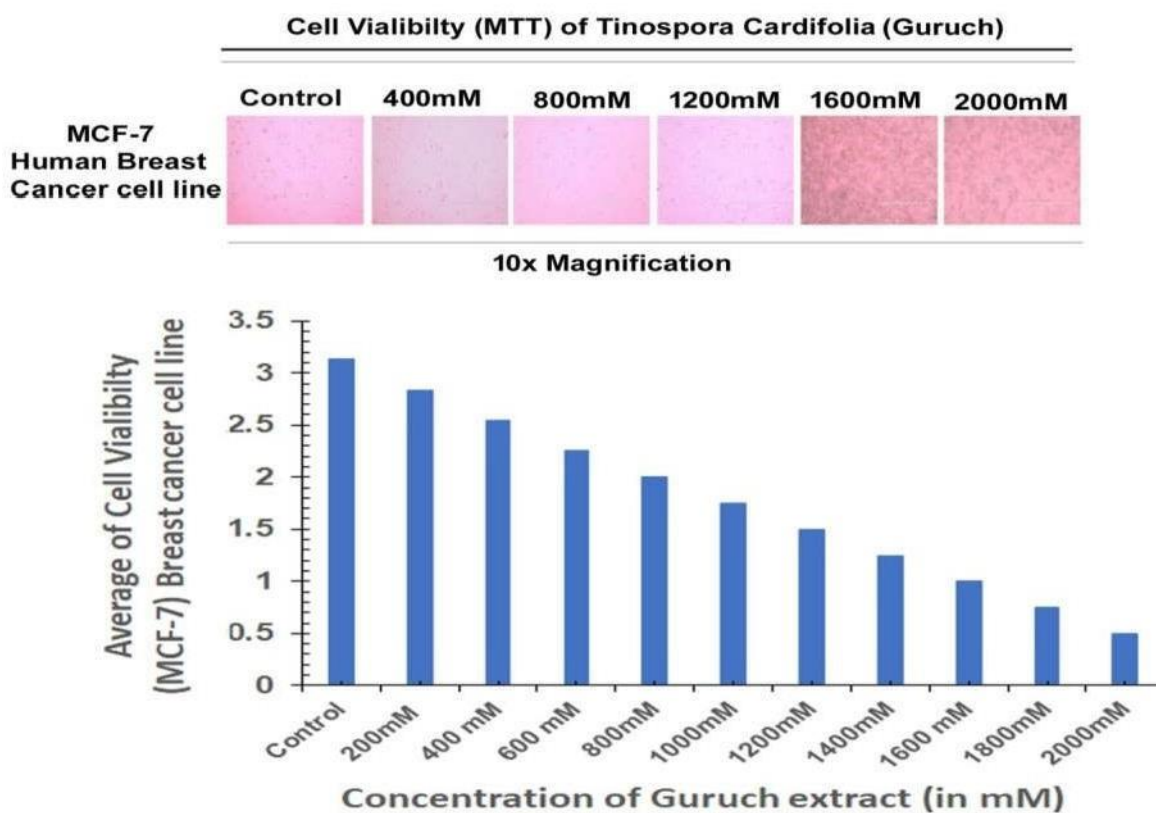


Figure: 1

Figure 1. (A) MTT assay on human breast adenocarcinoma (MCF-7) cells after 24 h incubation (B) Extent of alterations in morphology of MCF-7 cells upon treatment Scale bar: 200 μm

Despite enormous developments in medical technology for its diagnosis and treatment, cancer remains arguably the most debilitating and progressing disease in the world. The creation of medications that selectively eliminate tumor cells or at the very least restrict their multiplication without having negative side effects is essential for the efficacy of cancer chemotherapy. We extracted ECD from the stems of *T. cordifolia* plants, which are known to be abundant in diterpenoids generated from clerodane ECD triggered MCF-7. In this study, *T. cordifolia* was named because this shrub is native to India and easy to Search (Virginia and Premila, 2006). Its medicinal use in the treatment of breast cancer has applications in traditional medicine (Kumar et al., 2020). In older books like Sushruta Samhita and Charaka Samhita, it was called Guduchi (protection of conditions). The active ingredients of this plant found in medicinal products include glycosides, steroids, sesquiterpenoids, phenols, polysaccharides, diterpenoid lactones, aliphatic compounds and alkaloids (Upadhyay et al., 2010). Its leaf and stem been reported to have anti-inflammatory, anti-diabetic, anti-tumour, etc. (Ghosh and Saha, 2012). The attention-dependent induction of cell death by TCE was shown by the MTT experiment. The findings are consistent with those of Palmieri et al. (2019), Rao et al. (match), and Rao and Rao (2010), who

observed reduced cell viability in various cell lines. By inducing apoptosis, the fragment demonstrates anticancer action, as demonstrated by annexin V staining and verified by caspase stress testing. The attention-dependent remodeling of a mitochondrial membrane caused apoptosis. The outcomes are consistent with those reported by Mishra et al. in a glioblastoma cell line (Mishra and Kaur, 2013). One of the important routes in the genesis of cancer, the epithelial- mesenchymal transition, is regulated by this extract. The outcomes are consistent with those of Palmieri et al. (Palmieri et al., 2019), who found that the fragment had a comparable effect on the epithelial-mesenchymal junction in a fatal colon cancer cell line (MCF-7). But the metastatic genes were unaffected by this snippet. The findings conflict with those of Leyon et al. When the polysaccharide component was administered intraperitoneally to mice that had already been implanted with tumor cells, they found in their study that B16-F10 tumor cells' capacity to metastasize was significantly reduced (Leyon and Kuttan, 2004). This might be the result of a different active ingredient in the plant extract, which may have affected the phytochemical and therapeutic characteristics. Furthermore, different results might be obtained depending on how the examined cell line differs from others.

A rising population of cancer stem cells is in charge of metastasis, medication resistance, and washout. This is influenced by a variety of elements, and proteins that regulate stem cell behavior in healthy stem cells also affect cancer stem cells. Oct4, Sox2, Nanog, and CD34 are a few of them. Comparing gene expression in treated and untreated cells in the current study revealed that TcE treatment decreased the expression of the three crucial genes, Oct4, Sox2, and Nanog. In actuality, the values have decreased. In treated and stripped cells, expression was neither significant nor significant, presumably because the prevalence of cancer stem cells is significantly lower in the whole population of cell lines. After the cancer stem cell population has been separated from the cell lineage, this effect on cancer stem cells is anticipated to occur again. Berberine (BBR), which has been described by scintillating authors for its healing properties such as those for diabetes, gastroenteritis, dyslipidemia, heart disease, subversive conditions, and roundness, is responsible for the anticancer effects of TcE (Chen et al., 2014; Lahiri and Dutta, 1967; Liu et al., 2015; Sun et al., 1988). In labeled cell lines, this emulsion's anticancer effects have also been documented (Tillhon et al., 2012). According to studies conducted by Albring et al. (2013) and Chidambara Murthy i. (2012), BBR induces apoptosis by activating Janus kinase, the p38 mitogen-activated protein kinase pathway, suppressing Wnt/- catenin signaling, and inhibiting mitosis by generating a G1/S and G2/cell cycle arrest.

CONCLUSION

Tinospora cordifolia and its phytochemicals, including palmatin and berberine, have been found to be cytotoxic in various preclinical transplant culture models and strongly tinted deadly cell lines. TC and its components' capacity to promote free conformation and DNA damage in developing cells may be the cause of their cytotoxic effects. By include lipid peroxidation and lactate dehydrogenase, they also lower the antioxidant state of the developing cells. TC and its phytochemicals can have cytotoxic effects on developing cells by molecularly inhibiting the topoisomerases that cause DNA damage. Cytotoxicity in proliferating cells may be induced by inhibiting NF-B, COX-II, Nrf2, STAT3, Bcl-2, Ca release, cyclin-dependent kinase (CDK) 2, CDK4, cyclin B, cyclin D, and cyclin E. Proliferating cells would experience an increase in

apoptosis as a result of the activation of p53, Wee1 and CDk1, Bax, P27, procaspase-9, caspase-9, caspase-3, and poly(ADP-ribose) polymerase (PARP).

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