Original Research Article

Evaluation of antitumour activity of ethanolic extract from *Tribulus terrestris* in human breast cancer cells

Running title: Antitumor activity of ethanolic extract of *Tribulus terrestris* in MCF-7 cells

Type of article: Original Research Article

ABSTRACT

Introduction: Breast cancer is the form of cancer that occurs in the breast cells. After skin cancer, breast cancer is considered to be the most common cancer diagnosed in women. The number of deaths associated with this disease was increased in case of lack of early detection. *Tribulus terrestris* is an annual herb belonging to the Zygophyllaceae family. Flavonoids, alkaloids, and saponins are some of the main phytoconstituents of the herb. They are known for their pharmacological actions such as anti carcinogenic, anti inflammatory, antimicrobial, antioxidant properties.

Aim: The aim of the present study is to evaluate the antitumor activity of ethanolic extract from *Tribulus terrestris* in MCF-7 human breast cancer cells.

Materials and Methods: The effect of *Tribulus terrestris* on cell viability was measured by MTT assay carried against breast cancer cells and morphological changes were investigated with phase contrast microscopy to confirm its antitumor activity.

Results: the cell viability assay results indicate that 24hrs treatment with ethanolic extract of significantly reduces the cell viability in dose dependent manner. At 40 µg/ml of the *Tribulus terrestris* extract inhibits 50% cell viability and it has been fixed as IC_{50} value for further *Tribulus terrestris* experiments.

Conclusion: From the results, the extracts were cytotoxic to the human breast cancer cell and it might be a good therapeutic value for further investigations needed to understand the mechanisms to develop antitumor agents.

Keywords: Anti-tumour; MTT assay; *Tribulus terrestris*; Breast cancer; Cytotoxicity

1. INTRODUCTION

Breast cancer is a kind of cancer that develops in the breast cells. Breast cancer is the second most prevalent cancer diagnosed in women, after skin cancer. In the case of early discovery, the number of deaths connected with this condition is constantly decreasing. In the last decade, there has been significant progress in both the knowledge of breast cancer and the development of preventative therapies (1). Amongst all the malignant cancer types, breast cancer is considered as one of the main causes of death in post menopausal women for 23% of all cancer deaths (2). Screening mammography helps to identify breast cancer at prior stages of the disease (3). Breast cancer develops in the lobules or ducts. It can also arise in the breast's fibrous connective tissue or fatty tissue. Lymph nodes serve as a main route for cancer to spread to other regions of the body. Breast cancer symptoms include lumps, thickening, swelling, discomfort, dimpling, redness, and flaky skin. Breast cancer is classified into four types: ductal carcinoma in situ, invasive ductal carcinoma, inflammatory breast cancer, and metastatic breast cancer. Breast cancer is caused mostly by reproductive and hormonal causes. Other variables that contribute to breast cancer include ionising radiation exposure and a hereditary susceptibility. Long-term exposure to high levels of endogenous estrogens raises the risk of breast cancer in menopausal and postmenopausal women (4).

Tribulus terrestris is an annual herb belonging to the Zygophyllaceae family. Flavonoids, alkaloids, and saponins are some of the main phytoconstituents of the herb. They are known for their pharmacological actions such as anti-carcinogenic, anti-inflammatory, antimicrobial, antioxidant properties. The fruits and roots of the plant is constantly used as a folk medicine for more than thousand years (5). The preparations from the plant parts are specifically popular for health issues and diseases such as hormonal imbalance, sexual problems and other heart diseases (6). In recent years, the beneficial effects of the plant have been found and the use of the plant is gradually increasing in developing countries (7).

Ethanolic extract is considered as a single stream process which can be done under both warm and cold conditions. It can be used as a solvent of extraction at room temperature or under supercooled temperature. Different levels of ethanolic extract of *Tribulus terrestris* were tested to enhance reproductive performance and to reduce hormonal imbalance (8). Our team has extensive knowledge and research experience that has translate into high quality publications (9-42). The aim of the study is to evaluate the antitumor activity of ethanolic extract from *Tribulus terrestris* using MCF-7 human breast cancer cells.

2. MATERIALS AND METHODS

2.1. Chemicals:

Dulbecco's Modified Eagle Medium (DMEM) medium, 0.25% Trypsin-EDTA solution, sodium bicarbonate solution, bovine serum albumin (BSA), low melting agarose, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) from Sigma Chemicals Co., St. Louis, USA. Fetal bovine serum (FBS) and antibiotic/antimycotic solution, Dimethyl sulfoxide (DMSO) were from Himedia, Sodium phosphate monobasic and dibasic, sodium chloride, sodium hydroxide, sodium carbonate, hydrochloric acid and methanol were purchased from Sisco Research Laboratories (SRL) India.

2.2. Preparation of ethanolic plant extract:

The whole plant of *Tribulus terrestris* powder was commercially purchased from The Indian Medical Practitioners Co-operative Pharmacy and Stores Ltd (IMPCOPS), (Chennai, India) for the present study. 100gm of powder with 500ml of 95% ethanol mixed well and kept at room temperature for 3 days in a static condition. Then the extract was filtered and fixed into a soxhlet apparatus subjected to evaporation at room temperature till a semisolid mass and further it was concentrated in vacuum evaporate and immediately stored at 4°C for further experiment.

2.3. Cell line and culture:

The Human breast cancer cells, MCF-7 was procured from National Centre for Cell Science (NCCS) Pune, India. The cells were grown in T25 culture flasks containing DMEM high glucose medium provided with 10% FBS, Penicillin (100 IU/ml), Streptomycin (100 µg/ml) and Amphotericin B along with 7.5% sodium bicarbonate and incubated at 37°C in 5% CO_2 incubator. After 3 days, about 80-90% confluent monolayer (adherent) formation was confirmed by inverted microscope and it was carried for further experiment by using Trypsin-EDTA solution.

2.4. Cell proliferation (MTT) Assay:

This MTT assay is used to determine the IC_{50} concentration of *Tribulus terrestris* extract on MCF-7 cells (43). For MTT assay, cells were placed in 96-well plates at the density of 10000 cells/well and were incubated for 24 hours at 37°c in 5% CO_2 for attachment of cells. After 24 hours various concentrations (50, 100, 150, 200, 250, 300 µg/ml) of *Tribulus terrestris* were added to the cells and incubated for 24 hrs at 37°C. After incubation, the medium was replaced with 10 µl µl of MTT (5 mg/ml) dye in serum free medium was added per well and wrapped with aluminium foil and incubated for further 4 hours dark at 37°C. Then, 100 µl DMSO was added to the wells to solubilize the formazan crystals. The absorbance was measured at 570 nm. The percentage of cell inhibition was determined by following formula

$$\%\ of\ cell\ viability = OD\ of\ test / OD\ of\ control \times 100$$

2.5. Cell Morphological studies by Phase contrast microscope

Morphological changes in the cancer cells before and after *Tribulus terrestris* extract treatment can be studied with the help of phase contrast microscope. At the end of the experiment the cells were taken and observed under an inverted light microscope with 20x magnification.

2.6. Statistical analysis:

All the data from the results were analyzed by t-test and depicted as mean ± SD. The results were statistically analysed using one way ANOVA in SPSS software. The statistical significance was at $p<0.05$.

3. RESULTS AND DISCUSSION

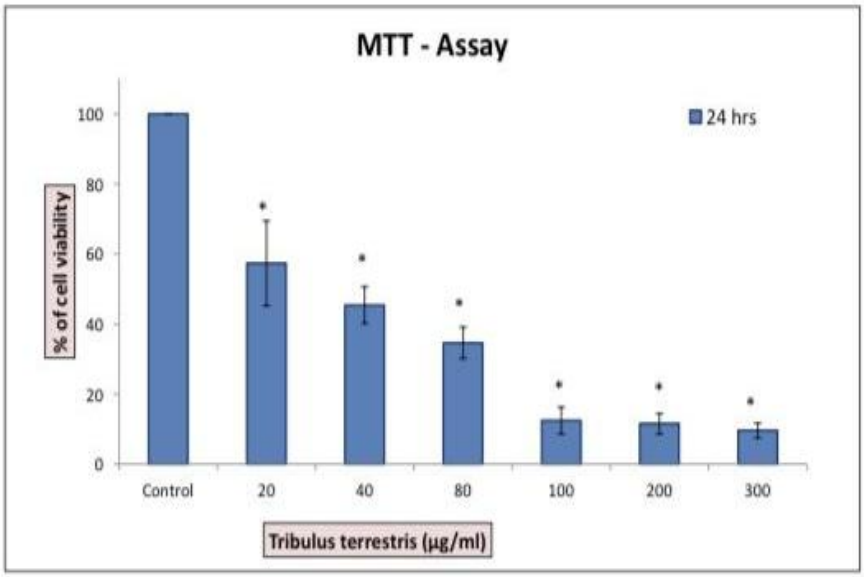

Figure 1: Bar graph representing the antitumor activity of *Tribulus terrestris* ethanolic extract via MTT assay at a regular interval of 24 hours. X axis represents the different concentrations of *Tribulus terrestris* ethanolic extract in (microgram/ ml) while Y axis represents the percentage of cell viability (in numbers). Data are shown as means ± SD (n = 3). * compared with the control-blank group, $p < 0.001$.

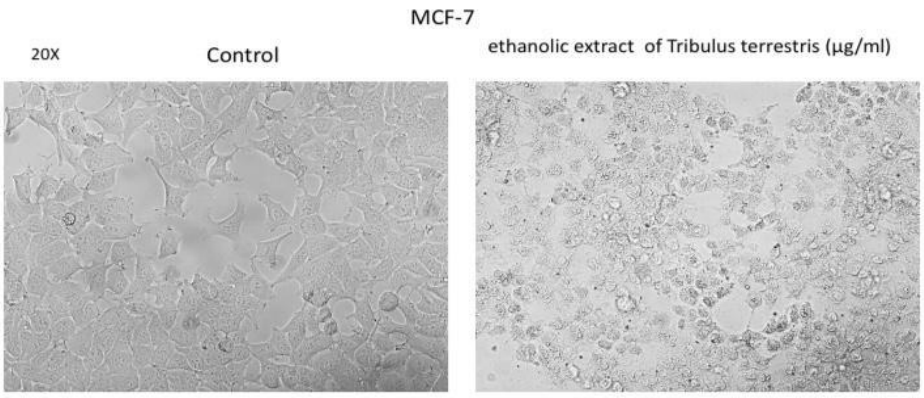

Figure 2: Figure representing the comparison of antitumor activity of *Tribulus terrestris* ethanolic extract against human breast cancer cells with the control where at 40 µg /ml concentration. Image viewed under phase contrast microscope at 20X magnification.

Tribulus terrestris is one of the plants which is used for a long time in treating various diseases (44). The plant is known for its anticancer, antidiabetic, antispasmodic, antifungal, antibacterial, anticariogenic and many more activities which make this a vital medicine for many ailments (45). Saponins present in the plant are found to be responsible for antifungal and antibacterial (46). The reason for choosing this plant is that it has a role in reducing hormonal imbalance and increasing reproductive function. Dysregulated proliferation of cells is seen in various types of cancer (47). In many countries, cancers are evolving as a reason for malignancy associated with death (48). Many drugs are being discovered for potent protective effects against cancer cells (49). In a previous study by Arumugam et al., it was discussed that cell cycle arrest by compounds can apoptitize cancerous cells and end proliferation of cells (50). In the present study, the cell viability assay showed that, cell growth inhibition was observed in dose dependent manner. The ethanolic extract of *Tribulus terrestris* treatment significantly reduces the breast cancer cell viability. At 40 µg/ml concentration of the extract arrests 50% of the human breast cancer cells proliferation. And also, *Tribulus terrestris* extract (40 µg/ml) treatment significantly induces the apoptosis, which was evidenced in phase contrast microscopic analysis. The cell morphological changes observed in *Tribulus terrestris* extract (40 µg/ml) treated reast cancer cells at 24hrs time point.

In the study by Apurva et al., the extract of the *Tribulus terrestris* plant seems to increase the caspase 3 activity of the human breast cancer cell line (51). In the study by Masoud et al., the herb is also found to have anticancer activity against prostate and colon cancer cell lines (52). It was inferred that the aqueous extract of the plant had a high cytotoxic effect and it can be suggested for marketing in the study by Claudio et al. (53). In the study by Farooq et al., it was inferred that saponins present were responsible for the anti tumor and anti proliferative function

of the plant (54). In the present study, it is clear that the anti cancer activity of the ethanolic extract of *Tribulus terrestris* plant is completely concentration dependent. At different concentrations, the activity of the extract differs. The limitation is that it is an *in vitro* study. Further experimental validation is required for establishment as an anticancer treatment against human breast cancer cells (24, 55-68).

4. CONCLUSION

From the above results, the *Tribulus terrestris* extracts were cytotoxic to the human breast cancer cell line MCF-7 at this dose dependent concentration. However more research is needed to understand the mechanisms of cytotoxicity and this study provides scope for future studies.

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