

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 from *Tribulus terrestris* in human breast cancer cells

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 were 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 *T.terrestris* significantly reduces the cell viability in dose dependent manner. At 40 µg/ml of the *T.terrestris* extract inhibits 50% cell viability and it has been fixed as IC₅₀ value for further 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: Antitumour; Innovative technique; Green synthesis; MTT assay; *Tribulus terrestris*; Breast cancer

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 is steadily declining in case of early detection. In the recent decade progress has developed in the understanding of breast cancer as well as preventive methods(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). The cancer forms in the lobules or ducts of the breasts. It can also occur in the fibrous connective tissue or the fatty tissue of the breast. Lymph nodes form a primary pathway for the cancer to move to other parts of the body. Lumps, thickening, swelling, irritation, dimpling, redness, flaky skin are some of the warning signs of breast cancer. Ductal carcinoma in situ, invasive ductal carcinoma, inflammatory breast cancer and metastatic breast cancer are the types of breast cancer. Reproductive and hormonal factors form the main reason for breast cancer. Exposure to ionising radiation and genetic predisposition are some of the other factors causing breast cancer. Long term exposure to high concentrations of endogenous estrogens increases the risk of breast cancer in pre and post menopausal women.

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(4). The preparations from the plant parts are specifically popular for health issues and diseases such as hormonal imbalance, sexual problems and other heart diseases(5). In recent years, the beneficial effects of the plant have been found and the use of the plant is gradually increasing in developing countries(6).

Ethanol 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(7). Our team has extensive knowledge and research experience that has translate into high quality publications(8–12),(13),(14),(8),(15),(16),(17),(18)(10,19,20),(21–25) (26) (27) (28) (29) (29,30) (31) (32) (33) (34) (35) (36) (37) (38) (39) (40) (41) (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.

Materials and Methods

Chemicals:

DMEM medium, 0.25% Trypsin-EDTA solution, sodium bicarbonate solution, bovine serum albumin (BSA), low melting agarose, MTT from Sigma Chemicals Co., St. Louis, USA. fetal bovine serum (FBS) and antibiotic/antimycotic solution, 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.

Preparation of ethanolic plant extract:

The whole plant of *Tribulus terrestris* powder was commercially purchased from 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 was concentrated in vacuum evaporate and immediately stored at 4°C for further experiment.

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% CO₂ 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.

Cell proliferation (MTT) Assay:

This MTT assay is used to determine the IC₅₀ 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% CO₂ for attachment of cells. After 24 hours various concentrations of *T. terrestris* 50, 100, 150, 200, 250, 300 µg/ml were added to the cells and incubated for 24 hrs at 37°C. After incubation, the medium was replaced with 10 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

$$\% \text{ of cell viability} = 100 - \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

Microscopic analysis

Phase contrast microscopic studies

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.

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.

Results and Discussion

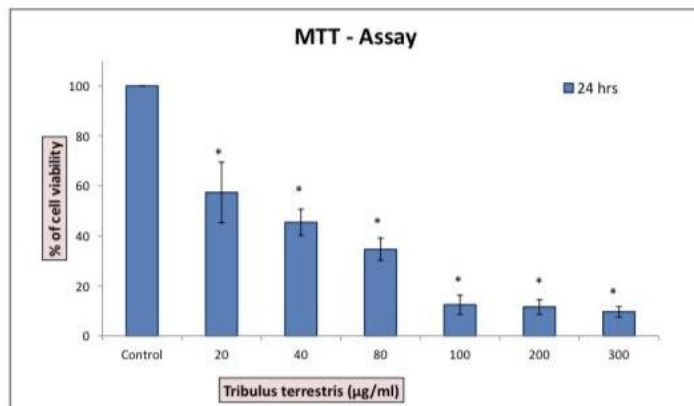


Figure 1: Bar graph representing the antitumor activity of Tribulus terrestris Ethanolic extract via MTT assay at a regular interval of 24 hours. X axis represents the different concentrations of Tribulus terrestris Ethanolic extract in (microgram/ ml) while Y axis represents the percentage of cell viability (in numbers)

Data are shown as means \pm SD (n = 3). * compared with the control-blank group, $p < 0.001$.

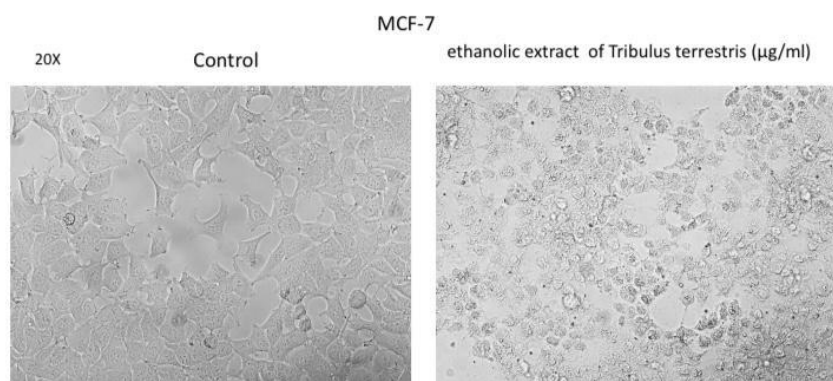


Figure 2: Figure representing the comparison of antitumor activity of Tribulus terrestris Ethanolic extract against human breast cancer cells with the control where at 40 microgram/ml concentration. Image viewed under phase contrast microscope at 20X magnification.

On observing the cell viability assay graph, it was found that increasing dose dependent ethanolic extract of *Tribulus terrestris* treatment significantly reduces the cell viability . At 40 µg/ml concentration of the extract arrests 50% of the human breast cancer cells proliferation.

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 makes 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 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) (56) (57) (58) (59) (60) (61)(62) (63) (64) (65) (66) (67) (68). This study can be used as a reference for future studies with the same plant extract.

Conclusion:

From the results, the extracts were cytotoxic to the human breast cancer cell line MCF-7 at this 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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