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Activity of Hydroethanolic Leaf Extract of *Tecoma stans* against Breast Cancer Cells Line - MCF - 7

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Cancer is abnormal growth of cells, which invade or spread to other parts of the body. There are over 200 types of cancers. Breast cancer is a cancer which develops in tissues of the breast, most commonly originating from the inner lining of milk ducts or lobules that supply ducts with milk. *Tecoma stans* commonly known as trumpet flower is a yellow trumpet shaped flower belonging to Bignoniaceae, and is native to Americas. It has anti cancer and anti proliferative properties.
Aim: Aim of the is to analyse the anti cancer activity of Tecoma stans against breast cancer cells MCF-7.
Materials and Methods: Breast cancer cell line (MCF-7) was purchased from National Centre for Cell Sciences (NCCS), Pune, India. Cell viability test was done by MTT assay. BcL2 and BclxL

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gene expression analysis was done by Real Time-PCR. The obtained data were analysed statistically by one-way analysis of variance and Duncan's multiple range test with Graph Pad Prism version 5 to analyse the significance. The significance was considered at $p<0.05$ level in Duncan's test.

Results: The results suggest Bcl2 mRNA gene expression is reduced on induction of 400, 500 ug/ml of *Tecoma stans* with significant difference in comparison with control. Bcl-xL mRNA gene expression is slightly reduced on induction of 400, 500 μg/mL of Tecoma stans with no significant difference in comparison with control.

Conclusion: From the obtained results, it can be concluded that hydroethanolic leaf extract of *Tecoma stans* possess anticancer activity against breast cancer cells with respect to Bcl2 mRNA gene expression as compared to Bcl xL mRNA gene expression. If complete research is done, we can use this drug to treat various cancers.

Keywords: Tecoma stans; cancer; gene expression; BcL2; BclxL; innovative.

1. INTRODUCTION

Cancer is abnormal growth of cells, which invade or spread to other parts of the body. There are over 200 types of cancers. Possible symptoms are cough, weight loss, abnormal bleeding and change in bowel movements. Tobacco usage is the main cause of about 22% of deaths of cancer. In the year 2015, about 90.5 million had been diagnosed with cancer [1]. As of 2019, about 18 million new cases occur annually as per the statistics [2]. 8.8 million people die annually due to cancer. There are four different types of cancer: carcinomas, sarcomas, leukemias and lymphomas. The most common types of cancer in males are colorectal cancer, lung cancer and stomach cancer. In females, the most common types of cancers are breast cancer, colorectal cancer, lung cancer and cervical cancer. Breast cancer ranked first among the most diagnosed cancers in India.

Breast cancer is a cancer that originates in the tissues of the breast. Breast cancer is diagnosed very commonly in women and rarely in men. In 2004, 5,19,000 people died due to breast cancer worldwide [3]. Cancer cells are formed from the normal cells due to a modification of DNA or RNA. Due to improper functioning of the immune system, cancer develops. Breast cancer develops mostly in cells that line the ducts (ductal cancer). Some of them develop in cells that line the lobules (lobular cancers). The first symptoms of breast cancer is appearance as an area of thickened tissue in the breast or a lump in the breast, or an armpit. Other symptoms include pain in armpits or breast which doesn't change with the monthly cycle, redness of skin of breast, rash around or one of the nipples, blood discharge from a nipple, or change occur in size occur in size or shape of the breast, and peeling, flaking, or scaling of the skin on the breast or nipple. Breast cancer can be treated stage wise. The experience from our previous studies [4-13] have led us to focus on the current topic.

Tecoma stans is commonly known as trumpet flower is a yellow trumpet shaped flower belonging to Bignoniaceae, and is native to Americas.Yellow, trumpet brush, yellow bells are few common names to the species. The genus *Tecoma* comprises [14] species of small trees. *Tecoma* is an ornamental plant which is fast growing in India [14] It is widely spread in an area of tropical and subtropical regions. It's native to Florida, West Indies and Mexico to South America. A leafed *Tecoma stans* infusion can be taken orally for treating diabetes and stomach pains. Most people are not aware that the species *Tecoma stans* have anticancer properties in their leaf extracts. Our team has research experience that has translate into good quality researches [15-34]. The aim of the present study is to analyse the activity of hydroethanolic leaf extract of Tecoma stans against breast cancer cells though m RNA gene expression of Bcl2 and Bcl xl genes.

2. MATERIALS AND METHODS

This is an *in-vitro* experimental study conducted in a private dental college and hospitals in Chennai.

2.1 Procedure

Dimethyl sulfoxide (DMSO),3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)were purchased from Sigma Chemical Pvt Ltd, USA. Trypsin-EDTA, fetal bovine serum (FBS), antibiotics-antimycotics, RPMI 1640 medium andphosphate buffered

saline (PBS) were purchased from Gibco, Canada. (5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazolocarbocyanine iodide) and Real Time PCR kit was purchasedTAKARA (Meadowvale Blvd, Mississauga, ON L5N 5S2, Canada).

2.2 Cell Lines and Cell Culture

Human breast cancer cell line (MCF-7) was purchased from National Centre for Cell Sciences (NCCS), Pune, India. Cells were cultured in DMEM medium (Thermo Fisher Scientific, CA, USA) containing 10% fetal bovine serum (Thermo Fisher Scientific, CA, USA), 100 U/ml penicillin and 100 µg/ml streptomycin (Thermo Fisher Scientific, CA, USA) at 37°C with 5% CO2.

2.3 Cell Proliferation Assay by Cell Viability

Cell proliferation was determined using the MTT assay. MCF-7 cells were seeded in 96-well plates with 5×103/200 µl and cultured overnight. Cell viability was assayed using a modified colorimetric technique that is based on the ability of live cells to convert MTT, a tetrazolium compound into purple formazan crystals by mitochondrial reductases (Mosmann, 1983). Briefly, MCF-7 cells(1 ×104/well) were exposed to different concentrations of hydroethanolic leaf extract of Tecomastans (100-500µg) with MCF-7 cells for 48 h. At the end of the treatment, 100 µl of 0.5 mg/ml MTT solution was added to each well and incubated at 37 ◦C for an hour. Then the formazan crystals formed were dissolved in dimethyl sulfoxide (100 µl) and incubated in dark for an hour. Then the intensity of the color developed was assayed using a Micro ELISA plate reader at 570 nm. The number of viable cells was expressed as the percentage of control cells cultured in serum-free medium. Cell viability in the control medium without any treatment was represented as 100%. The cell viability is calculated using the formula: % cell viability = [A570 nm of treated cells/A570 nm of control cells] × 100.

2.4 Gene Expression Analysis by Real Time-PCR: Procedure

Samples from each group were submerged in 2 ml Trizol (Invitrogen, Carlsbad, CA, USA) for RNA extraction and stored at −80°C until further processed. cDNA synthesis was performed on 2 µg RNA in a 10 µl sample volume using Superscript II reverse transcriptase (Invitrogen) as recommended by the manufacturer. Real-time PCR array analysis was performed in a total volume of 20 µl including 1 µl cDNA, 10 µl qPCR Master Mix 2x (Takara, USA) and 9 µl ddH2O. Reactions were run on an CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA) using universal thermal cycling parameters (95°C for 5 min, 40 cycles of 15 sec at 95°C, 15 sec at 60°C and 20 sec at 72°C; followed by a melting curve: 5 sec at 95°C, 60 sec at 60°C and continued melting). For quality control purposes, melting curves were acquired for all samples. The specificity of the amplification product was determined by melting curve analysis for each primer pairs. The data were analyzed by comparative CT method and the fold change is calculated by 2−ΔΔCT method described by Schmittgen and Livak (2008) using CFX Manager Version 2.1 (Bio Rad, USA).

2.5 Statistical Analysis

The obtained gene expression status of control and test groups of different dosages were analyzed statistically by one-way analysis of variance (ANOVA) and Duncan's multiple range test with a computer-based software (Graph Pad Prism version 5) to analyze the significance of individual variations among the control and experimental groups. The significance was considered at $p<0.05$ level in Duncan's test.

3. RESULTS

3.1 Effect of *Tecoma stans* on the Cell Viability

Cell viability of Human breast cancer cells (MCF-7 cells) was determined using MTT assay after administering the different doses of *Tecoma stans*. It was found to exhibit inhibition of breast cancer cells by decreasing the percentage of viability of cancer cells in a dose dependent manner when compared to control. It was found that maximum inhibition of cell growth was at concentration (100-400 µg/ml) used in this study when compared to control (Fig. 1).

3.2 Effect of Bcl-2 mRNA Expression on the MCF-7 Cancer Cells (Fold Change over Control)

The mRNA expression of Bcl-2 was assessed in a dose dependent manner. The cancer cells were significantly inhibited and it was found that there was significant reduction in mRNA

expression of Bcl-2 when compared to control at a dose of 400 µg/ml. Further there was significant reduction in mRNA expression of Bcl-2 when compared to control at a dose of 500 µg/ml. Thus the decrease in gene expression was in dose dependent manner.

3.3 Effect of Bcl-xl mRNA Expression on MCF-7 Cells (Fold Change over Control)

The mRNA expression of Bcl-xl was assessed in a dose dependent manner. The cancer cells were significantly inhibited and it was found that there was significant reduction in mRNA expression of Bcl-xl when compared to control at a dose of 400 µg/ml. Further there was significant reduction in mRNA expression of Bcl-xl when compared to control at a dose of 500 µg/ml. Thus interestingly, the decrease was in dose dependent manner.

Table 1. Percentage of cell viability

Concentration	Cell viability in %
0	100
100	90
200	80
300	65
400	50
500	48
500	45

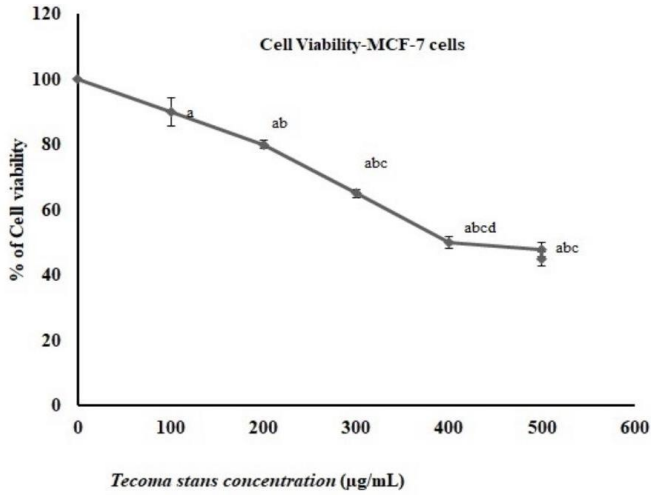

Fig. 1. Effect of hydroethanolic leaf extract of *Tecoma stans* on cell viability in MCF-7 cells. X axis represents different concentrations of Tecoma stans and the y axis represents the percentage of cell viability. There is a statistically significant difference observed in comparison with control in dose dependent manner with p< 0.05, a-compared with untreated control cells, b-compared with 100 µg treated cells, c-compared with 200µg treated cells

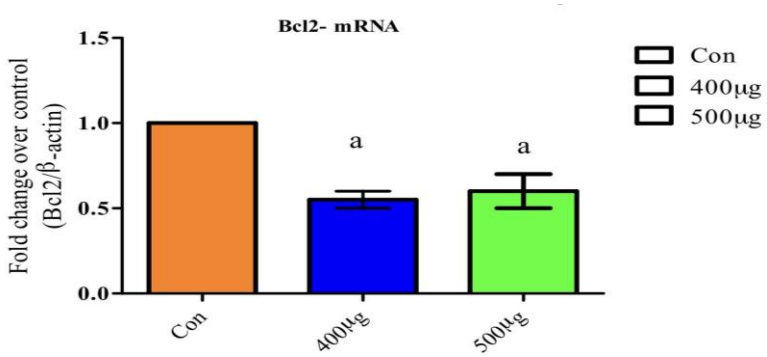

Fig. 2. Effect of hydroethanolic leaf extract of *Tecoma stans* on Bcl-2 mRNA expression in MCF-7 cells. X axis represents different concentrations of Tecoma stans and Y axis represents the fold change over control. A-compared with untreated control cells with 400µg/ml and 500µg/ml treated MCF-7 cells. There is a statistically significant difference between the test and control groups with p value < 0.05

Bcl-xl mRNA expression (Fold change over control)

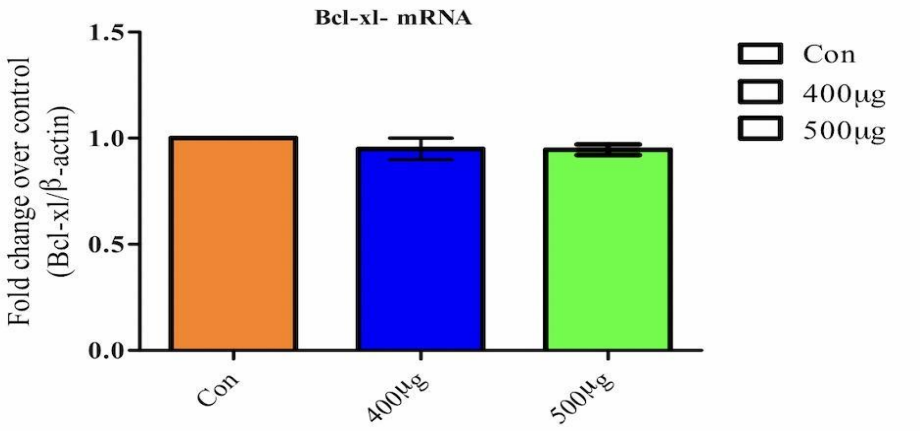

Fig. 3. Effect of hydroethanolic leaf extract of *Tecoma stans* on Bcl-xL mRNA expression in MCF-7 cells. X axis represents different concentrations of Tecoma stans and Y axis represents the fold change over control. A-compared with untreated control cells with 400µg/ml, b-compared with 500µg/ml treated MCF-7 cells. There is no significant difference between the test and control groups with p value < 0.05

4. DISCUSSION

Tecoma stans is a species on which different extracts of the plant are used for treating many diseases. This flower is taken to analyse the anti cancer activity against breast cancer cells. Two gene variables namely Bcl2 and BclxL are taken. Bcl2 gene on induction of increase in dosage from 400 µg/ml to 50 0µg/ml there was an increase in the anticancer activity in comparison with control. BclxL gene induction of increase in dosage from 400 µg/ml to 500µg/ml there was no significant increase in the anticancer activity in comparison with control.

The aqueous extract of flowers has anti-proliferative and antioxidant properties [35]. The methanolic extract of leaves has antispasmodic property [36]. Aqueous extracts of leaves possess antidiabetic activity by streptozotocin induced diabetes in Sprague dawley rats method [37]. The ethanol, methanol and aqueous extracts of leaves possess anti microbial activity and antioxidant activity [38]. The ethanol, methanol and aqueous extracts of heart wood of stem possess anti microbial activity against human pathogenic microbes [39,40]. The alcoholic leaf extract of flowers possess antifungal activity [41,42].

Cancer is one of the ailments which cannot be completely cured by chemotherapy. Research on *Tecoma stans* is very limited to generalise the activity in breast cancer cells. Although there are many new innovative approaches for discovery of drugs, such as combinatorial chemistry and computer based molecular modelling design, not even one of them cannot replace the importance of natural ailments [43,44].

Bcl2 mRNA gene expression is reduced on induction of 400, 500 µg/ml of *Tecoma stans* of significant difference in comparison with control. The article in Bcl2 in breast cancer is a favourable prognostic marker of molecular subtypes and independent adjuvant therapy is received [45]. Instead of Mcl-1 alone, or in the combination with other anti-apoptotic Bcl2 family members, may be an essential driver of tumor progression and mediator of therapeutic existence in breast cancer. *Tecoma stans* in Bcl2 gene have anticancer properties against breast cancer cells and can be treated or cured with natural ailments.

Bcl xL mRNA gene expression is slightly reduced on induction of 400, 500 µg/ml of *Tecoma stans* with no significant difference in comparison with control. The article is targeting Bcl-xL improves the efficacy of bromodomain and extra terminal protein inhibitors in triple negative breast cancer by eliciting the death of senescent cells [46]. Bcl xL gene should be predictive in tumour responses. There are no previous articles of anticancer properties of *Tecoma stans*. *Tecoma stans* in Bcl-xL have anticancer properties against breast cancer cells and can be cured without synthetic drugs and side effects.

The sample size used for the study is very less and cannot be generalised for a research to be done. It needs a large scale study. Large-scale study should done on *Tecoma stans* for anticancer activity against breast cancer in in-vivo animal studies and clinical trials.

5. CONCLUSION

From the obtained results, it can be concluded that hydroethanolic leaf extract of *Tecoma stans* possess anticancer activity against breast cancer cells with respect to Bcl2 mRNA gene expression as compared to Bcl xL mRNA gene expression. To generalize the result the study should be conducted with large sample size and invivo studies.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study is approved by the institutional review board.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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