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APOPTOSIS INDUCED IN MCF-7 BREAST CANCER CELL LINES BY BETA-SITOSTEROL ISOLATED FROM *ABUTILON INDICUM LINN.* ROOTS

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Abstract

Breast cancer is a prevalent malignancy among women. Numerous studies have indicated that the likelihood of developing breast cancer increases with age. Furthermore, the prevalence of breast cancer varies according to different types of biased traditional Chinese medicine constitution, with the Qi-depressed constitution being highly concomitant and increasing the prevalence of breast cancer. Consequently, the Qi-depressed constitution is considered a distinct risk factor for breast cancer. Previous research has demonstrated that the ethanol extract of *Abutilon indicum* and its components possess potential as an anticancer agent against MCF-7. In the present study, beta-sitosterol extracted from *Abutilon indicum* was examined in MCF-7 human breast cancer cell lines using resazurin assays and flow cytometry. Resazurin experiments demonstrated its ability to inhibit cell proliferation and trigger apoptosis, and flow cytometry was utilized to examine this capacity. Beta-sitosterol was found to inhibit MCF-7 breast cancer cell proliferation and exhibit apoptotic activity proportional to the dose. The findings of this study suggest that beta-sitosterol may be employed as a cancer treatment.

Keywords: *Abutilon indicum*, Apoptosis, Beta-sitosterol, MCF-7, Breast cancer

INTRODUCTION

Breast cancer is a significant cause of cancer-related fatalities, particularly in impoverished nations, ranking as the fifth-leading cause of such deaths and one of the highest causes of mortality worldwide. The International Agency for Cancer Research (IARC) focuses on 20 geographically diverse regions across the globe. According to the International Agency for Research on Cancer, breast cancer is the leading cause of cancer-related deaths among women[1].

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In 2018, there were 18.1 million new cases of cancer and 9.6 million cancer-related deaths, indicating that one in five men and one in six women worldwide were affected by the disease. The statistics reveal that one in eleven women and one in eleven men died of cancer. Breast cancer has the highest incidence rate among women of all types of cancer[2]. Based on data from GLOBOCAN (IARC) in 2012, new cases of breast cancer accounted for the highest percentage of cases based on age control and the highest percentage of fatalities (after controlling for age), at 12.9%. Women are more susceptible to breast cancer than men, with a mortality rate of 42.1 per 100,000 people[3]. Breast cancer in younger age groups has been found to exhibit more aggressive biological behavior and worse clinical outcomes than breast cancer in older age groups[4].

In recent years, there has been significant progress in the field of anticancer chemical research. Numerous researchers have conducted investigations into the potential of bioactive compounds derived from plants as prophylactic agents to mitigate carcinogenesis. Several chemopreventive chemicals have been identified based on their capacity to regulate specific chemical processes. The identification of potent herbs and a comprehensive comprehension of their mechanisms of action could lead to the development of complementary and alternative methods for cancer prevention and treatment.

Abutilon indicum Linn. (Malvaceae) is a typical weed along street sides and other waste places up to a height of 1200 m[5].The various parts of this plant are widely used by various Indian tribal communities and forest dwellers for the treatment of variety of ailments. The plant is documented to possess beneficial effects as sweet, cooling, digestive, laxative, expectorant, diuretic, astringent, analgesic, anti-inflammatory, anthelmintic, demulcent and aphrodisiac. The leaves and seeds are crushed with water to a form paste which is applied to the penis to cure syphilis. The leaves are used as eye and mouth wash, in catarrh and bilious diarrhoea. A leaf paste is taken orally to treat piles and legs pains[6]. The bread prepared from the mixture of leaf powder and wheat flour is taken daily during night for about one month overcome uterus displacement[7]. The leaf juice mixed with jaggery is taken orally as an antidote to counteract snakebite. The fruits are prescribed to combat piles, gonorrhea and cough. The fruit decoction mixed with ammonium chloride is given orally to appease haemorrhagic septicemia. Seed powder is administered orally with water as aphrodisiac and laxative. The root of the plant is recommended to mitigate gonorrhea and leprosy. A root infusion is drunk to normalize fever, dry cough and bronchitis.

MATERIALS AND METHODS

Extraction and separation

10 g of ethanol root extract were chromatographed using silica gel column (100-200 mesh) with the help of various solvents with increasing polarity. The admixture was packed on a silica gel column (Merck, India) and elution starts with 100% Hexane and then polarity of the solvents was increased in the order Chloroform, Ethyl acetate, Methanol and Ethanol. A total of 20 eluates were collected. Similar fractions were pooled together. Isolated sterols were verified thin layer spray with Anisaldehyde - sulphuric acid and the plate was incubated at 120°C for ten minutes, the

purple color spots were confirmed as sterols. Further purification is carried out using preparative TLC. Finally eluate AI yielded a single spot when subjected to TLC using solvent system Chloroform: Methanol (8:0.6), then spray with H2SO4 (10 % in ethanol) and heat in oven at 110 degree Celsius for 5-10 minutes. Pink spot is obtained having the Rf 0.34 which confirmed the presence of beta-sitosterol (AI) in the ethanolic extract.

Culture Cell and Treatment

The MCF-7 cell line of breast cancer was utilized with the help of Jawaharlal Nehru Cancer Hospital and Research, Bhopal. The antibacterial agents, streptomycin and penicillin, were procured from Sigma. The growth medium utilized for the cancer cell line was RPMI-1640, which was enriched with 10% fetal bovine serum sourced from the IISER, Bhopal. Additionally, beta-sitosterol was added to the cell culture medium in varying concentrations and incubated for a period of 24 hours.

Sensitivity test

Cell proliferation was assessed through a colorimetric resazurin assay at varying concentrations of beta-sitosterol. Resazurin is basically used to evaluate cell metabolism and has been a reliable indicator of oxidation-reduction (redox) reactions for an extended period. Resazurin is a non-fluorescent blue dye that is reduced to a deep fluorescent pink resorption form. The change in color from blue (resazurin) to pink (resorufin) indicates a reduction by cells. Resazurin transforms from a non-fluorescent dye to a red fluorescent dye called resorufin in response to the chemical reduction of the growth media caused by cell growth. Growth inhibition maintains an oxidized environment, while sustained cell growth does the opposite. As a result of growth-related reduction, the redox indicator changes from an oxidized (blue, non-fluorescent) to a reduced (red, fluorescent) state[8]. Additionally, fluorescence (590 nm) or absorbance at 570 nm can be used to detect reduced resazurin. The fluorescent or colorimetric signal is inversely correlated with the viable cell count of the sample.

APOPTOTIC ACTIVITY DETERMINATION BY FLOW CYTOMETRY

Reagent used for Flow Cytometry

1. Annexin V binding buffer
2. Propidium Iodide (PI)
3. FITC V Kit

Coloring or staining of cells

1. The cells underwent two washes with cold PBS and were subsequently suspended in binding buffer at a concentration of 1 x 10-6 cells/ml.
2. Subsequently, 100 µl of a solution containing 1 x 10^5 cells was transferred to either a 2 ml or 1.5 ml Eppendorf tube.

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3. Additionally, 5 µl of FITC Annexin V and PI were added to all cells that had been subjected to the test material and positive control.
4. In the case of control cells:

a. Cells that were not stained with annexin and PI were treated.
b. Cells were treated with FITC Annexin V staining.
c. Cells were treated with PI staining.
d. FITC Annexin V and PI stained cells were gently agitated and incubated in the dark at room temperature for 15 minutes.

5. Subsequently, 400 µl of binding buffer was added to each tube, and the results were analyzed using flow cytometry within 1 hour[9].

RESULTS AND DISCUSSION

Beta-sitosterol isolation and identification

In this study, the most important chemical compound in *Abutilon indicum* root active fraction was shown to be beta-sitosterol. After purification from the active fraction of the root of this plant, beta-sitosterol is obtained as a bioactive compound. The chemical structure of beta-sitosterol isolated from *Abutilon indicum* is shown below (Figure 1).

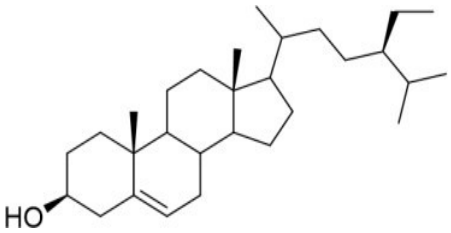

Figure 1. Structure of beta-sitosterol isolated from *Abutilon indicum*

Inhibition of MCF-7 cells proliferation by Beta-sitosterol

Beta-sitosterol was tested for its ability to slow down the growth of MCF-7 cells. The treatment with beta-sitosterol resulted in a dose-dependent inhibition of cell growth, as shown by the resazurin assay. After 48 hours of exposure to beta-sitosterol, the MCF-7 cells showed reduced proliferation. The effects of beta-sitosterol on the morphology of MCF-7 cells are illustrated in Figure 2.

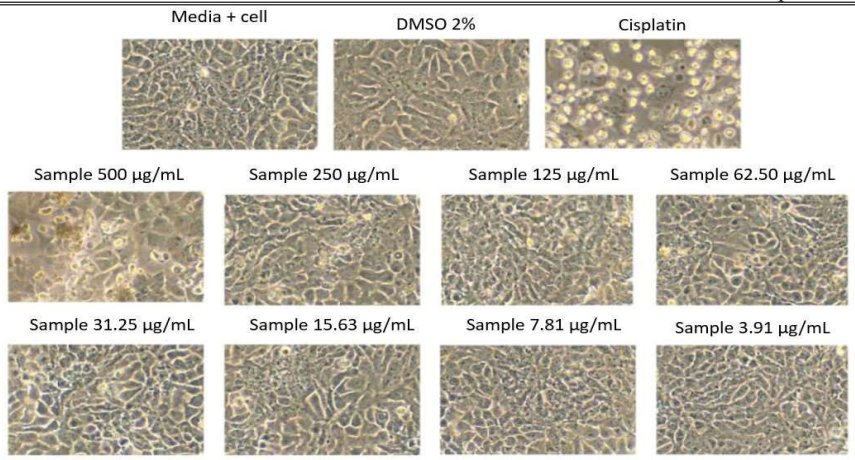

Figure 2. Effect of beta-sitosterol on the morphological characteristics of MCF-7 cells at various concentrations

Induction of apoptosis by Beta-sitosterol on MCF-7

Annexin V functions as an anticoagulant that specifically attaches to phosphatidyl (PS) when calcium is present. During the early stages of apoptosis, PS is relocated to the cell surface. Annexin V is labeled with fluorescein isothiocyanate (FITC), allowing it to be detected using flow cytometry. When cells undergo necrosis, their membranes become permeable and they also bind to Annexin V-FITC. To distinguish between surviving, early apoptotic cells and necrotic or late apoptotic cells, propidium iodide is used. Propidium iodide is excreted from viable and early apoptotic cells. Late apoptotic cells, which undergo necrotic-like cell disintegration, are stained with both FITC and propidium iodide. The treatment of MCF-7 cells with beta-sitosterol induced apoptosis in these cells following a 24-hour exposure[9].

Furthermore, the result of the beta-sitosterol test curve on MCF- 7 cells was obtained as follows (Figure 3):

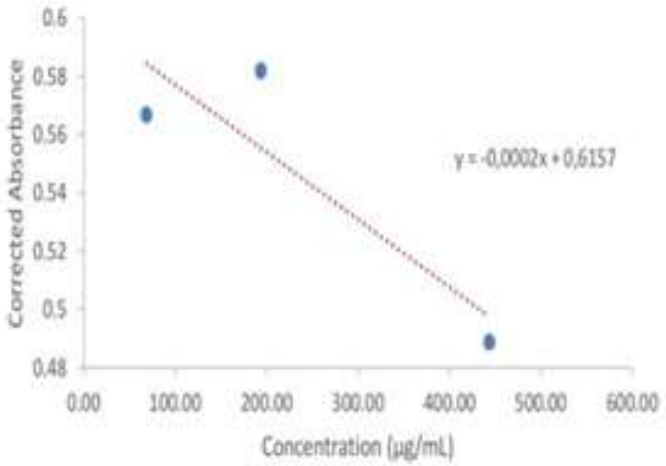

Figure 3. Curve for beta-sitosterol effect on MCF-7 cells

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The outcome of the test examining the apoptotic activity of beta-sitosterol and determining the type of cell death through flow cytometry using Annexin-Propidium Iodide (PI) is shown in Figure 4.

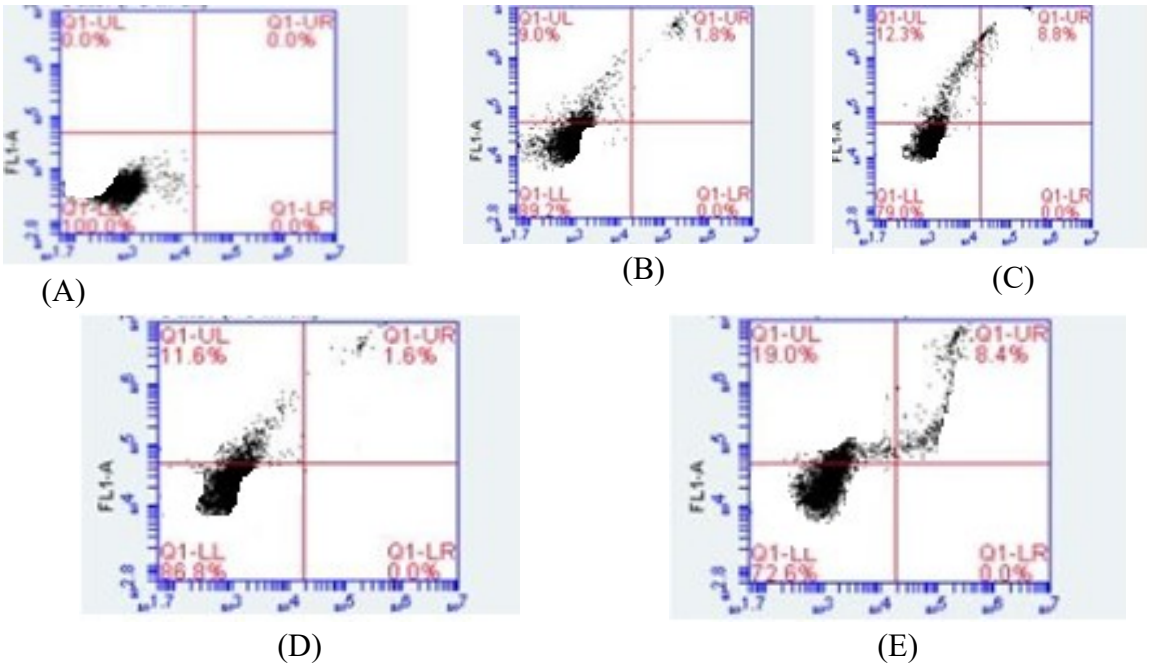

Figure 4. Apoptotic activity of the beta-sitosterol of Abutilon indicum roots: A) control, B) cell control, C) cisplatin (2.85 ppm), D) beta-sitosterol (250 ppm), E) beta-sitosterol (1000 ppm).

The results indicate that beta-sitosterol at a concentration of 1000 ppm exhibited the highest apoptotic activity compared to 250 ppm. Additionally, beta-sitosterol increased apoptotic activity when compared to cisplatin, a positive control.

Previous studies have shown that A. wilkesiana has antioxidant, antimicrobial, and cytotoxic effects, as well as antibacterial, antifungal, and antimalarial properties. It has also been found to have anti-inflammatory, analgesic, and anticancer effects.

Our previous research demonstrated that extracts and fractions of A. wilkesiana had anticancer activity against MCF-7 breast cancer cells, inhibiting their proliferation.

In this study, beta-sitosterol was found to inhibit the proliferation of MCF-7 cells in a dose-dependent manner. Furthermore, the research also indicates that beta-sitosterol induces apoptosis through flow cytometry.

Overall, these results suggest that beta-sitosterol triggers cell death via the apoptotic pathway in MCF-7 breast cancer cells, making it a potential candidate for future development as an anticancer agent.

CONCLUSION

Beta-sitosterol isolated from the roots of *Abutilon indicum* found to have apoptosis activity by inhibiting proliferation in MCF-7 breast cancer cells.

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CONFLICT OF INTEREST
None

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