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Research Article

Investigation of Proapoptotic Potential of *Ipomoea carnea* Leaf Extract on Breast Cancer Cell Line

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Abstract

Cancer remains to be a major global healthcare burden as 18.1 million new cases and 96 million death occurred 2018 on the basis of report from the international agency for research on cancer Breast cancer is the most common cancer diagnosed in women causing the highest morbidity. *Ipomoea carnea* leaf extracts have been used as a chemotherapeutic agent due to their antioxidant, anti-inflammatory, anti-bacterial, and anti-tumor effect. The aim of the study is to evaluate the proapoptotic potential of *Ipomoea carnea* leaf extract on breast cancer cell lines (MCF-7). The proapoptotic potential of *Ipomoea carnea* leaf extract was assessed using an MTT assay. The cell morphological changes in *Ipomoea carnea* leaf extract-treated cells were observed under a phase-contrast microscope. The proapoptotic effect of *Ipomoea carnea* leaf extract was examined using DAPI staining. The MTT assay results showed a significant reduction in the viability of breast cancer cells after treatment with different concentrations of *Ipomoea carnea* leaf extract (5-80 µg/ml) for 24h. We observed the inhibitory concentration at 20 µg/ml. Morphological changes such as reduction in the number of cells, cell shrinkage, and cytoplasmic membrane blebbing indicate the hallmark features of apoptosis were observed in *Ipomoea carnea* leaf extract-treated cells. DAPI staining results showed that apoptotic nuclei are stained intensely, fragmented, and have condensation chromatin in treated cells. Therefore it can be concluded that *Ipomoea carnea* leaf extracts have a pro-apoptotic potential in the breast cell cancer lines (MCF-7).

Keywords: *Ipomoea carnea*, Breast cancer, MCF-7, Apoptosis

INTRODUCTION

Cancer is a disease of gene disorder that occurs in the normal processes of cell division and is one of the most dreaded diseases worldwide¹. Cancer remains to be a major global healthcare burden as 18.1 million new cases and 96 million death occurred 2018 on the basis of report from the international agency for research on cancer (IARC). Most common types of cancers occurring in humans are lung, female, breast and colorectal. There are high chances of death in these cancer patients on factors based on early detectionchemo resistance or tumourheterogenicity as well as chances of metastases.²

Breast cancer is the most common cancer overall among women in both developed and less developed regions of the world, representing 25% of new cancer cases among women, and the second cause of death in developed regions after lung cancer. Breast cancer is cancer formed in the breast cells. Breast cancer is the most common malignancy in women, but it can occur less frequently in men. The incidence of breast cancer is increasing in the last decades of life. 75% occur in women over the age of 50, while 50% occur in women over the age of 65^{3,4}. In recent years, the

cancer research field has made splendid improvements in technology.

The current study indicates the important role of apoptosis with cancer cells. Apoptosis or programmed cell death is a process of eliminating normal cells without any inflammatory response which is initiated by intrinsic or extrinsic signals⁵. It plays an important role in cancer development and treatment, thus the ability of bioactive compounds to increase the sensitivity of cancerous cells towards cellular damage and activate the proapoptotic response is the most desirable. Induction of apoptosis in tumor cells is the most common anticancer mechanism in the treatment of cancer cells in the body⁶. Chemoprevention research has led to the identification of many phytochemicals which are as effective as potential chemopreventive agents⁷.

Breast cancer can be usually treated by chemotherapeutic agents if diagnosed at early stages. Nowadays, many plant products are effective as chemotherapeutic agents. The main benefits of using natural formulas are their minor side effects, inexpensive and easy accessibility as compared to chemical drugs⁸. The use of medicinal plants for cancer

treatment is improving fast in the last few years. Plant extracts contain materials such as flavonoids and aromatic compounds, which can be effective in decreasing the oxidative stress present in cancerous cells⁹. Many natural plant extract and phytochemicals have been reported to induce apoptosis in cancer cell lines^{10,11}. One of the most conventionally used plant-derived products is *Ipomoea carnea*.

In our present study, *Ipomoea carnea* leaf extracts were selected for the evaluation of proapoptotic potential on breast cancer cell lines (MCF-7). *Ipomoea carnea* belongs to the Convolvulaceae family. This plant contains various phytochemicals like glycosides, reducing sugars, alkaloids, flavonoids, esters, fatty acid, alcohol and tannins. The leaves possess alkaloids, hexadecanoic acid, saponins, stearic acid, 1, 2 diethyl phthalate, phenolic compounds, n-octadecanol, octacosane, hexatriacontane, tetracontane, 3-diethylamino-1-propanol, xanthoproteins and flavonoid. Some of these compounds have been known to have anti-diabetic, hypolipidemic, anti-inflammatory, anti-bacterial, hepatoprotective and anti-cancerous properties. Previous studies also reveal that *Ipomoea carnea* extracts cause the formation of DNA laddering in the MCF-7 breast cancer cell lines. The aim of the study is to evaluate the proapoptotic potential of *Ipomoea carnea* leaf extract on breast cancer cell lines (MCF-7)^{12,13}.

MATERIAL AND METHODS

Reagents

DMEM (Dulbecco's Modified Eagle Medium), Phosphate Buffered Saline (PBS), Trypsin-EDTA, Fetal bovine serum (FBS), were purchased from Gibco, Canada. Dimethyl sulfoxide (DMSO), [3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT), DAPI, were purchased from Sigma Chemical Pvt Ltd, USA. All other chemicals used were extra pure of molecular grade and were purchased from NCL, India.

Cell line maintenance

Estrogen-dependent (MCF-7) breast cancer cell lines were obtained from the NCCS, Pune. The cells were grown in T25 culture flasks containing DMEM supplemented with 10% FBS and 1% antibiotics. Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. Upon reaching confluency, the cells were trypsinized and passaged.

Preparation of the Herbal Extract

Ipomoea carnea leaf powder obtained from IMPCOPS (Chennai, India) was used for the present study. About 50g of *Ipomoea carnea* leaf powder was soaked in 500 mL of 95% ethanol and kept at room temperature for 3 days in a static condition. Then the solution was filtered with crude filter paper followed by whatmann paper. Fine filtrate was subjected to rota evaporation after that 3g of the material was obtained. The total ethanol extract was concentrated in a vacuum evaporate and immediately stored at 4°C.

Cell viability (MTT) assay

The percentage of breast cancer cell viability after treatment with *Ipomoea carnea* leaf extract was assessed by MTT assay. The assay is based on the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically active cells. MCF-7 cells were plated in 48 well plates at a concentration of 2x10⁴ cells/well and incubated 24h, then the cells were washed twice with 500µl of serum-free medium and starved by incubating the cells in serum-free medium for 3 hours at 37°C. After starvation, cells were treated with *Ipomoea carnea* leaf at different concentrations

(0, 5, 10, 20, 40, 60 and 80 µg/ml) for 24 hours. At the end of treatment, the medium from control and *Ipomoea carnea* leaf treated cells were discarded and 200µl of MTT containing DMEM (0.5 mg/ml) was added to each well. The cells were then incubated for 4h at 37°C in the CO₂ incubator. The MTT containing medium was then discarded and the cells were washed with 1x PBS. The crystals were then dissolved by adding 200µl of solubilization solution and this was mixed properly by pipetting up and down. Then the formazan crystals formed were dissolved in dimethyl sulfoxide (200µl) 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 a 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.

Morphology study

Based on MTT assay we selected the optimal doses (IC-50: 20µg/ml) for further studies. Analysis of cell morphology changes by a phase-contrast microscope. 3×10⁴ cells were seeded in 6 well plates and treated with *Ipomoea carnea* (20µg/ml) for 24h. At the end of the incubation period, the medium was removed and cells were washed once with a phosphate buffer saline (PBS pH 7.4). The plates were observed under a phase-contrast microscope.

Determination of nuclear morphological changes of cells (DAPI staining)

For the nuclear morphological analysis, the monolayer of cells was washed with PBS and fixed with 3% paraformaldehyde for 10 min at room temperature. The fixed cells were permeabilized with 0.2% Triton X-100 in PBS for 10 min at room temperature and incubated with 0.5µg/ml of DAPI for 5 min. The apoptotic nuclei (intensely stained, fragmented nuclei, and condensed chromatin) were viewed under a fluorescent microscope.

Statistical analysis

Statistical analyses were performed using one-way ANOVA followed by Student–Newman–Keul's (SNK) tests for comparison between treatment values and control values. Data were expressed as mean ± SEM. The level of statistical significance was set at p<0.05.

RESULTS

Effect of *Ipomoea carnea* leaf extract on cell viability of breast cancer cell line

The cytotoxic potential of *Ipomoea carnea* leaf extract in breast cancer cells was assessed by an MTT assay. The cells were treated with different concentrations (5-80 µg/ml) of *Ipomoea carnea* leaf extract for 24h. *Ipomoea carnea* leaf extract treatment significantly decreased the viability of MCF-7 cancer cells compared to control at 24 h time point (Figure 1). The percentage of cell viability reduced gradually with an increase in the concentration. We observed the 50% growth inhibition at (20 µg/ml) concentration. Hence, IC-50 dose (20 µg/ml) was considered for further experiments.

The effect of *Ipomoea carnea* on cell morphology

The cell morphological analysis of *Ipomoea carnea* leaf extract treated breast cancer cells was observed in an inverted phase-contrast microscope. The MCF-7 cells were treated with *Ipomoea carnea* leaf extract (20 µg/ml) for 24h, compared with the untreated cells, treated cells showed

significant morphological changes, which are characteristic of apoptotic cells, such as cell shrinkage and reduced cell density were observed in the *Ipomoea carnea* leaf treated cells (Figure 2). Cells undergoing apoptosis also displayed other types of morphological changes such as rounded up cells that shrink and lose contact with neighboring cells. Some sensitive cells were even detached from the surface of the plates.

Pro-apoptotic effect of *Ipomoea carnea* leaf extract in breast cancer cells (DAPI staining)

The induction of apoptosis in *Ipomoea carnea* leaf extract (20 $\mu\text{g/ml}$) treated cells were analyzed by DAPI staining. After a 24h treatment period, the cells were stained with nuclear staining (DAPI) and observed in fluorescence microscopy. The treated cells clearly showed condensed chromatin and nuclear fragmentation, which are characteristics of apoptosis compared to the control which showed clear round nuclei (Figure 3).

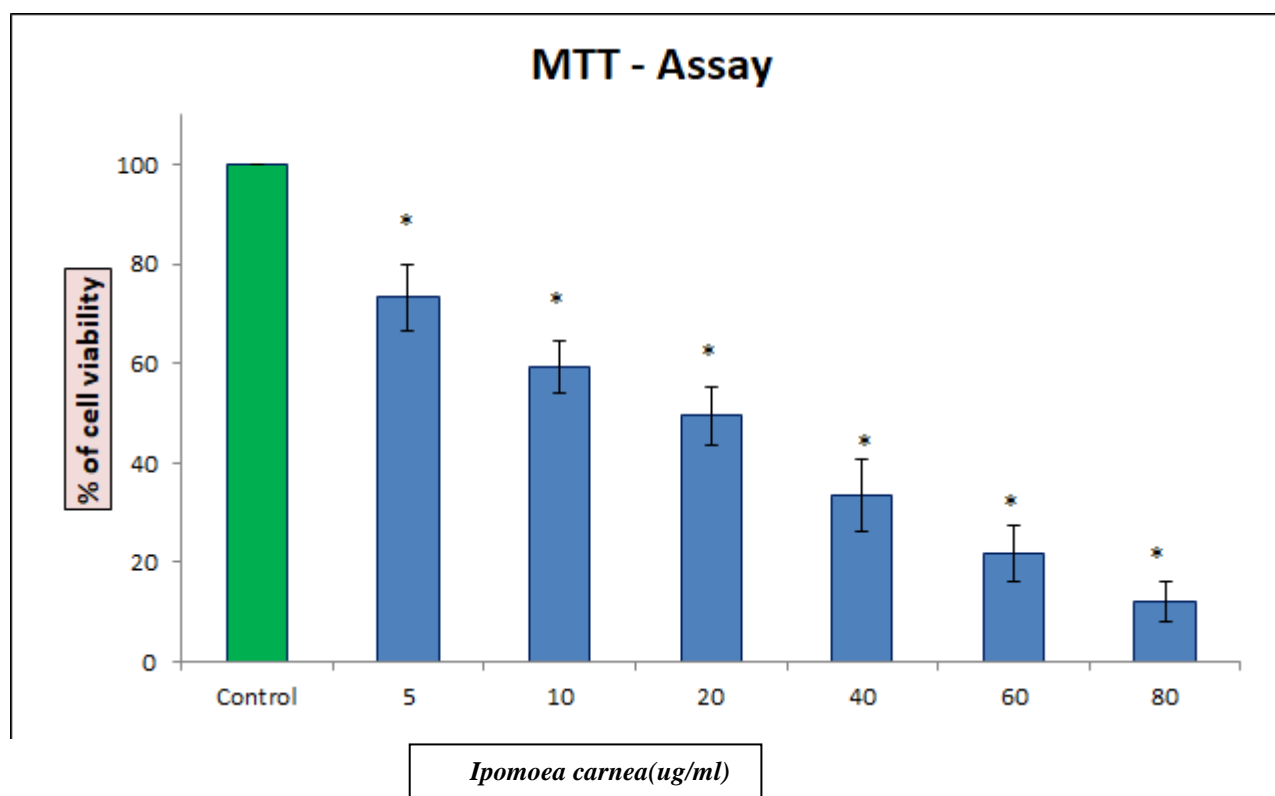


Figure 1: Effect of *Ipomoea carnea* leaf extract on the viability of breast cancer cells was determined by MTT assay. The cells were treated with different concentrations (0,5,10,20,40,60 and 80 $\mu\text{g/ml}$) for 24 hrs. Inhibitory concentration (IC-50) dose: 20 $\mu\text{g/ml}$ (p value: 0.0027). *represents statistical significance between control versus treatment groups at $p < 0.05$ level using Student's-Newman-Keul's test.

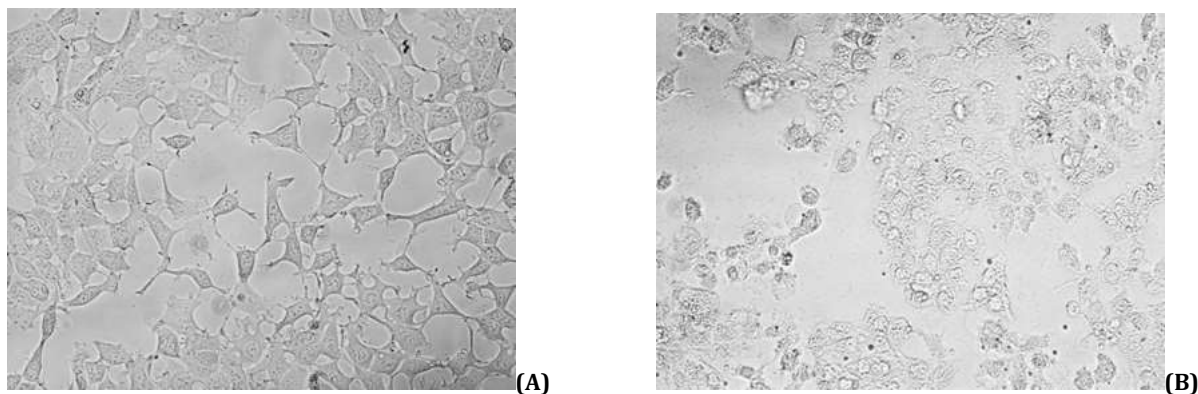


Figure 2: Effect of *Ipomoea carnea* extracts on cell morphological changes in breast cancer cell line (MCF-7) visualised in a phase-contrast microscope at 20x magnification. (A) Control cells; (B) *Ipomoea carnea* leaf extract (20 $\mu\text{g/ml}$). The number of cells decreased after the treatment and the cells exhibited cell shrinkage and cytoplasmic membrane blebbing.

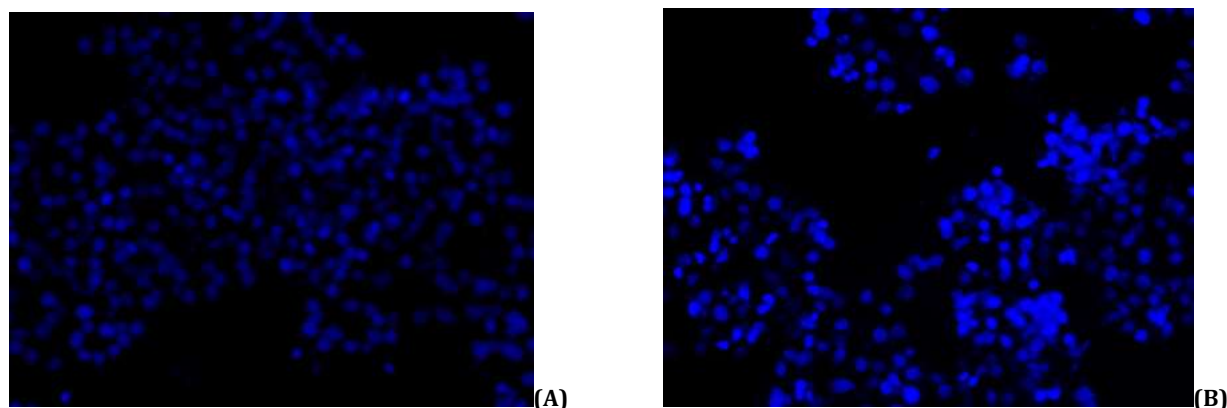


Figure 3: Induction of apoptosis in *Ipomoea carnea* leaf extract treated breast cancer cell line (MCF-7) visualised under a fluorescent microscope at 20 x magnifications. (A) Control cells ; (B) *Ipomoea carnea* leaf extract (20µg/ml).The nuclei were stained with DAPI and observed under a fluorescent microscope. The treated cells clearly showed condensed chromatin and nuclear fragmentation.

DISCUSSION

Apoptosis is a physiological process that functions as an essential mechanism of tissue homeostasis and the elimination of unwanted cells. Current trends include the use of medicine-derived plant products as the alternative for anti-cancer and chemotherapeutic agents. Screening plant-derived products as apoptotic inducers play a major role in anticancer research. Plants also have the opportunity to supply newer therapies. Recently, numerous treatments have been proposed for cancer, which uses plant-derived materials. Medical plants contain bioactive compounds which include tannins, sugars, hormones, and flavonoids. These compounds help in preventing carcinogenesis by blocking metabolic activation, increasing detoxification, or providing alternative targets for electrophonic metabolites¹⁴.

The present study focuses on the ability of *Ipomoea carnea* leaf extracts against breast cancer cell lines and the pro-apoptotic activity. The leaf extracts significantly inhibited the proliferation of breast cancer cell lines after an incubation period of 24 h and the pro-apoptotic potential was evaluated by DAPI staining. The MTT assay showed that the pro-apoptotic potential of *Ipomoea carnea* leaf extracts was selective towards MCF-7 cells. Morphological investigations reveal that apoptosis in the breast cancer cell line is characterized when *Ipomoea carnea* leaf extracts were at an inhibitory concentration of 20µg/ml (**Figure 1**). Morphologic changes observed showed cell shrinkage, cytoplasmic membrane blebbing, and collapse of cells into small membranes when viewed in a phase-contrast microscope (**Figure 2**). There is interplay of caspase substrate cleavage during apoptosis¹⁵. The membrane blebbing is due to the Rho effector protein ROCK I, which contributes to phosphorylation of myosin light chains, myosin ATPase activity, and coupling of actin-myosin filaments to the plasma membrane. It is cleaved during apoptosis to generate an active form. ROCK proteins are necessary and sufficient for the formation of membrane blebs and for the re-localization of fragmented DNA into blebs and apoptotic bodies¹⁶. These morphological changes could be seen after 24 h treatment with extracts and positive control from the surface.

These hallmark features of morphological changes suggested that *Ipomoea carnea* extracts caused early apoptotic changes in MCF-7 breast cancer cell lines¹⁷. The nuclei were stained with DAPI and observed under a fluorescent microscope.

The apoptotic nuclei were intensely stained, fragmented, and had condensed chromatin (**Figure 3**). AO/EtBr staining shows apoptotic rate was positively correlated with the cytotoxic activity of the extracts. Previous studies state that *Ipomoea carnea* extracts when assessed for antitumor activity using Ehrlich's ascites carcinoma (EAC) bearing swiss albino mice were found to bear good antitumor activity, which was supposed to be due to the increased antioxidant activity¹⁸. These effects could be due to the free radical-scavenging efficiency and reducing power, as a result of their phenolic and/or non-phenolic constituents in triggering different cellular mechanisms involved in cancer and apoptosis. The results of our study are supported by Teoh et al. confirming *Ipomoea carnea* leaf extracts as one of the most potent chemotherapeutic agents against breast cancer cell lines with pro-apoptotic potential.

CONCLUSION

Overall, the present study results demonstrated that the *Ipomoea carnea* leaf extracts were cytotoxic and induced apoptosis to the breast cancer cells at 20µg/ml concentration and 24h incubation period. However, more research is needed to understand the mechanisms of cytotoxicity of *Ipomoea carnea* extracts.

CONFLICT OF INTEREST

None.

FUNDING

None.

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