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Evaluation of anti-cancer activities of Carcino SC Mammae 200ch in breast cancer cell lines (MDA-MB-231)

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Abstract

Aim/Background: Breast Carcinoma is one of the most common cancers occurring mostly in adolescents and has a very high risk of malignancy. Despite of some challenges with the present therapeutic approaches, alternatives have gained popularity, such as ayurveda, homeopathy, siddha, unani, etc including the use of phytochemicals and various other anticancer compositions. Carcino SC Mammae 200CH is a homeopathic medicinal mother tincture which is not as much therapeutically known for its exceptional health benefits and have to be explored for its effective activities during breast cancer. The current study aimed at evaluating the anti-cancer properties of Carcino SC Mammae 200CH on MDA-MB-231 cell line for human breast cancer, by investigating its apoptotic activity through changes in cell viability.

Materials and Methods: Carcino SC Mammae 200CHwas achieved through local market in Aurangabad, Maharashtra. The MDA-MB-231 cells were treated with ity in the concentration range 3.125 µg/mL to 75µg/mL of the Carcino SC Mammae 200CH and incubated for 24, 48, and 72 hr. The percentage cell viability and IC50 values were obtained. The nuclear changes were examined. GraphPad Prism was used for statistical analysis with significant p-value at <0.05.

Results: The IC50 values obtained for Carcino SC Mammae 200CH were 75µg/mL, 25µg/mL, and 6.25µg/mL for 24, 48, and 72 hr respectively. Change in the nuclear morphology was observed following incubation with Carcino SC Mammae 200CH.

Conclusion: Our result suggests that Carcino SC Mammae 200CH have shown significant effects on MDA-MB-231 breast cancer cells and can be considered to supplement conventional therapeutic strategies.

Keywords: Apoptosis; Breast Cancer; Carcino SC Mammae 200CH; MDA-MB-231; DAPI staining; MTT assay

1. Introduction

Cancer of breast mesenchymal cells, hence known as malignant breast carcinoma, often metastasizes to various body parts, predominantly to the lungs and blood. In terms of treatment, surgical amputation was the first line of therapeutic setting before the 1970s, followed by chemotherapy. The National Cancer Institute (NCI) lists surgery, chemotherapy, radiation therapy, the use of samarium, and targeted therapy as standard routes of treatment, nevertheless coming with side effects and complications.

Breast Cancer is a common type of gyanic related cancer (nearly 90% in women and 10% in men) with a high metastatic rate as well as drug resistance. The American Cancer Society (ACS) statistics and Surveillance, Epidemiology, and End Results Program (SEER) put the diagnosis rate of breast cancer in women 82% in 2022.

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Conventionally, DNA damage of cancer cells brought by doxorubicin and apoptosis Induced through DNA adducts by cisplatin are useful in chemotherapeutic treatments. Other drugs and combinations are also used and are being studied for clinical intervention.

Many of these homeopathic dilutions like Carcino SC Mammae 200CH impart certain activities in cancer cells and tumors.14 A recently some research study showed cell cycle arrest and induction of apoptosis by ursalic acid in human breast cancer cells.15 Various studies on homeopathic dilutions have shown their potential ameliorating activities in many cancer cells and tumors. These medicines have the added advantage of acting on multiple apoptotic pathways, thereby effectively inducing the inhibition in cancer cells. "With a plethora of anti- cancer agents in Carcino SC Mammae 200CH, and the wide array of functions displayed by its various constitutes, there seems to be a need to establish studies exploring more uses for breast cancer treatment strategies. Although studies involving certain compounds found in anticancer compositions present on Carcino SC Mammae 200CH have been reported in breast cancer," the therapeutic activity of Carcino SC Mammae 200CH as a whole homeopathic dilution, in the same has not been reported to the best of our knowledge in this study, we have buyed Carcino SC Mammae 200CH from the local market in Aurangabad, Maharashtra for evaluation of its anti-cancer activities in the human breast cancer cell line, MDA-MB-231 by investigating its effects on the viability of the cancer cells, changes in membrane potential, etc.

Chemotherapeutic drugs are added to treatment complications due to side effects and increased recurrence rates while developing drug resistance, which are prompting researchers to develop alternate therapies. As traditional Indian and Chinese medicine are not new to the use of natural compounds for treatment, many researchers worldwide are directing their strategies to compounds and extracts from natural sources. With more than half of the anti-cancer drugs originating from homeopathic dilutions, exhibit an eclectic array of activities right from anti- microbial to anti-cancer and neuroprotective effects. Of anti-cancer effects, studies ranging from in- vitro assays to clinical trials have shown radical scavenging, anti-tumor, angiogenesis, anti- proliferative and apoptotic activities."

Looking at breast cancer alone, studies reporting the anti-cancer activities of homeopathic drug dilutions show various mechanisms and pathways involved in inducing these effects. A study recently showed that caspase 3/9 directed apoptosis and mitochondrial dysfunction in breast cancer cell lines through the activity of corosolic acid. Interestingly, it also prevents metastasis to the lungs by acting on signal transducer and activator of transcription-3 (STAT3) expression and exhibiting, tumor suppression and Immune-suppression activities in murine models.

Homeopathic dilutions are more preferred in combination therapies along with conventional drugs and treatment strategies as they amplify their therapeutic actions and act on various apoptotic pathways." Recommendations for a change in dietary habits are made for a cost- effective chemoprevention strategy. These are encouraged in clinical trials as well, to evade drug toxicity and the subsequent complications of drug resistance."

2. Material and methods

2.1. Materials

Homeopathic Dilution Carcino SC Mammae 200CH were procured from an authorized distributor (Pournima Medical, Aurangabad, Maharashtra, India), Dulbecco`s Modified Eagle Media (DMEM) with low glucose, Antibiotic – Antimycotic (100X) solution, and Fetal bovine serum (FBS) were purchased from Gibco; Thermo Fisher Scientific, MA, USA, MTT (3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide) and DAPI (4′,6-diamidino-2- phenylindole) were purchased from Hi Media Laboratories, India. Dimethyl sulfoxide (DMSO) and para-formaldehyde were procured from Qualigens, Mumbai, India. Annexin V- FITC Apoptosis Detection Kit was purchased from R&D Systems, Inc. USA.

2.2. Methods

2.2.1. Experimental Availability of Carcino SC Mammae 200CH Homeopathic Drug

This Experimental procedure is done on readily marketed homeopathic medicinal formulation known as Carcino SC Mammae 200CH which was brought from local market in Aurangabad, Maharashtra.

2.2.2. Cell Culture

Human Breast Cancer (MDA-MB-231) cell lines were procured from National Center for Cell Science (NCCS), Pune, India, were maintained in a CO_2 incubator (New Brunswick galaxy 170R, Eppendorf India Private Ltd., India) at 37°C in a

humidified environment of 5% CO_2. Dulbecco's Modified Eagle Medium (DMEM) was used as the standard culture medium along with 10% heat- inactivated fetal calf serum (FBS) and 1% Antibiotic – Antimycotic 100X solution.

2.2.3. In vitro Cell Viability Assay

Trypsinized MDA-MB-231 cells seeded in a 96-well plate (5 × 103 cells per well) and incubated overnight in a CO_2 incubator at 37°C in a humidified environment of 5% CO_2 were treated with Carcino SC Mammae 200CH at a concentration range of 75%µg/mL, 50%µg/mL, 25%, 12.5%µg/mL, 6.25%µg/mL, and 3.125%µg/mL and incubated for another 48 hrs. The untreated cells with culture medium were employed as control. After incubation, the culture media from each well was discarded and washed twice with phosphate-buffered saline (PBS). Following this, 20 µL of the 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) solution was added to the cells and incubated at 37°C for 4 hr, in dark. After aspirating this, 100 µL of dimethyl sulfoxide (DMSO) was added and agitated to dissolvethe insoluble formazan crystals. The absorbance was recorded at 570 nm using a microplate reader (Lisa plus microplate reader).18 The experiment was repeated three times mean ± standard deviation (SD) of the results was recorded. The percentage of cell viability was determined by using the following formula. Further, the Half-maximal inhibitory concentration (IC50) value was calculated by using Graph Pad Prism software (version 5; GraphPad Software, San Diego, CA).

Surviving cells (%) = Mean Optical Density of test compound /Mean Optical Density of Negative control ×100

2.2.4. Assessment of nuclear changes by DAPI staining

MDA-MB-231 cells cultured and fixed in a similar way as mentioned previously were incubated with 10 µL of DAPI (0.05µg/mL in PBS)in stock solution; Invitrogen D1306) in dark at room temperature for 5 min. After a PBS wash, the cells were observed under a fluorescence microscope at 20X magnification and visualized using Pro RES® Capture Pro software (Jena, Germany).20

2.3. Statistical Analysis

The data for each experiment in triplicates was expressed as ± standard error of the mean (SEM). Statistical analysis of data was done by using Graph Pad Prism software (Version 5; GraphPad Software Inc., CA, USA). "One-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test for MTT analysis and Bonferroni test for apoptosis assessment were used to compare the significance difference between the groups. Significant p values (significant probability value) at less than 0.05.

3. Results

3.1. Carcino SC Mammae 200CH Inhibited the Proliferation of MDA-MB-231 breast cancer cells

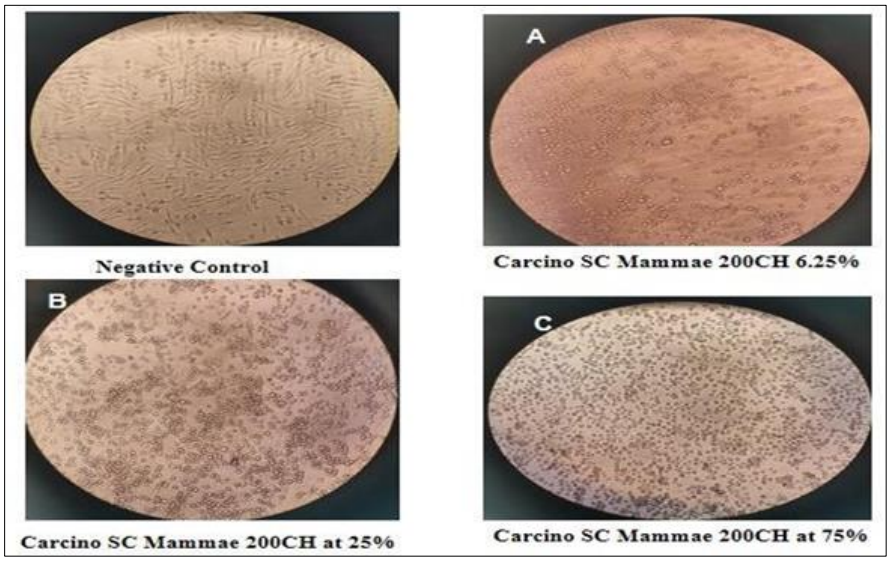


Figure 1 Resolutionary changes in MDA-MB-231 cell line after applying Carcino SC Mammae 200CH at Negative Control, A. Concentration at 6.25; B. Concentration at 25 and C. Concentration at 75

The cytotoxicity of Carcino SC Mammae 200CH in MDA-MB-231 cells was investigated using MTT. Figure 1 depicts the percentage of viable MDA-MB-231 cells after treatment with different concentrations of Carcino SC Mammae 200CH for various durations displaying a concentration-dependent cytotoxicityq. Further, the cytotoxicity increased in a time-dependent manner with 72 hr treatment showing slightly higher inhibition at all the tested concentrations as compared to 24 and 48 hr. The IC50 values of Carcino SC Mammae 200CH were 75µg/mL, 50µg/mL, 25µg/mL, 12.5µg/mL, 6.25µg/mL, and 3.125µg/mL respectively for 24, 48, and 72 hr incubation period.

Table 1 Cell Viability of MDA MB 231

Cell viability of MDA MB 231						
Concentration (%)	**Carcino SC Mammae 20^0c**			**Doxorubcin**		
75	35.73	35.38	35.55	10.74	11.35	10.95
50	36.60	35.90	36.08	13.58	14.26	14.32
25	43.43	42.91	43.26	15.88	15.95	16.08
12.5	51.09	51.04	51.61	21.15	21.55	21.42
6.2	54.00	54.82	54.99	22.64	23.04	23.45
3.125	69.88	69.18	69.35	27.23	27.36	27.64
Negative Control	100			100		
IC_{50} value (%)	24.22			1.81		
Standard Deviation	0.39			0.03		

3.2. Induced Changes in Nuclear Morphology

Fluorescence microscopic images depicting the apoptotic nuclear changes in MDA-MB-231 Breast Cancer Cells after treatment with Carcino SC Mammae 200CH for 24 hr are shown in Figure 3. The untreated cells with normal intact nuclei showed weak blue staining, while the Carcino SC Mammae 200CH treated cells displayed small nuclei with bright chromatin condensation. In addition, blebbing of cell membrane, nuclear fragmentation, and formation of apoptotic bodies were observed in the cells treated with high concentrations of Carcino SC Mammae 200CH. These results indicate that Carcino SC Mammae 200CH induced apoptosis in MDA-MB-231 Breast Cancer Cells.

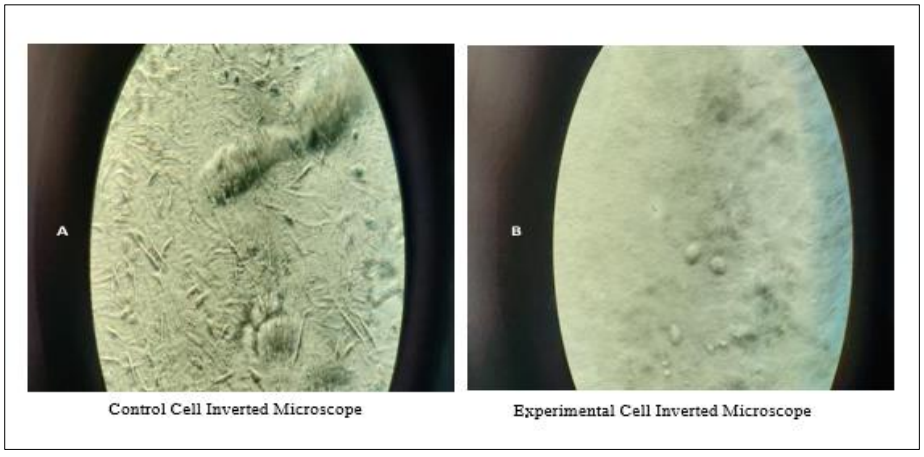
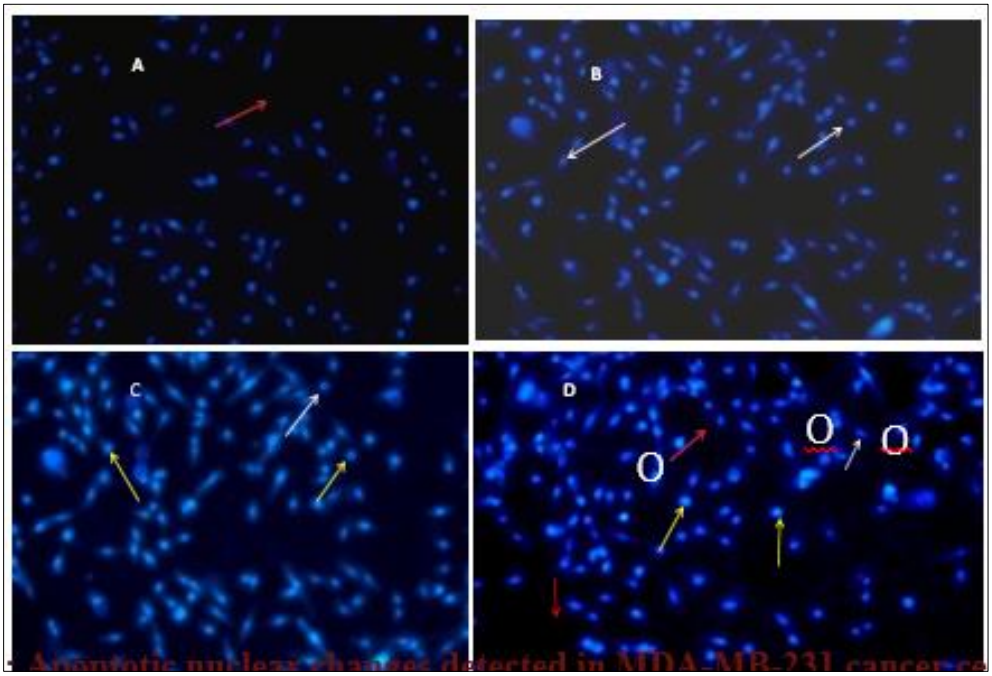


Figure 2 A. Control Cell Inverted Microscope and B. Experiment Cell InvertedMicroscope



(A) untreated control cells, (B) cells treated with 6.25µg/mL of Carcino SC Mammae 200CH; (C) cells treated with 25µg/mL of Carcino SC Mammae 200CH, and (D) cells treated with 75µg/mL of Carcino SC Mammae 200CH The white arrows show membrane blebbing, yellow arrows represent apoptotic bodies, red arrows show nuclear fragmentation and white circles represent the chromatin condensation.

Figure 3 Apoptotic nuclear changes detected in MDA-MB-231 cancer cells treated with different concentrations of Carcino SC Mammae 200CH by DAPI staining

4. Discussion

The Breast Cancer is generally treated with chemotherapy, 4 and other strategies like surgeries and radiation therapies are often faced with complications (NCI data). National Cancer Institute's data lists infertility, mood swings, and complications in learning and memory along with risks of developing other cancers like sarcoma or leukemia as some of the side effects. In an alternate strategy to tackle such complications, researchers are focusing on homeopathy having anti-cancer and chemo-sensitizing properties.22 Previously reported studies on some of the homeopathic drug dilutions have shown the presence of high-performance anti- carcinogenic compounds which can help to inhibit cancer cell growth and kill cancerical cells.8 Focusing on this anti-carcinogenic activities, these homeopathic drug dilutions have also been explored for their such therapeutic properties as an alternative option.

Studies evaluating subjects' urine and plasma content after Carcino SC Mammae 200CH consumption also indicate the presence of anticancer drugs compositions, that improve anti- oxidant performance when introduced in cell line.12 Since the effects of whole Carcino SC Mammae 200CH have not been priorly evaluated in breast cancer MDA-MB-231 breast cancer cell line, we attempt to do so, by applying such homeopathic drug dilution of Carcino SC Mammae 200CH.

Evaluation of MDA-MB-231 breast cancer cell line with 3-(4,5-dimethylthiazol- 2-yl)-2,5- diphenyltetrazolium bromide (MTT), after exposing them to varying dilution concentrations of the homeopathic drug Carcino SC Mammae 200CH shows a concentration - dependent and time-dependent inhibition. This cell viability evaluation is based on the fact that metabolically active cells break down these tetrazolium salts into formazan crystals.12 To facilitate the optical density measurements, these crystals are solubilized using Dimethyl Sulfoxide (DMSO). Half-maximal inhibitory concentration (IC50) determines the appropriate concentration required to kill about 50% of cells. Considering these concentrations obtained through IC50 for homeopathic drug Carcino SC Mammae 200CH treated MDA-MB-231 breast cancer cell line at 24, 48, and 72 hr respectively, further experiments were carried out with 75µg/mL, 50µg/mL, 25µg/mL, 12.5µg/mL, 6.25µg/mL, and 3.125µg/mL.

In this study, we assessed the early stage of apoptosis induced by homeopathic drug dilution Carcino SC Mammae 200CH on the MDA-MB-231 cells using fluorescent microscopy. Rhodamine 123, a cationic fluorescent dye used in this method generally accumulates the mitochondria of intactcells with highly polarized mitochondria which brightly stains the control cells. In homeopathic drug dilution Carcino SC Mammae 200CH treated MDA-MB-231 cells, the mitochondria

are de-polarized leading to leakage of this dye into the cytosol. This opening of mitochondria leads to the release of pro-apoptotic factors triggeringthe apoptotic pathway.26 The cells treated with an increasing concentration of homeopathic dilution drug Carcino SC Mammae 200CH at 75µg/mL, 50µg/mL, 25µg/mL, 12.5µg/mL, 6.25µg/mL, and 3.125µg/mL show a significantly decreasing pattern of the fluorescent intensity imparted by Rhodamine 123.

Further assessment of apoptotic changes to the nuclei was done using 4′,6-diamidino-2- phenylindole (DAPI). The dye binds to adenosine-thymidine regions in the nuclear DNA and enables its visualization using a fluorescent microscope. The concentration-dependent investigations of homeopathic drug dilution Carcino SC Mammae 200CH on breast cancer cells done in this study show that it induces blebbing, formation of apoptotic bodies, nuclear fragmentation as well as condensation. The condensation and apoptotic bodies observed could be as a result of a change in membrane potential, caspase-3 activation, and the subsequent apoptotic pathway, 27 as has been previously observed in the activities of ursolic acid.24

5. Conclusion

Our study involving homeopathic dilution of Carcino SC Mammae 200CH shows that it has significant effects on breast cancer cells. The inhibitory effect of Carcino SC Mammae 200CH dilution can be seen with the IC_{50} values (75%µg/mL, 50%µg/mL, 25%µg/mL, 12.5%µg/mL, 6.25%µg/mL, and 3.125%µg/mL) derived from the MTT assay at 24, 48, and 72 hr respectively. Staining the Carcino SC Mammae 200CH treated cells with DAPI showed changes in nuclear morphology including condensation, blebbing, fragmentation, and induction of apoptotic bodies. Carcino SC Mammae 200CH induces changes in the mitochondrial membrane potential and significant apoptosis in the human breast cancer, MDA-MB-231 breast cancer cell line. These investigations leave us with the potential anti- cancer activities imparted by Carcino SC Mammae 200CH as a whole. Its efficacy, bioavailability, and knowledge of the mechanism of action and pathways involved remain to be explored.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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