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The Cytotoxic Effect of PM 701 and its Fractions on Cell Proliferation of Breast Cancer Cells, McF7

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Abstract**—**Breast cancer is the most common malignancy in the world among women. Many therapies have been designed to treat this disease. Mamectomy, chemotherapy and radiotherapy are still the main therapies of breast cancer. However, the results were unsatisfactory and still far from the ideal treatment.

PM 701is a natural product, has anticancer activity. The bioactive fraction PMF and subfraction PMFK had been isolated from PM701. PM 701 and its fractions were proved to have a cytotoxic properties against different cancer cell lines. This article is directed for the further examination of lyophilized PM701 and its active fractions on the growth of breast cancer cells (MCF-7). PM 701, PMF or PMFK were adding to the cultural medium, where MCF-7 is incubated.

PM 701, PMF or PMFK were able to inhibit significantly the proliferation of MCF-7 cells, Moreover these new agents were proved to induce apoptosis of the breast cancer cells; through its direct effect on the nuclei.

Keywords**—**Anticancer agent, breast carcinoma, MCF-7 cell line, PM 701

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I. Introduction

THE management of malignancies in humans still constitutes a major challenge for contemporary medicine [1]-[4]. Although with progress in understanding cancer nature, many therapeutic anticancer have been developed which has relied on surgery, chemotherapy, radiotherapy, hormone therapy and more recently immunotherapy [5]. However, all are still far from the ideal treatment, which selectively kills the malignant cell and sparing the normal healthy tissue and functions of vital organs [6]- [7].

Breast cancer is the most common malignancy in the world among women. Over a million women are diagnosed every year and 370 000 were died due to breast cancer [8]. Many drugs and therapies have been designed to treat this disease. The identification and elucidation of the molecular components and signals that control different biological processes underlying the regulation of cell growth, differentiation and apoptosis of the mammary epithelium is important to lead to the development of new drugs and play an important role in designing an anti-cancer drug [6]-[8].

Hence, anti-cancer drug substitutes are actively sought after in the hopes of finding alternative ways to suppress the growth of breast cancer cells. The PM 701 an anticancer substrate [9]- [12] was used in this study to test its effect on MCF-7 human breast cancer cell line. In addition to screening of its active fraction PMF [13] as a novel anti-cancer compounds for human breast cancer, this study also seeks to determine the effect of PMFK subfraction [13] on the MCF-7 proliferation.

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The study also determined the inhibitory concentration, IC50, upon treatment of these compounds on the MCF-7 human breast cancer cell line.

So we present herein the capacity of PM 701, PMF or PMFK in inhibit the proliferation of MCF-7 cell line in vitro.

II. Materials and Method

A. Media

Dulbecco's modified eagle medium (DMEM) and Dulbecco's phosphate buffered saline (PBS) were purchased from MP Biomedicals Inc, USA. Fetal calf serum (FCS) was obtained from Gibco, Canada. Coomassie blue, pencillin-streptomycin, Trypan blue and Trypsin-EDTA were purchased from Sigma, USA.

B. Preparation of tested agents

The powder form of PM 701 and its fractions (PMF and PMFK) were prepared according to [13], each fraction was dissolved in DMEM, 2% FBS at a concentration of 2 Milligram (mg) / Milliliter (ml) before using. The solution was then filter sterilized using a 0.2 μ syringe filter to prepare the working solution.

C. Cell Culture

Breast carcinoma cells (MCF-7) were purchased from National Cancer Institute, Cairo University, Egypt.

MCF-7 cells were cultured in DMEM supplemented with 10% FCS, 100 U/Milliliter (ml) penicillin and 100 microgram (ug) / Milliliter (ml) streptomycin. Cells were grown in 75 cm2 tissue culture flasks in a 37˚C incubator with a humidified mixture of 5% Co2 and 95% air [14].

D. Cytotoxicity Assay

Cells were detached with 0.025% trypsin-EDTA. The cells were resuspended in 10 Milliliter (ml) of medium to make single cell suspension and viable cells were counted by trypan blue exclusion in haemocytometer and diluted with medium to give a final concentration of 1 x 105 cells/well. One Milliliter (ml)/ well of these cell suspensions were seeded in 24-well microtiter plates and incubated to allow for cell attachment.

E. Cell count using hemocytometer

A cell suspension fixed in a volume of cells (e.g. 1 Milliliter (ml)). 200 microliter (μL) of this suspension was mix with an equal volume of trypan blue. Mixed solution was transferred using a pipette to a hemocytometer and live cells were counted. The numbers of cells were calculated per Milliliter (ml), using the following formula.

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Cell viability = total viable cells (unstained) / total cells x 104 x dilution factor (suspension cells: Trypan blue) [15]-[16].

F. Treatment with lyophilized PM 701 or it's fractions

Cytotoxicity assays were performed using short incubation for only 24 hr in serials dilutions of examined drug, that were used to estimate the IC50 concentration. The long incubation for more than 72 hr, with a concentration lower or higher than IC50 concentration, was also used with only crude substrate (PM701) to confirm the cytotoxicity effect.

G. Short incubation for IC_{50} estimation

lyophilized PM 701 or PMF or PMFK were diluted in medium. Serial dilutions were prepared (5 and 7.5 microgram (µg) and 1 Milliliter (ml) of each concentration was added to each well of the plate in 3 replicates of cancer cells (treated), after the selected time of treatment, the medium was aspirated. Then cells washed with PBS, trypsinized and counted by two methods counter coulter and Hemocytometer using trypan blue dye exclusion test [9], [12], [15], [17].

H. Fixing and staining cells

Each group of cells were plated onto Petri dishes in DMEM media for 24 hr, then the media changed with examined media (with different concentrations) and control media and incubated at 37˚C for 24 hr.

Each group of cells were fixed in 4% formaldehyde for 5 min at room temperature after double washing with 1 X PBS each for 5 min. Then cells stained with Coomassie blue for 5-10 min followed by repeated washing with tap water [9].

III. Statistical Analysis

The data were expressed as the mean ± SD of the optical density obtained from three independent experiments (each experiment was performed in three replicate wells). Statistical analysis was performed with SPSS and graph pad statistical programs.

IV. Results

The MCF-7 human breast cancer cell line was treated with PM 701 and its fractions at different concentrations. The next tables and figures illustrate the comparison between the effect of PM 701, PMF and PMFK on MCF-7 cells. Cells were plated with 1x105 cells/ well in 24-well tissue culture plates and incubated in tested agent for 24h at 37˚C.

A. IC50 estimation (short incubation)

This experiment was preformed to estimate the IC50 concentration of all tested agents.

The cytotoxic effect of lyophilized PM 701 was studied by incubated the human breast cancer cells, MCF-7 for 24 hr in DMEM media with serial concentration of the drug. PM 701 and its fractions inhibited the proliferation of MCF-7 cells in a dose-dependent manner. The IC50 of and PM 701 is 0.35 microgram (µg) /Milliliter (ml) of media, which was significantly low as compared to IC50 of PMF and PMFK (3 and 3.5 microgram (ug) /Milliliter (ml) of media respectively).

The fixed and stained MCF-7 cells showed that they decreased in number when incubated in media containing the examined substrate for 24 hr.

B. Effect of lyophilised PM701 on MCF-7 Cells

First results indicated that lyophilized PM 701 inhibited the proliferation of cancer cells and the IC_{50} was about 0.35 microgram (µg) of PM701/Milliliter (ml) of media, Table 1; Figs 1; 4

TABLEI
STUDY THE EFFECT OF PM701 ON MCF-7

Conc.ug	N	Mean	Std. Deviation
Control	3	0.407	0.086
Conc. 5	3	0.307	0.040
Conc. 7.5	3	0.287	0.117

Table 1: The effect of lyophilized PM701 on the growth of MCF-7 cancer cells after 24hr of incubation comparing with non treated cancer cells (control).

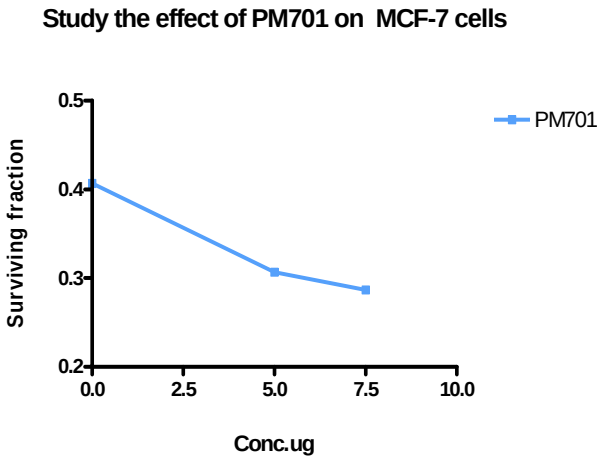


Fig. 1 The effect of lyophilized PM 701 on MCF-7 cell line after 24 hr of incubation, PM 701 appeared cytotoxic to the cancer cells in a dose-dependent manner.

C. Effect of PMF on MCF-7 Cells

Treatment of MCF-7 with serial concentrations of PMF fraction in incubated media showed inhibition of the cell proliferation with IC50 near the concentration 3 microgram (µg) of PMF /Milliliter (ml) of media, Table 2; Figs. 2; 4.

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TABLE II
STUDY THE EFFECT OF PMF ON MCF-7

Conc.ug	N	Mean	Std. Deviation
Control	3	3.967	2.301
Conc. 5	3	2.367	2.307
Conc. 7.5	3	2.233	0.737

Table 2.The effect of PMF on the growth of MCF-7 cancer cells after 24hr of incubation comparing with non treated cancer cells (control).

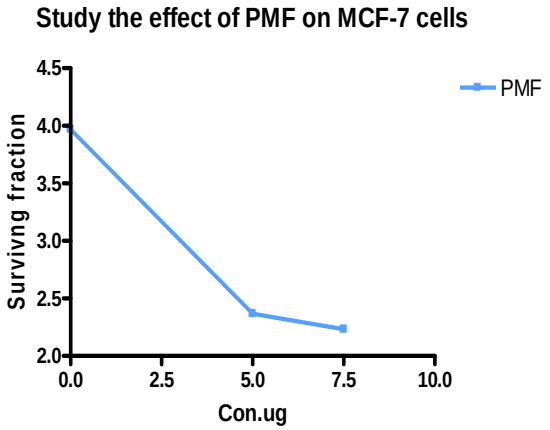


Fig 2: The effect of PMF on MCF-7 cell line after 24 hr of incubation, PMF appeared cytotoxic to the cancer cells in a dose-dependent manner.

D. Effect of PMFK on MCF-7 Cells

This part indicated that the serial concentrations of PMFK in incubated media inhibited the proliferation of MCF-7 cells and the IC50 was determined near the concentration 3.5 microgram (µg) of PMFK/Milliliter (ml) of media, Table3; Figs. 3; 4.

TABLE III
STUDY THE EFFECT OF PMFK ON MCF-7

Conc.ug	N	Mean	Std. Deviation
Control	3	4.2	1.95
Conc. 5	3	3.23	1.89
Conc. 7.5	3	2.6	1.55

Table 3: The effect of PMFK on the growth of MCF-7 cancer cells after 24hr of incubation comparing with non treated cancer cells (control).

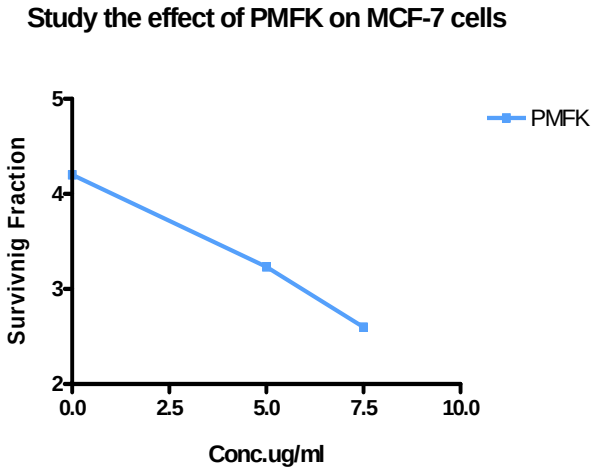


Fig. 3 The effect of PMFK on MCF-7 cell line after 24 hr of incubation, PMFK appeared cytotoxic to the cancer cells in a dose-dependent manner.

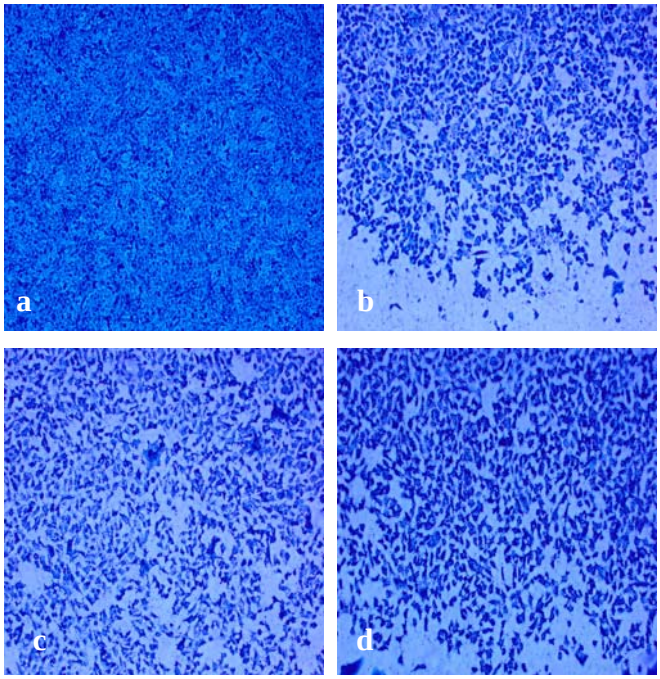


Fig. 4 MCF-7 cells imaged (x 10) after incubation with PM 701 (b), PMF (c) and PMFK (d) for 24 hr, fixed and stained with coomassie blue. The numbers of cells were decreased in treated cultures compared with the number of control non treated cells (a).

E. Long Incubation:

This experiment was carried on for only crude substrate PM 701 to confirm the cytotoxic effect of this agent against MCF-7 cells after long incubation for more than 96 hrs, while the same result could apply to PMF and PMFK that separated from PM 701.

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The incubation of MCF-7 cells in higher and lower concentrations than IC50 concentration of PM 701 confirmed the results of short incubation, long incubation of MCF-7 cells in 2 and 20 microgram (µg) of PM 701/Milliliter (ml) of media inhibited the proliferation of cancer cells in both concentration compared with control non treated cancer cells, whereas figs 5; 6 showed that MCF-7 cells decreased in numbers when PM 701 was added to the incubated media. It also showed that the cells incubated in 2 microgram (µg) died mostly after incubation for 48h.

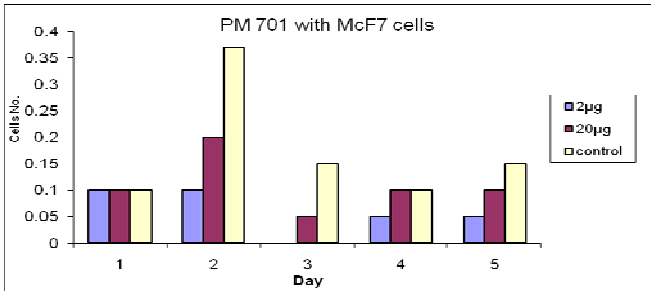


Fig. 5: The confirmed effect of lyophilized PM 701 on MCF-7 cell line, where PM 701 appeared cytotoxic to the cancer cells in both concentration lower and higher than IC_{50} concentration of PM 701 even after long incubation for 24, 48, 72; 96 hr.

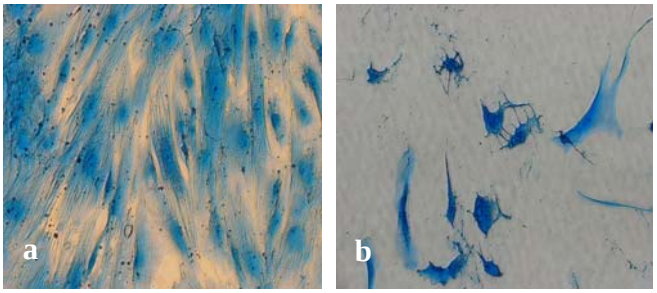


Fig. 6: Images illustrated Mcf7 incubated for 96h in control media (a) and in treated media with PM 701 (b) (10X), note the scattered non healthy cells, with attack nuclei in treated culture compared with healthy and well defined nuclei in non treated culture.

V. Discussion

The resistance of cancer cells to multiple chemotherapeutic agents poses a major problem in the successful treatment of breast cancer. Whether drug resistance is due to changes induced in the drug-exposed tumor cells (adaptation) or represents the selective growth of one or more drug-resistance clones present in initial tumor (selection) remains controversial. But some studies do provide evidence on the development of multidrug resistance (MDR) in a human breast cancer cell line, MCF-7, involving doxorubicin, vinblastine and colchicine [18]. In this study, PM 701 and its fractions were found to be cytotoxic to the MCF-7 cell line and was able to inhibit its proliferation. It proved that PM 701 and its fractions inhibited the proliferation rate of human breast cancer cells in vitro in a dose dependent manner. PM 701 or one of its fractions may use as alternative therapy or in combination with doxorubicin or other regime of therapies.

It is clear that PM701 showed cytotoxic effect on MCF-7 human breast cancer cell line and this suggests the possibility that PMF plays a role in the oxygen transport of breast cancer cells, where earlier studies showed that the treatment of A549 cells with PM701 increases the CH2 content [19]. The assumption would then be that PMF may helps the cancer cells revert back to normal cells by enhancing oxygen transport (turning on aerobic metabolism) and once the cells become normal, they go through the normal process of apoptosis.Other suggestion, that the chemoprevention and therapy by the use of PM 701 and its fractions may have offered new approaches to block tumor growth and progression via its polyphenolic component. Many studies reported that polyphenols are capable of inhibiting the growth of a variety of human cancer cells, via induction of apoptosis in vitro [20]-[23].On the other hand, apoptosis as an active process involves biochemical changes on three essential cellular components, DNA, protein and lipid. It should mentioned here that the amount of DNA decreases dramatically during the treatment of A549 lung cancer cell line with PM70 [19] , while chromatins condensation as a stage of apoptosis was also detected with PM701 treatment in that study that where previously published [19]. So the treatment of MCF-7 with PM 701 and its fractions resulted in the inhibition of both DNA synthesis and cell growth.It also may that the anticancer effect of PM 701 and its fractions related to the potentially toxic or carcinogenic chemicals in it, which promotes the excretion of therapeutic agents reducing the ability of carcinogenic signals to react with cancer cells and damage their nucleic acids and proteins [24].

Raouf [19] proved that PM 701 induced changes in plasma membrane permeability by altering the drug influx/efflux – due to change in membrane fluidity-system in cancerous cells, this result may implement on PM 701 fractions also. As a conclusion, this preliminary in vitro study suggests that PMF may be developed to be a potent natural compound with anti-cancer properties.

VI. Conclusion

In conclusion, the results showed that all tested fractions of PM701 inhibited the growth of the MCF-7 cells; therefore, these results may lead to a finding for a successful alternative drug for breast cancer cells.

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