EJEAFChe
Electronic Journal of Environmental, Agricultural and Food Chemistry

ISSN: 1579-4377

CYTOTOXIC ACTIVITY OF BIOACTIVE FRACTIONS FROM PM 701

Faten A. Khorshid[1], Abdel-Moneim M.Osman[2], Essam Abdel-Sattar[3]

[1]Department of Medical Biology, Faculty of Medicine, King Abdulaziz University, P.O. Box 80216, Jeddah 21589, [2]Department of Pharmacology, Faculty of Medicine, P.O. Box 80202, Jeddah, [3]Department of Natural Products, Faculty of Pharmacy, King Abdulaziz University, P.O. Box 80260, Jeddah 21589, Kingdom of Saudi Arabia

ABSTRACT

The cytotoxic activity of PM 701 (a clean and sterile natural product) and fractions obtained thereof was assessed against human cancer cell lines, namely, hepatocellular carcinoma (HEPG2), colon carcinoma (HCT 116) and human glioma (U251) cells by using sulforhodamine B assay. PM 701 was extracted with methyl alcohol to give PMF fraction that was chromatographed on a Si gel column to yield seven fractions (frs: M2-M8). PMF and its sub-fractions demonstrated a variable and notably high cytotoxic activity against the tested cell lines (IC_{50} 0.5-10 μg/mL). Fractions M2 and M5 were found to demonstrate the most potent activity against HEPG2 cell line (IC_{50} 0.54 μg/mL for each), followed by fraction M8 (IC_{50} 1.01 μg/mL). The effect of the different fractions against normal human foreskin cell line (HFS) was also evaluated. Isolation and identification of the active cytotoxic compounds are now in progress in our laboratories.

KEYWORDS
PM 701, cytotoxicity, HEPG2, HCT 116, U251, HFS.

INTRODUCTION

Cancer is a disease characterized by uncontrolled cellular proliferation and differentiation. Now, cancer became a very common disease with high incidence rate annually [1]. The treatment of cancer is either using surgery, radiotherapy or using chemotherapeutic agents [2]. Chemotherapy has been used in cancer treatment for more than five decades, sometimes in combination with or parallel to radiotherapy or surgery. The systematic use of chemotherapeutic agents leads to severe undesired side effects or resulted in multi drug resistance (MDR) [2]. Tumours can develop MDR to several drugs after a single drug has been administered due to change in genome or the stimulated expression of a specific key protein [3]. The development of MDR has been clinically observed among Vinca alkaloids, anthracyclines, antibiotics and epipodophyllotoxins [4, 5].

Natural products play an important role in our healthcare system [6-9]. They offer a valuable source of potent compounds with wide variety of biological activities and novel structures and provide important leads for the development of novel drugs [7, 10].

Previous investigations of PM 701 [11-16] proved its cytotoxic activity, it caused selective programmed cell death of cancer cells (apoptosis) of cultured human lung cancer cells, while it had reparative effect on normal human cell (foreskin) [11]. It showed the same effect on leukemia cells at tissue culture level. It was effective in limiting of metastasic spreading of leukemia cells in animal models [12, 13]. Light and electron microscopic histopathological study was carried on animal model, which proved the reparitive effect of this agent [16].

In a continuation of our interest on PM 701, a natural product readily available, sterile, cheap and non toxic [15], a more deep study was undertaken to isolate and define the active fraction in a biologically guided study.

MATERIALS AND METHODS

Materials. Silica gel 60 (0.063-0.2 mm) from Merck (Germany) was used in chromatographic separation. All solvents were of analytical grade. The hygroscopic lyophilized powder of PM 701 was prepared according to method reported by Khorshid [15].

Cell lines. Three human cell lines were used in this study, namely hepatocellular carcinoma (HEPG2), colon carcinoma (HCT 116) and glioma (U251) cell lines. These cell lines were purchased from National Cancer Institute, Cairo University, Egypt. Normal fibroblast cell line (HFS) was also used, and obtained from Tissue Culture Bank, King Fahd Medical Research Center, Jeddah, KSA. Cells were grown in RPMI media containing 10% heat inactivated fetal calf serum and 1% penicellin-streptomycin antibiotic. Modified Eagle' s media (MEM) was used for normal human foreskin cell line (HFS).

Cytotoxic activity of PMF and its fractions on the proliferation of Human Cancer Cell Lines

The cytotoxicity of the different fractions was tested using the method of Skehan *et al.* [17]. Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h in a humidified CO_2 incubator at 37°C. Different concentrations of the tested substance (0, 1, 2.5, 5 and 10 μg/ml) were added to the cells (triplicate wells were prepared for each individual concentration) and reincubated for additional 48 h at 37C° in a humidified 5%$C0_2$. After

the time of incubation, cells were fixed by gentle layering with 50 ul of cold 50% TCA (4^oc) on the top of growth medium in each well to produce final concentration of 10%. The cultures were incubated at 4 ^oC for 1 h and then washed five times with tap water to remove TCA. TCA fixed cells were stained for 30 min. with 0.4% (W/V) Sulforhodamine B dissolved in 1% acetic acid. At the end of staining period excess stain was rinsed four times with1% acetic acid to remove unbound dye. Bound dye was solubilizes with 10 mM unbuffered Tris EDTA (100 µl/well). Color intensity was measured immediately in an ELISA reader at wave length 490-530. The relation between surviving fraction and concentration is plotted to get the survival curve of each cell line after the specified period. Results are represented in Table 1 and Figure 1-12.

Cytotoxic activity on normal human foreskin cell line (HFS)

Cytotoxicity assay on HFS cell line was performed using the same previous procedures.

Extraction of PM 701

Water suspension of PM 701 was shacked with different solvents, such as $CHCl_3$, ethyl acetate, n-butanol or extracted with methyl alcohol. The best solvent for extraction was found methyl alcohol and coded fraction PMF.

Fractionation of PM 701

In a typical experiment, 30 g of PM 701 was extracted by sonication using MeOH (3X200 mL) to yield 4.5 g (15 %) of hygroscopic yellow powder (PMF).

Fr. PMF (1.5 g) was chromatographed on a Si gel 60 column (0.063-0.2 mm) using $CHCl_3$ and $CHCl_3$ containing increasing amounts of MeOH (10-60 %) and the column was finally washed with MeOH, to give seven fractions 250 mL each (M2 to M8). Each fraction was tested for its cytotoxicity using HEPG2 Cell line. Fr. M5 (120 mg) on concentration showed a white deposits of fr-10 (20 mg). TLC of fr. M8 showed two major spots, when sprayed with ninhydrin reagent followed by heating at 120^oC for 5 min. A trial for separation these two spots was performed using CHCl3-MeOH-H2O as solvent system and by chromatography on a Si gel column. Chromatographic separation resulted in isolation of two subfractions B-I and B-II (each with one major spot). The results of cytotoxic activity of the different fractions and subfractions isolated from PM 701 against HFS, HCT116, U251and HEPG2 human cell lines (IC_{50} (µg/ml) were presented in Table 1 and Figures 1-12.

RESULTS AND DISCUSSION

The lyophilized PM701 powder was extracted with methanol to give a bioactive fraction PMF. Fraction PMF was subjected to a bioguided fractionation on a Si gel column, which led to the isolation of seven subfractions (M2-M8). Fraction M5 on concentration showed a deposits of pure compound (fr-10). Fraction M8 was separated into B-I and B-II subfractions by chromatography on a Si gel column, and they were impure.

Cytotoxic activity of PMF and related fractions

To investigate the cytotoxic activity of the crude methanolic extract PMF and related fractions (M2-M8), an in vitro assay was performed on the following human cancer cell lines, hepatocellular carcinoma (HEPG2), colon carcinoma (HCT 116) and human glioma (U251). In addition, all fractions were also evaluated against normal human foreskin (HFS) cell line. The method described by Skehan *et al.* [17] was applied using

Sulforhodamine B stain (SRB). The results are expressed as IC_{50} values (μg/ml) and are listed in Table 1 and illustrated in Figures 1-12. The conventional anticancer drug in clinical use, doxorubicin was used as positive control in the present study.

Table 1: Cytotoxic activity of the different fractions and subfractions isolated from PM 701 against HFS, HCT116, U251 and HEPG2 human cell lines (IC_{50} μg/ml).

Fraction No.	IC_{50} μg/ml		SI	IC_{50} μg/ml	SI	IC_{50} μg/ml	SI
	HFS	HEPG2		U251		HCT116	
PMF	> 20	1.28	15.6	7.38,	2.7	1.88	10.6
M2	> 20	0.54	37.0	NT	-	2.75	7.3
M3	> 20	2.21	9.1	NT	-	NT	-
M4	16.9	2.89	5.8	NT	-	NT	
M5	14.2	0.54	26.3	NT	-	3.62	3.9
M6	> 20	2.75	7.3	NT	-	NT	-
M7	> 20	2.55	7.8	NT	-	NT	-
M8	4.8	1.17	4.1	1.21	4.0	5.64	0.85
Fr-10	4.5	0.54	8.3	NT	-	3.49	1.3
B-I	> 20	2.42	8.2	1.48	13.5	4.7	4.2
B-II	> 20	2.08	9.6	1.07	18.7	3.62	5.5
DOX	5.6	0.60	9.3	NT	-	0.69	8.1

NT: not tested; SI: selectivity index.

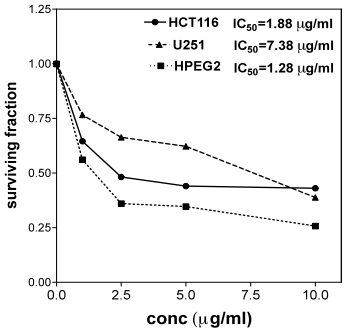

Fig 1: Cytotoxicity effect of fr PMF.

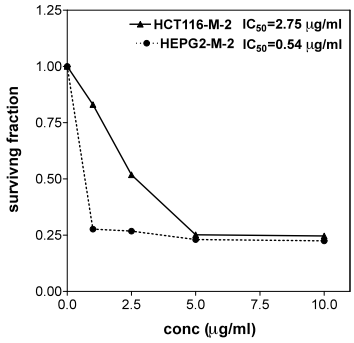

Fig 2: Cytotoxicity effect of fr M-2.

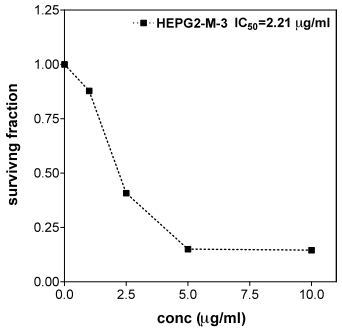

Fig 3: Cytotoxicity effect of Fr M-3.

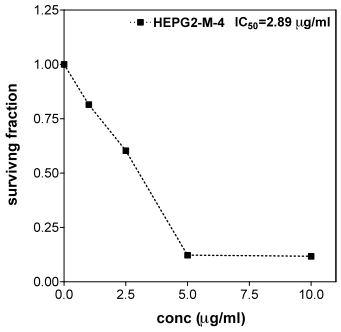

Fig 4: Cytotoxicity effect of Fr M-4.

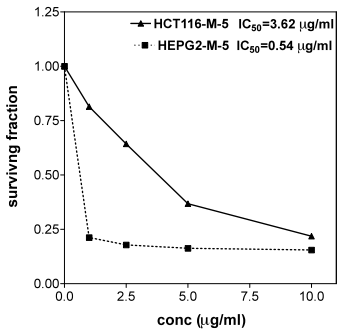

Fig 5: Cytotoxicity effect of Fr M-5.

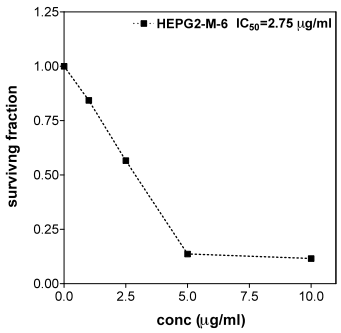

Fig 6: Cytotoxicity effect of Fr M-6.

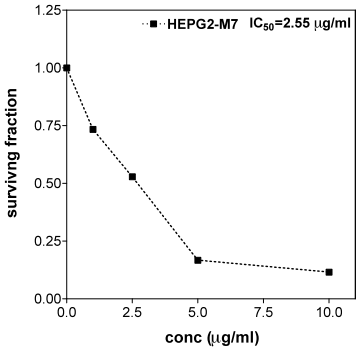

Fig 7: Cytotoxicity effect of Fr M-7.

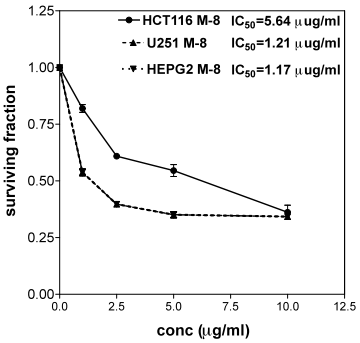

Fig 8: Cytotoxicity effect of fr M-8.

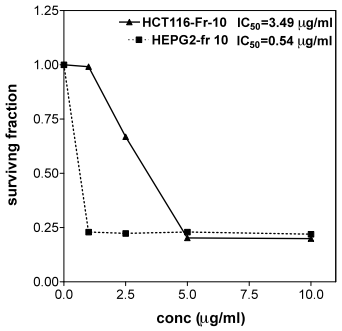

Fig 9: Cytotoxicity effect of fr 10.

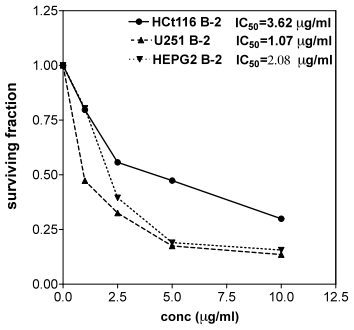

Fig 10: Cytotoxicity effect of fr B-II.

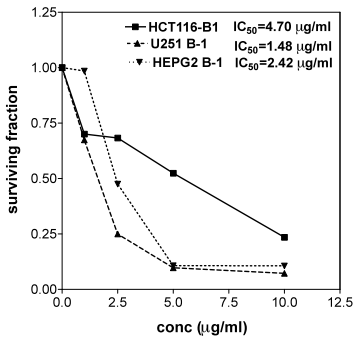

Fig 11: Cytotoxicity effect of fr B-I.

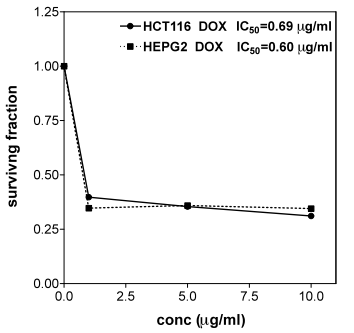

Fig 12: Cytotoxicity effect of DOX.

The American National Cancer Institute assigns a significant cytotoxic effect of extract for future bioguided studies, if it exerts an IC_{50} value ≤30 μg/mL [18]. On the other hand, we evaluated the SI; it is generally considered that biological efficacy is not due to cytotoxicity when SI ≥10 [19].

All the tested fractions exhibited different potency of cytotoxic activities in a concentration-dependent manner, although this effect varied among the cell types (see Table 1 and Figures 1-12).

Fr PMF showed a strong cytoxic activity against HPEG2 and HCT116 cell lines with IC_{50} 1.88 and 1.28 μg/ml, respectively. A weaker activity was observed against U251 cell line with IC_{50} 7.38 μg/ml. Fractionation of PMF fraction on a Si gel column, led to the isolation of seven subfractions (M2-M8). Among them, fractions M2, M5 and M8, showed the most potent activities against HEPG2 cell line with an IC_{50} 0.54, 0.54 and 1.17 μg/ml, respectively. The observed IC_{50} data of fractions M2 and M5 were comparable to that of the positive control doxorubicin, against HEPG2 cell line (see Table 1).

Fraction M5 on concentration showed a white deposits of fr-10. This deposits showed a strong cytotoxic activity against HEPG2 with an IC_{50} 0.54 μg/ml (Figure 9). The two impure subfractions B-I and B-II, were tested against U251 and HEPG2 cell lines. Fr B-I showed IC_{50} of 1.48 and 2.42 μg/ml against U251 and HEPG2, respectively, while fr B-II showed nearly similar activity with IC_{50} 1.07 and 2.08 μg/ml, respectively (see Table 1 and Figures 10 and 11). Fractions M3, 4, 6 and M7 were tested only against HEPG2 cell line and showed a moderate and nearly similar cytotoxic activity with an IC_{50} 2.21, 2.89, 2.75 and 2.55 μg/ml, respectively.

For host cells, cytotoxicity of all fractions was also evaluated against normal human foreskin cells (HFS). All fractions tested against HFS cell line showed IC_{50} more than 20 μg/ml, except for doxorubicin, M4, M5, M8 and fr-10 showed IC50 of 16.9, 14.2, 4.8 and 4.5 μg/ml, respectively. In addition, doxorubicin showed IC_{50} of 5.6 μg/ml. Fraction M2 was most selective (SI 37) followed by M5 (SI 26.3) using HEPG2 cell line, while M8 showed the least selectivity with SI 4.1.
Isolation, purification and identification of the active cytotoxic compounds are now in progress in our laboratories, in addition to the in vivo study of PMF fraction.

Acknowledgments – The authors gratefully acknowledged financial support of El-Zamel's scientific chair, Research and consultation institute, King Abdulaziz University, and authors also wish to thank Mrs Doaa Badr Mohamed for technical assistance, National Cancer Institute, Cairo, Egypt.

References

[1] Parkin D, Pisani P, Ferlay J. Global cancer statistics. CA: A cancer journal for clinicians 49, 33-64 (1999).
[2] Baquiran DC and Gallagher J. Lippincott's Cancer Chemotherapy Handbook, Lippincott-Raven Publishers-Philadelphia (1998).
[3] Ford JM. Modulators of multidrug resistance. Hematology/Oncology Clinics of North America, 9, 337-361 (1995).
[4] Beck WT, Cirtain MC, Danks MK, Felsted RL, Safa AR, Wolverton JS, Suttle DP and Trent JM. Pharmacological, molecular and cytogenic analysis of "atypical" multidrug-resistant human leukemic cells. Cancer Research, 47, 5455-5460 (1987).
[5] Danks MK, yalowich JC and Beck WT. Atypical multiple drug resistance in a human leukemic cell line selected for resistance to teiposide (VM-26). Cancer Research, 47, 1297-1301 (1987).
[6] Pezzuto JM. Plant-derived anticancer agents. Biochemical Pharmacology 53, 121-133 (1997).
[7] Cragg GM. Paclitaxel (Taxol®): A success story with valuable lessons for natural product drug discovery and development. Medicinal Research Reviews, 18, 315-331 (1998).
[8] Lee KH. Novel antitumor agents from higher plants. Med Res Rev., 19, 569-96 (1999).
[9] Schwartsmann G. Marine organisms and othet novel natural sources of a new drugs. Annals of Oncology, 11, 235-43 (2000).
[10] Vuorela P, Leinonen M, Saikku P, Tammela P, Rauha JP, Wennberg T and Vuorela H. Natural products in the process of finding new drug candidates. Current Medicinal Chemistry, 11, 1375-1389 (2004).
[11] Khorshid, Mosherf SS, Tawfiq NH. An Ideal Selective Anticancer Agent In Vitro, I- Tissue Culture Study of Human Lung Cancer Cells A549. JKAU- Medical Sciences, 12, 3-19 (2005).
[12] Khorshid FA, Moshref SS. In Vitro Anticancer Agent, I - Tissue Culture Study of Human Lung Cancer Cells A549 II - Tissue Culture Study of Mice Leukemia Cells L1210. International Journal of Cancer Research, 2, 330-344 (2006).
[13] Moshref SS, Khorshid FA, Jamal Y. The Effect of PM 701 on Mice Leukemic Cells:I - Tissue Culture Study of L1210 (In Vitro) II - In Vivo Study on Mice, JKAU- Medical Sciences, 13, 3-19 (2006).
[14] Abdel-Rauf G, Khorshid FA and Kumosani T. FT-IR Spectroscopy as a Tool for Identification of Apoptosis-Induced Structural Changes in A549 Cells Dry Samples Treated With PM 701. At the IBBI08–Isaoland Biosocial Interaction Conference. 13-18 April 2008 (Spain).
[15] Khorshid F. Preclinical evaluation of PM 701 in Experimental animals. Int. J Pharmacology, 4, 443-451 (2008).
[16] Moshref SS (2007): PM701 A Highly Selective Anti Cancerous Agent Against L1210 Leukemic Cells: II – In Vivo Clinical and Histopathological Study. JKAU- Medical Sciences, 14, 85-99.

[17] Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst.*, 82,1107–1112 (1990).

[18] Suffness M, Pezzuto JM. Assays related to cancer drug discovery. In: Hostettmann, K. (Ed.), Methods in Plant Biochemistry: Assays for Bioactivity, vol. 6. Academic Press, London, pp. 71–133 (1990).

[19] Vonthron-Sénécheau C, Bernard-Weniger B, Ouattara M, Tra-Bi F, Kamenan A, Lobstein A, Brun R, Anton R. In vitro antiplasmodial activity and cytotoxicity of ethnobotanically selected Ivorian plants. Journal of Ethnopharmacology, 87, 221–222 (2003)