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ORIGINAL ARTICLE

Cytotoxic activity of partial purified ExoT toxin produced by *P. aeruginosa* against MCF-7 and PC_3 cancer cell lines

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ABSTRACT

Background*: ExotoxinT (ExoT) is one of these exotoxins secreted through the type three secretion system (T3SS) produced by Pseudomonas aeruginosa.* ***Objectives:****This study aims to investigate the ability of exotoxin A extracted from Pseudomonas aeruginosa to inhibit cancer cell lines breast cancer (MCF-7) and prostate cancer (PC3).* ***Methodology****: seventy one isolates were isolated from 242 clinical samples. The isolates were identified using various microscopic, biochemical, and cultural diagnostic methods.The toxin was then extracted and purified from the productive isolate. ExoT was purified using ammonium sulfate, ion exchange, and gel filtration chromatography (Sephadex G-150). The MTT test was performed to study its inhibitory effects against cancer cell lines in vitro.* ***Results:*** *The results of the MTT assay refer to half-maximal inhibition concentration (IC50) of partially purified ExotoxinT aganist MCF-7,PC3 and HDFn cells was 84.72, 193.1, and 243.9 µg/ml, respectively. The toxic effect was more significant on breast cancer cells than on prostate cancer cells for the values of IC50 compared to normal cells (HDFn). The effect of partially purified ExotoxinT on some criteria or indicators of apoptosis was evaluated by high-content screening that showed a significant effect when exposed to concentrations of 200, and 100 µg/ml.* ***Conclusion:*** *The effect of partially purified exotoxin T was studied by MTT assay and apoptosis indices by high-content assay and showed significant effects.*

INTRODUCTION

Various bacterial products such as toxin, peptides, bacteriocins, spores and enzymes, have attracted interest as highly potential therapeutic agents for cancer therapy. Furthermore, bacteria have been employed either alone or with traditional approaches in cancer bacteriotherapy. Also, they have been employed as carriers for delivering genes, drugs, which have had favorable outcomes in terms of tumor regression and suppression[1]. *Pseudomonas aeruginosa* is frequently responsible for fatal infections acquired in hospitals. These bacteria employ many secretion systems to transport various virulence factors into host cells, facilitating the establishment of infections. *P. aeruginosa* possesses the remarkable ability to metabolize diverse resources from various conditions, outcompete other bacterial species, and acquire resistance to antibiotics. These attributes contribute to its exceptional success as a pathogenic organism[2-3]. Type III secretion system (T3SS) is an important component of *Pseudomonas aeruginosa* virulence, transporting the cytotoxin ExoT into host cells. It possesses amino-terminal GAP domains that inactivate Cdc42, Rac, and Rho, as well as carboxyl-terminal ADP-ribosyltransferases that modify specific host cellular proteins via ADP-ribosylation.[4] The activation of ExoS and ExoT has been considered critical due to the interaction of an amphipathic C-terminal helix in the 14-3-3 phosphopeptide binding groove. The crystal structures of the 14-3-3β, ExoS and -ExoT complexes, demonstrate a large hydrophobic interface that is capable of facilitating the formation of the complex and activating the toxin.[5] The ExoT protein disrupts the actin cytoskeleton, resulting in cell rounding and suppressing cell migration. It acts as an anti-internalization factor, preventing the uptake of substances into the cell. Additionally, it hinders cell division by interfering with cytokinesis at various stages and impedes wound healing. Another study has shown that ExoT is essential and effective for promoting apoptosis in HeLa cells, principally based on its ADPRT domain activity. However, the specific process by which ExoT triggers apoptosis in epithelial cells still needs to be understood.[6-7]

METHODOLOGY

Isolation and Identification of *P. aeruginosa*

In this study, 242 samples were taken from clinical cases from Ramadi Hospitals between November 2022 and December 2023. The cases included both male and female patients of different ages. By Gram staining, cultural characteristics, biochemical reactions and

Vitek2 compact system, species identification *P. aeruginosa was* confirmed[8].

ExotoxinT source

ExotoxinT was extracted and purified from 23 isolates *P. aeruginosa*. Which were the highest ExotoxinT produces out of 71 isolates obtained from Ramadi Hospital patients. ExotoxinT was identified by polymerase chain reaction (PCR) through the detection of the ExoT gene and concentration detection via the ELISA technique. The ExotoxinT was purified using ammonium sulphate, ion exchange chromatography (DEAE), and gel filtration chromatography (Sephadex G-150). The final product of the purification process was further concentrated by lyophiliziation and resuspension with appropriate amount of phosphate buffer saline to the required concentration for cytotoxicity experiments.[9-10]

Cellular culture

Cellular culture was done by three distinct cell lines specifically, breast cancer (MCF-7), prostate cancer (PC_3) and normal human dermal fibroblast (HDFn) cell lines. The cellular culture was conducted using PRMI-1640 medium, supplemented with 10% fetal bovine serum and 1% antibiotics (Streptomycin, Nystatin, Benzyl Penicillin). The culture was placed in tissue culture flasks and incubated at 37°C, with a carbon dioxide concentration of 5% until a monolayer cell line was formed. Cell densities were determined using the trypan blue exclusion method, resulting in a final quantification of 1 x 10^4 viable cells per ml.

Cytotoxicity assay

The cellular suspension was added into a 96-well plate at 1 X 10^4 cells per ml. The final volume of complete culture media in each well was 200 ml. After being covered with sterile parafilm, the plates were gently shaken, mixed, and incubated in a 5% CO_2 incubator at a temperature of 37 °C for 24 hrs. Following the incubation period, the medium was removed and 200µl of the produced concentration of partially purified ExotoxinT (400, 200, 100, 50, 25, µg /ml) were introduced into the culture wells of cell lines. A triplicate experiment was done for each concentration and control group. The plates were incubated in a 5% CO_2 incubator at a temperature of 37°C for 24 hrs. Following exposure to exotoxin T, 20µl of MTT solution was introduced into each well. Subsequently, the plate was placed in a 5% CO_2 incubator for 4 hours at a temperature of 37°C. Next, the medium was precisely taken out, and a 200µl of solubilization solution (DMSO) was added to each well. The wells were then incubated for 5 minutes. This study used ELISA devices to measure absorbance at a wavelength of 570nm. Based on the measurement of optical density, the IC50 values were calculated. Based on the given equation, Viability (%) = ratio of the optical density of the sample to the optical density of the control, multiplied by 100%. [11]

High content screening (HCS):

The purpose of this test was restricted to investigation of the impact of partial purified exotoxin T on the MCF-7 cell line, as determined by the results obtained from the MTT assay. This investigation includes the detection and monitoring of alterations in cellular viability, nuclear density, cell membrane permeability, mitochondrial membrane strength, and the release of cytochrome C from mitochondria. Distinct concentrations (25, 50, 100, 200µg/ml) were derived from the partial purified ExotoxinT. A volume of 50µl from each concentration was added into the well containing MCF-7 cancer cells, with three replicates for each well. Additionally, 50µl of culture media were exclusively supplied to the control wells. The cells were incubated at 37°C for 24 hours then follow the protocol of Al-Dulimi et al . [12]

Statistical analysis

The representation of all data was expressed as the mean ± standard deviation of the mean. An analysis of statistical data was conducted using GraphPad Prism (version 8).

RESULTS

The present study investigated the impact of partially purified ExotoxinT at various concentrations 400, 200, 100, 50 and 25 (µg/ml) on the growth rates of MCF-7, PC_3 cancer cells and normal cells (HDFn), it was assessed in vitro. The percentage of cell viability in the presence of ExotoxinT was determined using the MTT assay. The results demonstrated that viability rates of MCF-7 cells were 39.77%, 51.04%, 61.57%, 75.54%, 86.11% and PC_3 cells were 52.16%, 63.61%, 74.69%, 85.41%, 93.98% and HDFn cells were 73.99%, 86.22%, 93.63%,94.05, 95.25 in the presence 400, 200, 100, 50 and 25 µg/ml of partially purified ExotoxinT respectively also, the results of the MTT reduction assay showed that half-maximal inhibition concentration (IC50) of ExotoxinT agonist MCF-7, PC3 and HDFn cells was 84.72, 193.1, 243.9 µg/ml respectively, after 24 hours of incubation at 37 °C. The results showed that ExotoxinT had a significant effect on cancer cells including; breast cancer cells (MCF-7) and prostate cancer cells (PC3), compared to normal cells (HDFn) during an incubation period of 24 hours at 37 °C. The toxic effect was more significant on breast cancer cells than on prostate cancer cells for the values of IC50 as shown in figure 1.

The effect of partially purified ExotoxinT on some criteria or indicators that indicate programmed cell death was evaluated. The results of the high-content screening for MCF-7 cells showed a significant high effect on cell viability when exposed to concentrations of 200, and 100 µg/ml, while no significant effect was shown for exposure to concentrations of 50 and 25µg/ml .

The ExotoxinT has a role in inducing the programmed death process of MCF-7 cancer cells, as we noticed through these results a highly significant effect upon exposure to concentrations of 200, 100 of partially purified ExotoxinT for both total nuclear intensity and cytochrome c release from mitochondria and no significant differences for the effect of concentrations of 50, 25µg/ml compared to untreated cells. The release of cytochrome c is an effect of the mitochondrial membrane potential, as the results showed a highly significant effect for concentrations of 200, 100, and 50 µg/ml of partially purified ExotoxinT and exposure to a concentration of 25µg/ml did not show any significant difference. Only exposure to a concentration of 200 µg/ml of exotoxin T showed a significant effect on the permeability of the cell membrane and the other concentrations of 100, 50, and 25 did not significantly affect the permeability of the cell membrane compared to untreated cells at a probability $P \leq 0.05$ (fig 2).

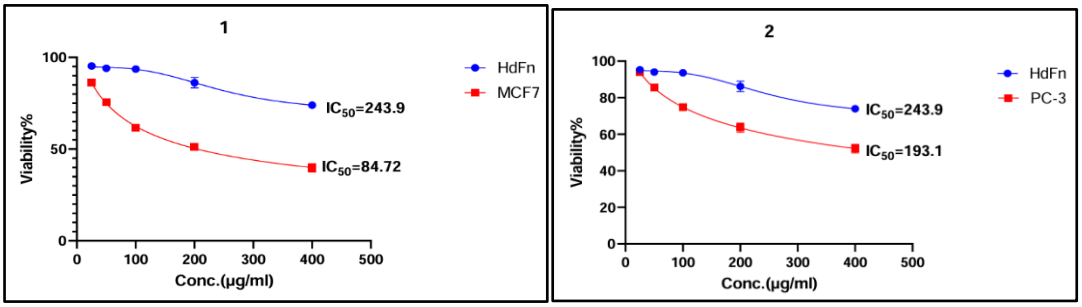

Fig. 1: Cytotoxicity effect of partial purified ExotoxinT on MCF-7, PC_3 and HDFn cell lines after 24 hours of incubation at 37ºC.

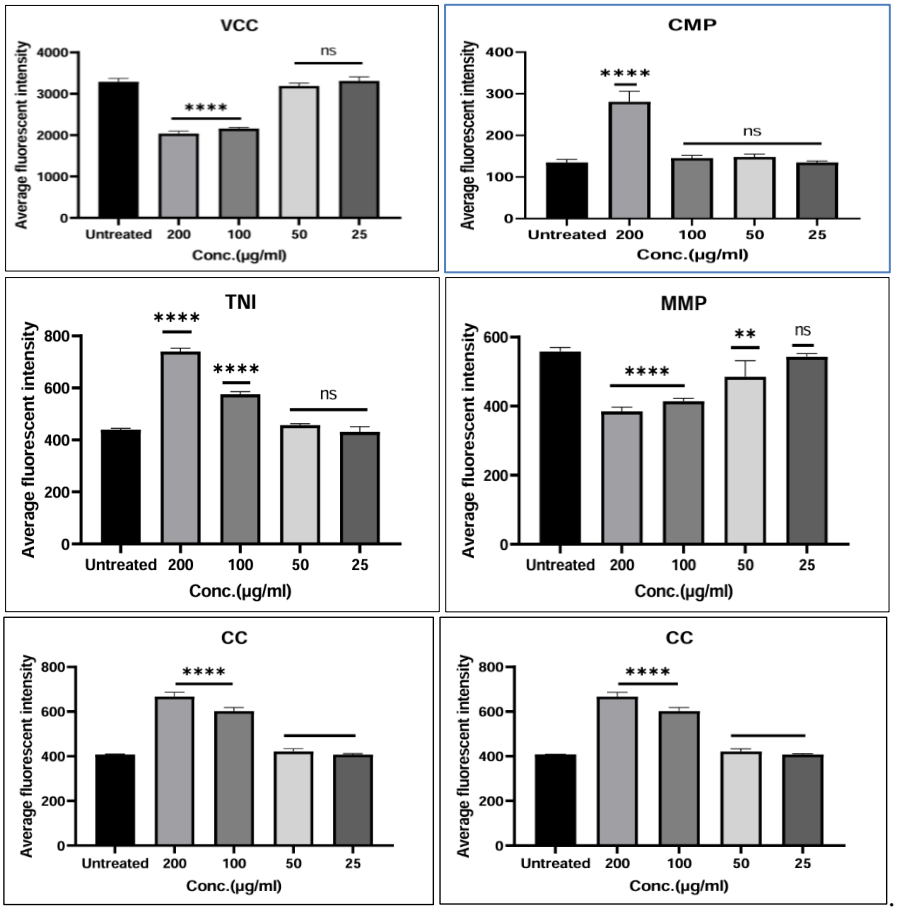

Fig. 2: Effect of different concentrations of partial purified ExotoxinT on the apoptosis of MCF-7 cells after 24 hrs of incubation at 37°C using HCS technique. VCC: Viable Cell Count; CMP: cell membrane permeability; TNI: Total Nuclear Intensity; MMP: Mitochondrial Membrane Potential; CC: Cytochrome C. ns: non-significant ; * : significant ; *** : highly significant ; $P \leq 0.05$

DISCUSSION

There are several studies in agreement with our results. These studies reported that ExoT can induce significant cytotoxicity in several resistant cancer cell lines and to generate a substantial decrease in tumor volume in an animal model of melanoma.[13] The potent cytotoxicity of ExoT in cancer is attributed to its capacity to trigger two different modes of apoptosis in cancer cells. ExoT triggers apoptosis by disrupting the integrin-mediated survival signalling process through its ADPRT domain and by inducing typical intrinsic (mitochondrial) apoptosis through its GAP domain[14]

The results suggest that ExoT has a strong inhibitory effect on the growth of melanoma cells. Experimental results demonstrated that toxin induces cell cycle arrest in the G1 interphase of melanoma cells by suppressing the G1/S checkpoint proteins. This study refer evidence that both the ADP-ribosyltransferase and GTPase activating protein (GAP) of the toxin play a role in causing G1 cell cycle arrest in melanoma. G1 cell cycle arrest in melanoma cells, triggered by ADPRT, is most likely associated with the Crk adaptor protein. Some studies have indicated the importance of these toxins and have further emphasized their therapeutic potential against tumors. [15]

CONCLUSION

The effect of partially purified exotoxin T was studied by MTT assay and apoptosis indices by high-content assay and showed significant effects.

Ethical approval:

The Research Ethical Committee of University of Anbar of had approved this research code No:96

Conflict of interest: None

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