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CYTOTOXIC POTENTIAL OF ARGINASE ENZYME FROM *PSEUDOMONAS AERUGINOSA*

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Abstract

In the study was based on studying the cytotoxic effect of arginase enzyme on breast cancer cell line (MCF-7), the purified arginase was showed highest inhibition for growth of breast cancer cell line (MCF7) with an IC50 of (116.0) µg/ml in comparison with an IC50 of (136.4)µg/ml for normal cell line. Five concentrations (200, 100, 50, 25, and (12.5) µg/ml of purified enzyme arginase was tested on breast cancer cell line (MCF-7) to detect the changes in six cellular parameters using high content screening system (HCS) (viable cell count VCC, nuclear intensity NI, cell membrane permeability CMP, mitochondrial membrane potential MMP and cytochrome C) reading result revealed that the concentration (200 µg/ml) have highest significant effect on the most parameters when compared with control.

Introduction

Enzymes are nature's catalysts molecule, they are biocompatible, biodegradable. Thousands of metabolic processes that sustain life which performed by enzymes, which consider a large biological globular protein molecule, and their function as catalysts to facilitate specific chemical reactions within the cell (Mori and Gotoh 2004).

L-Arginase (EC3.5.3.1) is an important enzyme in urea cycle hydrolyzed L-arginine by cleavage of guanidine group to urea and L-ornithine (Ash, 2004). L-arginine hydrolysis by L-arginase that will produce proline and polyamines, the last is important in cell proliferation and growth. These metalloenzyme is crucial in the urea cycle, which facilitates the organism in detoxifying ammonia and excretion of excess nitrogen from the system, and also regulates the cellular levels of arginine through these metabolic pathways, which lead to regulates the level of L-arginine for the production of nitric acid, regulates the level of L-ornithine for the production of L-proline and polyamines and facilitate cell growth and repair, respectively (Al-ezzy *et al*., 2022). There are critical relationship between the host and the pathogen to regulate arginase isoforms and the several infections. Arginine is a crucial amino acid that serves as one of the influencing factors which responsible for modulation of cellular immune response during infection. Arginine is also a common substrate for both inducible nitric oxide synthase (iNOS) and arginase. Arginine, also a common substrate for both inducible nitric oxide synthase (iNOS) and arginase (Dzik, 2014).

Cancer diseases showed by abnormal growth and spread of cells because of missing regulation of gene (Shalash *et al*., 2021). Normal cells are subjected to receive signals that the cell

should divide or die. Cancer cells altering those signals, resulting in randomly growth and proliferation (Hejmadi, 2010, Ad'hiah *et al*., 2018).High content screening (HCS) is a cellular imaging-established approach that played important role in the detection of toxicity and classification of compounds depending on observed patterns of reversible and irreversible cellular damage. HCS supply multi-parametric analysis of compound toxicity at the level of individual cells (Al-Ezzy *et al*., 2019; Mahmood *et al*., 2021).

Materials and methods

Arginase enzyme

Arginase enzyme ready to use from College of biotechnology/Al-Nahrain University used in this research.

Cytotoxic Effect of Arginase on a MCF-7 cell line: The cytotoxic effect method was performed by using MTT (Methylthiazolyldiphenyl-tetrazolium) ready to use kit to investigate the effect of arginase on MCF-7 cancer cell lines. In addition to normal cell line WRL-68. The cytotoxic effect of different concentrations (12.5, 25, 50, 100 and 200 µg/mL) of purified arginase enzyme (ready to use form Al-Nahrain biotechnology college) was performed using MTT ready to use kit (Intron Biotech /Korea) (Al-Saffar *et al.,* 2020; Hadi *et al.,* 2021).

High content screening assay: Five independent parameters including (Viable cell count (VCC), Total nuclear intensity (NI), cell membrane permeability (CMP), mitochondrial membrane potential (MMP) and cytochrome C releasing) which refers to the cell health. Different concentrations (12.5, 25, 50, 100 and 200 µg/ml) of the purified arginase of *Pseudomonas aeruginosa* were tested on MCF-7 cell lines. The assay was carried on at the Centre for Natural Product Research and Drug Discovery, Department of Pharmacology, Faculty of Medicine, University of Malaya Kuala Lumpur.

Statistical analysis: One mode examination of variance ANOVA (Duncan) was made to test whether group alteration was important or not, statistical significance was defined as $p \leq 0.05$. Data were expressed as mean± standard deviation and statistical significances were carried out using Graph Pad Prism version 6 (Graph Pad Software Inc., La J).

Results and Discussion

Cytotoxic effect of arginase enzyme in Vitro using MTT assay: The test of 3-(dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT) was carried out to estimate the cytotoxic effect of arginase produced by breast cancer cell (MCF-7) . MTT Assay was made to account the cells viability and inhibition rate on the tumor cell line by using different concentrations of arginase enzyme. The percentage viability of treated cells was calculated in a comparison with normal cell lineWRL-68. The cytotoxic effect of arginase detected in concentration ranged from (12.5-400) µg/ml on MCF-7.

The result in Table (1) there were a decrease in cell viability and the cell viability is reduced by increasing the concentration of arginase enzyme. The decreasing in MCF-7 cell viability (%) was noted by 400µg/ml (50.44 ± 6.79) while the highest MCF-7 cell viability at (12.5) µg/ml reached to (93.82 ± 0.17). The arginase showed significantly the most potent cytotoxic activity with IC50 value of (116.0) µg/ml. Furthermore an IC50 of 136.4 µg/ml was obtained from the effect of

arginase on WRI-68 normal cell line (Figure 2) with cell viability ranged from (94.13 ± 1.52 to 68.98 ± 3.63) from (12.5 to 400) µg/ml.

Table (1): Cytotoxicity effect of arginase on MCF-7 and WRL-68 cells after 24 hr. incubation at 37ºC. *: Mean ± SD.

Arginase concentrations (µg/ml)	Viable cell count of MCF-7 cell line *	Viable cell count of WRL-68 cell line *
400	50.44 ± 6.79	68.98 ± 3.63
200	59.25 ± 3.04	74.46 ± 0.85
100	74.07 ± 1.81	90.58 ± 3.93
50	86.92 ± 2.43	95.02 ± 0.87
25	93.44 ± 1.79	94.71 ± 1.87
12.5	93.82 ± 0.17	94.13 ± 1.52

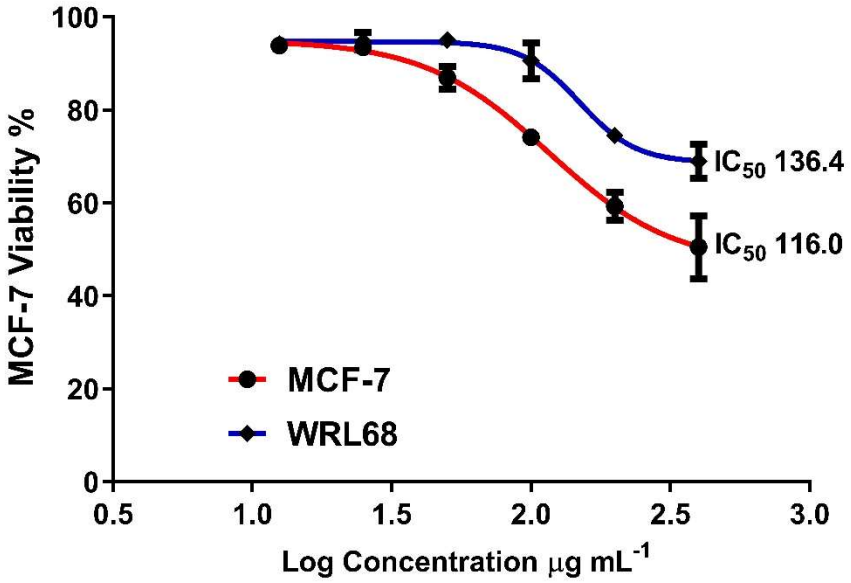

Figure (2): Cytotoxic effect of arginase on MCF-7 andWRL-68 cells after 24 hr. incubation at 37 ºC.

Specific type of cancers may be auxotrophic for a particular amino acid, and amino acid deprivation is one method to treat these tumors. Arginine deprivation is a novel approach to target tumors which lack argininosuccinate synthetase (ASS) expression. ASS is a key enzyme which important in arginine metabolism (Feun *et al.,* 2008). The importance of arginine in the cell because its contribution in multiple pathways that affected main important cellular functions such as nitric oxide production, creatine production and polyamine synthesis. In tumor cells, arginine influences their growth/proliferation (Yoon *et al*., 2012) and diet restriction has been shown to growth inhibition (Bowles *et al*., 2008). Arginase consider to be a particularly valuable source of

effective anti-proliferative, and cytotoxic agents against different cell lines. Therefore, they were made to cause metabolic stress and inhibit MCF-7 cells growth by inducing apoptosis and necrotic phenotypes (Al-ezzy *et al*., 2022)

High Content Screening of arginase enzyme on MCF-7 cell line: High-Content Screening (HCS) is a cellular imaging- depend on approach that played an important role in detecting the toxicity and classification of compounds, which may cause reversible and irreversible cellular injury. Furthermore, HCS provide analysis of compound toxicity at the level of individual cells (Abraham *et al*., 2008). Five concentrations (200, 100, 50, 25, and 12.5 µg/ml) of enzyme arginase was checked on MCF-7 cell line to detect the changes in six cellular parameters (viable cell count VCC, nuclear intensity NI, cell membrane permeability CMP, mitochondrial membrane potential MMP and cytochrome C) after 24 hr of exposure. Table (2) showed that (200) µg/ml has the highest significant effect on the most parameters when compared with control (untreated cell). Another concentration was (25, and 12.5) µg/ml showed results close to those of the untreated cells which represent the negative control with a very few significant differences.

The results of HCS are highly sustaining that of the MTT in which the reduction was dose dependent in cell count and the most significant reduction at 200 µg\mL. Cell viability known as the most frequent toxicity assay as it associated with the toxic effects of arginase (Zeng *et al*., 2013). Such effects may be attributed to down-regulate of telomerase enzyme by cytostatic or cytocide its activity. (Feun *et al*., 2008) founded that arginase had the ability to inhibit the growth of different cell line due its toxic effect upon cancer cell. Qiu *et al*., (2014) stated the activity of arginase represented cell cycle arrest with eventual apoptosis in breast cancer cells lines.

Table (2) mention that (200) µg/ml has the highest significant effect on the three parameters (nuclear intensity NI, cell membrane permeability CMP and cytochrome C) when compared with control (untreated cell). Also, (25 and 12.5) µg/ml reported that the results close to those of the untreated cells which represent the negative control with a very few significant differences. In Table (2), arginase shows significant increasing the nuclear intensity of MCF-7 cell line. This increasing was dose dependent (535.5±9.19, 481.5±9.192, and 437.5±24.75) for (200, 100, and 50) µg/ml, respectively. The highest percentage of increasing was (535.5±9.19) at 200 µg/ml when compared with control. In addition, (25 and 12.5) µg/ml did not appear any significant differences from control.

Nuclear condensation, nuclear fragmentation, cell shrinkage, formation and aggregation of apoptotic bodies are key role futures of apoptotic morphology of the cell (Al-Barazanchi *et al*., 2014). The results stated that mitochondrial membrane of MCF-7 cell was more permeable following treatment with the arginase at different concentration, so that led to death of cell by apoptosis or necrosis. These events can cause the mitochondrial membrane to lose its potential with the subsequent release of cytochrome C .Also arginase induces DNA damage in cancer cells which is consider an indicator of apoptosis (Husain *et al.*, 2017).

On the other hand mitochondrial membrane potential (MMP) listed in table (2) indicates that significantly decreased the MMP (561.0±19.80) for (200) µg/ml. Other concentrations (100, 50, 25, and 12.5) µg/ml did not revealed any significant differences from control. MPP measurement

was dependent on the mean intensity of (MMP) dye penetrating the mitochondria, and the highest effect upon mitochondria was the less fluorescent intensity. Apoptosis is often.dysregulated during cancer development and it is very significant to trigger proper apoptosis which occurs via the mitochondrial outer.membrane permeabilization (MOMP) and resulting in caspase activation and protein substrate cleavage (MOMP pathway) (Garrido, *et al*., 2006)

Also cell membrane permeability table (2) showed that all concentrations did not show any significant differences when compared with control. It has been reported that changes in cell membrane permeability are often associated with a toxic or apoptotic responses, and the loss of cell membrane integrity is a common phenotypic feature of marked cytotoxicity (Al-barazanchi *et al.*, 2014)

Cytochrome C releasing results in table (2) and represented non-significant changed in cytochrome C releasing in all concentration compared with control (484.5±19.09, 434.5±10.61, 428.5±16.26, and 441.0±19.80) for (100, 50, and 25, and 12.5) µg/ml respectively, except 200 µg/ml showed few increase (522.5±9.192). Cytochrome C was found within the nucleus of arginase-treated cells, especially at higher concentrations. When Cytochrome C releases, it cause to the activation of a cascade of caspase and cysteine proteases, which are consider the main players for the several events of the degradation and digestion of the cell from inside (Abraham *et al*., 2008).

Table (2): Cytotoxicity effect of arginase on multi cellular parameters after 24 hrs. of incubation at 37º C. *

Concentration µg/ml	Viable cell count	Nuclear intensity	MMP	Cell membrane permeability	Cytochrome C Releasing
Untreated	**3761±91.92**	**445.5±20.51**	**662.5±34.65**	**133.5±19.09**	**432.5±24.75**
200	**2673±173.2**	**535.5±9.19**	**561.0±19.80**	**133.5±12.02**	**522.5±9.192**
100	**3088±87.68**	**481.5±9.192**	**606.5±20.51**	**131.0±4.243**	**484.5±19.09**
50	**3551±176.8**	**437.5±24.75**	**653.0±4.243**	**142.5±7.778**	**434.5±10.61**
25	**3781±89.10**	**438.0±29.70**	**650.5±43.13**	**135.0±32.53**	**428.5±16.26**
12.5	**3834±15.56**	**443.0±21.21**	**644.5±17.68**	**133.0±11.31**	**441.0±19.80**

***: Mean ± SD.**

***Different letters in rows indicates significant differences (P ≤ 0.05).**

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