Evaluation of Antioxidant and Antiproliferative Activities of L-Asparaginase Produced by Pseudomonas aeruginosa

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Abstract

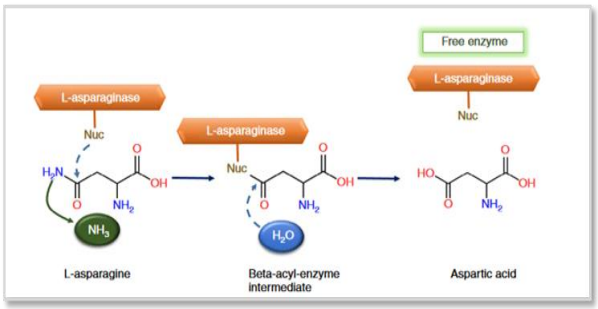
L-asparaginase is an antineoplastic enzyme that, is often used to treat acute lymphoblastic leukaemia (ALL). Pancreatic cancer, acute myeloid leukaemia, and lymphosarcoma. It had properties; such as antioxidant and anticancer. In the present study properties of purified L-asparaginase were analyzed in vitro. The effect of enzyme toxicity on human blood components was evaluated by hemolytic and in vitro cytotoxicity assays. Free radical scavenging potentials of the purified L-asparaginase against DPPH were confirmed based on its stable antioxidant effects. The in vitro antiproliferative activity of the purified L-asparaginase against breast cancer (MDA-MB-231cell line) with IC50 value 88 µgmL-1. The enzyme demonstrated increased activity, selectivity, and antiproliferative efficacy in cancerous cells and weak cytotoxicity in normal cells (WRL68). The findings of this study indicate that the purified L-asparaginase II could be utilized in medicinal applications.

Keywords: *L-asparaginase, cytotoxicity; MDA-MB-231 cells.*

1. INTRODUCTION

Employing enzymes in cancer treatment is the most significant therapeutic application. L-ASNase (E.C.3.5.l.l) a significant biocatalyst, caused L-Asparagine to hydrolyze in the presence of water, producing L-Aspartate and Ammonia as byproducts. [1].

Figure-1 The general mechanism of L-asparaginase catalyzed reactions [2]



In the 1970s the anti-proliferative properties of L asparaginase on leukemic cells were first identified and characterized clinically. Since then, L-ASNase became a powerful anticancer medication in the treatment of a wide variety of cancers particularly acute lymphoblastic leukaemia (ALL) and lymphosarcoma. Biochemically, L-ASNase causes a depletion of asparagine, which is an important amino acid for the development of malignant cells, and finally stimulates apoptosis by hydrolyzing the exogenous L-asparagine (Asn) that is present in the bloodstream. [3]. Aside from anticancer action, the enzyme can prevent or minimize the generation of acrylamide is considered a probable human carcinogen in baked or fried meals. At high temperatures, and low humidity free L-asparagine in raw vegetable and meat-

based food ingredients can react with reducing sugars. The use of ASNase before baking has been shown to dramatically minimize the generation of acrylamide [4]. Asparaginase had two isozymes: type I (cytosolic enzymes) and type II (periplasmic enzymes). Both types I and type II have been shown enzymatic activity for both L-asparagine and L-glutamine. Nevertheless, type II displayed higher specific action against L-asparagine. Thus, asparaginase II precisely shows antitumor activity and was utilized in chemotherapeutics [5]. The present study aimed to prove the probable antiproliferative activity of L-asparaginase against the human breast (MDA-MB-123) cell line, and assessment the antioxidant and hemolysis to human red blood cells in vitro to evaluate the toxicological impacts.

2. Materials and methods

2.1 In vitro hemolysis assay

Hemolytic toxicity of purified L-Asparaginase II was done according to K et al. with few modifications. In brief, 200 µL of the separated blood was diluted with 1600 µL normal saline. 200 µL of different concentrations of purified I-asparaginase (20, 40, 80 and 100) µgmL-1 was added to the blood after diluting it, along with the controls. In this case, distilled water was used as the positive control while normal saline was used as the negative control. For one hour, samples were kept in a water bath at 37OC. Following that, the samples were examined by using optical and fluorescence microscopy. After that, centrifuged for 5 minutes at 700 rpm. With the aid of a UV-Vis spectrophotometer, we determined the level of hemolysis by measuring the absorbance at 541 nm and plugging the results into the following formula: equation [6].

$$\text{Hemolysis}(\%) = \frac{\text{Ab. sample} - \text{Ab. negative control}}{\text{Ab. positive control} - \text{Ab. negative control}} * 100$$

Following that, the sediment (RBCs) was stained for 2 minutes with Leishman stain, dried, and rinsed with distilled water to form the blood film. A magnification of 40X. The blood film was examined using a light microscope.

2.2 Blood picture analysis:

The picture of blood had been conducted by utilizing a hematological auto-analyzer to examined numerous hematological parameters, this technique was performed by treating 1 ml of blood with 10 µL of the enzyme. Here, The positive control with (100% hemolysis), whereas the negative control with (0% hemolysis). The samples were incubated in the water bath at 37°C for 1 h and then centrifuged at 700 rpm for 5 min. The absorbance was measured at 541 nm with a UV-Vis spectrophotometer [7].

2.3 Determination of the antioxidant activity of purified L-Asparaginase II by DPPH

The antioxidant capacity for purified L-Asparaginase II had been measured with a DPPH assay. In this Assay, equal volumes (0.5 mL) of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and different concentrations of the enzyme (20, 40,60, 80, and 100 µg mL-1) have been mixed and remained for 30 min at room temperature. After that, absorbance had been read at 517 nm. Ascorbic acid (20-100 µg mL-1) was utilized as the positive control. [8]. An equation below was been applied to determine the percentage of the DPPH slowdown in the sample:

$$\boldsymbol{DPPH\ \ Scavenging\ \ Activity}\ (\%) = \frac{\mathbf{AControl - ASample}}{\mathbf{AControl}} * \mathbf{100}$$

2.3 Cytotoxicity assessments of the purified L-AsparaginaseII

The cytotoxicity effect of L-Asparaginase was tested against both normal cells (WRL68 cell line) and breast cancer (MDA-MB-231 cancerous cell line), 1-The MDA-MB-231 and WRL68 cells (1 × 105 cells mL-1) had been cultured in RPMIa1640 medium supplemented with 10% heat-inactivated (FBS), in an incubator of 5% CO2 at 37 °C in 96 – well flat-bottom culture plates. After 48 hours, then the cells had been treated with 200 µl of a series of ASNase concentrations (6.25, 12.5, 25, 50, and 100 □g mL -1). Control cells were treated with a serum-free medium and the plate was stamped with tape film and then incubated for 48hr in an incubator of 5% CO2 at 37 °C. The enzyme and medium were removed from the plate and 10µl from the MTT solution has been added. The plate was incubated for another 4hr at 37 °C with 5% CO2. Afterwards, supernatants were removed. The MTT crystals were dissolved in 100 µl of DMSO that was placed in each well, and then incubated for 5 minutes. A transmitting wavelength of 550 nm was used to get a reading of the optical density of each well [9]. The equation that was used to compute the inhibitory rate of cell growth was as follows:

$$\textit{Inhibition Rate}\ (\mathbf{IR})\% = \frac{\mathbf{AControl} - \mathbf{ATest}}{\mathbf{AControl}} * \mathbf{100}$$

2.4 Statistical analysis

One-way ANOVA was used to analyze the data using SPSS v.24 at a significance level of 0.05. Results were shown as a mean ± SD, and Graph Pad Prism version six (Graph Pad Software Inc., La Jolla, CA). All experiments have been carried out in triplicate.

3. Results and discussions

3.1 In vitro hemolysis assay

The Images of red blood cells after being treated with pure L-ASNaseII at five different concentrations 20, 40,60,80, and 100 µg mL-1 for one hour were been shown in figure (1), figure (2). the purified enzyme showed hemolysis values below the threshold of 3%. The results show A percentage of hemolysis of more than 5% indicates that the test enzyme causes damage to RBCs. According to these findings, all breakdown rates were within the typical range of less than 5%, These findings indicated that the purified P. aeruginosa L-ASNaseII is not toxic to erythrocytes, which supports the fact of its safety and feasibility [10,11].

Figure 1: Photograph of of Hemolysis % of human red blood cells after incubation with L-ASNaseII .with purified L-ASNaseII at different concentrations (20, 40, 80, and 100 µg mL-1).

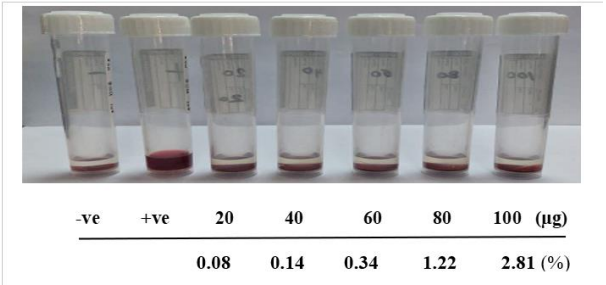


Figure 2: Light microscopic images showing the hemolytic Activity of purified L-ASNaseII at two concentrations (20 and 100 µg mL-1), as compared to +ve and –ve controls.

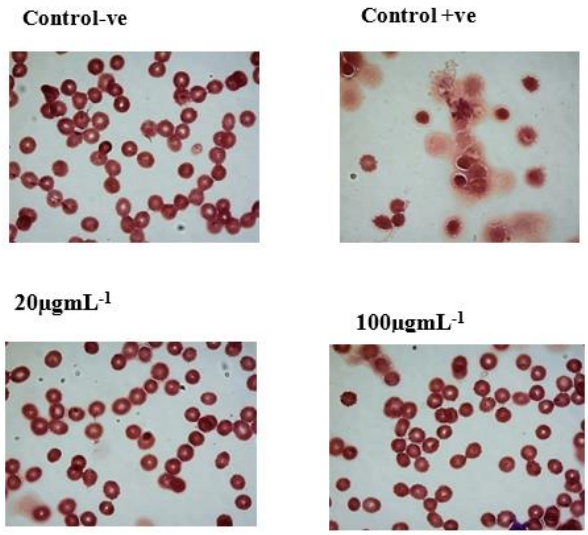


Table (1) displays some hematological Parameters, Samples treated with purified L-ASNaseII showed fewer changes. Hemoglobin will be released from RBCs after breaking the cell membrane. However, results from the present investigation show that the purified enzyme is very compatible with human blood and has a wide range of potential uses, especially in tumour therapy [10].

Table 1: Hematological characteristics of human blood samples treated with purified L-ASNaseII

Blood Picture	Controls		Sample
	-VE	+VE	
WBC	7.9 ± 0.4	2.7 ± 0 .5	5.4 ± 1.6
RBC	4.9 ± 0.5	2.1 ± 0 .3	3.9 ± 0.1
HGH	13.5 ± 0.5	4.5 ± 1.08	11.4 ± 0.4
PLT	239.3 ± 34.2	86.0 ± 34.0	178.6 ± 25.7

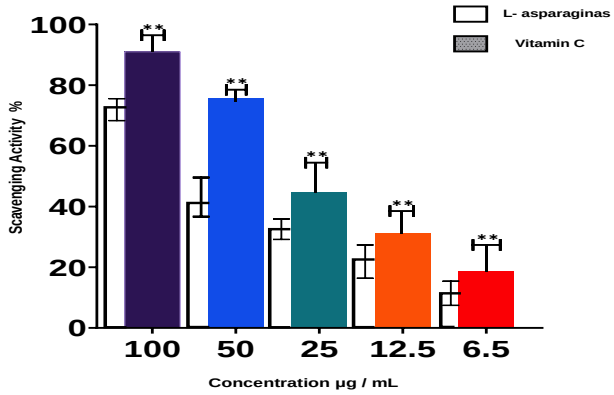
WBC: White blood cells (109 /l); RBC: Red blood cells (1012/l); HGB: Hemoglobin (g/dl); PLT: Platelets (109 /l).

3.2 Determination of the antioxidant activity of purified L-Asparaginase II by DPPH

Figure 3 depicts the antioxidant activity of pure bacterial L-ASNaseII at five different concentrations. The results showed that bacterial L-ASNaseII had enhanced free radical scavenging activity and that inhibition was concentration-dependent. Accordingly, our findings illustrated that purified L-ASNaseII from P. aeruginosa has high antioxidant activity against DPPH, indicating that this enzyme might play an important role in helping in the elimination and neutralization of several forms of endogenous oxidative stressors. These findings are consistent with those of Rani et al., who found that L-ASNaseII generated by Aspergillus flavus (KUFS20) has significant

antioxidant activity against a variety of free radicals [11]. In another study, El-Gendy et al. (2021) the extracellular L-ASNaseII AHMF4 from Fusarium equiseti has been shown to have effective radical scavenging against DPPH [12]. Aspergillus sp. L-ASNaseII showed promising scavenging and antioxidant properties [13].

Figure 3: The DPPH activity of purified ASNase at different concentrations (6.25, 12.5, 25, and 100 µg mL-1). All values were presented as ±SD



3.3 Cytotoxicity assessments of the purified L-AsparaginaseII

Table 2 showed the results tested by MTT colorimetric assay for the MDA-MB-231 cell line after 48 hrs of exposure with different concentrations of purified L-ASNaseII (ranging from 6.5 to 100 gmL-1). As shown by the results, L-ASNaseII significantly suppression growth IR % for the tumor cell line; and it has a dose-dependent effect. The IC50 was determined; a value represents the concentration at which cell growth is inhibited by 50%. The IC50 of purified L-ASNaseII was 88 g mL-1 against the MDA-MB-231 cell line. L- asparaginase's anticancer action is predicated on the hydrolytic deamination of L-asparagine, which normal cells may synthesize. However, because these cancerous cells lack asparagine synthase function, it becomes a necessary amino acid [14]. Mechanistically; the Purified L-ASNaseII triggered apoptosis by stopping the cell cycle in the G0/G1 phase and by disrupting mitochondrial integrity [15,16]. P. aeruginosa L-ASNaseII have weak cytotoxicity to normal cells (WRL68) and exhibited no hemolytic impact on erythrocytes.

Table 2: Inhibition Rate (IR) % of Purified L-ASNaseII on different cell lines.

Concentration µgmL^{-1} / Cell line	100	50	25	12.5	6.25
MDA-MB-231	85.00 ± 1.00	65.00 ± 5.00	42.40 ± 2.50	21.00 ±3.60	13.33 ±1.52
WRL68	20.80 ± 1.49	15.20 ±1.41	11.43 ±2.066	6.10 ± 1.61	6.50 ± 1.61

* Growth inhibition of MDA-MB-231cell line treated with different concentrations (6.25,12.5,25,50,100 µgmL-1) of Purified L-ASNaseII. The values were the M ± S.D from three independent experiments expressed as percent with control.

Ethics

All operations that involved human participants in this study were carried out in compliance with the ethical norms of the University of Technology, and Al-Nahrain University-Biotechnology Research Center in Baghdad, Iraq.

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