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PROPERTIES OF ALPHA-L-FUCOSIDASE FOR SERUM OF PATIENTS WITH HEPATOCELLULAR CANCER AND CYTOTOXICITY ON SOME CANCER CELL LINES

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Alpha-L-fucosidase (FUCA) degrades many fucosylated glycans and has long been recognized as a tumor marker associated with the early detection of some cancers. This study aimed to purify and characterize alpha-L-fucosidase from the serum of patients with hepatocellular cancer and estimate its toxic effect against hepatocellular carcinoma HepG2, prostate cancer PC3 cell lines and the standard hepatocyte WRL-68 cell line. SDS-Page Electrophoresis technique was used to determine the purity of the purified alpha-L fucosidase and estimate its molecular weight. Three purification steps were used for FUCA purification: precipitation with 65% ammonium sulfate saturation, DEAE-cellulose ion exchange, Sephadex G-75 gel filtration. The procedure resulted in 54% recovery of the enzyme with 27.5-fold purification and 14 U/mg specific activity. It was demonstrated that FUCA purified from the serum of HCC patients showed a more toxic effect on HepG2 cells (IC_{50} of 65.74 µg/ml) than on PC3 prostate cancer cells (IC_{50} of 111.5 g/ml) and less toxic effect against standard hepatocyte WRL-68cells (IC_{50} of 214.5 µg/ml). We can conclude that the inhibitory effect of the purified FUCA on hepatocellular carcinoma is more than its effect on prostate cancer cells. Also, the purified FUCA may be used in studies on anticancer drug development in liver cancer.

K e y w o r d s: alpha-L-fucosidase, hepatocellular cancer, HepG2 and PC3 cell lines, ion exchange chromatography, gel filtration.

Hepatocyte cancer (HCC) is the fifth and most common cause of death and the third cause of death associated with cancer [1-3]. Despite the progress made in recent decades, liver cancer (HCC) is still the most common disease and cause of death globally and in both sexes. According to the Cancer Society report in the United States for the year 2013, it was found that liver cancer ranks fifth in the world and that the number of deaths is 14,890, which is the ninth common disease in the world with approximately 6,780 deaths [4]. To improve survival, it requires early detection and detection of HCC [3]. Most cases of HCC are in patients with cirrhosis or chronic liver disease that cause the release of inflammatory factors [2]. Metamorphosis and penetrance of the metastasis are characterized by increased attachment to fucose.

Fucosylation of oligosaccharides on glycoproteins or glycolipids is one of the most common oligosaccharide modifications. Fucosylation is the process of attaching a fucose residue to N-glycans, O-glycans, and glycolipids. O-Fucosylation is a form of fucosylation that isn't found anywhere else. A specific type of fucosylation is required for Notch signaling – the legal framework. Fucosylation mechanisms are tricky. Fucosyltransferases come in a variety of forms. The GDP-fucose production route, as well as the GDP-fucose transporter, are both involved in the process. Fucosylation regulation Fucosylation levels are higher in a recent study. Inflammation and cancer are two examples of unhealthy conditions [5, 6].

The lysosomal enzyme α-L-fucosidase (EC 3.2.1.51; FUCA) removes terminal L-fucose residues from the glycoconjugate oligosaccharide

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chains [4, 7-13]. The lipid enzyme FUCA, commonly found in mammalian cells, has been associated with the degradation and disturbance of alpha fucose levels. Whereas, the disorder in the level of fucose is not only associated with an increase of deformation of fucoglycones containing fucose such as glycoproteins, glycolipids, and mucopolysaccharides present on the surface of the cell, only with an increase or change of viable cells but also associated with an increase or change of the viability of the cell. Fucose is associated with tumor formation, lymphomagenesis, and vascular attack; therefore, FUCA can play an essential role in cancer diagnosis [14-16]. Given the importance of FUCA, α-L-fucosidase's effectiveness has been studied in many sources [1, 4, 17-24]. Alpha-L-fucosidase degrades many fucosylated glycans and has long been recognized as a tumor marker associated with early detection of HCC and colorectal cancer [8, 12, 13, 22, 25-27].

The characteristics of α-L-fucosidase can be extracted, purified, and tested in HCC serum as a valuable early detection and illness progression indicator. On the other hand, α-L-fucosidase activity has previously been reported as a useful marker for detecting illness in primary serum hepatocytes [11, 36-40]. The results of the FUCA features of tissues from HCC tissues were remarkably similar to the serum FUCA characteristics of HCC patients [22].

Our study aimed to purify and characterize α-L-fucosidase as a medically relevant enzyme isolated from the serum of patients with HCC. Moreover, the SDS- Page Electrophoresis technique was used to study the purity of the purified α-L fucosidase and estimate its molecular weight. The study of the cytotoxic effect of the tetrazolium cultivated (TCC), prostate (PC3), and standard hepatocyte lines were also conducted using cultured tetrazolium (MTT) (WRL-68).

Material and Methods

Population study. The study was conducted on 40 patients with hepatocellular cancer of both sexes, 22 males and 18 females, whose ages ranged from 28 to 70 years, and for the period between December 15, 2020, and March 25, 2021. Samples were taken from patients attending the oncology hospital in Mosul.

All patients diagnosed by specialists underwent a physical and clinical examination of the disease. In this study, patients with any other malignant tumors were excluded.

According to the study protocol by the Ethics Committee at the University of Mosul/College of Science and the Health Department in Nineveh, the study was conducted. All participants agreed to conduct the study

Sample collection. Five milliliters of venous blood were drawn. After coagulation for 20 min at 37°C, it was centrifuged for twenty minutes at 3000× g. Serum was collected and stored at 80°C.

Assay of α-L-fucosidase concentration/content/amount and protein concentration

The enzyme-linked immunosorbent assay (ELISA) method was used to determine α-L-fucosidase activity. This BioAssay(TM) kit uses a sandwich ELISA to assess FUCA in human serum, plasma, and other biological fluids in vitro. The manufacturer's instructions were followed when conducting the test (Bioscience). All samples were diluted in a dilute solution at a ratio of one to fifty before being measured.

Sandwich-ELISA is used as the test method in this BioAssay(TM) ELISA kit. An antibody specific to human FUCA has been pre-coated on the micro ELISA plate included in this kit (α-L-Fucosidase, Tissue). Standards or samples are mixed with the specified antibody in the corresponding micro ELISA plate wells. After that, each microplate well is incubated with an Avidin-Horseradish Peroxidase (HRP) mix and a biotinylated antibody specific for human FUCA (α-L-fucosidase). Components that aren't needed are washed out. The substrate mixture is placed into each well. Blue will only appear in the wells containing human FUCA, biotinylated detecting antibody, and Avidin-HRP conjugate. The enzyme-substrate reaction is interrupted when Stop Solution is added, and the color turns yellow. The optical density (OD) is determined using spectrophotometry at a wavelength of 450 nm 2 nm. The OD is proportional to how much human FUCA is present in the sample. The concentration of human FUCA can be measured by comparing the OD of the samples to the standard curve.

Bradford method was used to determine protein concentration at each step of FUCA purification, using Coomassie bright blue G-250 and cow serum albumin as standard protein [28].

Purification of α-L-fucosidase

Precipitation of FUCA. Solid ammonium sulfate was gradually added at 65% saturation to 50 ml crude enzyme (serum) with a slow magnetic stirring procedure for 60 min. Then the supernatant was

discarded after chilled centrifugation at 300× g for 20 min [29]. The precipitate was dissolved in an appropriate volume of a citrate solution of pH 5.0.

Ion Exchange Chromatography. FUCA precipitated with 65% ammonium sulfate was passed through a DEAE-Cellulose column (2.5 cm × 35 cm).

A DEAE-Cellulose column was made using the modified procedure [22] by dissolving 20 g of resin in 1 L of distilled water. The beads were then allowed to settle before being cleaned with distilled water many times until they were completely clear. The suspension was filtered through Whatman No. 1 using a Buchner funnel after discharge. The resin was resuspended in a 0.25 M sodium chloride and sodium hydroxide solution. The suspension was filtered and washed many times with 0.25 M hydrochloric acid solution and then distilled water before being equilibrated with 0.05 M Tris-HCl buffer pH 8.0 as previously described. The ammonium sulfate precipitates were then passed over a DEAE-Cellulose column (2.5 cm × 35 cm) previously equilibrated with 0.05M Tris-HCl buffer pH 8.0. The column was then washed with an equivalent volume of the same buffer, and the bound proteins were eluted in steps with increasing sodium chloride concentrations (0.1-1 M). The flow rate was 1 ml/fraction throughout the column, and the absorbance of each fraction was measured at 280 nm with a UV-VIS spectrophotometer. The α-L-fucosidase activity in each fraction was assessed as described in the assay of α-L-fucosidase activity and protein concentration. Fractions with α-L-fucosidase activity were pooled and saved for subsequent purification processes [22].

Gel Filtration Chromatography. Pharmacia Fine Chemicals Company provided the instructions for making Sephadex G-200. Sephadex G-200 was dissolved in 0.01 M Tris-HCl buffer pH 8, degassed, and packed into a glass column (2 cm × 55 cm) with the same buffer. The ion exchange stage loaded concentrated α-L-fucosidase into the column. The elution flow rate was 2 ml/fraction, and the equilibration buffer was the same. Each fraction's absorbance was measured at 280 nm. The test of α-L-fucosidase activity and protein concentration was used to assess the alpha-L-fucosidase activity in each fraction. Fractions containing α-L-fucosidase activity were pooled and saved for further purification [30, 31].

Determine the purity and molecular weight of α-L-fucosidase

SDS-Page electrophoresis technique was used; protein standards were used to determine the molecular weight of FUCA after electrophoresis, and migratory protein distances were calculated [22, 32].

The purity of α-L-fucosidase was determined using the SDS-Page electrophoresis technique; in reference, proteins with known molecular weights in the range of 50 to 100 kDa and migrating protein distances were estimated.

SDS polyacrylamide gel (8 cm × 8 cm, 0.75 mm) in the existence of reference proteins with known molecular weights and migrating protein distances were estimated. The distances of protein migration were determined after electrophoresis to determine the enzyme molecular weight using the modified approach [22, 32]. The presence of subunits was evaluated in 12% polyacrylamide gels (8 cm × 8 cm, 0.75 mm) containing 0.1% SDS [22].

In brief, 5% stacking and 12% running gels were run at room temperature for 30-40 min at 80 V and 1-1.5 h at 100 V in 25 mmol/l Tris-HCl, 0.2 mol/l glycine buffer (pH 8.3) containing 0.1 percent SDS. FUCA samples (20-25 g/lane) were electrophoresed after 3 min in a boiling water bath with 2% SDS, 5% 2-mercaptoethanol, 25% glycerol, and 0.1 percent bromophenol blue in 60 mmol/l Tris-HCl (pH 6.8). Preserved molecular weight standards were used and stained under the same conditions. The gels were stained in glacial acetic acid for 3 h: methanol: distilled water (10:45:45, v/v/v) with 0.25 percent Coomassie brilliant blue R-250, then glacial acetic acid: methanol: distilled water (10:45:45, v/v/v) without Coomassie brilliant blue R-250 for 20 min per time[22].

The cytotoxic effect of α-L-fucosidase

The cell lines of each hepatocellular carcinoma HepG2, prostate cancer PC3 cell line, and the standard hepatocyte cell line WRL-68 were obtained from the University of Malaya Kuala Lumpur/College of Medicine/Department of Pharmacy/Center for Natural Products Research and Drug Discovery in Malaysia for this analysis. At Baghdad's CAC. Center for Research and Biotechnology, cancer streak cells were preserved, created, and examined.

The cytotoxic effect of α-L-fucosidase on cell lines was determined using the MTT assay. Methylthiazolyldiphenyl-tetrazolium (MTT) solution (10 ml) was added to each well of a 96-well plate, which was then incubated for four h at 37°C with the research sample (the solution converted yellow) (the solution converted yellow). After that, each well was filled with 0.2 ml of DMSO (dimethyl sulfoxide) and shaken for 5 min (the DMSO solution converted

purple). Following complete dye dissolution, the absorbance of the red-stained solution was measured at 575 nm using an ELISA reader. The average absorbance was calculated for each group of iterations. Cell Viability %= [Absorbance of treated sample/Absorbance of non-treated sample] × 100 (Non-treated cultures contained only the medium).

Statistical analysis. The findings of this research were expressed as mean ± SD. Using Minitab software was used to interpret the data.

Results and Discussion

α-L-fucosidase activity (EC 3.2.1.51) was studied in several sources, due to its significance in organism and metabolism disorders [1, 4, 17-24]. The mean age of patients was 63.5 ± 11.2 years.

α-L-fucosidase was purified from the serum of HCC patients under optimal conditions; the three purification steps are summarized in Table 1. The specific activity of FUCA at the precipitation stage was 0.92 U/mg protein

DEAE-cellulose chromatography purification improved enzyme activity even further. The graph below (Fig, 1) depicts the enzyme activity and specific activity for serum FUCA. According to the findings, the first peak, which occurred in a washing step with fractions numbers from 34 to 41, included enzyme-specific activity (1.3 U/ml). The specific activity of the second peak (eluted at 0.5 NaCl) was 5.9 U/ml at fractions numbers from 125 to 138, with a purification fold of 11.6 and a yield recovery of 60.4%.

After purification by ion exchange, the final step in the purification of FUCA from sera patients with hepatocellular cancer was the gel filtration chromatography technique, which yielded a high specific activity of 14 U/mg with a 27.5 purification fold and yield recovery of 54% (as shown in Fig. 2). Li et., 2006 found that the specific activity of the FUCA, which isolated from hepatocellular tissue carcinoma, a closed from our result [22].

Also, Fig. 3 depicts the advanced α-L-fucosidase purification steps (from crude to purify enzyme) in SDS-PAGE chromatography for serum hepatocellular cancer alpha-L-fucosidase. According to the results, the purified alpha-L-fucosidase has a single band with a molecular weight of 56 kDa, similar to other studies [22, 33]. While different from other previous studies [23, 24, 26, 27, 34, 35]. The fact source of the enzyme studied differed in some studies may explain the discrepancy in the findings.

The MTT assay was used to determine the cytotoxic effect of purified FUCA on each of heterogeneous human hepatocellular carcinoma HepG2, prostate cancer PC3 cell lines, the standard hepatocyte cell line WRL-68 for the comparison. To determine the characteristics of the cytotoxicity effect for purified FUCA on cancer cells, three cell lines were selected to be investigated; hepatocellular carcinoma HepG2, prostate cancer PC3 cell lines, and the standard hepatocyte cell line WRL-68 for the comparison. The effect of purified FUCA on cancerous cell line HepG2 and PC3 as well as the control non-cancerous WRL-68 cell line. The cytotoxicity of purified FUCA was measured as a percentage of cell viability calculated by MTT assay in each of HepG2, PC3 cells, respectively, in a dose-dependent manner after incubation with the purified enzyme for 24 h at 37°C. The results (Table 2 and 3) showed that purified FUCA at different concentration (25-400 mg/ml had a cytotoxic effect on HepG2 cell line higher than that of PC3 cell line and this effect increased with the decreasing in concentration. The IC_{50} which is the half maximal inhibitory concentration for cell growth constructing adose-response curve was measured. Purified FUCA showed IC_{50} 214.5 µg/ml against HepG2 cell line, whereas an IC_{50} of 182.1 µg/ml was obtained from the purified FUCA against PC3 cell line. At different concentrations of purified FUCA (25-400 µg/ml) and incubate it with cancer and standard cell lines at 37°C for 24 h. Cell inhibitory reached to 94.65 ± 0.85% using 25 µg/ml concentration of purified FUCA this finding suggest the sensitivity of HepG2 cell line to purified enzyme in a dose dependent manner which is due to cell death (Fig. 4 and 5). However, purified FUCA did not show significant toxicity on normal cell tested WRL-68 cell line (Fig. 4 and 5).

The MTT assay was used to determine the toxic effect of purified alpha-L-fucosidase from the serum of patients with hepatocellular cancer on studying the cytotoxic anticancer effect of α-L-fucosidase against HepG2, PC3, and WRL-68 cell lines were done by using the different concentrations of purified FUCA (25-400 µg/ml) and incubate it with cancer and standard cell lines at 37°C for 24 h. It was found that FUCA has a toxic effect on the HepG2 cell line more than PC3 when compared with normal cell line WRL-68. Purified FUCA was revealed to inhibit HepG2 cancer cell line growth with an IC_{50} value of 65.74 µg/ml compared to an IC_{50} of 214.5 µg/ml for purified FUCA against standard WRL-68 cell line.

T a b l e 1. Purification steps for α-L-fucosidase from hepatocellular cancer patients' serum

Purification steps	Volume, ml	FUCA activity, U/ml	Protein concentration, mg/ml	Specific activity, U/mg	Total activity, U	Purification, folds	Yield, %
Crude enzyme	50	28	55	0.51	1400	1	100
Ammonium sulfate precipitation 65%	35	33	36	0.92	1155	1.8	82.5
Ion exchange chromatography DEAE-cellulose/ washing fraction no. (19-23)	8	25	19	1.3	200	3.4	14.3
Ion exchange chromatography DEAE-cellulose/ elution fraction no. (60-71)	13	65	11	5.9	845	11.6	60.4
Gel filtration Sehadex G-200/ elution volume at (40-52) ml	12	63	4.5	14	756	27.5	54

Note: α-L-fucosidase (U/ml) activity is defined as the amount of enzyme required to remove a nanomole of fucose for a minute. The specific activity (U/mg) of the α-L-fucosidase (U/mg) is defined as the amount of enzyme required to remove nanomolar fucoside per milliliter for a minute per milligram of protein.

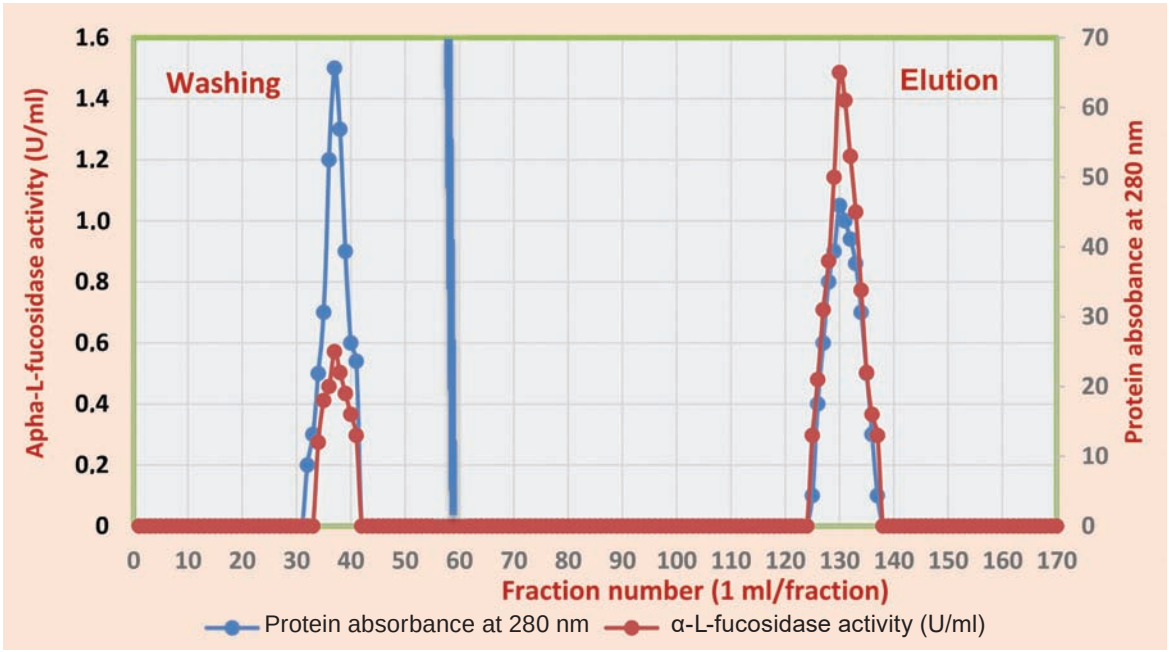

Fig. 1. Ion exchange DEAE-cellulose column (2.5 cm × 35cm) chromatography with a flow rate of 1 ml/fraction for α-L-fucosidase produced from the serum of patients with hepatocellular cancer (HCC) patients

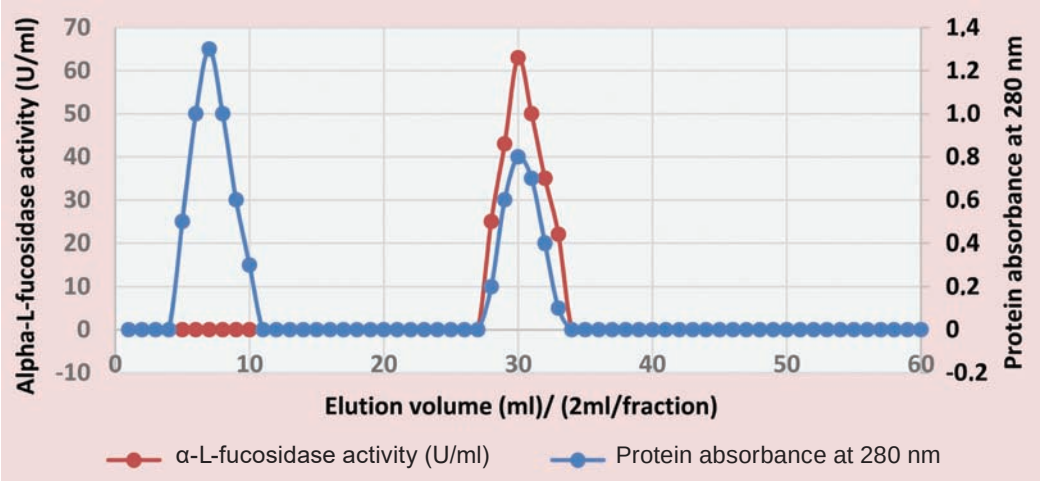

Fig. 2. Gel filtration chromatography column (2 cm × 55 cm)/Sephadex-G-75 with a flow rate of 2 ml/fraction for α-L-fucosidase produced from the serum of patients with hepatocellular cancer patients

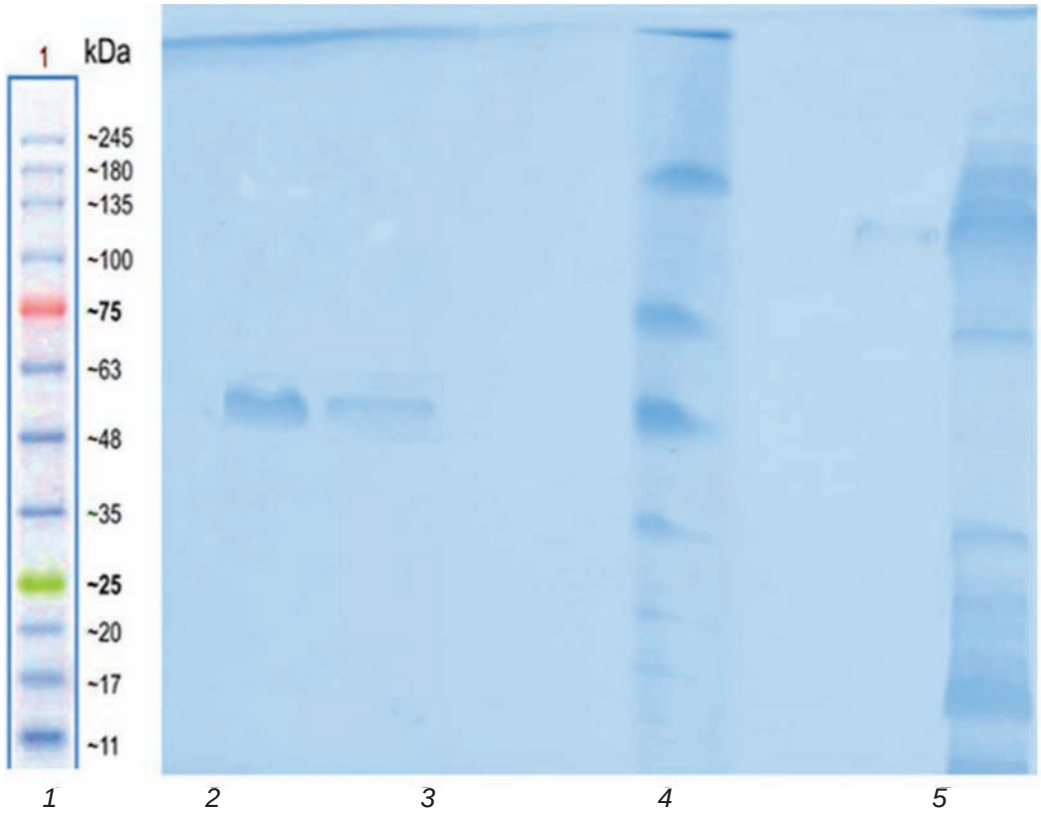

Fig. 3. SDS polyacrylamide gel electrophoresis of purified and crude α-L-fucosidase produced by sera patients with hepatocellular cancer: 1 – proteins markers from the manufacture; 2 – protein band after gel filtration step; 3 – proteins bands after ion exchange step; 4 – proteins after precipitation of enzyme with 65%; 5 – proteins bands in the crude filtrate

In contrast, the purified enzyme showed less inhibition growth of PC3 cancer cell line with IC_{50} of 111.5 µg/ml compared to an IC_{50} of 182.1 µg/ml for purified FUCA against standard WRL-68 cell line. Besides, this effect increases at low concentrations of 25 µg/ml and decreases with increasing concentration, as shown in Tables 2 and 3.

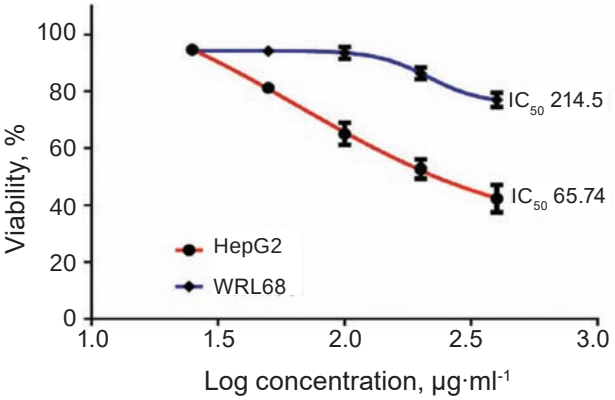

Fig. 4. Cytotoxic activity of purified α-L-fucosidase produced from the serum of patients with hepatocellular cancer on HepG2 and WRL-68 cell lines using MTT test after 24 h and 37°C

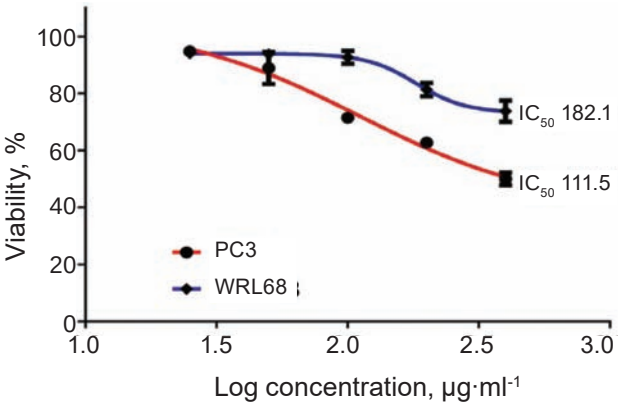

Fig. 5. Cytotoxic activity of purified α-L-fucosidase produced from the serum of patients with hepatocellular cancer on PC3 and WRL-68 cell lines using MTT test after 24 h and 37°C

Table 2. Inhibition ratio for HepG2 cancer cell line and standard cell line WRL-68 in incubation for 24 h at 37°C by purified α-L-fucosidase (FUCA) at different concentrations

Concentration of purified FUCA(μg/ml)	Inhibition (%) by purified FUCA on HepG2 cell line	Inhibition (%) by purified FUCA on WRL-68 cell line
	Mean ± SD	Mean ± SD
400	42.38 ± 4.85	76.97 ± 2.58
200	52.66 ± 3.33	86.34 ± 2.09
100	64.97 ± 3.85	93.60 ± 2.10
50	81.14 ± 0.82	94.17 ± 0.44
25	94.65 ± 0.85	94.44 ± 0.87

Table 3. Inhibition ratio for prostate cancer cell line PC3 and standard cell line WRL-68 in incubation for 24 h at 37°C by purified α-L-fucosidase (FUCA) at different concentrations

Concentration of purified FUCA(μg/ml)	Inhibition (%) by purified FUCA on PC3 cell line	Inhibition (%) by purified FUCA on WRL-68 cell line
	Mean ± SD	Mean ± SD
400	50.00 ± 2.20	73.69 ± 3.76
200	62.65 ± 1.16	81.33 ± 2.37
100	71.41 ± 1.22	92.67 ± 2.30
50	88.90 ± 5.63	93.75 ± 1.42
25	94.75 ± 1.08	94.02 ± 1.69

The α-L-fucosidase properties can be isolated, purified, and examined in HCC serum as a helpful marker for early detection and a progressional disease indicator. In contrast, α-L-fucosidase activity was previously reported as a valuable marker to detect disease in primary serum hepatocytes [11, 36-40]. The serum FUCA characteristics of HCC patients were very close to the results of the FUCA characteristics of tissues from HCC tissues [22]. This means that it is possible to study the proper-

ties of α-L-fucosidase in the serum of patients with liver cancer; You can give us information about the enzyme in liver cancer tissue.

The study results showed that FUCA purified from the serum of HCC patients showed a more toxic effect on the growth of HepG2 cells than its inhibitory effect on the development of PC3 prostate cancer cells. This provides a benefit that the enzyme could be used in the study of anti-liver cancer drugs

The inhibitory effect of FUCA purified from HCC patients' blood varied depending on its concentration, presumably due to the impact of cancer cell receptors with variable concentrations of the purified FUCA enzyme, which plays an essential role in apoptosis [41]. The findings of our study revealed that the higher the concentration of the purified enzyme, the lower the rate of inhibition of cancer cells, implying an inverse relationship between concentration and rate of inhibition. This condition is known as antagonistic in the effect of dosing (Hormesis), and it is a common biological phenomenon in toxicology and pharmacology. This phenomenon is employed in the treatment of incurable diseases like Alzheimer's and cancer with low quantities of harmful substances or pollutants, as tiny concentrations of specific compounds can destroy cancer cells without damaging normal cells [42]. This could be due to the enzyme purified alpha-L-fucosidase inhibiting the release of particular components from cancer cells, such as cytokines. At low concentrations, it has a more substantial inhibitory impact. According to Hamodat study 2021, the cytoxity effect of purified adenosine deaminase-2 on breast cancer line (MCF-7) increased at decreasing the concentration [43]. Also, Arshad et al. (2014) found growing the content of methanolic extract of Prosopis cineraria leaves reduced the percentage of cell inhibition of the cancer cell line MCF-7 [44]. The inhibitory effect of purified alpha-L-fucosidase was based on lower concentrations rather than high concentrations, and this depends on several factors, including the substance's concentration and molecular weight, as the higher the substance's concentration is low, the easier it is to penetrate the outer membrane in the cell wall, but not to reduce the dilution that makes it possible [44].

Conclusion. It is possible to conclude that the isolated enzyme differs in its properties according to its source. The inhibitory effect of the purified enzyme is more on hepatocellular carcinomas than on prostate cancer cells. The purified enzyme could be used in studies of the development of anticancer drugs in liver cancer. Also, the efficacy is more significant at a lower concentration. These results help in using purified alpha-L-fucosidase as an anticancer drug for the liver, which indicates that the purified enzyme can be used as an anticancer drug for the liver.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ВЛАСТИВОСТІ АЛЬФА-L-ФУКОЗИДАЗИ ІЗ СИРОВАТКИ КРОВІ ПАЦІЄНТІВ ІЗ ГЕПАТОЦЕЛЮЛЯРНОЮ КАРЦИНОМОЮ ТА ЇЇ ЦИТОТОКСИЧНИЙ ЕФЕКТ НА ДЕЯКІ ЛІНІЇ РАКОВИХ КЛІТИН

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Альфа-L-фукозидаза (FUCA) це ензим, що розщеплює фукозильовані глікани і визнаний пухлинним маркером, пов'язаним із раннім виявленням деяких видів раку. Метою цього дослідження було очистити та охарактеризувати альфа-L-фукозидазу із сироватки крові пацієнтів із гепатоцелюлярною карциномою (ГЦК) та оцінити її токсичний ефект щодо гепатоцелюлярної карциноми HepG2, клітинних ліній раку передміхурової залози PC3 та стандартної лінії гепатоцитів WRL-68. Для визначення чистоти виділеної альфа-L-фукозидази та оцінки її молекулярної маси використовували SDS-ПААГ електрофорез. Очищення FUCA проводили в три стадії: осадження сульфатом амонію, іонообмінна хроматографія на DEAE-целюлозі, гель-фільтрація на Sephadex

G-75. У результаті було виділено ензим із 27,5-кратним очищенням, питомою активністю 14 од/мг і виходом 54%. Показано, що FUCA, отримана із сироватки крові пацієнтів із ГЦК, виявляла більш токсичний ефект на клітини HepG2 (IC_{50} 65,74 мкг/мл), ніж на клітини раку передміхурової залози PC3 (IC_{50} 111,5 мкг/мл) та менш токсичний ефект щодо клітин гепатоцитів WRL-68 (IC_{50} 214,5 мкг/мл). Ми можемо зробити висновок, що інгібуючий ефект очищеного FUCA на гепатоцелюлярну карциному більше, ніж його вплив на клітини раку передміхурової залози. Крім того, очищену FUCA можна використовувати в дослідженнях щодо розробки протипухлинних препаратів при раку печінки.

Ключові слова: альфа-L-фукозидаза, гепатоцелюлярна карцинома, клітинні лінії HepG2 та PC3, іоннообмінна хроматографія, гель-фільтрація.

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