EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

PHYSIOLOGY & MOLECULARBIOLOGY

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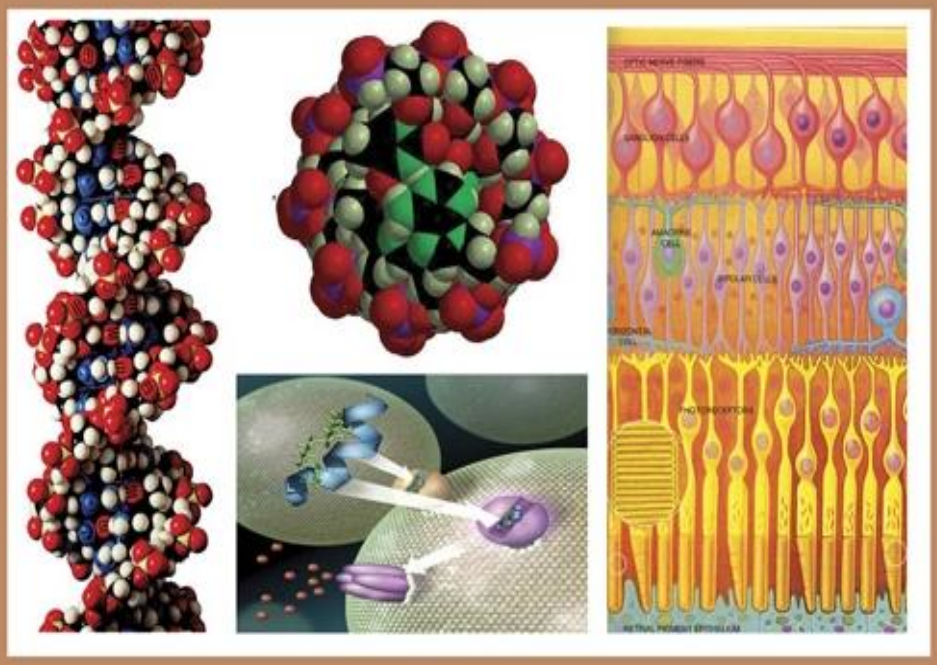

ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 14 No. 1 (2022)

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Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 14(1) pp107-119 (2022)*
DOI: 10.21608/EAJBSC.2022.215688

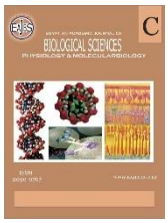
Egypt. Acad. J. Biolog. Sci., 14(1):107-120 (2022)

Egyptian Academic Journal of Biological Sciences

C. Physiology & Molecular Biology

ISSN 2090-0767

www.eajbsc.journals.ekb.eg



Characterization of White Button Mushroom and Its Biomedical Applications

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ARTICLE INFO

Article History

Received:2/1/2022

Accepted:21/1/2022

Available:24/1/2022

Keywords:

White button mushroom, antiproliferative, Gene expressions, HepG2Bax,Q-Sepharose, biomedical applications.

ABSTRACT

The biological contents of the white button mushroom including(lectin and polysaccharides*)* were extracted from its dried fruiting bodies and purified through Q-Sepharose column which was considered a strong anion for adsorption of lectin with high performance. The purified lectin has a great hemagglutinating activity (HA) that was considered a specific feature for lectin and inhibited by GlcNAc which was a specific binding sugar receptor. In this study, we reported that lectin agglutinated all blood groups without specificity, was active below 70 and 80°C and it had no activity at that temperature degrees. The best pH media for HA within range (6 to 9) for 24hr the purified lectin had molecular weight 16KDa that was determined by SDS PAGE ELECTROPHORESIS. Gene expressions by RT-PCR of the white button mushroom showed that the lectin upregulated Bax (pro-apoptotic gene). Lectin had apoptotic activity on HepG-2 cell line. The cell toxicity assay showed that mushroom's lectin has high toxicity on malignant cells with very low toxicity on WI-38 (normal cells). White button mushroom lectin had antiproliferative & toxic effects on HepG-2 with IC_{50}; 41.90 ug/ml while had very weak toxicity on WI-38(normal cells) with IC_{50} (98.54 ug/ml). The lectin of edible mushroom can be utilized in human eating regimens and recommended for malignancy with a safety margin in clinical pathological research with different concentrations starting from 200g fruiting bodies mushroom & 98.44 ug/ml lectin concentration.

INTRODUCTION

The common mushrooms could be an amazing wellspring of a wide range of nutraceuticals and may be utilized straightforwardly in human eating regimens and to advance wellbeing for the synergistic impacts of all the bioactive mixes present for example; Carbohydrates are present in high extents in the white button mushroom, including chitin, glycogen, trehalose and mannitol (Valverde, Hernández-Perez and Paredes-López, 2014). White button mushroom is considered a medicinal mushroom because of its biologically active substances(lectin and polysaccharides)utilized as an antitumor effect(Xu *et al.*, 2011). Lectins are a heterogeneous group of nonenzymatically carbohydrate-binding proteins that particularly perceive and bind reversibly to specific mono and oligosaccharides on cell surfaces(Sarup, Kaur and Kennedy, 2018), the extracellular framework and emitted glycoprotein (Evellyne de Oliveira Figueirôaa, 2017).

In this study, Lectin as a biological extract substance subjected to many purification steps including IEC which was considered a strong anion for adsorption of the lectin with high performance. the Thomsen Friedenreich (TF) antigen is a disaccharide, galactosylβ-1,3-N-Acetylgalactosamin(Galβ-1-3 GalNAc) connected to either threonines or serines on cell membrane glycoproteins and hidden in normal cells while appearing in high levels on human malignant cells, edible mushroom lectin has high affinity to TF, so it highly attached to malignant cells causing its aggregations then apoptosis while it couldn't attach to normal cells as TF-antigens were hidden (Carrizo *et al.,* 2005). We obtained that: white button mushroom has a toxic effect on malignant cells with low toxicity on normal cells.

MATERIALS AND METHODS

Purification:

The purpose of this test is to extract and purify lectin from mushrooms to be used in the applications. This purification process was performed according to (Panchak and Antonyuk, 2011). Agaricus bisporus (White button mushroom) was purchased from Polishea Company, Egypt (200 g). The fruit bodies were ground using a meat chopper. The resulting mass was processed to liquid using a press. The liquid (120 ml) was cleared by cooling centrifugation at 14000 r.p.m for 15 min. The supernatant was cooled in a refrigerator to 4°C, and then two volumes of 95% ethanol (240ml) (Sigma Aldrich), preliminarily cooled to -18°C, were added to it. The precipitant was gathered through centrifugation (15min, 14000 r.p.m). Add 1M NaCl (250ml) (Sigma Aldrich) to the formed precipitant to thaw the lectin. The precipitant was blended for 15 min. The undissolved precipitant was eliminated by centrifugation, again 1 M NaCl (100 ml) was added and after centrifugation the supernatants were mixed. The mixed liquid which enclosed the lectin was cooled again to 4°C and once again two volumes of 95% ethanol cooled to –18°C were added to the mixed liquid. The precipitant was sorted through centrifugation (15min., 14000 r.p.m) then dissolved in 1M NaCl (80 ml). The undissolved precipitant was eradicated by centrifugation. The supernatant was furthermore filtered by a filter paper (Whatman 1, GE Healthcare co.) three times**.** Add ammonium sulphate 90% saturation (10ml) to the supernatant with stirring in ice-water for 15 min and then kept overnight at 4°C. The ppt sample was centrifuged at (15min, 14000 r.p.m). Add 2 mL of PBS (0.1 M, pH 7.2) to the formed pellet to dissolve it Collect the supernatant-containing lectin and the dissolved pellets for dialysis against PBS (0.1 M, pH 7.2) at 4°C for 24h. The purified lectin adsorbed on IEC, Q-Sepharose; Anion exchanger by washing through elute buffer (1 M NaCl dissolved in 10 mM Tris-HCl buffer pH 7.3) collecting the fluid into a cleaned tube for extra applications as indicated by the manufactures' instructions(Sigma-Aldrich, 2012). The purification process was summarized in Table (2). The total protein was determined by Lowry's method.

Assay of Hemagglutinating Activity:

This assay is a characteristic feature for lectin that agglutinates red blood cells. According to(Wang, Ng and Liu, 2002) in the assay for lectin (Hemagglutinating) activity, a serial twofold dilution of the lectin solution in micro-titre U-plates,96 well (50 µL) was mixed with 50 µL of a 2% suspension of human red blood cells in phosphate-buffered saline pH 7.0 (Oxford Lab Chem. India) at 20°C. The outcomes were perused after around 60 minutes when the blank had been completely sedimented. The most dilution displaying agglutination is the titre and can be calculated as one unit (Ng *et al.*, 2009)(µg/µl). The agglutination was observed through the consistency of mat was a true action, whereas the consistency

of button at the base of the cavity was a false action, shown in Figure (2).

Sugar Specificity Assay:

The purpose of this assay is to detect the lectin specificity towards sugars through hemagglutinating inhibition of red blood cells. According to (Singh *et al.*, 2010a) the following procedure was performed: Put 20 µL of *A.bisporus* lectin (half the lowest concentration causes visible agglutination) with the similar volume of tested sugar solution which is responsible for repression within wells of the microplate (96 wells), making tow fold serial dilutions. After 30 minutes of incubation at 25°C put 40 µL of 2% RBCs suspension to every well then, the plate was again incubated for 30 minutes at 25°C. The positive control contains 20 µL of PBS instead of ABL while the negative control contains 20 µL of PBS was added instead of sugar solution. The plate was incubated at 4°C for 2–3 hrs. The consistency of button in the presence of sugar marks specific action and the consistency of mat marks nonspecific Action Figure (4). The tested sugars for the lectin specificity were obtained from Sigma Aldrich; D – Ribose, D + Xylose, L + Arabinose, GlcNAc, D+ Mannose, D + Sucrose, D - Lactose.

The Effect of Temperature on Lectin Activity:

The purpose of this assay is to detect the effect of temperature degree on the activity of lectin. This test was performed according to (Singh *et al.*, 2010b)The serial dilutions of ABL were incubated with 2% RBCs suspension at different degrees of temperature from -10 to 80°C in a water bath for 10 minutes then the samples were cooled in ice and titrated, so the ideal temperature can be detected, Figure (5). *A. bisporus* lectin activity at any tested temperature was explicit as a rate relative activity contrasted with control tests that incubated at 4°C.

The Effect of pH on Lectin Activity:

The purpose of this test is to detect pH at which lectin activity on hemagglutination is declined. This test was performed according to (Singh *et al.*, 2010b) Use the different buffers ranging from pH 2.0-12.5 at 25°C through agglutinating assay Figure (6). The stabilized pH of ABL was determined through incubating the specimen (50 µL) in the range of different pH at 4°C. The used Buffers during pH assay HCl-KCl buffer (0.1M pH 2.0), Glycine-HCl buffer (0.1 M pH 3.0),Sodium acetate-acetic acid buffer (0.1 M, pH 4.0–5.0),Citrate buffer (0.1 M, pH 6.0),Phosphate buffer saline (0.1 M, pH 7.2),Potassium phosphate buffer (0.1 M, pH 8.0),Trizma buffer (0.1 M, pH 9.0),Glycine-NaOH (0.1 M, pH 10.0),Carbonate/bicarbonate buffer (0.1 M, pH 10.8),Potassium phosphate buffer (0.1 M, pH 12.7).*A.bisporus* lectin activity was assayed at 0, 2, 4 and 12 hr. The samples were balanced before the hemagglutination assay. The lectin activity was contrasted with control samples incubated with PBS (0.1 M, pH 7.2) and defined as a Hemagglutinating titre.

SDS-PAGE-Gel Electrophoresis:

The Determination of the molecular weight of ABL was fulfilled through the method of SDS-PAGE (Laemmli, 1970), Figure(7).

Cell Culture and Cell Toxicity Assay:

The purpose is to detect the toxicity of *A. bisporus* lectin on HepG-2 cell line. The cell line was purchased from the cell cultures department, VACSERA Table (1). This assay was performed according to (Wang, Ng and Liu, 2002)The tested cell line was grown in culture T-flasks 175 cm^2 in a CO_2-incubator at 37°C with 5% CO_2. The cells were developed in Dulbecco's Modified Eagle Medium (DMEM) medium accompanied with 0.1mM non-essential amino acid solution, 100 U/mL penicillin, 0.1 g/mL streptomycin, as well as 10% fetal bovine serum (FBS) &10 mM of sodium bicarbonate. The objective cells were preserved in the laboratory for additional requests wherever growth medium is poured, and 0.025 trypsin solution was added for three minutes. The trypsin is drawn off and the cell lines were incubated till the whole dissociation of cells. A portion of the kept cell lines is sub-cultured in 96 well plates or tissue culture flasks. The cytotoxic influence of specimens is appraised when cells are associated. The passage number of HepG-

2, WI-38(normal cells) was 14, 13 respectively. The cell lines were cultured in 25 cm^2 tissue culture flasks (TPP-Swiss) for tentative determinations. The best cell concentration as strongminded through the progress outline of the cell lines was 2 $\times 10^5$ cells/ml of HepG2 and W1-38 cells. The Cells were left 24 hr. for cohesion before the applied treatments. All cell work was fulfilled in a tissue culture hood (Shell Handling Biological Safety Cabinet, Class II, Italy) using only sterile tools in direct contact with the cells. Put MTT3-[4,5 -dimethylthiazol-2-yl]2,5 diphenyltetrazolium bromide 5 mg/ml to the pre-treated cells as 50 µl /well, plates are incubated (Jouan –France) at 37˚C for 4 hrs. in the dark. The MTT stain was poured, and stained cells are washed twice using PBS solution (Oxford Lab Chem. India). The Developed formazan complex crystals are dissolved using 0.4% acidified isopropanol as 0.05 ml/well. The optical density (OD) of dissolved crystal was measured at 570 nm using ELISA plate reader (Biotek-ELx-800, USA) at 24hrs,48hrs and 72hrs The OD is plotted contrary to the concentration of white button mushroom Lectin. The two flasks of cultured cells HepG-2 and WI-38 were treated with the IC_{50} values for 24hrs which had been calculated through GraphPad Prism, Figure (8). The Cells were inspected using an inverted microscope (Hund –Germany). The morphologic alterations of cell lines were observed. The flasks of tested cell lines were kept as a negative control without ABL treatment. The affected cell lines were collected and sedimented by using a cold centrifuge (Jouan-Ki 21-Franc). The Precipitated cells were washed using Phosphate Buffer Saline (PBS) (Oxford Lab Chem.India). The washed cells were dispensed in two Eppendorf tubes for gene expression by RT-PCR assay.

Table (1) the identification of the cell lines

Item	HepG2	WI-38 (normal cell)
organism	Homo sapiens human	
Tissue	Liver	Lung
Cell type	Epithelial	Fibroblast
Culture properties	**Adherent**	
Disease	Hepatocellular carcinoma	**Normal**
ATCC	HB-8065	CCL-75
Media	**DMEM**	

Gene expressions by RT-PCR assay:

The purpose is to detect apoptosis through measuring gene expressions of Bax and housekeeping gene GAPDH

Primers (BAX 5'CCAGCCCATGATGGTTCTGAT-3', 5'CCCGAGAGGTCTTTTTCCGAG-3',
GAPDH 5'GGAGTGGGTGTCGCTGTTG-3',5'TGCCAAATATGATGACATCAAGAA-3')

RT-PCR Was Performed in Three Steps:

1-The Extraction of Total RNA from cell lines by RNeasy Mini Kit (Qiagen).

2- The Reverse transcription of the total RNA Through a high-capacity cDNA Transcription kit (Applied Biosystems).

3-the Power Sybr Green RT-PCR Master MIX kit (Applied Biosystems). Set up the reaction PCR Master mix (25 µl) for each gene (GABDH, Bax and Bcl2)12.5 µl- 2X Sybr Green PCR Master Mix ,2.5 µl- 1µM primer mix,2µl dscDNA(sample) (5 ng / µl),8 µl Nuclease free water, Real-Time PCR Performed on 10ng of dscDNA. The amplification procedure started according to the standard protocol of (7500-Applied Biosystems) via applying thermal

cycling program for RT-PCR as the following: 95 ^{0}C for 10 min (activation process), 95 ^{0}C for 15 sec. (denaturation process), 60 ^{0}C for 1 min. (annealing process). Note: AmpliTaq is bound to its modifier causing its inactivation. It dissociates at high temperatures, so AmpliTaq is active (Biosystems, 2005).

Comparative quantitation is the performance that studies the variations in the level of the gene expression in a treated specimen comparative with a control one. Calculate C_T (ΔΔ CT) to define the fold change of two tests.

ΔΔ C_T = ΔC_T Treatment - ΔC_T Control

Fold=$2^{\wedge-\Delta\Delta C_T}$

RESULTS

Purification:

The lectin activity of the crude sap was 32-unit titre mg as an initial lectin activity, while we used ethanol as a first solvent the activity increased to 64 units with 2 purification factors that (PF) was acquainted as the proportion of lectin activity after to lectin activity before the purification step. When we used Ammonium sulphate as a second solvent, the specific lectin activity was reached 256 U titre/mg with 8 folds than the first extraction. The purification fold of lectin through the IEC was 16 times that recovered from 200 g dried fruiting bodies purified from *A. bisporus* Table (2).

The refined lectin adsorbed on a column, Q-Sepharose was washed through elute buffer (1 M NaCl dissolved in 10 mM Tris-HCl buffer pH 7.3). The three fractions containing lectin were collected into cleaned tubes. Each fraction was performed for lectin activity at λ_{280} forming three peaks.

The lectin activities were enriched in the second (Q2) of the three formed fractions Q1-3 Figure (1). The total protein contained in the collected fractions was 9.744 mg/ml with specific lectin activity 512 U titre/ mg that was calculated after finalizing the purification steps of lectin through IEC. The fractions containing lectin were assayed for lectin activity and protein content.

The specific hemagglutinating activity of purified lection through IEC was quantified 512 U titre/mg.

Table 2: purification assay.

Stage	V, ml	protein content mg/ml	Total protein (mg)	Specific Lectin activity (titre) Unit(titre/mg)	Total activity of lectin (mg)	P F
Crude Extract	120	1.14	136.8	32	4378	1
1stethanol step	360	0.987	355.32	64	22740	2
2ndethanol step	450	0.982	442	64	28288	2
Filter paper	80	0.941	75.28	128	9635	4
Amm.sulfate	30	0.875	26.25	256	6720	8
IEC	15	**0.812**	**9.744**	**512**	4989	16

Titre is defined as the reciprocal of the end-point dilution exhibiting the hemagglutination "IEC=Ion Exchange Chromatography, PF (Purification Factor).

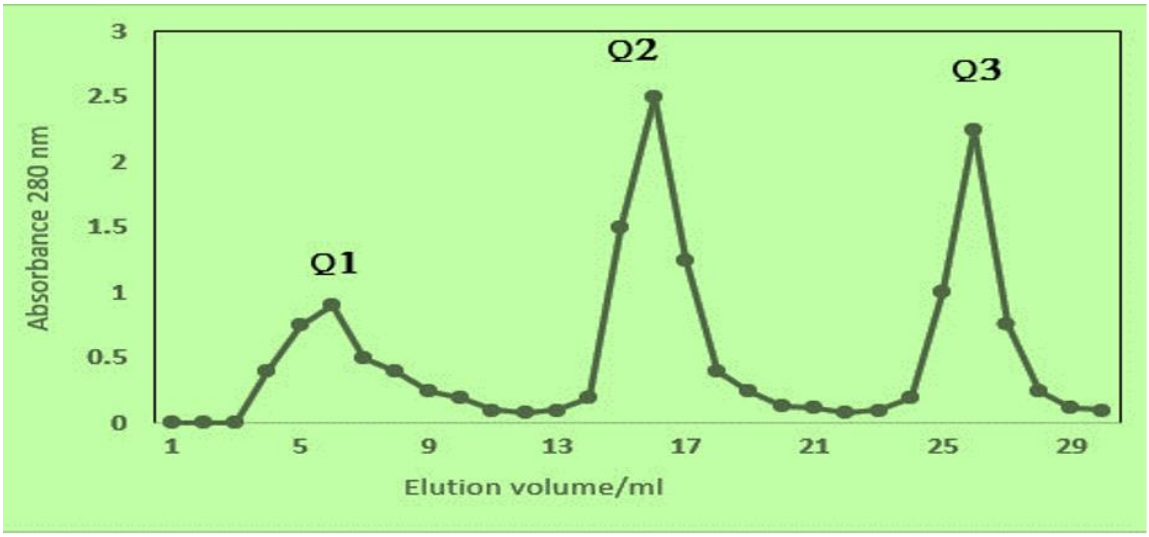

Fig. 1: The Purification of *Agaricus bisporus* lectin (ABL) by chromatography on Q-Sepharose (1 cm × 5 cm); three fractions (Q1, Q2, Q3) peaks from Elution of IEC**.**

Assay of Hemagglutinating Activity:

The Most of lectins agglutinate red blood cells of all human blood bunches with no perceptible particularity and are alluded to as nonspecific lectins. Such lectins link to carbohydrate sites allocated on the membrane of all red blood cells, regardless of blood gather determinants. The hemagglutinating activity is a characteristic feature of lectin. ABL agglutinated the red blood cells as a mat formation (true test), The erythrocytes were aggregated by binding of its sites to receptors of ABL while button formation is considered as (false test) that resembled control well (unapplied lectin) illustrating no agglutination occurred as shown in Figure (2). The purification step through Ion Exchange Chromatography; IEC showed hem-agglutinating activity was 512 units (titre/mg) that represented the highest dilution of lectin. *A. bisporus* lectin, ABL can agglutinate a wide range of red blood cells, (A, B, AB & O groups) with no specificity as indicated in Figure (3).

Sugar Specificity Assay:

The hemagglutinating activity of *Agaricus bisporus* lectin was suppressed by its binding to some sugars that caused no agglutination. The white bottom mushroom has no affinity to ribose, xylose, arabinose, mannose, lactose & sucrose while having a high affinity to GlcNAc. The consistency of button in the presence of sugar marks specific action and the consistency of mat marks nonspecific action, so the binding of GlcNAc to ABL was considered as a specific reaction (button formation) as shown in Figure (4).

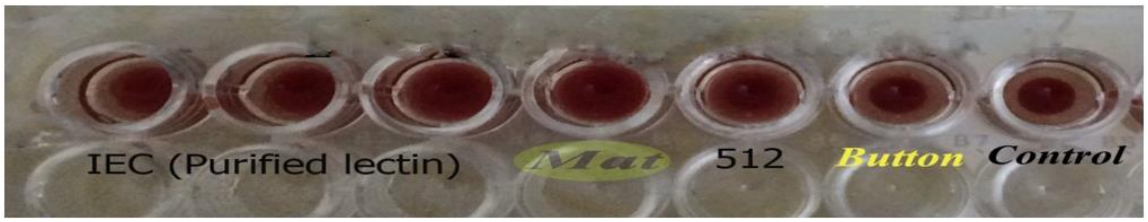

Fig. 2: Shows the **button** and the **mat** formation of **hemagglutination activity**. The mat formation shows agglutination of the RBCs (positive test) while the button formation shows no agglutination (negative test). The experiment was repeated three times and the results were reproducible.

Fig. 3: Shows mat formation compared to blood sample control that indicated ABL agglutinated all blood groups without specificity.

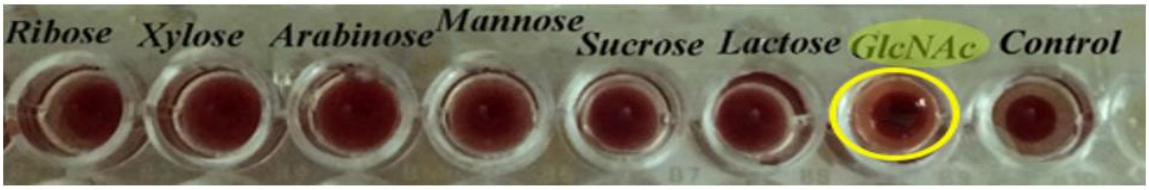

Fig.4: Shows control = RBCs added to phosphate buffer saline forming button formation (no agglutination). GlcNAc=N- Acetyl-glucosamine has button formation that indicates its high affinity to ABL while mat formations (agglutination occurred) are formed with Ribose, Xylose, Arabinose, Mannose, Sucrose and Lactose that indicates ABL has no affinity to these sugars.

The Effect of Temperature on Lectin Activity:

A. bisporus lectin had no hemagglutinating activity at 70 & 80 ºC Figure (5). The activity was detected by the ability to agglutinate red blood cells which were characterized by mat formation at 65 ºC while button formation was observed at 70 & 80 ºC.

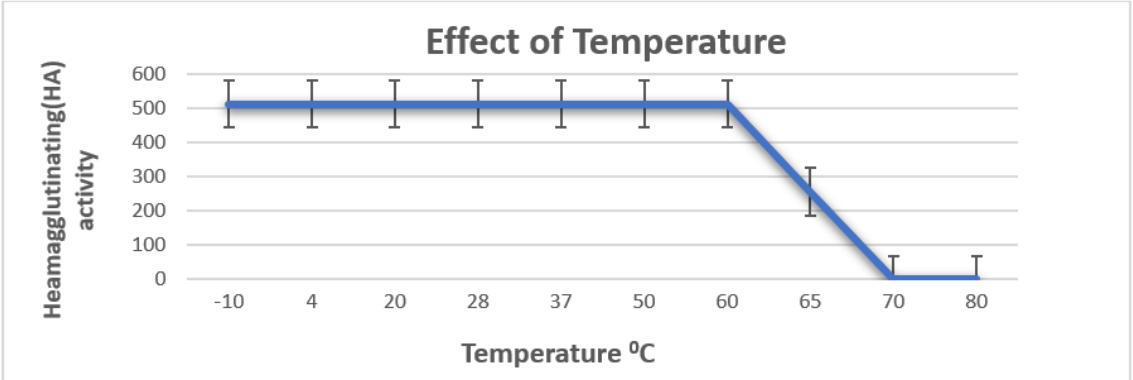

Fig.5: Effect of temperature on lectin activity

The Effect of pH on Lectin:

The pH assay was performed in the different buffers for 12hr & 24hr to detect the hemagglutinating activity (HA) of *A. bisporus* lectin. The lectin activity was 256 U titre/mg at pH in range (2 to 5) for 24hr

then increased to 512U titre/mg at pH (6 to 9) for 24hr but started to decrease to 128 U titre /mg at pH 10 for the same period. The activity declined to 64 U titre/mg at pH 10.8 for 12 hr. We observed that the best pH media for HA within range (6 to 9) compared to the control sample that had pH 7.2 neutral medium. Figure (6) indicated that the effect of pH media on the hemagglutinating activity of *A. bisporus* for 12hr & 24hr increased at pH 6 until to pH 9; i.e., ABL was active in acidic & alkaline media for 24hr.

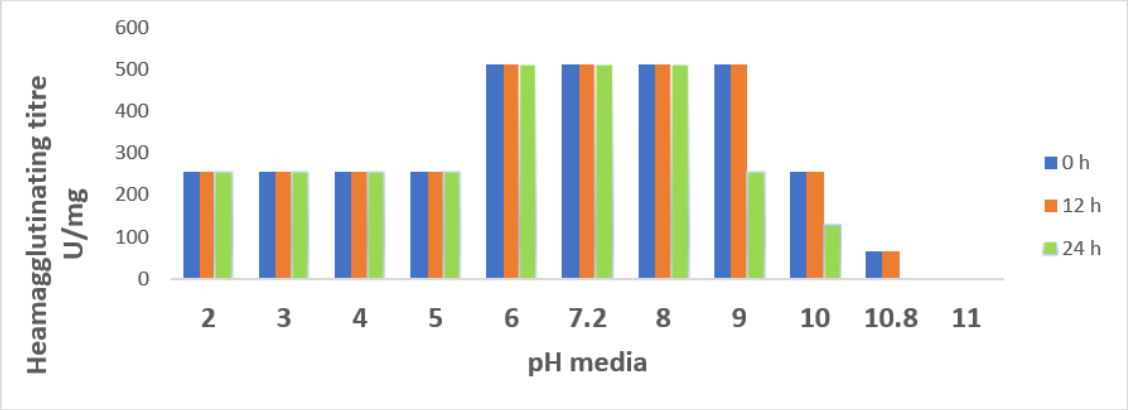

Fig.6: shows the effect of pH media on *A. bisporus* lectin for 12hr and 24hr through hemagglutinating activity titre U/mg**.**

SDS-PAGE

The purpose of this test is to determine the molecular weight of *A. bisporus* lectin. SDS-PAGE analysis showed a single band for purified lectin (lane 3) as shown in Figure (7) with molecular weight 16KDa.

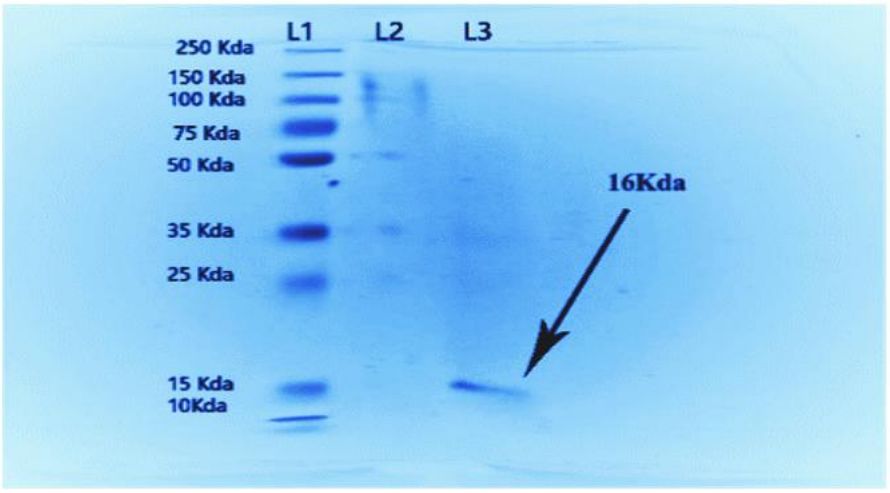

Fig.7: SDS-PAGE of Agaricus bisporus lectin. Lane 1 marker protein (10-250KD), Lane 2 Crude extract (unpurified lectin, Lane 3 IEC sample (Purified Agaricus bisporus lectin) 16KDa. SDS-PAGE revealed that molecular weight of the subunits was 16KDa these results allowed us to conclude that the protein is composed of four identical subunits.

Cell Toxicity & Statistic Results:

The reduction of tetrazolium salts is commonly recognized as a dependable approach to investigate cell viability. MTT test was done to appraise the effect of *A. bisporus lectin* (as a treatment) on HepG2 & WI-38 proliferations. IC_{50} which value is an effective dose needed to suppress the proliferative response by 50%. The tissue culture

results showed that ABL has antiproliferative & toxic effects on cell lines with IC_{50} 41.90 ug/ml for HepG2 while was 98.54 ug/ml for and WI-38 (normal cells) Figure (8).

The statistical analysis was analyzed by using SPSS 18, a one-sample t-test with a value of 100 for the highest % viability. Table (3), p-value was < 0.05 for all tested cell lines, i.e., there were significant differences among means of cell lines emphasizing the toxic effect of *A. bisporus* lectin on malignant cells. WI-38 had the highest cell viability that means *A. bisporus* lectin had a low toxic effect on normal cell.

Table 3: Shows the Statistical analysis

Cell line	N	Mean ± S.E.M	T value =100			
			Std.deviation	*-t-*	*Df*	*Sig-2 tailed*
HepG2	8	48.898±10.587	29.9473	-4.826	7	.002
WI38	8	91.332±2.698	7.63110	-3.212	7	.015

Apoptosois:

A. bisporus lectin has an apoptotic effect on the HepG2cell line compared to its controls. *A. bisporus* upregulated Bax (pro-apoptotic gene) on tested cell line as shown in Figure (9), which indicated the apoptosis effect of ABL. *A. bisporus* lectin increased Bax gene to, 27.58 for, HepG2 cell line Table (4).

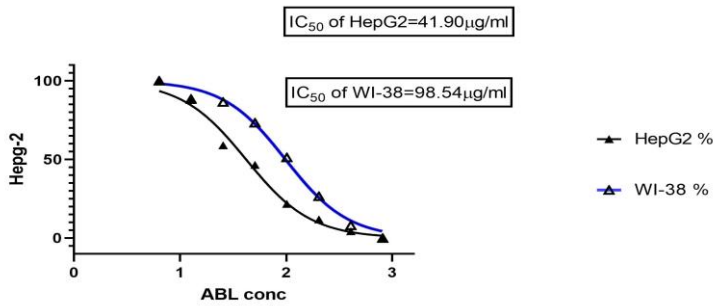

Fig.8: shows IC_{50} of HepG-2 & WI-38 had been calculated through graph pad prism.

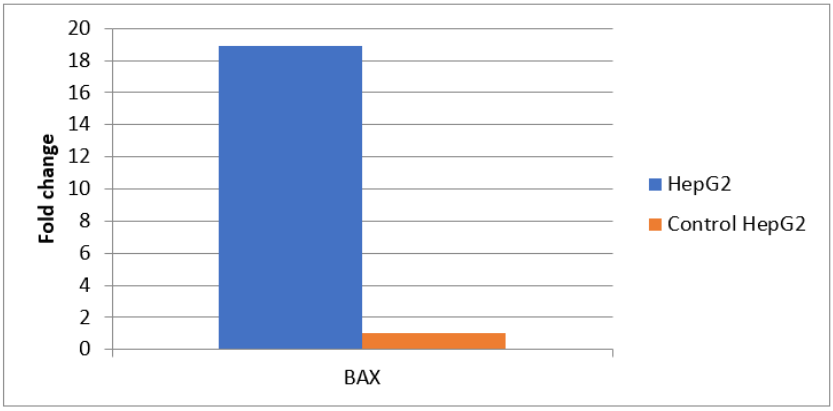

Fig.9: Gene expression of Bax shows an increase of Bax gene
Note: Amplification & melting curves had been preceded.

Table 4: BAX (C_T) values

Sample	BAX							
	Control cells			Treated cells (Tr)				FLD
	GAPDH	Bax	$\Delta C_T C$	GAPDH	**Bax**	$\Delta C_T Tr$	$\Delta\Delta C_T$	$2^{\wedge -\Delta\Delta C_T}$
	$C_T G$	$C_T B$	$C_T B$-$C_T G$	GC_T	BC_T	BC_T-GC_T	$\Delta C_T Tr$-$\Delta C_T C$	
HepG2	26.59	31.33	4.74	27.08	**27.58**	0.5	-4.24	18.895
Cont.HepG2	26.59	31.33	4.74	26.59	31.33	4.74	0	1

FLD=Fold change, cont.=control

DISCUSSION

Navegantes-Lima *et al.*, 2020 mentioned that the White button mushroom has antimicrobial and antiproliferative activities. *Agaricus bisporus* is considered a medicinal mushroom because of its biologically active substances(lectin & polysaccharides) utilized as an antitumor effect, our results agreed with (Wang *et al.*, 2021).

Lectins are a heterogeneous group of non-enzymatically carbohydrate-binding proteins that particularly perceive and bind reversibly to specific mono and oligosaccharides on cell surfaces, the extracellular framework, and emitted glycoprotein as was issued by (Hashim, Jayapalan and Lee, 2017).

The activity of lectin was increased by increasing the purification steps. The lectin activity of the crude sap was 32-unit titre/mg as an initial lectin activity, while we used ethanol as a first solvent, the activity increased to 64 unit/mg with **2** purification factors (PF) which were calculated as the proportion of lectin activity after to lectin activity before the purification step. When we used Ammonium sulfate as a second solvent, the specific lectin activity reached 256 U titre/mg with 8 folds than the first solvent. The purification fold of lectin through the IEC was 16 times that recovered from 200g dried fruiting bodies purified from *A. bisporus* IEC which was considered a strong anion for adsorption of lectin with high performance that was matched with (Singh *et al.*, 2015). White button mushroom was adsorbed on Q-Sepharose column which has high-affinity binding process due to ABL was dissolved in acidic medium; therefore, the total net charge of ABL was a positive charge that was adsorbed to anion exchange medium supported by elution process via elute buffer that washing all adsorbed matrices as a purified lectin. The total protein contained in the collected fractions was 9.744 mg/ml with specific lectin activity 512 U titre/ mg that was calculated after finalizing the purification steps of lectin through IEC**.**

White button mushroom lectin hemagglutinates erythrocytes as a characteristic feature for lectin, so its activity was measured by titre which is defined as the equal of the endpoint dilution displaying the hemagglutination. In this study, ABL agglutinated the red blood cells as a mat formation (+ve test); The erythrocytes were aggregated by binding of its sites to receptors of ABL while button formation is considered as (-ve test); no agglutination has occurred. *A.bisporus* lectin agglutinated all blood groups without specificity these results were agreed with(Singh, Bhari and Kaur, 2010). We proved that the hemagglutinating of ABL was inhibited only by GlcNAc that was considered as a specific sugar this result was matched with (Hassan *et al.*, 2015) while D (-) Ribose, L(+) Arabinose, D (+) Mannose, D (+) Xylose, D (-) Sucrose & D (-) Lactose were not specific sugars for ABL.

White button mushroom lectin was active at different temperatures ranging from -10 up to 65^{o}C. The hemagglutinating activity of lectin was at 512 U titre/mg at -10 ^{o}C up to 60 ^{o}C but decreased to 256 U titre/mg at 65 ^{o}C. *A. bisporus* lectin had no hemagglutinating

activity at 70 & 80 ^{o}C. The lectin activity was 256 U titre/mg at pH in range (2 to 5) for 24hr then increased to 512 U titre/mg at pH (7 to 9) for 24h but started to decrease to 128 U titre /mg at pH 10 for the same period. The activity declined to 64 U titre/mg at pH 10.8 for 12 hr. we can conclude that *A. bisporus* lectin was active at acidic & alkaline as well as neutral media. The best pH media for HA was within range (6 to 9) compared to the control sample that had pH 7.2 neutral medium.

In the present study, we proved that the molecular weight of *Agaricus bisporus* lectin was 16Kda as a specific character for White button mushroom lectin.

Witzell and Martín, 2014 reported that the Anti-cancer drugs offered in the present market are not objective-specific and have numerous side effects and complications faced in the clinical management of different forms of cancer, which concentrates the essential necessity for novel effective &less-toxic therapeutic attempts. The malignant cells can also propagate to different parts of the body and deliver new tumors. In case the propagation of cells winds up uncontrolled, it can prompt death. So, it is vital in cancer drugs that the treatment seeks only the malignant cells, without affecting the normal cells, which is extremely hard, particularly in chemotherapies; this is considered the main important issue.

A*.bisporus* lectin has a high affinity to N- acetyl glucosamine; GlcNAc, as well as the TF-antigen, So A.bisporus lectin, has dual binding sites which are found on the cell surface that facilitate aggregation of cells the results were verified by (Gao *et al.,* 2020). Carrizo *et al.,* 2005 proved that the Thomsen Friedenreich (TF) antigen is a disaccharide, galactosylβ-1,3-N-Acetylgalactosamin (Galβ-1-3GalNAc) connected to either threonines or serines on cell membrane glycoproteins. ABL has a high affinity to TF, so *A. bisporus* is highly attached to malignant cells because of appearing TF- antigen in high levels on human malignant cells causing its aggregations then apoptosis while ABL couldn't attach to normal cells because of TF- antigen was hidden inside the normal cells.

ABL aggregated malignant cells leading to apoptosis that was matched with the study ('Role of edible mushrooms as functional foods- A review', 2016). The tested cell line was treated with varying concentrations of *A.bisporus* lectin for 24hr.& 48hr. IC_{50} which value is an effective dose needed to suppress the proliferative response by 50%. The tissue culture results showed that White button mushroom lectin has antiproliferative & toxic effects on cell line HepG2 with IC_{50}; 41.90 ug/ml while it has low toxicity on WI-38 with IC_{50} (98.54 ug/ml). Hassan *et al.,* 2015 mentioned that IC_{50} of *Agaricus bisporus* lectin on HT-29(colon cancer) was 50 ug/ml

The suppressor impacts of the white button mushroom on mitogen- and antigen- prompted DNA synthesis is controlled through its carbohydrate-specific binding domain The inhibitory impacts of *Agaricus bisporus* lectin (ABL) is believed to be a result of the particular obstructing of the nuclear localization signal (NLS) dependent protein import, basic for cell working that was mentioned by (Singh, Kaur and Kanwar, 2016). de Oliveira Figueiroa *et al.,* 2017 illustrated that *A. bisporus* lectins link to carbohydrate-binding receptors present on the cell membrane and excite diverse metabolic pathways that actuate inconsistency in mitochondria bringing about high quantities of reactive oxygen species (ROS) and liberating of pro-apoptotic factor Bcl-2- related X protein (BAX) causing suppression of B-cell lymphoma2

protein (Bcl2), so the apoptosis procedure is enacted. Cytochrome C is released into cytosol that combines with Apaf1 & procaspase 9 to form apoptosome which activates procaspase 3 into caspase 3.

A.bisporus upregulated Bax(pro-apoptotic gene) on tested cell line HepG-2 our result was matched with (Adams *et al.*, 2008). *A. bisporus* lectin increased Bax gene to 27.58 for HepG2 cell line compared to the control gene.

The Statistical analysis was analysed by using SPSS 18, a one-sample t-test with a value of 100 for highest % viability, p-value was < 0.05 for all tested cell lines, i.e., there were significant differences among means of cell lines emphasizing the toxic effect of *A. bisporus* lectin on malignant cells. WI-38(normal cells) had the highest cell viability that means *A. bisporus* lectin had a low toxic effect on NORMAL CELLS. Very few studies e.g. (Ferriz-Martinez *et al.*, 2010) focused on That result; the white button mushroom has high selectivity for normal cells. *Agaricus bisporus* lectin has a toxic effect on HepG2; this was a new era for the effectiveness of ABL on hepatocellular carcinoma.

Conclusion

ABL has a toxic effect on malignant cells HepG2 as ant proliferative activity & an apoptotic effect, while it has very low toxicity on WI-38 (normal cell) which represents normal cells. White button mushroom lectin can be used as a discrimination tool for malignant and normal cells in practical applications. The purified lectins are used to determine blood type because of the specificity of carbohydrate structures present on the cell surface of red blood cells. Lectin can be used in isolation of specific membrane and serum glycoconjugates. The specificity of lectins for sugars makes them good tools in glycobiology, such as lectin array The recommendation is to use lectins from different mushroom species in clinical pathological research to produce a novelle anticancer drug with high selectivity to normal cells. Lectin can be used in wound healing applications in future aspects. The effect of ABL on Hepatocellular carcinoma needs more assays.

Acknowledgements

The authors like to thank the Department of Molecular Biology, Medical Research Centre, Faculty of Medicine, Ain Shams University for providing needed facilities to implement the work.

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