

The Agglutinating And Humoral Immunomodulating Activities Of Oryza sativa L. Cucumis melo, and Panicum miliaceum L dry Seed Lectins

Ibrahim M. S. Shnawa*

*Department of Biology, College of Science, Babylon University
Hilla, P. O. Box(4)

Oda M. Al-Zamily**

and Senan Al-Mashta*

**Department of Chemistry, College of Science, Babylon University
Hilla, P. O. Box(4)

Abstract :

The *Oryza Sativa* L, *Cucumis melo*, L and *Panicum meliaceum*, L dry seed lectins were separated and partially characterized. As they were salted out by trichloroacetic acid, ammonium sulfate and Alcohole. They were biurate positive, carbohydrate binder with cell agglutinating potentials.

Cucumie melo lectin (CML) agglutinate human erythrocytes A, B and H. CML agglutmate, also, BT lymphoid avian cells and it did so to 8.8% of K pneumoniae clinical isolates. At low concentration it was found of anti Egg Albumin (EA) enhancing titres in lapin model, and it was of titre inhibiting potential at high concentration.

Panicum meliaceum L (PML), agglutinate human erythrocyte B, H and avian T lymphoid but not B cells. PML was of non agglutinating ability to K. pneumoniae isolates. It enhanced anti EA antibody titres at low and suppress at high concentration. Thus, CM and PM lectins were immunomodulator.

الخلاصة:

جرى الفصل والوصف الجزئي لكل من لكتينات الرز والبطيخ والدخن. ووجد بانها تترسب بثالث كلوريد حمض الخليك وسلفات الامونيوم و الكحول. وكانت محاليل هذه اللكتينات موجبة البايوريت وترتبط بالسكريات ولها اثر ملزن للخلايا الحية. وقد لزن بذور البطيخ كريات دم البشر من نوع A و B و H. وتلزن الخلايا اللمفاوية الثانية والبائية للطيور وكانت ملزنة لـ 8.8% من سلالات K. pneumoniae السريرية. وفي موديل الارنب تبين بان التراكيز الواطنة لها اثر محفز للمناعة الخلطية المتخصصة لمستضد آح البيض وتصبح مثبطة لهذه المناعة بالتراكيز العالية. وظهر بان محلول لكتين الدخن ملزن للكريات الحمر للبشر من نوع B و H وملزن ايضا للخلايا اللمفاوية الثانية للطيور وليست البائية منها. ولم يلزن أي من العزول السريرية لـ K. pneumoniae وكان اثره على الاستجابة المناعية الخلطية في الارنب والمتخصصة لمستضد آح البيض محفز بالتراكيز الواطنة مثبط في التراكيز العالية. ولهذا فان هذه اللكتينات قد تعمل عمل محور مناعي.

Introduction :

Phytolectins were globular glycoprotein, nonenzyme, nonimmune, nonantibody carbohydrate binding molecules and antibody like cellular agglutinins⁽¹⁻³⁾. Such lectins, however, have preferential ability to agglutinate embryonic and neoplastic cells. Some which exhibits cell mitogenic activity⁽⁴⁾. Other are usable in human erythrocyte typing and lymphocyte culture for genetic studies⁽⁵⁾. Shnawa⁽⁵⁾ have been documented the separation, partial purification and functional characterization of five plant seed lectins.

The objectives of the present work were to perform;

1. Separation of Oryza sativa (OS) Cucumis melo (CM) and Panicum meliaceum (PM) seed lectins.
2. Some biological and biophysical lectin characterization.
3. Assessment for agglutinating and Immunomodulating activities for CM, PM lectins.

Materials and Methods:

Dry seeds of Oryza sativa L Cucumis melo L and Panicum melaceum L were cleaned and made in fine powder mill forms. One gram from each of these fine powder mills was processed as in Shnawa⁽⁵⁾.

Sugar binding assay was done by coating tanned erythrocytes with lectin solutions and titrated against sugar solutions of 1.25 to 10 mg/ml by microhaemagglutination try⁽⁶⁾.

Preconditioning of rabbits with graded concentrations of CM and PM lectin solutions (Table 1).

Passive haemagglutination test was done by titration of egg albumin coated erythrocyte against rabbit immune sera⁽⁶⁾ to assess the humoral immunomodulating activity of CM and PM lectins using Oral dosage immunization programme that follow the preconditioning manue.

Results :

I- Partial Characterization;

Both watery and Alcoholic separated solutions were salted out by Trichloroacetic acid & ammonium sulfate as well as alcohol precipitation. The best of which was the trichloroacetic acid, but it interferes with the agglutination reactions. They were protein in nature with sugar binding and cell agglutinating potentials. They with stand pH of up to 8.0 and temperature of up to 40 C⁰ but not more. (Table 2).

II- Agglutininations;

Variable degrees of agglutinating ability were noted among these lectins. Some of which agglutinate K pneumoniae cells other were not. Most agglutinate thymus and bursa, lymphoid cells of chicken, few didn't do so. (Table 3).

III- Humoral Immunomodulation;

The lapin humoral immune model has shown that at certain CM & PM lectin concentrations they could act as immunostimulators and at others they acted as immunosuppressors. (Table. 4).

IV- Cucumis melo lectin (CML);

CM solution showed protein concentration of 82.8 mg/ml, binds galactose, tolerate pH of neutral and alkaline range of up to 8.0. It withstand temperature of up to 40 C^o but it lacks activity at 60 C^o and 90 C^o. It agglutinate; B and T avian lymphoid cells, A B and H human erythrocytes.

CM lectin could act as immunomodulant at concentration of 24.1 & 56.4 mg/ml. Both stimulant at 24.1 and suppressant at 40.2, 56.4 mg/ml respectively.

V- Panicum meliaceum L (PML);

PML solution showed 8.42 mg/ml protein. It binds non of mannose galactose and glucose. It tolerates pH of up to 8, but its best functioning at neutral pH. It was reduced to half of its activity at 40 C^o and lose it completely at 60 & 90 C^o. It agglutinates non of K. pneumoniae cells and agglutinate avian T but not B lymphoid cells. It was immunostimulating at 2.5, 4.2 mg/ml and immunosuppressant at 5.7 mg/ml.

VI- Oryza sativa (OSL);

OSL solution gave protein concentration of 12.83 mg/ml. It binds mannose and glucose. Watery lectin solution agglutinate human A, B and H erythrocyte. OSL with stand temperature of up to 40 C^o but not 60 or 90 C^o as well as pH of up to 8.0 .

OSL agglutinate 22.22% K. pneumoniae cells as well as T and B avian lymphoid cells.

Discussion :

The OS, CM and PM solutions were proved to be lectin solutions (Table 1-4). This finding is parallel to that of others reports(7-8,5).

OS, CM and PM lectin solution were showing different human erythrocyte, avian leukocytes, and K. pneumoniae agglutinability as well as different sugar binding abilities(8-9,5,10-12).

There was an inverse relationship between the increase in temperature and decrease in the direct hamagglutinability(8).

Variable lectin agglutinability was noted among K. pneumoniae cells which may be depend on lectin type and isolate nature, and parallel to that made on C. Fetus using standard lectin preparations(12-13,11,5).

Lectin lymphocyte agglutinability had been reported by Sharon(1) and documented by Shnawa(5) on Cicer orientum seed lectin. Parallel to these studies. The alcoholic extracts showed T and B lymphoid cell agglutinins (Table 3).

The immunomodulating effects of CM and PM lectins were in agreement with what have been reported on mushroom lectins(15).

Thus on summing up one may conclude the following:

1. *Oryza sativa*, *Cucumis melo* and *Panicum meliaticum* dry seed lectins were separated and partially punned.
2. OSL, CML and PML were proteins, carbohydrate binder and cell agglutinins with potential use in bacterial and erythrocyte lectin typings.
3. CML and PML were lapin humoral immunostimulator and mmunosuppressors and the effect was concentration dependent (table 4). Such findings may indicate their possible use as a candidate, safe, cheap and handable immunomodulator after eligible refinement and evaluations.

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Table 1: Preconditioning and Immunization Protocols.

Control: Three animals receiving 5ml saline solutions through oral rout same as test groups.	
Preconditioning	
CML	PML
Four successive graded oral doses* 24.3, 40.2, 56.4 mg/ml spaced by 2 days between each of the doses. Followed by three days leave.	Four successive graded doses of 2.5, 4.2 and 5.7 spaced by 2 days between each of the doses. Followed by three days leave.
Immunization; Three oral 5 ml doses of 2% egg albumin solution spaced in between by five days a part. Following the final dose are one week leave then bleed.	
* Three animals for each of the concentrations per each lectin.	

Table 2: The biological Properties of *Oryza sativa* L, *Cucumis melo* L and *Panicum meliaceum* L lectin solutions.

Properties

I- Salting out

Name	Salting agent	Protein conc. Mg/ml	HA titre
<i>Oryza sativa</i> L. rice	TCAA 36%	12.83	256
<i>Cucumis melo</i> L melon	AS 40%	82.66	16
<i>Panicum meliaceum</i> L miuet	Ethyl alcohol 99.9%	8.42	4

II- Direct Haemagglutination

	A		B		H	
	H*	A**	W	A	W	A
OSL	32	4***	16	4	64	16
CML	0	4	0	8	16	16
PML	4	4	4	16	4	16
* water A**alcohol ***titre						

III- Suggar Binding

	Mannose	Galactose	Glucose
OSL	6.25*	0	10
CML	0	10	0
PML	0	0	0
* median concentrations mg/ml			

IV- Temprature effect

	25	40	60	90
OSL	32	32	0	0
CML	8	4	0	0
PML	4	2	0	0

V- PH EFFECT

	PH			
	2.5	5	7	8
OSL	4	8	32	16
CML	2	4	8	8
PML	0	2	4	2

TABLE 3: The lectin cellular agglutinins of prokaryotic and eukaryotic cells

I- Bacterial Agglutinins of *K. pneumonia*

Lectin Type	Lectin Agglutination
OSL	8/36 (22.22%)
CML	3/36 (8.33%)
PML	0/36 (0%)

II- Avian lymphoid cell agglutinins

Avian lymphoid cell	OSL		CML		PML	
	W	A	W	A	W	A
B	-	+	-	+	-	±
T	±	+	-	+	-	+
W= water , A= Alcohol						

Table 4: Humoral Immunomodulation via anti EA titres in lapin model

Preconditioning concentrations	Antigen 2% EA	Anti EA Haemagglutinin Titres
CML mg/ml		
24.3		4096*
40.2		24
56.4		24
PMLmg/ml		
2.5		4096
4.2		4096
5.7		32
No preconditiong	256	
*mean of three readings		