ISSN (Online) 2249-6084 (Print) 2250-1029

International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)
[Impact Factor – 0.852]

Journal Homepage: www.eijppr.com

Research Article *Article ID: 413*

Hemagglutinating activity of Chickpea extracts for lectin

Sameer S. Bhagyawant[1], Ajay Gautam[1], S. K. Chaturvedi[2] and Nidhi Shrivastava[3*]

[1]School of Studies in Biotechnology, Jiwaji University, Gwalior 474011, India
[2]ICAR-Indian Institute of Pulses Research, Kanpur, 208024, India
[3]Department of Bioscience & Biotechnology, Banasthali University, 304022, India

***Corresponding author Email:** nidhishrivastava1234@gmail.com

Article info

Article History:
Received 28 October 2015
Accepted 28 December 2015

Keywords:
Lectin, Chickpea, Hemagglutination

Abstract

Lectins are carbohydrate-binding proteins of non-immunoglobulin nature and are ubiquitous in nature. Lectins of some pulses exhibit various applications that includes anticancer, mitogenic and anti HIV-1 reverse transcriptase activity. Objective of this study is to determine the lectin levels in chickpea seeds using blood coagulation methods. A total of 50 different chickpea seed genotypes were screened for lectin composition using human and rabbit erythrocytes. Trypsinised rabbit erythrocytes gave considerable lectin activity under *in-vitro* conditions. A titre of such activity was observed in a range of 2-2048 HAU with a protein concentration of 1.57 to 6.32 mg/ml of seed extract. This lectin assay was stable for about 2-4 h. However, the isolated *Cicer* lectin does not inhibit simple sugar moieties. Though it could inhibit a complex sugar like desialated fetuin only. Based on this screening data, further investigations are in progress for purification and characterization of chickpea lectin from wild *Cicer* species.

1. INTRODUCTION

Lectins are carbohydrate-binding proteins of non-immunoglobulin nature and are ubiquitous in nature. The word lectin comes from the Latin word *legere*, which means “to select” by William Boyd[1]. Lectins have the ability to bind carbohydrates. Nowadays, proteins that can agglutinate red blood cells with known sugar specificity are referred to as lectins. The name hemagglutinin is used when the sugar specificity is unknown. Plant lectins are a heterogeneous group of carbohydrate-binding proteins. Some of the legume lectins have been found in vegetative tissues like tissues, stems, bark, roots etc. Legume lectins are dimeric or tetrameric proteins, each subunit (25-30kDa) consisting of one carbohydrate binding site. They can bind to the carbohydrate moieties on the surface of erythrocytes and agglutinate the erythrocytes, without altering the properties of the carbohydrates [2]. Plants commonly synthesize some antinutrients as part of protection against their predators and/or as a means to survive under adverse growing conditions, this includes lectin also. Moreover, two approaches are familiar for lectin classification. They can be classified on the basis of their carbohydrate specificity. They can also be categorized according to the overall structures into merolectins, holoectins, chimerolectins and superlectins, or be grouped into different families (legume lectins, type II ribosome-inactivating proteins, monocot mannose-binding lectins, and other lectins).

Chickpea (*Cicer arietinum* L.*)* is a legume and belongs to the family *Leguminoseae.* There exists different assortment of chickpea types viz. desi, kabuli and wild etc. Leguminous plants are known to contain lectins abundantly in various tissues such as seed, bark, stem, leaf, flower, and root. Vegetative lectins are present at much lower levels than seed lectins and they have been less characterized [3]. Lectins with specific carbohydrate specificity have been purified from various plant tissues Lectins exhibit a diversity of therapeutic applications vis-à-vis anti-

microbial, antitumor, immunoregulatory, and HIV-1 reverse transcriptase inhibitory activities [4]. Lectins from several plant species were able to agglutinate erythrocytes, with assorted levels in the same species. Some lectins having hemagglutination activity were isolated earlier from *Cicer arietinum* [5, 6]. A wide range of improved chickpea verities/germplasms are now available. The lectin contents are low in some varieties and high in other varieties. The objective of this paper was to determine erythrocytes agglutinating activity of the lectin extracts from the seeds of chickpea.

2. MATERIALS AND METHODS

2.1 Seed material

Total fifty chickpea genotypes including wild were used for agglutination experiments (Table 1). The mature and dry seed material was obtained from Indian Institute of Pulses Research, Kanpur, India under MTA understanding.

2.2 Lectin extraction

Lectin in the seeds was isolated using procedure as described by Gurjar *et al.* and Bhagyawant *et al.*[7,6]. The dry matured seeds were finely ground in warring blender. Seed meal (50 g), was added to 250 ml of Tris-HCl extraction buffer (20 mM Tris-HCl pH 7.2, containing 150 mM NaCl). The suspension was agitated for 12 h at 4 ºC in cold and filtered through muslin cloth. The filtrate was subsequently centrifuged at 10,000 rpm for 20 min. at 4 ºC. The clear supernatant was saved and used for hemagglutination assay.

2.3 Hemagglutination assay

The titer assay was initially performed using normal and trypsinised human erythrocytes (A, B, AB, and O) and rabbit erythrocytes. Fresh rabbit erythrocytes were separated from plasma by centrifugation at 3000 rpm for 4 minutes at 5-10 ºC and washed extensively with 10mM Tris-HCl buffer, pH 7.2, containing 150 mM NaCl (TBS). Finally, 3% suspension was prepared in TBS and used in hemagglutination assays. Hemagglutination tests were performed in standard microtitre plate by the two-fold serial dilution method. A 50 ml aliquot of the erythrocyte suspension was mixed with 50 ml of serially diluted lectin. Agglutination assay was examined visually after incubation for one hour at room temperature. Lectin free sample was used as a control. The unit of hemagglutination activity (U) termed as titre expressed as the reciprocal of the highest dilution of the lectin that showed complete agglutination. The specific activity of the lectin is defined as the titre of hemagglutination per mg of protein.

2.4 Preparation of trypsin treated erythrocytes

Trypsin treated erythrocytes for hemagglutination assay were prepared by the method of Lis *et al.* and Sharon *et al*. [8, 9]. Fresh rabbit erythrocytes were centrifuged at 2000 rpm for 10 minutes. The serum was removed and the erythrocytes were repeatedly washed with TBS. The RBC suspension (3%) was incubated with 0.05% (w/v) trypsin at 37 ºC for 1 h. After incubation, erythrocytes were re-washed with TBS to remove trypsin and finally suspended in TBS at a concentration of 3% and used for hemagglutination assay.

2.5 Protein estimation

Protein content in the seed extracts was estimated by the Folin-Lowery method [10] using BSA as a standard. Lectin specific activities were expressed as titre over milligrams of protein as hemagllutinaing Units (HU) per mg of protein.

2.6 Carbohydrate Inhibition assay

Hemagglutination inhibition tests were performed as described in hemagglutination assay, except that serial dilutions of the sugar solution (25ul) were pre-incubated at room temperature with 25ul of the lectin (minimum concentration showing titer) for 15 minutes. 50ul of rabbit erythrocyte suspension was added, mixed and the plates were read after one hour. The concentration of all the sugars used was 500mM and that of the glycoprotein used was 10mg/ml. The various sugars were employed in the inhibition studies (Table 2). The assay was carried out at room temperature in a 96 well microtitre plate.

Table 1. Eryrothrocytes agglutination by chickpea seed extract

S.N.	Chickpea genotypes	Type	Protein conc. (mg/ml)	Hemagglutinatin units (HU)	Specific activity (HU/mg)
1	ICC-4495	Desi	2.81	128	45.55
2	IPC-12-99	Desi	1.64	128	78.0
3	IPCK-12-291	Kabuli	1.87	2	1.11
4	JG-130	Desi	1.98	1024	517.17
5	IPCK-12-289	Kabuli	2.83	1024	361.8
6	ICC-1882	Desi	2.77	512	184.8
7	ICCV-07109	Desi	2.59	512	197.68
8	ICCV-07117	Desi	2.88	128	44.4
9	ICCV-12-286	Desi	2.02	512	253.4
10	ILWC-292	Wild	2.91	1024	351.8
11	IPC-7-28	Desi	2.79	1024	367.0
12	IPC-10-181	Desi	3.45	1024	296.8
13	IPC-11-83	Desi	2.99	1024	342.44
14	ICC-6816	Desi	2.62	1024	390.8
15	ILWC-142	Wild	3.01	1024	340.1
16	IPC-12-82	Desi	3.62	1024	282.8
17	IPC-10-137	Desi	3.10	1024	330.3
18	IPC-11-30	Desi	2.80	512	182.85
19	IPCK-12-284	Kabuli	2.91	1024	351.8
20	BGD-112	Desi	3.20	1024	320.0
21	JGG-1	Desi	3.65	1024	280.5
22	JGK-3	Desi	4.10	1024	249.7
23	RADHEG	Desi	5.32	2048	376.8
24	JGK-43	Desi	4.56	1024	224.5
25	JSC-45	Desi	5.21	2048	393.0
26	RGG-888	Desi	5.32	2048	384.9
27	ICC-16269	Desi	6.01	2048	340.7
28	ICC-6263	Desi	2.39	512	214.2
29	JGK-3	Desi	3.05	1024	335.7
30	ICC-12028	Desi	5.22	2048	392.3
31	ICC-6537	Desi	3.90	1024	262.5
32	ICC-14199	Desi	2.99	1024	342.4
33	ICC-9848	Desi	3.89	1024	263.2
34	ICC-2	Desi	5.30	2048	386.4
35	ICC-800	Desi	5.39	2048	379.9
36	JG-379	Desi	6.32	4096	648.10
37	ICC-17186	Wild	2.93	512	174.7
38	UJJAIN-21	Desi	6.01	2048	340.7
39	L-550	Desi	5.30	2048	386.4
40	PHULE-5	Desi	2.99	512	171.2
41	JG-63	Desi	5.36	2048	382.0
42	JG-11	Desi	3.45	1024	296.8
43	ICC-13441	Desi	5.08	2048	403.1
44	ICC-15618	Desi	3.66	1024	279.7
45	ICC—6537	Desi	4.65	1024	220.2
46	ICC-12537	Desi	5.33	2048	384.2
47	ICC-12028	Desi	6.01	2048	340.7
48	BCP-17	Desi	5.66	1024	180.9
49	ICC-12155	Desi	3.24	1024	196.1
50	ICC-15518	Desi	5.22	2048	392.33

3. RESULT

In the present investigation, lectin extracted from desi, kabuli and wild type chickpea seeds were used for erythrocytes agglutination assays. Of the 50 desi, kabuli and wild type were screened. Hemagglutination activity performed in 96 well microtitre plate with 3% rabbit blood in PBS (pH-7.2), all the 50 seed types gave agglutination activity. Human erythrocytes either of the blood group A,B, AB and O nor rabbit erythrocytes, did not show any agglutinating activity at any of the concentrations that were tested. Only the trypsin treated rabbit erythrocytes were able to show positive agglutinating activity. This is evident by the formation of carpet layer on the bottom of a microtitre plate wells. The reciprocal of dilution is calculated as titer value, which reflects lectin activity. Higher the titer value the higher is the lectin activity. On the other hand, absence of lectin marked a distinctive red button on the bottom of microtitre plate well. Considerable variation was shown in protein composition of the different seed extracts of chickpea. Protein content varied lowest of 1.6 mg/ml in IPC-12-99 to highest of 6.3 mg/ml in JG-379.

In desi chickpea seeds, the highest hemagglutination and specific activity was observed in accession JG-379 (4096 HU) having specific activity of 648.10 HU/mg. (protein conc. 6.32 mg/ml) in figure 1. On the other hand, lowest activity was found in IPC-12-99 exhibiting 128 HU and 78.0 HU/mg hemagglutination and specific activity in respectively (Protein conc. 1.64 mg/ml). In kabuli chickpea seeds, the highest hemagglutination and specific activity was observed as 1024 HU and 351.8 HU/mg in IPCK-12-284 respectively (protein conc. 2.91 mg/ml). While lowest activity was found as 1.06 HU and 2 HU/mg in ICC-12-291 respectively (protein conc. 1.87mg/ml). In wild chickpea, (ILWC-292) the highest hemagglutination activity was set up as 1024 HU and 351.8 HU/mg in respectively (protein conc. 2.91 mg/ml). While the lowest activity was found in ICC-17186 as 512 HU and 174.7 HU/mg respectively (protein conc. 2.93 mg/ml) in figure 2. A significant variation was found in the units of hemagglutination where in the minimum of 2 units were detected in IPCK-12-291 cultivar and the maximum 4096 units were observed in JG-379 (Table 1).

Inhibition of agglutination was studied by using various monosaccharides, disaccharides, oligosaccharides, polysaccharides and glycoproteins. None of the sugars like glucose, mannose, galactose, their derivatives, disaccharides, disaccharides and tetra saccharides inhibited the hem-agglutinating activity (Table 2). Only desialated fetuin at a concentration of 100 ug/ml inhibited the hemagglutination indicating complex nature of the lectin.

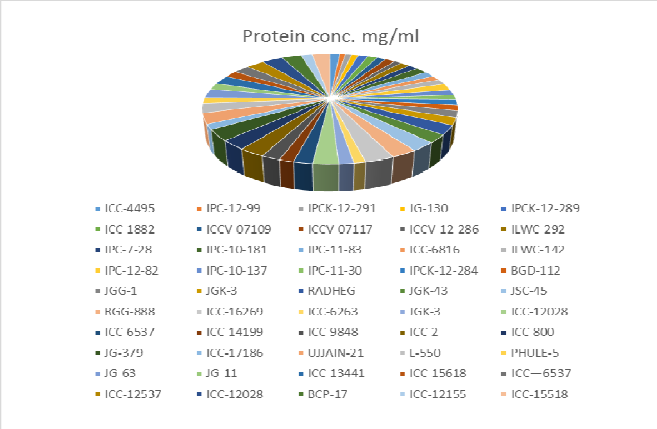

Figure 1: Distribution of protein content in different seeds of chickpea

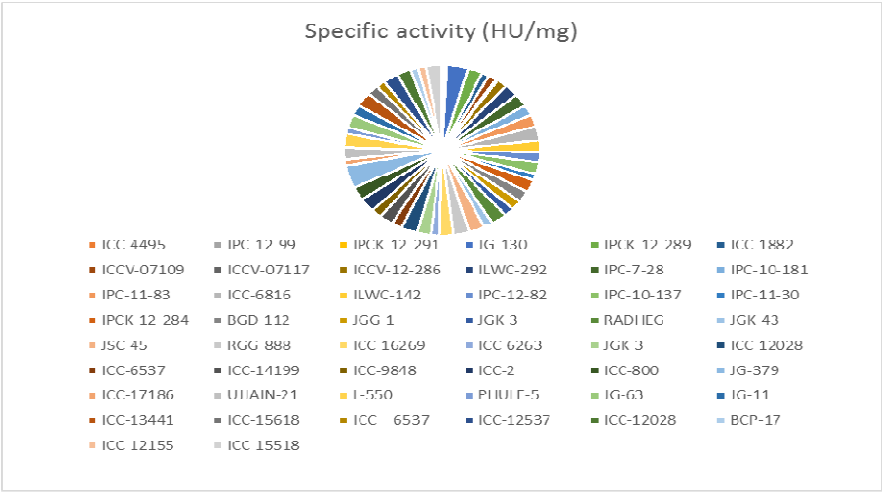

Figure 2: Representation of specific activity for lectin

Table 2. Carbohydrate inhibition assay

S.N.	Sugar type	Inhibition
1	Galactose	NI
2	Glucose	NI
3	Mannose	NI
4	L-fucose	NI
5	D-fucose	NI
6	Xylose	NI
7	Rhamnose	NI
8	Galactosamine	NI
9	Glucosamine	NI
10	Mannosamine	NI
11	GalNAc	NI
12	GlcNAc	NI
13	Man Nac	NI
14	Sialic acid	NI
15	Fibrinogen	NI
16	Desialated Fibrinogen	NI
17	Feutin	NI
18	Desialated Feutin	100 ug
19	Ovalbumin	NI
20	RNAse	NI

4. DISCUSSION

The lectin activity greatly varies between the seeds that were investigated, although they belong to closely related species. This is likely due to the different lectin composition in these seed types. The rationale behind this is different amounts of tertiary structures, hydrophobic and hydroxyl groups which are the parameters that may determine agglutination [11]. Desi seed of JG-379 has produced a positive reaction gave a maximum titer (648 Hu/mg) amongst all the genotypes that are tested. In present investigation, trypsinised rabbit blood gave significantly higher lectin activity compared to other blood groups that are tested. Specificity is influenced by the limited number of contacts with carbohydrates and depth of the sugar binding sites [11]. In addition, any modification or substitution to a binding site can influence binding specificity [12]. The observed differences in *Cicer* species lectin activity with different blood groups may be due to differences in carbohydrate-lectin binding interactions which can be attributed to differences in carbohydrates presented on the cell surfaces of the different blood groups [13].

The carbohydrates on the erythrocyte cellular surfaces are distinguishable among the four different blood groups [14]. There are reported monosaccharide determinants in the different blood groups, fucose in horse [15], galactose in human [16-18] and mannose in rabbit erythrocytes [19]. Horse, human and rabbit red blood cells may contain carbohydrate components on the cellular surface binding sites that are relatively less recognized by the *Q. fusiformis* lectin binding site. On the other hand, the carbohydrates found on the cellular surface of sheep red blood cells may contain carbohydrate units in a structure and position more specific and with higher affinity for the binding of *Q. fusiformis* lectin, subsequently increasing sheep erythrocyte agglutination. Moreover, lectin characteristics such as, multivalence may determine cross-linking interaction in binding recognition. Spatial distribution of multivalence among lectin structures may produce a higher level of specificity [20, 21].

Different erythrocytes react in a different way with lectin. Reports of chickpea producing a certain amount of agglutinating activity with cow erythrocytes are reported. Ruby *et al*. (2015) isolated and characterized lectin activity in Texas Live Oak (*Quercus fusiformis*). Quercus fusiformis lectin activity is similar to other plant lectins that are also non-blood group specific. *Erythrina speciosa* lectin was characterized as a non-blood group specific. Its lectin activity was examined in the human blood ABO system and animal blood groups, rabbit, mouse, sheep and horse [22]. Likewise, the blood group specificity for leaf lectin in *Kalanochoe crenata* was characterized as a non-blood group specific lectin, agglutinating to the different types of human blood red cells of the ABO system [23]. *Artocarpus incisa* seeds were also examined in a wide range of blood groups including human ABO system and animal blood groups, cow, goat, rabbit, pig and sheep. *Artocarpus incisa* seed lectin resulted in non-blood group specificity in humans ABO system, while rabbit blood group activity was not different from human ABO and the other four blood groups were significantly different from human and rabbit agglutination [21].

In our previous study, lectin from desi chickpea (*Cicer arietinum* L.) cultivar BDN 9-3 was purified and crystalized. *Cicer arietinum* L. lectin i;e CAL possessed complex-sugar specificity. The molecular weight of the native protein as determined by gel filtration using HPLC is 43 000 Da. It has been identified as a homodimer of subunit molecular weight 21 500 Da by SDS-PAGE both in the presence and in the absence of B-mercaptoethanol. The lectin was basic in nature (pI 9.0) and is a glycoprotein containing 4.5% neutral sugars. Neither human (blood groups A, B and O) nor rabbit erythrocytes are agglutinated by this lectin; however, pronase-treated versions of both have been successfully agglutinated. None of the sugars, such as glucose, mannose or galactose, nor their derivatives, disaccharides, trisaccharides and tetra saccharides has any effect on the agglutination activity of this lectin. The evidence for the complex specificity of CAL comes from the observation that the haem agglutination activity of 1mg lectin inhibited using about 10ug desialated fetuin. The present study also reports the non-blood group specificity of the *C. arietinum* lectin and in line with earlier reports. At present, our aim is to purify and characterize the lectin from wild *Cicer* species which is not reported so far. Based on this screening data, further studies vis-à-vis purification, characterization and pharmacological studies in animal model from wild seeds are in progress.

5. ACKNOWLEDGEMENT

Authors are thankful to University Grant Commission (UGC), New Delhi for the financial support. Mr. Ajay Kumar Gautam is highly indebted to UGC- New Delhi for providing research fellowship. Thanks are also due to Director, Defense Research Development and Establishment (DRDE) Gwalior and Mr. P.C. Jatav, Technical officer-D for providing rabbit blood.

REFERENCES

1.Boyd WC., Shapleigh E. Specific precipitation activity of plant agglutinins (lectins). Science. 1954, 269: 9429-9435.

2.Van Damme EJ, Peumans WJ, Barre A, Rougé P “Plant lectins: a composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles” Crit Rev Plant Sci, 1998, 17: 645-662.

3.Agrawal P, Kumar S, Jaiswal YK, Das HR, Das RH “A Mesorhizobium lipopolysaccharide (LPS) specific lectin (CRL) from the roots of nodulating host plant, Cicer arietinum” Biochimie, 2011, 93: 440-449.

4.Lam SK, Ng TB “Lectins: production and practical applications” Appl Microbiol Biotechnol, 2011, 89:45–55.

5.Katre UV, Gaikwad SM, Bhagyawant SS, Deshpande UD et al. “Crystallization and preliminary X-ray characterization of a lectin from Cicer arietinum (chickpea)” Acta Crystallographica Section F, 2005 61:141-143.

6.Bhagyawant SS, Srivastava N “Assessment of antinutritional factors and protein content in the seeds of chickpea cultivars and their divergence” Journal of Cell and Tissue Research, 2008**,** 8:1333-1338**.**

7.Gurjar MM, Khan MI, Gaikwad SM “alpha-Galactoside binding lectin from Artocarpus hirsuta: characterization of the sugar specificity and binding site” Biochem. Biophys.Acta, 1998, 1381: 256-264.

8.Lis H, Sharon N “Erythrina lectins” Methods in Enzymol., 1987, 138:544-551.

9.Sharon N, Lis H “Lectins as cell recognition molecules” Science, 1989, 246(4927): 227-234.

10.Lowry OH, Rosebrough NJ, Farr AL, Randall RJ “Protein measurement with the Folin phenol reagent” J. Biol. Chem., 1951, 193: 265-275.

11.Drickamer K, “Multiplicity of Lectin-Carbohydrate Interactions” Nature Structural Biology, 1995, 2:437-439.

12. Drickamer K, Taylor E “Glycan Arrays for Functional Glycomics” Genome Biology, 2002, 3, Reviews1034.1- Reviews1034.4.

13.Ynalvez RA, Cruz CG, Ynalvez MA “Isolation, Partial Purification and Characterization of Texas Live Oak (Quercus fusiformis) Lectin” Advances in Bioscience and Biotechnology, 2015, 6:470-484.

14.Khan MMH, Rahman ATM, et al “Purification and Characterization of Lectins from Jute (Chorchorus olitorius) Leaves” Journal of the Chinese Chemical Society, 2008, 55: 1171-1177.

15.Wu AM, Lisowska E, Duk M and Yang Z “ Lectins as Tools in Glycoconjugate Research” Glycoconjugate Journal, 2009, 26: 899-913.

16.Kusui K and Takasaki S “Structural Study of N-Linked Sugar Chains of Sheep Erythrocyte Membrane Glycoproteins” Glycoconjugate Journal, 1998, 15: 3-10.

17.Shibuya N, Goldstein IJ, Van Damme EJM and Peumans WJ “Binding Properties of a Mannose-Specific Lectin from the Snowdrop (Galanthus nivalis) Bulb” Journal of Biological Chemistry, 1988, 263: 728-734.

18.Yaga F, Iwaya T, Haraguchi T, et al. “The Lectin from Leaves of Japanese Cycad, Cycas revoluta Thunb. (Gymnosperm) Is a Member of the Jacalin-Related Family?” European Journal of Biochemistry, 2002, 269: 4335-4341.

19. Loris R “Principles of Structures of Animal and Plant Lectins” Biochimica et Biophysica Acta, 2002, 1572: 198-208. Sacchettini JC, Baum LG, Brewer CF “Multivalent Protein-Carbohydrate Interactions. A New Paradigm for Supermolecular Assembly and Signal Transduction” Biochemistry, 2001, 40: 3009-3015.

20.Konozy EHE, Mulay R, Faca V, et al “Purification, Some Properties of a D-Galactose-Binding Leaf Lectin from *Erythrina indica* and Further Characterization of Seed Lectin” *Biochimie*, 2002, 84:1035-1043.

21.Moreira RA, Castelo-Branco CC, Monteiro ACO, et al. “Isolation and Partial Characterization of a Lectin from *Artocarpus incisa* L. Seeds” Phytochemistry, 1998, 47:1183-1188.

22.Adenike K, Eretan OB “Purification and Partial Characterization of a Lectin from the Fresh Leaves of Kalanchoe crenata (Andr.) Haw” Journal of Biochemistry and Molecular Biology, 2004, 37:229-233.