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EXTRACTION AND CHARACTERIZATION OF LECTINS FROM DIFFERENT LEGUMES

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Abstract- Lectin, the carbohydrate-binding proteins have been isolated from different types of Legumes (*Phaseolus lunatus*, *Phaseolus vulgaris*, *Phaseolus aureus (Vigna radiata)*, *Glycine max* and *Pisum sativum*). Purified Lectins were tested for different biological activities. The Lectins were of C-type. Phytohemagglutinin (PHA) activities were tested and the Lectins from *Phaseolus vulgaris* were found to have the maximum activity. Other biological activities like thermal stability, insecticidal activities and genotoxic effects including agglutination was compared against commercial PHA (gibco). Domination of mitogenic activities similar to the commercial product proved various activities of lectins. The detailed study broadened the idea of the Lectins having different beneficial activities of great biological values.

***Keywords*: Lectins, legumes, Phytohemagglutinin (PHA), thermal stability, insecticidal activity, genotoxicity.**

I. INTRODUCTION

Lectins are widely distributed carbohydrate-binding proteins, present in microorganisms, viruses, animals and higher plants. It has highly variable amino acid sequences with different structures, tissue localization and functions.[1] According to Vandamme et al.1998 plant lectins were classified into seven families; legume lectins family is one among them.[2] The literature review revealed that legume lectin is the largest and thoroughly studied family for this particular protein which helped legumes to occupy a special position among others. They exhibited remarkable conservation in their primary, secondary and tertiary protein structure but varied in quaternary structure, [3,4] which led to diverse physiological roles including mitogenic, anti-tumor, anti-viral, insecticidal, antifungal activities. Lectins recognize carbohydrates from the cell membrane forming variable glyco-conjugates, which has been used as a molecular tool to study drug delivery and targeting.[5-10].

Generally, plant lectins have been isolated from different parts of plant like flowers, leaves, seeds, roots, rhizomes, barks etc. Phytohemagglutinin (PHA) is a specific type of lectin found in plants especially in beans, which is broadly classified as legume lectin. Legume lectins are the largest family of carbohydrate binding proteins. It is further categorized into two subdivisions on the basis of distinguishing property of subunits like indistinguishable or almost indistinguishable subunits. In the Protein Data Bank, the legume lectins were also classified into two groups as ligand free or apo group and sugar bound or holo group, which have more than forty different reported varieties. Amino acid sequencing of plant lectins shows sequence homology among wild type legume lectins with domesticated form which helps to confer evolutionary relationship and provide information about the evolution of carbohydrate binding ligads [11-13].

In the present study lectin has been isolated from five commonly used beans in Indian cushine namely*Phaseolus vulgaris*, *Phaseolus lunatus*, *Vigna radiata*, *Glycine max*, *Pisum sativum* and characterized for their different biological properties such as Mitogenic activity, Hemagglutination assay, Larvicidal activity and genotoxic effect. All the selected plants belong to Fabaceae family. The study revealed that the source of lectin is wide in several legumes and their function may be beyond the mitogen in artificial cellular culture, as it has larvicidal as well as genotoxic property. The in depth study of legume lectins shall widen the spectrum of biological activities of this phyto-constituent in near future.

2. MATERIALS AND METHOD

2.1 Collection of samples –

Phaseolus lunatus, *Phaseolus vulgaris*, *Phaseolus aureus (Vigna radiata)*, *Glycine max*and *Pisum sativum* were collected from local markets of Kolkata and properly identified.

2.2 Chemicals used –

Commercial PHA (gibco), all other chemicals and reagents used were of analytical grade.

2.3 Extraction of Lectin –

About 20g of each type of beans was washed thoroughly and soaked in distilled water overnight at 37±0.50 C. The excess water was then removed and beans were blended in 30ml of 0.85% NaCl solution to obtain a fine pulp. Further 70ml NaCl was added to the pulp and leftovernight at 4˚C. The pulp was then spinned down at 5,000rpm for an hour and the collected supernatant was further centrifuged at 5,000rpm for one more hour. This finally collected supernatant was filtered through Whatman filter no. 1(Sigma-Aldrich) and stored at 40 C in sterile aliquots for further usage.

2.4Protein Estimation –

The protein content of extracted lectins was estimated by Lowry method using Bovine Serum Albumin (Lowry et al., 1951)[14]

2.5Mitogenic Activity –

Human leukocyte culture media was established and 0.1ml of extracted lectin was added to it as mitogen. Commercial PHA (gibco) was taken as control. Mitotic index of all the cultures were studied.

2.6 Hemagglutination Assay –

Serial two-fold dilutions of the lectin solution in microtiter v-plates (25 µL) was mixed with 25 µL 2% Human peripheral red blood cell suspension in saline (pH 7.2). Readings were recorded after about 30 minutes at room temperature, when the blank had fully sedimented. The hemagglutination titer, defined as the reciprocal of the highest dilution exhibiting hemagglutination, was treated as one hemagglutination unit.

2.7 Thermal Stability

1ml of extracted lectin was diluted to 1:10 ratio and incubated at 200C for 15 minutes. With every 100 C increase in temperature hemagglutination assay was done till it reaches 1000 C. The Themagglutination activity was measured by the method adopted in the previously mentioned hemagglutination activity section.

2.8 Insect Growth Retardation Activity –

1ml of extracted lectin at three different concentrations (Crude Extract, 1:5 dilution, 1.10 dilution) was mixed with normal food of *Drosophila melanogaster* and then flies were cultured in it, thereafter the growth of larvae was studied and the hatching time of eggs, expansion of first, second and third instar stages, pupation time and finally emergence of adult flies observed in comparison with positive and negative control. For the confirmation of the experimental result perform the experiment in triplicate.

2.9 Larvicidal Activity

1ml of extracted lectin at three different concentrations (Crude Extract, 1:5 dilution, 1.10 dilution) was taken in watch-glass and five healthy *Drosophila melanogaster* third instar larvae were released in each of the experimental unit. Observe the activity of the larvae in an one hour internal for 12 hours. For the confirmation of the experimental result perform the experiment in triplicate. .

2.10Genotoxic Effect –

1ml of extracted lectin at three different concentrations was mixed with normal food of *Drosophila melanogaster* and then flies were cultured in it. Salivary glands were dissected out from well fed third instar larvae and polytene chromosome preparation was done as conventional orcein staining method. For the confirmation of the experimental result perform the experiment in triplicate.

3. RESULTS

3.1 Protein Estimation

The protein content of extracted lectins was estimated by Lowry method using Bovine Serum Albumin and the result depicted in **Table: 1.** *P. vulgaris* shows the highest amount of proteins and *P. lunatus* shows the lowest amount of proteins.

Table 1: Estimation of protein in lectins extracted from five different plants.

Samples	**Optical Density (OD)**	
	at 1:5 dilution	**at 1:10 dilution**
Control(Commercial PHA)	2.13	1.39
P. vulgaris	2.00	1.30
P. lunatus	1.16	0.66
V. radiata	1.48	0.82
G. max	1.98	1.25
P. sativum	1.31	0.89

3.2 Mitogenic Activity –

Metaphases of each field was observed under microscope at 10x and then confirmed at 40x and 100x respectively. In the present study, lectins extracted from *P. vulgaris* shows the highest property of mitogenic activity followed by *P. sativum, P. lunatus, V. radiate* and *G. max* shows the lowest mitogenic property. The result shows in **Table: 2**.

Table 2: Detection of mitogenic activity in lectins extracted from five different plants

Samples	**Mitogenic Activity**
Control (Commercial PHA)	6.30±0.02
P. vulgaris	5.80±0.11
P. lunatus	5.19±0.09
V. radiata	5.00±0.03
G. max	4.80±0.01
P. sativum	5.60±0.27
± = Mean SD, N=3	

3.3 Hemagglutination Assay

The RBCs clotted immediately when concentrated extract of lectins was added, but when diluted to 1:5 and 1:10.ratio the agglutination time was found to be less than the concentrated one. However, agglutination time for lectin extracted from *P. vulgaris* and commercial PHA are showing almost similar activity. The properties of Hemagglutination gradually decreases respectively as *G. max, V. radiate, P. sativum* and Lastly *P. lunatus.*

3.4 Thermal Stability –

The hemagglutination activity declined after incubation above 500 C. When temperature was raised above 600 C the activity reduced to 50%; and finally when temperature was above 800 C no hemagglutination activity was shown by any of the samples. The reseut of Thermal stability has been shown in **Table-3**.

Table 3: Observation of thermal stability in lectins extracted from five different plants

Samples	**Temperature's effect on hemagglutination activity**								
	20˚C	30˚C	40˚C	50˚C	60˚C	70˚C	80˚C	90˚C	100˚C
Control (Commercial PHA)	N	N	N	N	D	D	D	NAF	NAF
P. vulgaris	N	N	N	N	D	D	D	NAF	NAF
P. lunatus	N	N	N	N	D	D	D	NAF	NAF
V. radiata	N	N	N	N	D	D	D	NAF	NAF
G. max	N	N	N	N	N	D	D	NAF	NAF
P. sativum	N	N	N	N	D	D	D	NAF	NAF
N= Normal activity; D= Decreasing activity; NAF= No activity found									

3.5 Insect Growth Retardation Activity

The extracted lectins has been shown concentration dependent inhibition of larval growth and significant mortality. Most effective larvicidal activity was shown by extracted lectins from *P. vulgaris, P. sativum* and *P. lunatus*. The insect growth retardation activity of other lectins has been depicted in **Table: 4.**

Table 4: Observation of the insect growth retardation activity in lectins extracted from five different plants.

Samples	**Concentrations**		
	Crude extract	**1:5 dilution**	**1:10 dilution**
Control (Commercial PHA)	+++	+++	+++
P. vulgaris	+++	+++	++
P. lunatus	+++	++	+
V. radiata	++	+	--
G. max	++	+	--
P. sativum	+++	++	+
+++ = Extremely retarded; ++ = Medium; + = Almost normal:			

3.6 Larvicidal Activity –

The extracted lectins has been shown concentration dependent inhibition of larval growth and significant mortality. Most effective larvicidal activity was shown by extracted lectins from *P. vulgaris*, *P. sativum* and *P. lunatus*. The larvicidal activity of other lectins has been depicted in **Table: 5**.

Table 5: Observation of the larvicidal activity in lectins extracted from five different plants

Samples	**Concentrations**		
	Crude extract	**1:5 dilution**	**1:10 dilution**
Control (Commercial PHA)	+++	+++	+++
P. vulgaris	+++	++	++
P. lunatus	++	++	+
V. radiata	++	++	+
G. max	++	++	+
P. sativum	+++	++	++
+++ = High; ++ = Moderate; + = Few			

3.7 Genotoxic Effect –

The crude extracted lectins have been reported to exert genotoxic effect when directly exposed to larvae. The exposed larvae shows ectopic pairing and unusual puffing when exposed for 3 hours at 22±10 C, but when exposed for 1 hour, only unusual puffing was observed. The negative control does not show any type of genotoxic effect. The positive control and extracted Lectins in different experimental groups in different concentration shows observable genotoxicity. The experimental result clearly depicted in **Table: 6.**

Table 6: Observation of the larvicidal activity in lectins extracted from five different plants

Samples	**Concentrations**					
	Crude extract		**1:5 dilution**		**1:10 dilution**	
	1Hr	**3Hr**	**1Hr**	**3Hr**	**1Hr**	**3Hr**
(+)Ve Control (Commercial PHA)	UP + EP	UP + EP	UP + EP	UP + EP	UP	UP + EP
P. vulgaris	UP + EP	UP + EP	UP + EP	UP + EP	UP	UP + EP
P. lunatus	UP	UP + EP	UP	UP + EP	UP	UP
V. radiata	UP	UP + EP	---	UP + EP	---	UP
G. max	UP + EP	UP + EP	UP + EP	UP + EP	---	UP
P. sativum	UP + EP	UP + EP	UP + EP	UP + EP	UP	UP + EP
(-)Ve Control	---	---	---	---	---	---
UP= Unusual Puffing ; EP= Ectopic Pairing						

4. DISCUSSION

Plant tissues contain several proteins which have different biological functions. Proteins like lectins are sugar binding proteins that are highly specific for their sugar moieties and they play a key role in biological recognition phenomena involving cells and proteins. Sometimes this is used by some viruses for attachment of the cells to the host organism during infection with themselves. Mostly lectin are non-enzymatic and non-immunogenic in action, and occur ubiquitously in nature and binds to the soluble carbohydrate or carbohydrate moiety as a part of glycoprotein or glycolipid, thereby changing the physiology of the membrane, causing agglutination, mitosis and other biochemical changes in the cell. They typically agglutinate certain animal cells or precipitate as a glycol-conjugate without altering the covalent structure of any of the recognized glycosyl ligands [15-17].

According to carbohydrate specificity lectins are categorized as fructose, mannose, n-acetylglucosamine, n-acetylgalactosamine and glycan complex. Selectivity of binding depends upon bonds like hydrogen bridges, vanderwaals and hydrophobic interactions between sugar and lectin site. The compact globular structure, molecular aggression and glycosylation help in structural stability of lectins. In some plant having multiple molecular forms of lectins with different electrophoretic mobility is a frequent phenomenon within species, such lectins are called isolectins [18-19].

Hemagglutination activity is the most common assay for the detection of lectin from a sample as it ensures easy visualization of agglutination. Hemagglutinin activity is defined as the reciprocal of the highest dilution of isolated sample, promoting full agglutination of erythrocytes, by binding lectins to the erythrocyte surface carbohydrates. This assay also helps to access the stability of lectin to different pH and temperature values which can determine the condition of biotechnological applications of lectins. Additionally the assay may determine the lectin-carbohydrate specificity as conjugated carbohydrate, more effectively inhibiting the hemagglutination activity [20].

Mitogenic activity has been identified in many leguminous species, especially in beans. Phytohemagglutinin (PHA) is a kind of lectin extracted from *P. vulgaris* which is the main source of mitogen used *in-vitro* cell proliferation experiments [21-22]. The concentration of PHA used in cell proliferation assay differs according to the cultivar and breeding location. In our present study lectin extracted from *P. vulgaris* exhibited maximum percentage of mitogenic activity which is almost similar to that of the commercial PHA among five used legume lectins. The percentage of mitogenic activity gradually decreases from P. vulgaris, P. lunatus, V. radiata, P. sativum, G. max.

Legume lectins have a wide range of toxicity in insects and exert harmful effect in any developmental stage of their life cycle including larval and adult stage. The literature review revealed that the insecticidal effect of legume lectins observed in different order like Coleoptera, Diptera, Lepidoptera, Hymenoptera, Isoptera, Neuroptera and Homoptera under class insect [23-24]. It also effects the emergence of adult from pupa and the fecundity of the female flies. Generally lectins exhibit insecticidal effect by binding with brush border microvilli of epithelial cells and peritrophic membrane, peritrophic gell of food bolus leading

change of charge and dimemsion of pore of peritrophic membrane. On the other hand insect without peritrophic membrane, it directly interact with glycoconjugates of epithelial membrane of insect gut. Insect gut contains varied range of glycoconjugats for their specialized functions which provide several binding site of lectins for there larvicidal activity. Lectin prohibited the degradation of gut enzyme by persisting for a long time within the gut as it posses wide range of pH stability. Whereas, in intracellular space, it also bind with several new targets leading interruption of many physiological pathways. In the present study, the extracted lectins also have the observable insect growth retardation activity and larvicidal activity. Here also *P. vulgaris* exhibited maximum percentage of mitogenic activity which is almost similar to that of the commercial PHA among five used legume lectins. The other legumes also exhibited potential growth retardation and larvicidal property as comparable to *P. vulgaris.*[25-27].

Genotoxicity is a destructive effect on a cellular genetic material affecting its integrity. As lectin binds with carbohydrates present in the cellular membrane in a variable way, it may alter the pathway of cellular gene interaction or membrane bound second messenger system. After binding with cellular ligands, lectins promote uncontrolled cellular replication and erroneous DNA repair mechanism resulting loss of genetic integrity which may the pioneer cause of genotoxic property of lectin. The exact molecular mechanism of genotoxic interaction is under study [28]. Potential genotoxic activity has been observed in our current study and here also lectin extracted from *P. vulgaris* leeds the others.

5. CONCLUSION

Legume lectin is very essential part of *in-vitro* cellular culture for its mitogenic activity. It has great importance for its hemaglutinin activity and sugar binding property. On The basis of protein- carbohydrate interactions with variable specificities, legume lectin could be used as a potential candidate for lectin mediated drug delivery on target specific cells which leads promising role in the treatment of cancer and disease related to microbial infections. The insecticidal property and insect growth retardation property of lectins in wide range of insects could have great prospects in sustainable insect pest management, agriculture and economic crop production in future. More research and in depth study is necessary to understand the molecular features of lectin, its effect in gene modulation and protein interaction for its clinical as well as various other applications.

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