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Identification and Characterization of Indigenous Dietary Plant Lectins

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Abstract: ***Introduction: Lectins are sugar binding proteins of non-immune origin which agglutinate red blood cells. The objectives of the present study were to identify and characterize some indigenous dietary plant lectins which have haemagglutination property with human erythrocytes of varied types. Materials and Methods: Twenty four different dietary seeds of leguminosae family were selected, in which fourteen seeds exhibited agglutination activity against any one of the blood groups of human population tested at different level of dilution in the serology laboratory of the department of Physiotherapy, Guru Nanak Dev University, Amritsar, Punjab, India. Results: The haemagglutination reactions showed weak agglutination of the lectins Celastrus paniculatus, Zea mays and Hordeum vulgare with A blood group, no agglutination of the lectins Dolichous biflorus, Triticum aestivum and Sesamum indicum, weak agglutination of the lectins Celastrus paniculatus and Zea mays with blood group B, weak agglutination of the lectin Lens culinaris with the blood group AB and no agglutination of the lectins Lens culinaris and Dolichous biflorus with the blood O. Conclusion: It may be concluded from the findings of the present study that three lectins agglutinated weakly with A blood group, two with B blood group, one with AB blood group, whereas no agglutination was recorded in three lectins with B blood group and two lectins with the O blood group. Thus, lectins can be used for blood groups typing and detection of secretor status.***

Keywords: Indigenous dietary plant lectins. Haemagglutination. Human ABO blood types

1. Introduction

1) Interest in haemagglutination by plant extracts started about a century ago. As early as in 1888, Stillmark noted that saline extracts of the castor bean, *Ricinus communis*, had the property of agglutinating animal red cells. He called the agglutinating factor 'Ricin'. Later, in 1891, Hellin discovered a similar phytoagglutinin in the seed extracts of *Abrus precatorius* and named it 'Abrin'. Due to their toxicity, they were collectively called 'Phytotoxins'. Weinhaus (1909) introduced a new term 'phasin' for non-toxic plant agglutinins. The term 'Lectin' which is derived from the Latin word 'Legere' meaning 'to pick out' or 'to choose' or 'to select' was introduced by Boyd and Shapleigh (1954). Uhlenbruck and Krupe (1963) coined the term 'phytohaemagglutinins' for 'extractable globin like substances of vegetable origin'. Gold and Phelps (1972) discussed the use of term 'antibody like substances' and concluded that the similarity of lectins to antibodies was rather superficial. Later, Gold and Balding (1975), considering their functional approach, called these substances as receptor-specific proteins'.

2) Goldstein et al. (1980) defined lectin as a sugar binding protein of non-immune origin which agglutinates red blood cells and/or precipitates glycoconjugates. King and Stansfield (1985) proposed lectins as proteins capable of agglutinating certain cells, especially erythrocytes, by binding to specific carbohydrate receptors on the surface of the cells. Lectin research is finding much application today in many areas including serology, cell biology, microbiology and immunochemistry. Lectin-studies are scanty in Indian indigenous plants. Thus, the present study was planned with the objectives to identify and characterize the indigenous dietary plant lectins of Punjab.

2. Materials and Methods

1) Selection of Lectins

Twenty four different dietary seeds of leguminosae family were selected, in which fourteen seeds, namely, *Vigna unguiculata*, *Vigna radiata*, *Lens culinaris*, *Glycine max*, *Pisum sativum*, *Phaseolus vulgaris*, *Celastrus paniculatus*, *Dolichous biflorus*, *Triticum aestivum*, *Zea mays*, *Hordeum vulgare*, *Sesamum indicum*, *Oryza sativa* and *Abrus pectorius* exhibited agglutination activity against any one of the blood groups of human population tested at different level of dilution in the serology laboratory of the department of Physiotherapy, Guru Nanak Dev University, Amritsar, Punjab, India.

2) Preparation of Plant Lectins

In the preparation of the lectin, all essential steps were the same as described by Dunsford and Bowley (1967). The seeds were ground to a fine powder and mixed with normal saline in the ratio of 1:9. The mixture was then allowed to stand at ambient temperature for four hours, with occasional stirring. After this period the slurry was centrifuged at 3000-4000 rpm. for 30 minutes. The clear supernatant was subsequently separated and stored under refrigeration with sodium azide added to it, in the ratio of 1:10,000 parts, as preservative.

3) Collection and Processing of Human Blood

To characterize the lectin, hemagglutination assay was performed by using human erythrocyte suspension (A^+, B^+, AB^+ and O^+). Normal blood samples were collected, using the finger-prick technique. All blood samples were washed thrice in physiological saline and re-suspended at a concentration of 2% in normal saline. For ABO typing, standard serological procedure were followed.

4) Hemagglutination technique

The frozen seed extracts were thawed at room temperature just before the beginning of the experiments. Blood grouping slides with 12 cavities were used for the hemagglutination tests, 0.25µl red blood was added to an equal amount of seed extract. After 20-25 minutes results were recorded.

3. Results

1) Table 1 showed the reaction patterns of different lectins with blood group A. The results exhibited that with human A blood group, the lectin *Abrus pectorius* showed the hemagglutination reaction with the strength (+++), the lectin *Pisum sativum* with the strength (++), *Vigna unguiculata, Vigna radiata, Oryza sativa* with the strength (+) upto 1:128 dilution, while *Phaseolus vulgaris* and *Dolichos biflorus* showed hemagglutination reaction with the strength (+) upto 1:64 dilution, and *Glycine max* and *Triticum aestivum* showed reaction with the strength (+) upto 1:32 dilution, and *Lens culinaris* showed the hemagglutination reaction with the strength (+) upto 1:8 dilution, while *Celastrus paniculatus, Zea mays* and *Hordeum vulgare* showed the reaction with the strength (+) upto 1:2 dilution.

2) Table 2 showed that with human B blood group, the lectin *Abrus pectorius* showed the hemagglutination reaction with the maximum strength (+++), the lectins *Vigna unguiculata, Vigna radiata, Pisum sativum* and *Phaseolus vulgaris* with the strength (+) upto 1:128 dilution, *Glycine max* and *Hordeum vulgare* with the strength (+) upto 1:32 dilution and lectin *Oryza sativas* with the strength (+) upto 1:8 dilution, while *Celastrus paniculatus* and *Zea mays* showed the hemagglutination reaction with the strength (+) upto 1:4 dilution.

Table 1: Reaction patterns of different lectins with blood group A

Lectins	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Vigna unguiculata	+++	+++	+++	++	++	+	+	+
Vigna radiata	+++	+++	+++	+++	+++	++	++	+
Lens culinaris	++	++	++	+	—	—	—	—
Glycine max	+++	+++	+++	++	++	+	—	—
Pisum sativum	+++	+++	+++	+++	+++	+++	+++	++
Phaseolus vulgaris	+++	+++	+++	++	++	+	+	—
Celastrus paniculatus	+	+	—	—	—	—	—	—
Dolichous biflorus	+++	+++	+++	+++	++	+	+	—
Triticum aestivum	+	+	+	+	+	+	—	—
Zea mays	+	+	—	—	—	—	—	—
Hordeum vulgare	+	+	—	—	—	—	—	—
Oryza sativa	++	++	+	+	+	+	+	+
Abrus pectorius	+++	+++	+++	+++	+++	+++	+++	+++

3) Table 3 showed that with human AB blood group, the lectin *Abrus pectorius* showed the hemagglutination reaction with the maximum strength (+++), *Pisum sativum* showed the hemagglutination reaction with the strength (++) and the lectins, *Vigna radiate* and *Phaseolus vulgaris* showed the hemagglutination reaction with the strength (+) upto 1:128 dilution, while *Triticum aestivum, Hordeum vulgare* and *Oryza sativa* showed the hemagglutination reaction with the strength (+) upto 1:64 dilution. *Vigna unguiculata, Glycine max, Celastrus paniculatus, Dolichos biflorus* and *Sesamum indicum* showed hemagglutination reaction with the strength (+) upto 1:32 dilution, *Zea mays* with the strength (+) upto 1:16 dilution, while *Lens culinaris* with the strength (+) upto 1:2 dilution.

Table 2: Reaction patterns of different lectins with blood group B

Lectins	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Vigna unguiculata	+++	+++	++	++	+	+	+	+
Vigna radiata	+++	+++	+++	+++	++	++	+	+
Lens culinaris	—	—	—	—	—	—	—	—
Glycine max	+++	+++	+++	+	+	+	—	—
Pisum sativum	+++	+++	+++	+++	++	++	+	+
Phaseolus vulgaris	+++	+++	+++	+++	+++	++	++	+
Celastrus paniculatus	++	+	+	—	—	—	—	—
Dolichous biflorus	—	—	—	—	—	—	—	—
Triticum aestivum	—	—	—	—	—	—	—	—
Zea mays	++	+	+	—	—	—	—	—
Hordeum vulgare	++	++	++	++	+	+	—	—
Sesamum indicum	—	—	—	—	—	—	—	—
Oryza sativa	++	++	+	+	—	—	—	—
Abrus pectorius	+++	+++	+++	+++	+++	+++	+++	+++

4) Table 4 showed that with human O blood group, the lectin *Abrus pectorius* showed the hemagglutination reaction with the maximum strength (+++), *Vigna radiata ,Pisum sativum, Phaseolus vulgaris* and *Oryza sativa* with the strength (+) upto 1:128 dilution, while *Triticum aestivum, Celastrus paniculatus* and *Hordeum vulgare*, showed hemagglutination reaction with the strength (+) upto 1:64 dilution, *Vigna unguiculata, Glycine max* and *Sesamum indicum* showed hemagglutination reaction with the strength (+) upto 1:32 dilution, *Trachyspermum ammi* with the strength (+) upto 1:16 dilution, while *Zea mays* showed hemagglutination reaction with the strength (+) upto 1:8 dilution. No reaction was recorded with *Vigna aconitifolia, Cicer arietinum, Arachis hypogaea, Picrorhiza kurrooa, Cajanus cajan, Pennisetum glaucum* and *Brassica nigra* with ABO blood groups.

Table 3: Reaction patterns of different lectins with blood group AB

Lectins	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Vigna unguiculata	+++	++	++	+	+	+	—	—
Vigna radiata	+++	+++	+++	+++	+++	+++	++	+
Lens culinaris	++	+	—	—	—	—	—	—
Glycine max	+++	+++	+++	++	++	+	—	—
Pisum sativum	+++	+++	+++	+++	+++	+++	++	++
Phaseolus vulgaris	+++	+++	+++	+++	+++	+++	++	+
Celastrus paniculatus	+++	++	++	+	+	+	—	—
Dolichous biflorus	+++	+++	+++	+++	++	+	—	—
Triticum aestivum	+	+	+	+	+	+	+	—
Zea mays	+++	++	++	+	+	—	—	—
Hordeum vulgare	++	++	++	++	+	+	+	—
Sesamum indicum	+++	++	++	++	+	+	—	—
Oryza sativa	++	++	++	++	+	+	+	—
Abrus pectorius	+++	+++	+++	+++	+++	+++	+++	+++

5) Table 5 showed that with the effect of temperature on hemagglutination reaction of lectin showed that *Vigna unguiculata* reacted upto 40°C, *Pisum sativum, Celastrus paniculatus* and *Oryza sativa* reacted upto 50°C, *Vigna radiata, Glycine max, Zea mays, Hordeum vulgare, Phascolus vulgaris* and *Dolichous biflorus* reacted upto 70°C, *Arachis hypogaea* reacted upto 90°C, *Abrus pectorius* reacted upto 100°C, *Vigna aconitifolia, Cicer arietinum,*

Lens culinaris, Picrorhiza kurrooa, Cajanus cajan, Triticum aestivum, Sorghum bicolar, Pennisetum glaucum, Sesamum indicum, Brassica nigra and *Trachyspermum ammi* showed no reaction with temperature.

Table 4: Reaction patterns of different lectins with blood group O

Lectins	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Vigna unguiculata	+++	+++	++	++	+	+	—	—
Vigna radiata	+++	+++	+++	+++	+++	++	+	+
Lens culinaris	—	—	—	—	—	—	—	—
Glycine max	+++	+++	++	++	+	+	—	—
Pisum sativum	+++	+++	+++	+++	++	++	+	+
Phaseolus vulgaris	+++	+++	+++	+++	+++	++	++	+
Celastrus paniculatus	+++	+++	++	++	+	+	+	—
Dolichous biflorus	—	—	—	—	—	—	—	—
Triticum aestivum	++	++	++	++	++	++	++	—
Zea mays	+++	++	++	+	—	—	—	—
Hordeum vulgare	+++	+++	++	++	++	++	+	—
Sesamum indicum	+++	++	++	++	+	+	—	—
Oryza sativa	+++	+++	++	++	++	+	+	+
Abrus pectorius	+++	+++	+++	+++	+++	+++	+++	+++

4. Discussion

1) Lectins are agglutinins found in plants as well as in animals. They have an affinity for carbohydrates and they can agglunitate cells of various types. In the present study, the lectins from *Vigna unguiculata, Vigna radiata, Glycine max, Pisum sativum, Celastrus paniculatus, Phaseolus vulgaris, Zea mays, Hordeum vulgare, Oryza sativa, Abrus pectorius* showed the hemagglutination reaction with human A, B, AB, O blood groups. The lectin *Triticum aestivum* showed the hemagglutination reaction with human A, AB, O blood groups.Whereas, the lectin *Lens culinaris* and *Dolichous biflorus* showed the hemagglutination reaction only with human A, AB blood groups. The lectin *Sorghum bicolar* and *Trachyspermum ammi* showed hemagglutination reaction only with human O blood group.The lectin *Sesamum indicum* showed the hemagglutination reaction with human AB, O blood groups. The first blood group specific plant reagent was that from *Phaseolus limensis* which according to Boyd and Reguera (1949) agglutinated human red cells of type A only. Makela and Makela (1956) reported anti-B specific property in the seed extracts of *Bandeiraea simplicifolia.* An agglutinin giving reaction of anti-H was first reported by Renkonen (1948) in the seed extracts of Cytisus sessilifolius, Laburnum alpinum and Lotus tetragonolobus.

Table 5: Properties of lectins: Effect of temperature on haemagglutation

Name of lectins	30°C	40°C	50°C	60°C	70°C	80°C	90°C	100°C
Vigna unguiculata	+	+	—	—	—	—	—	—
Vigna radiate	+	+	+	+	—	—	—	—
Glycine max	+	+	+	+	—	—	—	—
Pisum sativum	+	+	+	+	—	—	—	—
Phaseolous vulgaris	+	+	+	+	+	—	—	—
Celastrus paniculatus	+	+	+	—	—	—	—	—
Dolichous biflorus	+	+	+	+	+	—	—	—
Zea mays	+	+	+	+	—	—	—	—
Hordeum vulgare	+	+	+	+	—	—	—	—
Oryza sativa	+	+	+	—	—	—	—	—
Abrus pectorius	+	+	+	+	+	+	+	+

2) Sharon (1987) illuminated on some important applications of lectins. Lectins can be used for blood groups typing and detection of secretor status. They may be a unique tool for identification and separation of cells, isolation of glycoprotein and glycopeptides, detection and identification of cell surface sugars and characterization of membrane organization. They may be applicable to investigate the structure of carbohydrates also useful in studies of protein-carbohydrate interactions. To serve mankind lectins are used in a wide range of applications now-a-days.

5. Conclusion

It may be concluded from the findings of the present study that three lectins agglutinated weakly with A blood group, two with B blood group, one with blood group AB, whereas no agglutination was recorded in three lectins with B blood group and two lectins with the blood group O. Thus, lectins can be used for blood groups typing and detection of secretor status. The studies on lectins are getting tremendous now-a-days for their beneficial effects on humankind.

References

[1] Boyd WC. And Reguera RM. Haemagglutinating substances for human cells in various plants. J.Immunol. 1949; 62: 333.
[2] Boyd WC, Shapleigh E. Specific precipitating activity of plant agglutinins (lectins). Science, 1954; 119: 419.
[3] Dunsford I. and Bowley CC. Techniques in Blood grouping Vol.II . (Oliver and Boyd Ltd. Edinburgh), 1967.
[4] Gold ER, P Balding. Receptor – specific proteins: Plant and animal lectins. American Elsevier: New York, 1975; pp. 151 – 236.
[5] Gold ER, CF Phelps. *Z. Immun. Forsch*, 1972; 143: 430.
[6] Goldstein IJ, RC Hughes, T Monsigny, Osawa, N Sharon. What should be called a lectin ? Nature, 1980; 285: 66.
[7] Hellin, H. Thesis, University of Dorpat Estonia. Cited from Sandhu, RS, Reen, RS: Phytolectins a review. In Annu. Rev. Plant. Sci., 1980; Vol. 2, (Ed.) CP Malik, Kalyani Publishers: New Delhi.
[8] King, RC, WD Stansfield. *A Dictionary of Genetics*, Oxford University Press: Londan, 1985.
[9] Makela O. AND Makela P. Some new blood group specific phytoagglutinins. Ann. Med. Eup. Biol. Fenn. 1956; 34: 402.
[10] Renkonen KO. Studies on haemagglutinins present in seeds of some representatives of the family of leguminosae. Ann. Med. Exp. Fenn. 1948; 26: 66.
[11] Sharon N. Lectins: An overview. In Vertebrate lectins (Eds.), Olden K. Parent JB. Van-Nostrand Reinhold Company Inc. New. York, 1987.
[12] Stillmark, H. 1888. Uber Ricin, ein giftiges Ferment aus den samen Von *Ricinus Communis* L. und einigen anderen Euphorbiaccen. Inaug. Dissert. Dorpat. Cited from Makela, O., 1957 : Studies in haemagglutinins in Leguminosae seeds. *Ph.D. Thesis, Helsinki*.
[13] Uhlenbruck, G, M Krupe. Weitere serologische Eigonschaften von mucoidenaus Ronderery throzytenstrome: Speciflosche Reakinafahigkeitmit

phytagglutinin *aus Solanum tubersum* and *Ricinus communis*. Sowie mit anti-pneumokokken Type. XIV. Pferdesserum. *Eschr. Immunit. Forsch.*, 1963; 125: 285.
*[14]*Weinhaus, O. Des phasins *Biochem. E*., 1909; 18: 228.