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Study of Edible Lectins in Patients with Type 2 Diabetes Mellitus

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Abstract: ***Introduction: Lectins with carbohydrate specificity have been purified from various plant tissues and other organisms. Objectives of the present study were to identify and characterize some indigenous edible lectins which have hemagglutination property with erythrocytes of various types in patients with type 2 diabetes mellitus. Materials and Methods : Thirteen different dietary seeds of leguminosae family were selected, in which ten seed extracts exhibited agglutination activity against any one of the blood groups of patients with diabetes mellitus tested at different level of dilution in the serology laboratory of the institute. Results: The hemagglutination reaction showed agglutination of the lectins Cicer arietinumm, Phaselous vulgaris, Lens culinaris, Helianthus annus, Abrus precatorius, Glycine max, Dolichus biflarus, Salvia hispanica and Brassica nigra with bolld group A. Lectin Nigella sativa showed no reaction with blood group A in patients with type 2 diabetic disease. The lectins Glycine max, Dolichus biflarus, Abrus precatorius, Brassica nigra, Nigella sativa, Helianthus annus and Phaselous vulgaris reacted with blood group B. Lectin Cicer arietinum, Lens culinaris and Salvia hispanica showed no reaction with blood group B in patients with type 2 diabetic disease. Lectins Helianthus annus, Nigella sativa, Phaselous vulgaris, Glycine max, Abrus precatorius, Dolichus biflarus and Brassica nigra reacted with AB blood group. Lectins Cicer arietinum, Lens culinaris and Silvia hispanica showed no reaction with blood group AB in patients with type 2 diabetic disease. Lectins Glycine max, Abrus precatorius, Silvia hispanica, Brassica nigra, Nigella sativa and Phaselous vulgaris reacted with diabetic O blood group. Lectins Dolichus biflarus, Cicer arietinum and Lens culinaris showed no reaction with blood group O in patients with type 2 diabetic disease. Conclusion: From the findings of the present study, it has been found the certain lectins reacted with the erythrocytes of diabetic patients differently.***

Keywords: Indigenous dietary lectins. Hemagglutination. Human ABO blood types. Type 2 diabetes mellitus.

1. Introduction

1) Toward the end of the 19th century, evidence started to accumulate for the presence in nature of proteins possessing the ability to agglutinate erythrocytes. Such proteins were referred to as hemagglutinins, or phytoagglutinins, because they were originally found in extracts of plants. In 1888, Stillmark marked the beginning of lectin research, he noted that saline extracts of the common castor bean, *Ricinus communis*, responsible for the agglutination with human erythrocytes was a protein and gave it the name "Ricin". Landsteiner (1902) tested the reversibility of agglutination reactions of plant agglutinins with erythrocytes. Landsteiner and Raubitschek (1908) showed the lectin specificity towards the red blood cells.

2) It is known that lectins are inhibited by simple sugars of one kind or the other. Since the red blood cells of patients with diabetes mellitus contain variable amount of glucose, it was thought that the agglutinability of such cells with a given lectin would differ with that from cells which did not have this sugar. In 1954, Boyd and Shapleigh named them "lectins" from the Latin word legere meaning "to choose" or "to select." This definition was further broadened by Goldstein et al. (1980) which stated that lectin was "a sugar-binding protein or glycoprotein of non-immune origin, which agglutinated cells and/or precipitated glycoconjugates."

3) Lectins are proteins found in a diversity of organisms. They possess the ability to agglutinate erythrocytes with known carbohydrate specificity since they have at least one non-catalytic domain that binds reversibly to specific monosaccharides or oligosaccharides (Lam and Ng., 2011). In most plants, lectins are present as carbohydrate binding proteins, especially seeds and tubers like cereals, potatoes and beans. It is now well established that many lectins are toxic, inflammatory, resistant to cooking and digestive enzymes and present in much of our food and sometimes cause "food poisoning."The global pattern of varying prevalence of diseases such as coeliac disease, autoimmune diseases, rheumatoid arthritis, obesity, cardiovascular disease and insulin dependent diabetes mellitus, suggests that some dietary factors specific to plant foods could initiate these diseases.(Hamid and Masood, 2009).

4) In the present study, an attempt will be made to identify the indigenous dietary lectins taken by the patients with diabetes mellitus and cardio-vascular diseases from north India, along with their characterizations.

2. Materials and Methods

1) Selection of Lectins

Thirteen different indigenous plant lectins as well as dietary seeds of leguminosae family were selected in which ten seeds namely *Cicer arietinum, Lens culinaris, Glycine max, Dolichos biflarus, Abrus precatorius, Salvia hispanica, Nigella sativa, Brassica alba ,Helianthus annus and Phaseolus vulgaris* exhibited agglutination activity against any one of the blood groups of patients with type 2 diabetes mellitus 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 Lectins

The lectins were prepared following the methods described by Dunsford and Bowley (1967). The seeds were grounded to a fine powder and were 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 the 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: 10000 parts, as preservative.

3) Collection and Processing of Human Blood

To characterize the lectin ,hemagglutination assay was performed by using erythrocyte suspension of A, B, AB and O types of patients with type 2 diabetes mellitus. The 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 extracts. 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 A blood group of diabetic patients, the lectin *Cicer arietinum* and *Phaselous vulgaris* showed reaction upto the strength (++) 1:128, the lectin *Lens culinaris* showed the reaction upto the strength (+) 1:128, lectin *Helianthus annus* and *Abrus precatorius* showed maximum reaction upto1:64 (+) strength, lectin *Glycine max* showed reaction with the strength (++) upto 1:4, lectin *Ddolichus biflarus* showed hemagglutination reaction with the strength (+) upto 1:16. Lectin *Salvia hispanica* showed reaction with the strength (+) upto 1:4. Lectin *Brassica nigra* showed hemagglutination reaction with the strength (++) upto 1:16. lectin *Nigella sativa* showed no reaction with blood group A in patients with type 2 diabetic disease .

2) Table 2 showed the reaction patterns of different lectins with blood group B of diabetic patients, The lectin *Glycine max* and *Dolichus biflarus* showed hemagglutination reaction with strength (+) upto 1:64, *Abrus precatorius* showed reaction strength (++) upto1:8, lectin B*rassica nigra* showed a strong hemagglutination reaction with strength (+++) upto 1:128. Lectin *Nigella sativa* showed reaction strength (+++) upto 1:128, lectin *Helianthus annus* showed reaction strength (+) upto 1:32., lectin *Phaselous vulgaris* showed reaction upto the strength (+) 1:32, lectin *Cicer arietinum*, *Lens culinaris* and *Salvia hispanica* showed no reaction with blood group B in patients with type 2 diabetic disease.

3) Table 3 showed the reaction patterns of different lectins with blood group AB of type 2 diabetic patients. Lectin *Helianthus annus* and lectin *Nigella sativa* showed strong reaction with strength of (+++) upto 1:128, lectin *Phaselous vulgaris* showed reaction upto the strength (++) 1:32, lectin *Glycine max* showed reaction with the strength (++) upto 1:16, *Abrus precatorius* showed reaction strength (++) upto1:8. lectin *Dolichus biflarus* showed hemagglutination reaction with strength (+) upto 1:8. Lectin B*rassica nigra* showed hemagglutination reaction with strength (+) upto 1:16. Lectins *Cicer arietinum*, *Lens culinaris* and *Silvia hispanica showed* no reaction with blood group AB in patients with type 2 diabetic disease.

Table 1: Reaction pattern of different lectins with blood group A in patients with type 2 diabetes mellitus

Lectins	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Cicer arietinum	+++	+++	+++	+++	++	++	++	++
Lens culinaris	++	++	++	++	+	+	+	+
Glycine max	++	++	++	-	-	-	-	-
Abrus precatorius	+++	+++	+++	+++	++	+	+	-
Dolichus biflarus	+++	+++	+++	++	+	-	-	-
Salvia hispanica	+++	++	+	-	-	-	-	-
Brassica nigra	+++	+++	+++	++	++	+	+	+
Helianthus annus	+++	+++	+++	++	+	+	+	-
Nigella sativa	-	-	-	-	-	-	-	-
Phaseolus vulgaris	+++	+++	+++	+++	+++	+++	++	++

Table 2: Reaction pattern of blood group B in patients with type 2 diabetes mellitus

Lectins	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Cicer arietinum	-	-	-	-	-	-	-	-
Lens culinaris	-	-	-	-	-	-	-	-
Glycine max	+++	+++	+++	++	++	+	+	-
Abrus precatorius	+++	++	++	++	-	-	-	-
Dolichus biflarus	+	+	+	+	+	+	+	-
Salvia hispanica	-	-	-	-	-	-	-	-
Brassica nigra	+++	+++	+++	+++	++	++	+	+
Helianthus annus	+++	++	+	+	+	+	-	-
Nigella sativa	+	+	+	+	+	+	+	+
Phaseolus vulgaris	+++	+++	+++	+++	+++	++	-	-

Table 3: Reaction pattern of blood group AB in patient with type 2 diabetes mellitus

Lectins	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Cicer arietinum	-	-	-	-	-	-	-	-
Lens culinaris	-	-	-	-	-	-	-	-
Glycine max	+++	+++	++	++	++	-	-	-
Abrus precatorius	+++	++	++	+	-	-	-	-
Dolichus biflarus	++	++	++	+	-	-	-	-
Salvia hispanica	-	-	-	-	-	-	-	-
Brassica nigra	+++	+++	+++	+++	+++	++	++	-
Helianthus annus	+++	++	++	+	+	+	+	+
Nigella sativa	+	+	+	+	+	+	+	+
Phaseolus vulgaris	+++	+++	+++	+++	+++	++	-	-

4) Table 4 showed the reaction patterns of different lectins with blood group O of type 2 diabetic patients. Lectin *Glycine max* showed reaction with the strength (+) upto 1:8, Lectin *Abrus precatorius* showed reaction *strength* (+) upto1:4. Lectin *Silvia hispanica* and lectin *Brassica nigra* showed hemagglutination reaction with strength (++) upto 1:64. Lectin *Nigella sativa* showed strength (+) upto 1:8. Lectin *Phaselous vulgaris* showed reaction upto the strength (+) 1:32. Lectins *Dolichus biflarus*, *Cicer arietinum* and *Lens culinaris* showed no reaction with blood group O in patients with type 2 diabetic disease.

Table 4: Reaction pattern of blood group O in patients with type 2 diabetes mellitus

Lectins	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Cicer arietinum	-	-	-	-	-	-	-	-
Lens culinaris	-	-	-	-	-	-	-	-
Glycine max	++	++	++	++	-	-	-	-
Abrus precatorius	+++	++	++	+	-	-	-	-
Dolichus biflarus	-	-	-	-	-	-	-	-
Salvia hispanica	+++	++	+	+	+	+	+	-
Brassica nigra	+++	+++	+++	+++	++	++	+	-
Helianthus annus	+++	++	++	+	+	+	+	-
Nigella sativa	+	+	+	+	+	+	+	+
Phaseolus vulgaris	+++	+++	+++	++	+	+	-	-

4. Discussion

1) Lectins are carbohydrate-binding proteins of non-immune origin. Lectins are used in extensive relevance such as agglutination, blood typing, mitogenic stimulation, toxicity to cells, inhibition of fungal, bacterial and viral growth, insecticidal property, anti-HIV property, anti-cancer, neurosciences, and recently reported to have nuclease like activity (Sharon, 1975).
2) Thirteen different dietary seeds of leguminosae family were screened for identification of lectins against four individual blood groups, these are A, B, AB, O of type 2 diabetic patients. The experimental results showed the lectins *Clerodendrum inermi, Brassica alba, Momordica charantia* exhibited no agglutination activity with any one of the blood groups, where as ten lectins were found agglutination activity against any one of the diabetic blood groups which are tested at different level of dilution.
3) In blood group A, *Cicer arietinum, Lens culinaris, Brassica nigra* and *Phaseolus vulgarius* showed strong reaction (+++) upto 1:128, Lectin *Helianthus annus, Abrus pectorious* showed reaction (++) upto 1:64. Lectin *Nigella sativa* showed no reaction with the blood group.
4) In blood group B, *Brassica nigra* and *Nigella sativa* showed strong reaction (+++) upto 1:128. Lectin *Glycine max* and *Dolichus biflarus* showed reaction (++) upto 1:64. Lectins *Cicer arietinum, Lens culinaris,* and *Silvia hispanica* showed no reaction with the blood group .
5) In blood group AB, lectins *Helianthus annus and Nigella Sativa* showed strong reaction (+++) upto 1:128, lectin *Brassica Nigra* showed reaction (++) upto 1:64. Lectins Cicer arietinum, Lens culinaris and Salvia hispanica showed no reaction with the blood group.
6) In blood group O, lectins *Silvia hispanica, Brassica nigra* and *Helanthus annus* had reaction (++) upto 1:64. *Cicer arietinum, Lens culinaris* and Dolichus biflarus showed no reaction with the blood group.
7) From the findings of the present study, it has been found the certain lectins reacted with the erythrocytes of diabetic patients differently.

5. Conclusion

This research is an initiative steps towards the sports nutrition. It needs more attention and more research on it. Mostly seeds are consumed as raw in their daily routine diet without knowing the effect of lectins of seeds. It is advised to consume all the seeds properly denatured with on their respective temperature and then should be consumed. The studies on lectins are getting tremendous now a days for their beneficial effects on humankind.

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