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Original Research Article

Identification and Characterization of Lectins from Leguminosae Plants

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ABSTRACT

Introduction: Since lectins are widely found in Leguminosae family due to their high protein content, in the present study, an attempt has been made to identify such legume lectins which have the agglutination properties with the red blood cells from normal individuals.
Methods: 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.
Results: In the results, various lectins showed the hemagglutination reaction pattern with human ABO blood groups. The lectin reacted with various blood groups with the strength of 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128.
Conclusion: The research findings were considered as the first step towards the field of sports nutrition. The lectin seeds are collected from the daily routine of the sports person, without knowing the effect of seeds on the body. Properly denaturized seeds should be consumed.
Key words: Lectins. Hemagglutination. ABO blood types.

INTRODUTION

The term 'Lectin' is derived from the Latin word 'Legere' this means 'to pick out' or to 'choose' or 'to select'. The term was first introduced by Boyd and Shepleigh. [1] Dixon [2] explained lectins as proteins with at least one carbohydrate binding site. Sharon and Lis [3] described lectins as having the existence of additional hydrophobic sites. Hydrophobicity is the main interacting force of lectins with carbohydrates, through the carbonate binding sites.

Lectins were first described in 1888 (as extracts) by Stillmark working with castor bean extracts. They have an affinity for carbohydrates and they can agglutinate cells of various types. Lectins are ubiquitous natural proteins that hydrophobically bind carbohydrates with characteristic specificities. They have the ability to induce cell agglutination phenomena. Some proteins we now regard as animal lectins were discovered before plant lectins, though many were not recognized as carbohydrate binding proteins for many years after being first reported. As recently as 1988, most animal lectins were thought to belong to one of two primary structural families, the C-type and S-type (presently known as galectins) lectins. However, it is now clear that animal lectin activity is found in association with an astonishing diversity of primary structures. At least 12 structural families are known to exist, while many other lectins have structures apparently unique amongst carbohydrate-binding proteins, although some of those ''orphans'' belong to recognized protein families that are otherwise not associated with sugar

recognition. Furthermore, many animal lectins also bind structures other than carbohydrates via protein–protein, protein–lipid or protein–nucleic acid interactions. While animal lectins undoubtedly fulfill a variety of functions, many could be considered in general terms to be recognized molecules within the immune system. More specifically, lectins have been implicated in direct first-line defense against pathogens, cell trafficking, immune regulation and prevention of autoimmunity.

Two major discoveries made in the early 1960s were instrumental in bringing lectins into the limelight. The first of these was by Nowell [4] at the University of Pennsylvania, Philadelphia, who found that the lectin of the red kidney bean (Phaseolus vulgaris), known as phytohemagglutinin (PHA), is mitogenic, that is, it possesses the ability to stimulate lymphocytes to undergo mitosis. This discovery had a revolutionary impact on immunology in that it shattered the view, held until then, that lymphocytes are dead-end cells incapable of dividing or differentiating further. Within a short time, several other lectins were proven to be mitogenic. Of special significance was the finding that concanavalin A acts as a mitogen because, in contrast to PHA, its activity could be inhibited by low concentrations of monosaccharides, for example, mannose. This finding provided proof that mitogenic stimulation is the result of binding of lectins to sugars on the surface of the lymphocytes and was among the earliest demonstrations for a biological role of cell surface sugars. Mitogenic lectins soon became tools for the study of signal transmission into cells and for the analysis of the biochemical events that occur during lymphocyte stimulation *in vitro*. Lectins are resistant to gastrointestinal digestion. Lectins are also ubiquitous in our food supply, mainly in legumes, like beans and soybean, which can vary greatly in lectin content. Other legumes may contain 20 g of lectin per 100 g of flour. In addition to legumes, other foods may contain considerable amounts of lectins, like amaranth (8.3%) [5] and corn (0.8%). [6] These also include cereal type foods (187.2 μg of lectin/g) and texturized protein flour (12.9 μg lectin/g). Meat substitutes are free of active lectin, and milk substitutes and bakery products have low levels. [7] Commercial preparations of wheat germ also contain lectin (13–53 μg of WGA/g).

A chemical reaction occurs between our blood and the foods we eat. This reaction is part of our genetic inheritance. It is amazing that, our immune and digestive systems still maintain favoritism for foods that our blood type ancestors ate. Now in the twenty first century, we know this because of lectins. Lectins, abundant and diverse proteins found in foods, have agglutinating properties that affect our blood and the lining of our digestive tract. Lectins are a powerful way to attach themselves to other organisms. Our immune systems use this super glue to their benefit. The cells of bile ducts have lectins on their surfaces to help snatch up bacteria and parasites. Bacteria and other microbes also have lectins on their surfaces, works like suction cups, which can attach to the slippery mucosal linings of the body. Often the lectins used by viruses or bacteria can be blood type specific which makes them a stickier pest for people of that blood type. In the last two decades, there has been increased interest in the potential health benefits of bioactive proteins from plants, including lectins from legumes because of the extensive studies which showed that lectins exhibit anti-proliferative, anti-tumor, immune-modulatory, anti-fungal, anti-viral, and HIV-1 reverse transcriptase inhibitory activities. [8-9] Even for lectins with homologous amino acid sequences as those from legumes, a common function cannot be ascribed to them because individual parameters such as carbohydrate specificity and other influencing factors differ. In addition to increasingly sophisticated descriptions of the occurrence and structural characteristics of lectins, their potential to enhance health status has been a driving

force for the expanding interest in lectinology.

In cancer research, lectins are used in the fields of biochemistry, cell biology, and immunology, as well as for diagnostic and therapeutic purposes. [3,10] Lectins can bind reversibly with free sugars or with sugar residues of polysaccharides, glycoproteins, or glycolipids. There are some groups of lectin which play a major role in the defense systems of plants are the extremely cytotoxic lectins, including ricin, abrin, mistletoe lectin I (ML-I), and modecin. The B-chains of these lectins bind to mammalian cell surface receptors and promote the uptake of the A-chain into the cell. The A-chain then cleaves the *N*-glycosidic bond of the adenosine residue of rRNAs, thereby inactivating all eukaryotic ribosomes in the cell. Among other functions, lectins are responsible for innate immunity and defense mechanisms in plants. As many foods are of plant origin, the daily ingestion of lectins by both humans and animals is significant. In the present study, an attempt has been made to identify some legume lectins which have the agglutinating property with the normal RBC and to study their properties.

MATERIALS AND METHODS

Instrumentation

The instruments used were-centrifuge (Khera Research Centrifuge KI 199), spectrophotometer (Khera UV Spectro), automatic blood analyzer cobas c 111, shaker, hot air oven and other instruments were used for isolation and characterization of lectins.

Human blood sample for hemagglutination

To characterize the lectin, hemagglutination assay was performed by using human erythrocyte suspension (A, B, AB and O types).

Collection and processing of human blood

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

ABO typing

For ABO typing, standard serological procedure [11] was followed. The finger was sterilized and pricked with sterilized lancet. 0.5 ml of blood was collected in 0.85% NaCl (physiological saline) in test tube. RBCs pellet was washed thrice with physiological saline by centrifuging it at 2500-3000 rpm. 2% RBCs suspension was prepared in physiological saline. ABO typing was done by normal serological method using 2% RBCs suspension and with anti-A, anti-B and anti-D.

Preparation of Plant Lectins

In the preparation of the lectin, all essential steps were the same as described by Dunsford and Bowley. [12] 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.

Table 1: List of lectins studied

Common name	Scientific name	Family
Kabli chana	Cicer arietinum	Leguminosae
Mung	Vigna radiate	Leguminosae
Peas	Lathyrus sativa	Fabaceae
Soya bean	Gycine max	Leguminosae
Dhania	Coriendum sativum	Apiceae
Rajma	Phaseolus vulgaris	Leguminosae
Ground nut	Arachis hypogoea	Leguminosae
Kali dal	Vigna mungo	Fabaceae
Arhar	Cajanus cajan	Leguminosae
Wheat	Triticum aestivum	Leguminosae
Maize	Zea mays	Leguminosae
Rongi	Vigna unguiculata	Leguminosae
Barley	Hordeum vulgare	Leguminosae
Rice	Oryza sativa	Leguminosae
Ajvain	Trachyspermum ammi	Leguminosae
Beans	Phaseolus lunatus	Leguminosae
Sesame	Sesamum indicum	Leguminosae
Black mustard	Brassica niagra	Fabaceae
Masoor	Lens culinaris	Leguminosae
Sorghum	Sorghum bicolar	Leguminosae
Matar	Pisum sativum	Leguminosae
Kulthi	Dolichos biflorus	Leguminosae
Ratti	Abrus pectorius	Leguminosae
Yellow mustard	Brassica nigra	Leguminosae
Moth	*Vigna aconitifolia*	Leguminosae

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. 25μl red blood was added to an equal amount of seed extract. After 20-25 minutes, results were recorded as per Dunsford and Bowley, [11] noted below:

C – Complete agglutination, ++++ 12.

V – Visual agglutination, several agglutinates being clearly visible without using microscope, +++ 10, ++ 9.

++ – Very large agglutinates visible under the microscope, ++ 8, + 7.

+ – Large agglutinates visible under the microscope, +5.

(+) – Small agglutinates visible under the microscope, ±3.

W – Small, but definite agglutinates, -2.

O – No agglutination.

Effect of temperature or thermal stability

The thermal stability of the crude lectins was done by the method described by Chowdhary et al. [13] 0.5 m1 of the lectin (1mg/10ml), in NaCl (pH 7.4) was incubated separately for 30 minutes in a range of temperatures between 30-100°C with an increment of 10°C. Aliquots of 100 µl were withdrawn, cooled and hemagglutination titre was determined against the A, B, AB and O blood group cells, as described earlier.

RESULTS

Table: 2 showed the hemagglutination reaction pattern of various lectins with human ABO blood groups. The lectins reacted with various blood groups with the strength of 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128. Some of the lectins reacted with ABO blood group up to the strength of 1:128, some lectins reacted up to 1:64, some reacted up to 1:32, some reacted up to 1:16, some reacted up to 1:8, some reacted up to 1:4, some reacted up to 1:2, some reacted up to 1:1 and some lectins showed no reaction .

Table: 3 showed the effect of temperature on hemagglutination reaction of lectins with different temperatures. Temperature selected from 30 degree Celsius to 100 degree Celsius. Above 80 degree Celsius there were no reactions of lectins with human blood. Very few had reacted in 70 degree Celsius.

Table 2: Hemagglutination reaction pattern of various lectins with various blood groups

Lectins	A	B	AB	O
Cicer arietinum	1:128	1:64	1:64	1:32
Vigna radiate	1:128	1:128	1:128	1:128
Lathyrus sativa	1:128	1:64	1:64	1:32
Gycine max	1:64	1:64	1:16	1:8
Coriendum sativum	_	_	_	_
Phaseolus vulgaris	1:128	1:64	1:64	1:32
Arachis hypogoea	_	_	_	_
Vigna mungo	_	_	_	_
Cajanus cajan	_	_	_	_
Triticum aestivum	1:1	1:1	1:2	1:4
Zea mays	1:2	1:1	1:4	1:4
Vigna unguiculata	1:128	1:128	1:128	1:128
Hordeum vulgare	1:2	1:8	1:16	1:16
Oryza sativa	1:1	1:4	1:8	_
Trachyspermum ammi	_	_	_	_
Phaseolus lunatus	1:128	1:64	1:64	1:32
Sesamum indicum	1:1	1:1	1:2	1:2
Brassica niagra	_	_	_	_
Lens culinaris	_	_	_	_
Sorghum bicolar	_	_	_	_
Pisum sativum	1:128	1:128	1:128	1:128
Dolichos biflorus	1:1	1:1	1:1	1:1
Abrus pectorius	1:128	1:128	1:128	1:128
Brassica nigra	_	_	_	_
Vigna aconitifolia	_	_	_	_

DISCUSSION

The extract of many seeds have the property to agglutinate red blood cells caused by some remarkable proteins called lectins. They are found mostly in seeds from which they may be extracted by salt solution. They may also exist in leaves, barks, roots, tubers, etc., agglutination activity have been detected in Leguminosae family.

Sports person's food contains lectins and they are therefore consumed in their native form when foods are eaten raw for their food supplement to enhance their performance. This study is the first steps of its kinds in the field of sports nutrition to find out any lectins which are helpful to enhance the performance of athletes, and to identify the some lectins which uses as raw in nature which create toxic activity into the

gastro intestinal system those are responsible for mood or we can say stress enhancer which play important role in their game.

In humans, lectins have been reported to cause damage, including mass food poisoning from uncooked kidney beans and it also cause hemolytic anemia and jaundice from Mexican fava beans. Lectins may cause acute gastrointestinal symptoms including nausea and vomiting leading to dehydration. They bind to the luminal surface of absorptive erythrocyte in small intestine. This may cause severe damage to the microvilli of the intestine, thus disrupting the digestion and absorption. Lectins can also promote growth of harmful bacteria in the gut. Lectins also disrupt proteins and carbohydrate malabsorption. In protein malabsorption, gut lectins bind to erythrocytes, cause inflammation which blocks the production of enterokinase, a protein enzyme. In case carbohydrate malabsorption, it reduces intestinal glucose uptake by 50%. Wheat germ agglutinin and other lectins can even bind to insulin receptors on cells, disrupting glucose metabolism. Grains will have high content of lectins, which may cause inflammatory bowel and celiac diseases in humans. [14]

These lectins selectively bind carbohydrate moieties of the glycoprotein that decorate the surface of most of the animal cells. Structurally, these lectins have a diverse class of proteins, which have the ability to bind carbohydrates with considerable specificity. [15]

Table 3: Effects of temperature on hemagglutination of various lectins

Lectin	30	40	50	60	70	80	90	100
Cicer arietinum	_	_	_	_	_	_	_	_
Vigna radiate	+	+	+	+	_	_	_	_
Lathyrus sativa	+	+	_	_	_	_	_	_
Gycine max	+	+	+	+	_	_	_	_
Coriendum sativum	+	+	+	_	_	_	_	_
Phaseolus vulgaris	+	+	+	+	+	_	_	_
Arachis hypogoea	_	_	_	_	_	_	_	_
Vigna mungo	_	_	_	_	_	_	_	_
Cajanus cajan	_	_	_	_	_	_	_	_
Triticum aestivum	_	_	_	_	_	_	_	_
Vigna unguiculata	+	+	_	_	_	_	_	_
Zea mays	+	+	+	+	+	_	_	_
Hordeum vulgare	_	_	_	_	_	_	_	_
Oryza sativa	+	+	+	_	_	_	_	_
Trachyspermum ammi	_	_	_	_	_	_	_	_
Phaseolus lunatus	+	+	+	+	_	_	_	_
Sesamum indicum	+	+	+	+	+	+	+	+
Brassica niagra	_	_	_	_	_	_	_	_
Lens culinaris	+	+	_	_	_	_	_	_
Sorghum bicolar	_	_	_	_	_	_	_	_
Pisum sativum	+	+	+	+	_	_	_	_
Dolichos biflorus	_	_	_	_	_	_	_	_
Abrus pectorius	+	+	+	+	+	+	+	+
Brassica nigra	_	_	_	_	_	_	_	_
Vigna aconitifolia	_	_	_	_	_	_	_	_

In-vitro, effects lymphocyte mitogenesis, both stimulating and inhibitory effects, with the lymphocytes of gastro intestine tract (GIT) being most susceptible. They possess the ability to aggregate immunoglobulins, to trigger the alternative complement pathway, to inhibit fungal growth and also to induce histamine release from basophilic and mast cells. Lectins are relatively resistant to both heat (at 70^{O}C more than 30 min) and digestion. Some of the lectins are highly resistant to gastric acid and proteolytic enzymes (Rocca, 2004).

In the present study, twelve different dietary seeds of Leguminosae family were selected, which are being used by all cases of human population in daily life, were screened for identification of lectins against four individual blood groups, that is A, B, AB, O. The experimental results showed the

seven seed extracts exhibited good agglutination activity against any one of the blood groups tested at different level of dilution.

It was observed that *Pisum sativum* lectins reacted strongly in hemagglutination reaction (+++) which were seen at level upto 1:32 in A and AB but B and O blood groups showed strong reaction (+++) of hemagglutination only up to 1:16. Further the rate of reaction in all the four blood groups gradually decreased as the rate of dilution ratio increased. Agglutination reaction was reported upto the temperature of 60^{O}C and with further increase in temperature, the lectins are denatured.

In *Vigna mungo,* strong reaction (+++) was observed in blood group A, and O upto dilution ratio of 1:2, while B and AB blood group showed strong reaction (+++) upto 1:4. Further, the rate of reaction in all the four blood groups gradually decreased as the rate of dilution ratio increased. In blood groups A and B, reaction has been observed to occur upto 1:64 and 1:32 respectively, while AB and O blood groups showed reaction upto 1:128 . It has been observed that reaction occurred upto 50^{O}C. On further increasing temperature, lectins are denatured.

In *Dolichos biflorus,* reaction (+++) was observed in blood groups A, and AB upto dilution ratio of 1:8, while B and O blood groups showed no reaction. Further, the rate of reaction in both two blood groups gradually decreased as the rate of dilution ratio increases. In blood group A reaction has been observed occurred upto 1:64 while AB blood groups showed reaction upto 1:32. It has been observed that reaction occurred upto 70^{O}C. Further with the increase in temperature, lectins are denatured.

Pisum sativum lectin reacted with A and AB blood groups with the strength (++) upto 1:128 dilution and with B and O blood group with the strength of (+) upto 1:128.

Phaseolus vulgaris lectin reacted with A , B and AB blood groups with the strength (+) upto 1:128 dilution and with O blood group with the strength of (+) upto 1:32.

Vigna unguiculata lectin reacted with A blood groups with the strength (+) upto 1:128 dilution and with B blood group strength of (+) upto 1:64, AB and O blood group with the strength of (+) upto 1:32.

Vigna radiate lectin reacted with A, B, O and AB blood groups with the strength (+) upto 1:128 dilution.

Glycine max lectin reacted with A and AB blood groups with the strength (+) upto 1:64 dilution and with B blood group the strength of (+) upto 1:32 and O blood group with the strength of (+) upto 1:128.

Vigna mungo lectin reacted with A blood groups with the strength (+) upto 1:64 dilution and with B blood group strength of (+) upto 1:32, AB and O blood group with the strength of (+) upto 1:128.

Dolichos biflorus lectin reacted with A blood group with strength (+) upto 1:64, with AB blood group strength of (+) upto 1:32 and no reaction was recorded with B and O blood groups.

The effect of temperature on lectins of *Pisum sativum*, *Phaseolus vulgaris*, *Vigna unguiculata* upto, *Vigna radiate* upto, *Glycine max*, *Vigna mungo*, *Dolichos biflorus* reacted with blood groups and hemagglutination activity was observed from 30^{O}C to 70^{O}C, however no reaction was occurred with *Vigna aconitifolia*, *Lens culinaris*, *Cicer arietinum* (white & red), *Cajanus cajan* in any case.

CONCLUSION

The research findings were considered as the first step towards the field of sports nutrition. The lectin seeds are collected from the daily routine of the sports person, without knowing the effect of seeds on the body. Properly denaturized seeds should be consumed.

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