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HIGHLY SPECIFIC HEMAGGLUTINATION ACTIVITY OF PLANT LECTINS IN SPECIFIC SPECIES: CASE OF FABACEAE AND SOLANACEAE

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Abstract

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Lectins are carbohydrate-binding proteins present in most of the plants and in some animals. They possess the ability to agglutinate erythrocytes with known carbohydrate specificity since they have at least one non-catalytic domain that binds reversibly specific monosaccharides or oligosaccharides. This study investigated the presence of lectins in the plant species of the family Fabaceae and Solanaceae. The results of our study have shown that 6 of 10 plant lectins caused agglutination, and 4 of them did not cause agglutination of human erythrocytes. Blood agglutination activity against A, B, AB and 0 groups was shown after exposing blood to lectin extracts obtained from 80% of tested plants in family Fabaceae, and 20% of tested plants in family Solanaceae. The highest degree of agglutination was obtained in *Phaseolus vulgaris* (+4) and the highest protein concentration was obtained in *Lens esculenta* within the family Fabaceae. There was no statistically significant difference for protein concentration and specific lectin activity between two compared families ($p>0.05$).

Key words: agglutination activity; agglutination degree; blood groups; plant lectins; protein content
List of abbreviations: d.a. – Degree of agglutination, M – Molar, PHA-E – Phaseolus vulgaris erythroagglutinin, PHA-L – Phaseolus vulgaris leucoagglutinin, Rh factor – Rhesus factor, UV/VIS – Ultraviolet and visible

Introduction

Lectins are proteins or glycoproteins of non-immune origin with specific binding affinity for the carbohydrate moiety of glycoconjugates (Goldstein et al., 1980; Monira et al., 2009). Lectins are also known for their ability to agglutinate erythrocytes *in vitro* and because of that, they are also called agglutinins. They are highly diverse in structure, molecular weight, composition, and in the number of sugar binding sites present per molecule (Laija et al., 2010; Rudiger et al., 2000; Van Damme et al., 1998). Lectins have been classified according to their sugar-binding specificity to monospecific and polyspecific and they can interact with more than one sugar (Neutsch et al., 2012).

✓ Generally, lectins are classified into four groups, based on their affinity to bind with:
- ✓ Glucose/mannose
- ✓ Galactose and N-acetyl-D-galactosamine
- ✓ L-fucose
- ✓ Sialic acids (Peumans et al., 2010).

Many of the plants and animal lectins are resistant to both heating and digestion (digestive enzymes and acids). While some of the lectins are degraded and others pass through the gut, about 1–5% are re-absorbed into the bloodstream in animals, which is considered a significant amount, sufficient to cause an immune response (Rini, 1995).

Plant lectins exhibit a variety of biological activities, including cell agglutination, mitosis, toxicity and cell growth

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inhibition (Kjeldsen, 1988). It has also been shown that local (gut) and systemic metabolism of rats is altered by consumption of raw bean (*Phaseolus vulgaris* L. var. athropurpurea) and these changes have been attributed to be probably mediated primarily by lectins in raw bean diet (De Moya et al., 2003). Studies show that lectins have been recognized as useful probes for structural investigations of polysaccharide and complex carbohydrates on cell surfaces. Some lectins have been used for blood group typing because of their specificity to particular blood group. Certain lectins differentiate malignant from normal cells (Sela et al., 1970; Sharon and Lis, 1989, 2004). Several plant lectins have been shown to induce cell death in cancer cells, suggesting that they may have applications in cancer treatment. Cancer cells have been shown to express and/or secrete glycoconjugates with an aberrant glycan structure. Thus, lectins may detect such changes, leading to their use in the cancer-specific treatment (Kjeldsen,1988).

Mature seeds are the main source of plant lectins, but they are also found in other vegetative tissues such as leaves, fruits but merely roots (De Hoff et al., 2009). Legume lectins are the best-known lectin family (Sharon and Lis, 1990). It has been shown that lectins from peanuts, soybean, beans, lens, potato, tomato agglutinate human erythrocytes of A, B, AB and 0 blood types (Hamid and Masood, 2009; Nachbar and Oppenheim, 1980; Zubčević et al., 2016). Numerous plant species of the family Solanaceae and Fabaceae are used daily in a human diet and because of that the aim of the research is to determine which species from these families cause agglutination of human erythrocytes and to determine whether is lectin titre and protein concentration in correlation with the degree of agglutination.

Materials and Methods

Two plant families (Fabaceae and Solanaceae) were used in this research to test the effects of plant lectins on human erythrocytes agglutination, including the analysis of the total protein concentration and agglutination titer. Plant families were chosen based on the fact that many species among them have agglutination properties and are mainly used in the daily diet. Used plant species and plant parts are presented in Table 1.

Lectins extraction

We used 2.5 g of plant tissue/seeds that was macerated/grounded into powder with an addition of 5 mL 0.9% NaCl solution. The mixture was filtered through 80 mesh grit and then centrifuged for 10 minutes at 10 000 rpm. Supernatant that contained lectins was separated and stored at 4°C and was used for further analysis.

Table 1
Plant species and used plant parts for analysis

	Species	Vernacular name	Part used
Fabaceae	*Arachis hypogaea*	Peanut	Seed
	Glycine max	Soy	Seed
	Lens esculenta	Lens	Seed
	Phaseolus vulgaris	Beans	Seed
	Pisum sativum	Pea	Seed
Solanaceae	*Solanum tuberosum*	Potato	Tuber
	Solanum lycopersicum	Tomato	Fruit
	Solanum melongena	Eggplant	Fruit
	Capsicum annuum	Pepper	Fruit
	Capsicum longum	Cayenne pepper	Fruit

Blood collection and preparation of erythrocytes suspension

Different blood types (A, B, AB, O) and different Rh (Rh+ and Rh-) were used in this study. Erythrocytes were separated from plasma and washed in sterile physiological solution, and centrifuged at 1000 rpm for five minutes. This was repeated for three times.

Erythrocyte agglutination and degree of agglutination

After mixing the erythrocytes suspension with the lectin extract (1:1), the determination of erythrocytes agglutination was analyzed by light microscopy (Digital Microscope Olympus DP12 including 3.34 megapixel Olympus camera to create images). Control samples were used by mixing the erythrocyte suspension with 0.9% NaCl solution. The degree of the agglutination was recorded in terms: no agglutination 0; small erythrocyte aggregates +1; medium size aggregates +2; several large aggregates +3 and one solid aggregate +4.

Hemagglutination activity assay (agglutination titer)

Serial two-fold dilutions of the lectins in 0.15 M NaCl solution (25 μL) were performed in microtiter plates with U-shaped bottoms (Cooke Engineering, Co., Alexandria, Va.). Erythrocytes suspension (25 μL) was added to each well. After thorough mixing on a Cooke Micro shaker, the covered plates were incubated for 2 h at 37°C and read by using a Cooke microtiter mirror. Visible aggregation of particles was seen in a case of a positive reaction; negative reactions appeared as unchanged cloudy suspensions. The agglutinating activity was expressed as the titer, the reciprocal of the greatest dilution at which visible agglutination could be detected. The specific activity was expressed as titer/mg of protein.

Estimation of the protein level

Estimation of protein content was carried out by Biuret method (Human, Wiesbaden, Germany). The absorbance of

sample and standard against the reagent blank were measured at 546 nm using a spectrophotometer (Taupe Elmer, Lambda 25, UV/VIS spectrophotometer).

Statistical analysis

Statistical analysis was done by SPSS Version 17.0. software to estimate mean values and statistically significant difference between compared parameters. 95% confidence interval and 5% absolute precision were used through the research.

Results

Erythrocytes agglutination, the degree of agglutination (d.a.) and the percentage ratio (+/-) within Fabaceae and Solanaceae family of different blood types are presented in Table 2.

Species from Solanaceae family caused agglutination of 40% blood types, in comparison to species from Fabaceae family that caused 80%. The highest degree of agglutination (+4) was determined in *Phaseolus vulgaris* (Fabaceae). Other plant species of the family Fabaceae had the degree of agglutination of +3, except species *Arachis hypogaea*, which did not cause the agglutination. *Solanum tuberosum* had the degree of +3, while *Solanum melongena* had the degree of +1. Other species of the family Solanaceae did not cause the agglutination. Examples of different agglutination degrees are shown in Figure 1.

Table 2
Percentage ratio and degree of agglutination of erythrocytes

	Species	A	d.a.	B	d.a.	AB	d.a.	O	d.a.	+	–
Fabaceae	*Arachis hypogaea*	–	0	–	0	–	0	–	0	80%	20%
	Glycine max	+	3	+	3	+	3	+	3		
	Lens esculenta	+	3	+	3	+	3	+	3		
	Phaseolus vulgaris	+	4	+	3	+	4	+	4		
	Pisum sativum	+	3	+	3	+	3	+	3		
Solanaceae	*Solanum tuberosum*	+	3	+	3	+	3	+	3	40%	60%
	Solanum lycopersicum	–	0	–	0	–	0	–	0		
	Solanum melongena	+	1	+	1	+	1	+	1		
	Capsicum annuum	–	0	–	0	–	0	–	0		
	Capsicum longum	–	0	–	0	–	0	–	0		

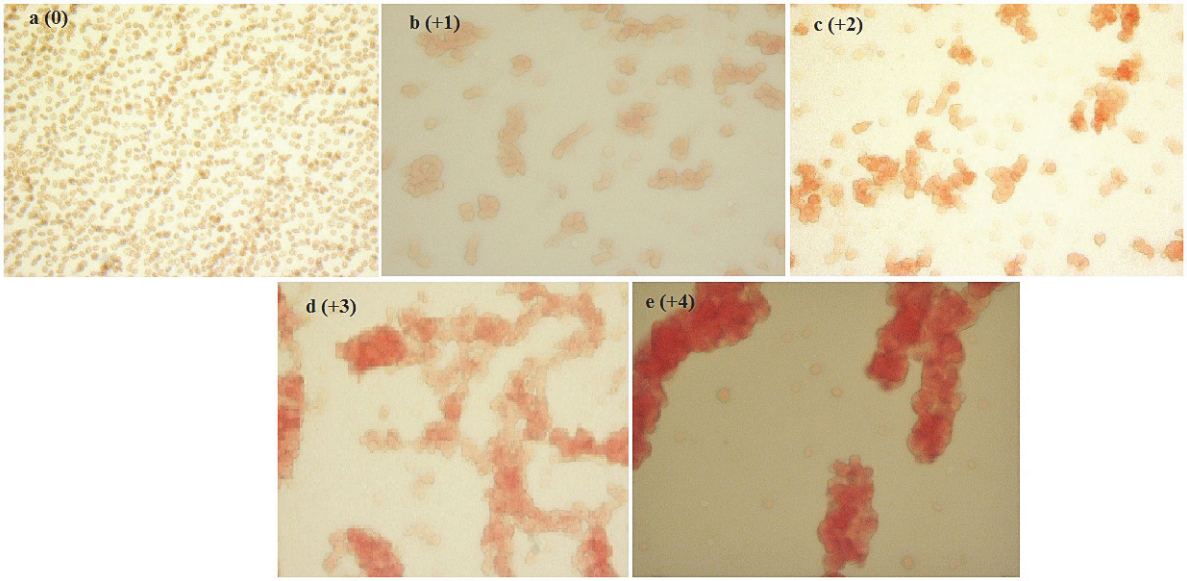

Fig. 1. Different agglutination degree: a – no agglutination (*Arachis hypogaea)*; b – small erythrocyte aggregates (*Solanum melongena*); c – medium size aggregates; d – several large aggregates (*Solanum tuberosum*) and e – one solid aggregate (*Phaseolus vulgaris*)

Hemagglutination activity of species in the Fabaceae family is shown in the Figure 2. Plants thatrevealed higher agglutination degree showed also higher hemagglutination activity. *Phaseolus vulgaris* had the highest hemagglutination activity (512 titer/mL). For plants that had agglutination degree of +3 (*Glycine max*, *Lens esculenta* and *Pisum sativum*), hemagglutination activity was 64 titer/mL.

Hemagglutination activity of species in the Solanaceae family is shown in Figure 3. *Solanum tuberosum* had the highest agglutination properties of +3 and hemagglutination activity was 64 titer/mL. *Solanum melongena* caused agglutination despite the fact that it had the lowest agglutination level (+1). *Solanum melongena* showed different agglutination activity for the different blood types. Hemagglutination activity for the A blood type was 8 titer/mL, for B blood type 16 titer/mL, while hemagglutination activity for the AB and the O blood type was 32 titer/mL.

Values of lectins activity (including total and specific activity) and total protein concentration are shown in Table 3. The highest concentration of protein was measured in *Lens esculenta* (13.75 mg/mL) in the family Fabaceae. In the family Solanaceae the highest protein concentration was determined in *Solanum lycopersicum* and *Capsicum annuum* (11.00 mg/mL). Regardless on the fact that *Lens esculenta*

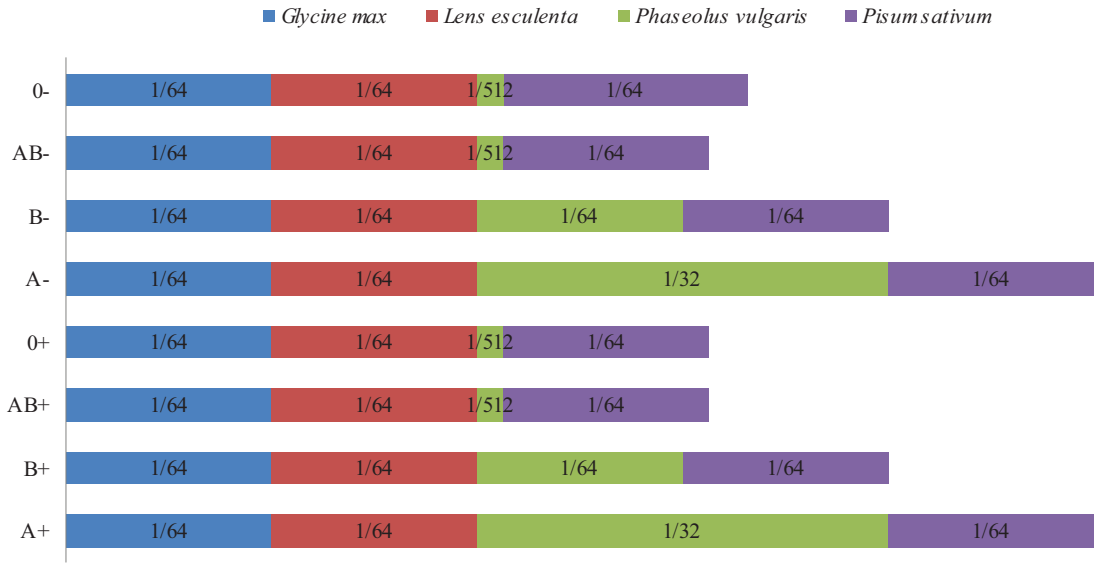

Fig. 2. Hemagglutination activity of the Fabaceae family

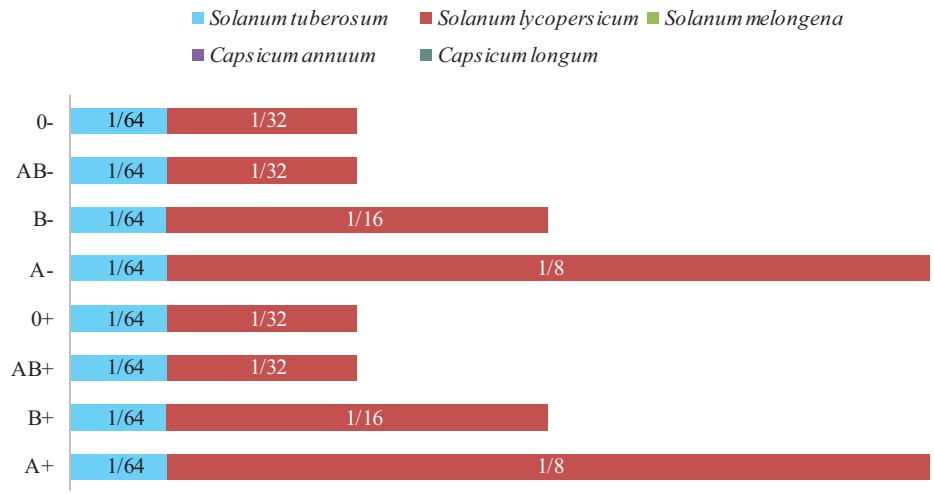

Fig. 3. Hemagglutination activity of the Solanaceae family

Table 3
Lectin activity and concentration of total protein

	Species	Lectin activity (titer/ml)	Total activity (titer)a	Protein concentration (mg/mL)	Total protein (mg)	Specific lectin activity (titer/mg)b
Fabaceae	*Glycine max*	64	480	2.75	20.62	23.28
	Lens esculenta	256	1920	13.75	103.12	18.62
	Phaseolus vulgaris	512	3840	5.5	41.25	93.09
	Pisum sativum	64	480	8.25	61.87	7.76
	Arachis hypogaea	–	–	2.75	20.62	–
Solanaceae	*Solanum tuberosum*	64	480	8.25	61.87	7.76
	Solanum melongena	32	240	5.5	41.25	5.82
	Solanum lycopersicum	–	–	11	82.5	
	Capsicum annuum			11	82.5	–
	Capsicum longum			5.5	41.25	

[a]The total activity of lectin is defined as the activity of lectin multiplied by a total volume
[b]The specific activity of the lectin is defined as haemagglutinating units divided by the total protein concentration

had the highest value of total protein, the highest specific protein activity was established in *Phaseolus vulgaris*. Plant species in Fabaceae family had the higher content of the total proteins and specific activity.

Mean values, standard deviation, range and significant differences for protein concentration and specific lectin activity are shown in Table 4. Higher protein concentrations and lower standard deviation were established for Solanaceae in comparison to Fabaceae. However, lectins specific activity was significantly higher in Fabaceae with high standard deviations. Statistically significant values for protein concentration and specific lectin activity were not obtained between two families.

Table 4
Significant values for protein concentration and specific lectin activity

Protein concentration	Family	Mean±stdv	Range	Sig.
	Fabaceae	6.60 ± 4.60	2.75-13.75	0.687
	Solanaceae	8.25 ± 2.88	5.50-11.00	
Lectins specific activity	Fabaceae	35.70 ± 38.81	7.76-93.09	0.99
	Solanaceae	6.80 ± 1.37	5.82-7.76	

Discussion

Lectins present in the diet cause subclinical effects in humans, especially when used in large quantities (Tommy et al., 2005). Allergenic proteins present in the food may contain components that induce either the food intolerance or food allergy (Venkata Raman et al., 2012). Our research confirmed the agglutination of erythrocytes caused by plant lectins, more exactly the agglutination of erythrocytes with the specific membrane antigens. A high percentage (60%) of lectins from utilizedplants, especially plant species from the Fabaceae family (soybeans, lentils, beans and peas) agglutinated erythrocyte of all blood groups. These results are consistent with the earlier studies by Venkata et al. (2012), Power (1991), Zubčević et al. (2016) and Bashir et al. (2010). Lectins from peanut (Fabaceae), as well as tomato, pepper and hot pepper (Solanaceae), didn't cause agglutination of erythrocytes. Potato and eggplant (Solanaceae) agglutinated erythrocytes of all blood groups. On the other hand, we found that agglutination does not depend on Rh factor of the blood group type. Studies have shown that the lectin inactivity on blood groups has a great evolutionary significance in the diversified tolerance on these types of food (Raman et al., 2012).

Bean lectin consist two closely related proteins called leucoagglutinin (PHA-L) and erythroagglutinin (PHA-E). PHA-E agglutinates erythrocytes, whereas PHA-L agglutinates lymphocytes and is a mitogen for the mammalian cells (Borrebaeck, 1984). Soybean lectin leads to the decrease in the activity of trypsin while protein levels and amylase activity increases in the pancreatic juice (Hemalatha, 2011). The degree of agglutination depends on the part of the plant in which lectins are found. All plants lectins of the Fabaceae family which have caused agglutination were isolated from seeds, except for peanuts. However, erythrocyte agglutination for the Solanaceae family was different; lectins isolated from potato tubers caused agglutination, while fruit lectins did not cause agglutination, except for eggplant. This shows that Fabaceae lectins are most abundant in the seeds, while the Solanaceae lectins are distributed in different parts. Most plant lectins are present in seeds, tubers and fruits, while in roots, stems

and leaves are less presented (Nachbar and Oppenheim, 1980). The highest degree of agglutination was presented in beans (Fabaceae, +4) and in potato (Solanaceae, +3). The reason for this various degree of agglutination is the different affinity of isolated lectins on the carbohydrates present on erythrocyte surface. Thus, a large degree of agglutination is characteristic for the species from Fabaceae family. Species with a higher degree of agglutination show higher hemagglutination activity (titer).

The largest titer was found in beans as also claimed by Schiefer (1974) and Venkata et al. (2012). However, eggplant had the lowest degree of agglutination (+1) and different haemagglutination activity for different blood group. Interestingly, the protein concentration does not correlate with agglutination rate. For example, chili pepper had a protein concentration of 11 mg/mL and did not cause agglutination, while potato had a lower concentration of proteins (8.25 mg/mL) and it did agglutinate all blood groups. The highest concentration of lectins was found in the lens as confirmed by Sultana (2014). Beans had the lowest protein concentration and showed the highest specific activity. Because of their oligosaccharide-binding specificities, lectins have been studied as powerful biorecognition weapons. They could also be used as drugs or can be used for targeting of other therapeutically active molecules.

Conclusion

Because of the increasing consumption of food containing lectins that cause mild inconvenient symptoms to visible destructive disorders, the importance of lectin research in the future will become more in focus. An interesting fact is that some plants species have allow protein concentration but a high degree of agglutination and specific activity. This particularly refers to many species of the Fabaceae family. Agglutination ability also depends on the part of the plant being consumed and is particularly important for proper blood-dependent nutrition. Since lectins play a key role in encoding biological information through cell-cell interaction and cell–routing, new overviews in disease diagnosis, prophylaxis and treatment are being continuously explored in an attempt to identify newer therapeutic approaches beneficial to mankind.

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