ABSTRACT

Plant lectins are carbohydrate binding proteins or phytohaemagglutinins present in most plants, especially seeds and tubers, which include cereals, potatoes and beans. Lectins have great significance in the diet because of their involvement in gastrointestinal difficulties and erythrocyte agglutination. Blood agglutination activity against A, B, AB and O groups was shown after exposing blood to extracts obtained from 55% of tested plants, while in 45% of plants, agglutination was absent. The results of our study have shown that in humans, 40% of plant extracts exhibited activity against A, 40% of plant extracts exhibited activity against B, and 50% of plant extracts exhibited activity against AB and O groups in humans. The concentration of plant lectins depends on the part of the plant. Lectins from the seeds of certain plants cause the greatest percentage of erythrocyte agglutination, while the lowest agglutination was caused by plant bulbs and leaves. However, lectins derived from all plant species of the family Fabaceae agglutinated erythrocytes of all blood types to some extent.

Keywords: lectins, agglutination, blood groups

INTRODUCTION

Lectins are carbohydrate binding proteins present in most plants and in some animals. Lectins do not cause any antigenic stimulation within the immune system, but they have the basic capacity to bind analogously to an antibody (1). The specific capacity of lectins to bind with the cell surface mainly depends on the monosaccharides or simple oligosaccharides, which, when present, inhibit lectin associated reactions. They are involved in cellular interactions (2-3) and the phenomenon of biological recognition such as the binding of microorganisms to target tissues, protein sorting, control of morphogenesis, cell differentiation, fertilization, leukocyte adhesion, metastasis and inhibition of natural killer cell activity against healthy cells (4).

These proteins are widely distributed in living organisms such as algae, animals, microorganisms, fungi and plants (5-7). Plant lectins have mostly been found in seeds and in almost all types of vegetative tissues, including fruits, bulbs, leaves, stems and roots (8-11). Plant lectins from the Fabaceae family that are not effectively degraded by digestive enzymes and that have
an affinity for the surface epithelial cells of the gastrointestinal tract may be toxic (12). On the other hand, some lectins are slightly absorbed in the gastrointestinal tract and are relatively non-toxic in moderate concentrations (13). Lectins bind to glycosyl groups on the membranes of cells lining the digestive tract; this lectin binding is used as a potential tool for the specific targeting of drugs and for bioadhesive applications. Areas of epithelial cells and even whole zones are necrotized, which can be observed in biopsies of the mammal (14) and insect (15) intestines. However, when cells are treated with lectins in vitro, even in high doses, necrosis is not observed, although many other responses have been noted including mutagenesis (16), formation of vacuoles (17) and inhibition of exocytosis.

Over the past few decades, it has been reported that many lectins are toxic and inflammatory. Lectins are resistant to both heat and digestion. Some lectins are highly resistant to gastric acid and proteolytic enzymes (18). According to some studies, some foods containing lectins pass through the intestinal wall, which can result in the deposition of lectins in distant organs (19). Lectins are very active if consumed in fresh food, although activity does not decrease with heat treatment. Haemagglutination activity has been found in processed wheat germ agglutinin, peanuts and dry cereal (20), tomato lectin (12), and navy bean lectin (21), and lectins have been recovered intact in stool (20-21). Wheat gliadin, which causes celiac disease, contains a lectin like substance that binds to the human intestinal mucosa (22). Nephropathy might be caused or aggravated by wheat lectins (23). Wheat lectins are known to induce hyperplasia and hypertrophy of the small intestine and cause changes in body weight and in intestinal function in experimental rats (24). At high dietary levels, lectins cause severe damage to the structure of the small intestine (25-26) and lead to hypersensitivity of the immune system (27).

Recent studies have shown different lectin reactions toward the ABO blood type (28), while other studies show that lectins preferential for a particular ABO type are not found in food and that lectins with ABO specificity are more frequently found in non-food plants or animals (29-30). Despite these criticisms, there seems to be a correlation between diet and blood type. Therefore, when food containing lectin proteins that are incompatible with one’s blood type antigen are eaten, the lectins begin to agglutinate blood cells.

**MATERIALS AND METHODS**

**Blood collection from human subjects**

The blood samples for this analysis were obtained by venipuncture from healthy individuals of both sexes who have blood types A, B, AB and O (n=24).

| Table 1. Percentage of agglutination ratio between blood types |
|-------------------|---|---|---|---|---|
|                  | A | B | AB | O | total |
| Negative         | 12 | 12 | 10 | 10 | 45 % |
| Positive         | 8 | 8 | 10 | 10 | 55 % |
| %                | 40 | 40 | 50 | 50 |     |

**Preparation of extracts**

A total of 20 different plant species which are found in the human diet were purchased. The plant materials were first thoroughly washed with tap water and then rinsed with distilled water. Different plant parts that were being used for isolating lectins were collected from the available sources and taxonomically identified. Portions (1 g) of the edible portions of each food were placed in a warring blender. Two millilitres of 0.9 % NaCl was added and the contents were homogenized for 1 to 3 minutes. The resulting suspension was filtered through cheesecloth, and the filtrate was then centrifuged at 10000 rpm for 10 minutes to obtain a clear extract. The supernatant was separated and stored.

**Erythrocyte agglutination**

The samples were analysed using an “Olympus CH2” microscope, while digital pictures were taken using an “Olympus DP12 Digital Microscope”. A drop of blood of a specific blood type was mixed with a drop of extract and then observed using a microscope (100x magnification). A sample containing a drop of extract and a drop of 0.9 % NaCl was examined as a control.

**RESULTS**

The percentage ratio of agglutination and the total number of plant species extracts with and without agglutination ability are presented in Table 1. The results showed that lectins from 55% of the plant species studied caused the agglutination of a particular blood group, while lectins from 45% of plant species showed no agglutination. From the 55% of plant species where lectins were capable of agglutination, 40% agglutinated blood types A and B, while the remaining 50% agglutinated blood types AB and O.

The studied plant species and plant parts as well as presence or absence of agglutination is presented in Table 2. It was found that the following nine plant species do not cause agglutination (45%): grape, tomato, banana, walnut, carrot, pumpkin, lemon, garlic and onion. The lectins from beetroot caused blood type O agglutination, while those from apple caused blood type AB agglutination. Furthermore, the lectins from cucumber caused blood type AB and O agglutination. Cashew, melon, strawberry, soy, bean, pea, eggplant and potato caused agglutination in all cases. Plant species whose seeds were used for the extraction of lectins, such as soy, bean and pea (with the exception of...
pumpkin), caused agglutination of erythrocytes of all blood types, while the plants whose fruit were used for the extraction of lectins showed different ability to cause agglutination. Plants such as watermelon, cashew, strawberry and eggplant agglutinated erythrocytes of all blood types, while lemon, carrot, banana, tomato and grape did not cause agglutination of erythrocytes. The bulbs used for the extraction of lectins (garlic and onion) did not cause agglutination of erythrocytes, and the lectins from the leaf of beetroot agglutinated blood type O, as shown in Figure 1.

The plants from the Fabaceae and Anacardiaceae families agglutinated red blood cells in all four blood types

<table>
<thead>
<tr>
<th>PLANT NAME</th>
<th>VERNACULAR NAME</th>
<th>FAMILY</th>
<th>PART USED</th>
<th>BLOOD TYPE AGGLUTINATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Allium cepa</td>
<td>Onion</td>
<td>Amaryllidaceae</td>
<td>bulbs</td>
<td>- - - -</td>
</tr>
<tr>
<td>2. Allium sativum</td>
<td>Garlic juice</td>
<td>Amaryllidaceae/Liliaceae</td>
<td>bulbs</td>
<td>- - - -</td>
</tr>
<tr>
<td>3. Anacardium occidentale</td>
<td>Cashew</td>
<td>Anacardiaceae</td>
<td>fruit</td>
<td>+ + + +</td>
</tr>
<tr>
<td>4. Beta vulgaris</td>
<td>Sugar Beet</td>
<td>Chenopodiaceae</td>
<td>leaf</td>
<td>- - - +</td>
</tr>
<tr>
<td>5. Citrullus vulgaris</td>
<td>Watermelon</td>
<td>Cucurbitaceae</td>
<td>fruit</td>
<td>+ + + +</td>
</tr>
<tr>
<td>6. Citrus limon</td>
<td>Lemon</td>
<td>Rutaceae</td>
<td>fruit</td>
<td>- - - -</td>
</tr>
<tr>
<td>7. Cucumis sativus</td>
<td>Cucumber</td>
<td>Cucurbitaceae</td>
<td>fruit</td>
<td>- - + +</td>
</tr>
<tr>
<td>8. Cucurbita pepo</td>
<td>Pumpkin</td>
<td>Cucurbitaceae</td>
<td>seed</td>
<td>- - - -</td>
</tr>
<tr>
<td>9. Daucus carota</td>
<td>Carrot</td>
<td>Apiaceae</td>
<td>fruit</td>
<td>- - - -</td>
</tr>
<tr>
<td>10. Fragaria vesca</td>
<td>Strawberry</td>
<td>Rosaceae</td>
<td>fruit</td>
<td>+ + + +</td>
</tr>
<tr>
<td>11. Glycine max</td>
<td>Soyabean</td>
<td>Fabaceae</td>
<td>seed</td>
<td>+ + + +</td>
</tr>
<tr>
<td>12. Juglans regia</td>
<td>Walnut</td>
<td>Juglandaceae</td>
<td>kernel</td>
<td>- - - -</td>
</tr>
<tr>
<td>13. Malus domestica</td>
<td>Apple</td>
<td>Rosaceae</td>
<td>fruit</td>
<td>- - + -</td>
</tr>
<tr>
<td>14. Musa paradisica</td>
<td>Banana</td>
<td>Musaceae</td>
<td>fruit</td>
<td>- - - -</td>
</tr>
<tr>
<td>15. Phaseolus vulgaris</td>
<td>Black kidney bean</td>
<td>Fabaceae</td>
<td>seed</td>
<td>+ + + +</td>
</tr>
<tr>
<td>16. Pisum sativum</td>
<td>Pea</td>
<td>Fabaceae</td>
<td>seed</td>
<td>+ + + +</td>
</tr>
<tr>
<td>17. Solanum lycopersicum</td>
<td>Tomato</td>
<td>Solanaceae</td>
<td>fruit pulp</td>
<td>- - - -</td>
</tr>
<tr>
<td>18. Solanum melongena</td>
<td>Eggplant</td>
<td>Solanaceae</td>
<td>fruit and seed</td>
<td>+ + + +</td>
</tr>
<tr>
<td>19. Solanum tuberosum</td>
<td>Potato</td>
<td>Solanaceae</td>
<td>bulb</td>
<td>+ + + +</td>
</tr>
<tr>
<td>20. Vitis vinifera</td>
<td>Green grapes</td>
<td>Vitaceae</td>
<td>fruit pulp</td>
<td>- - - -</td>
</tr>
</tbody>
</table>

Figure 1. Plant species and agglutination in different blood types
(100%), while the plants from the Amaryllidaceae, Rutaceae, Apiaceae, Juglandaceae, Musaceae and Viaceae families had no effect on erythrocyte agglutination (0%), as shown in Figure 2.

**DISCUSSION**

Some of the dietary lectins are heat-stable and react with the gastrointestinal tract, causing subclinical effects in humans, especially when used in large quantities (31). Lectins in excess can cause gastrointestinal damage, type-2 IgG immune responses, mild allergies and haemagglutination. It is possible that lectins may not be responsible for short term toxicity caused by the consumption of a raw meal (32). Therefore, lectins play a key role in the daily life of people based on their geographic existence, genetic constitution and adaptability to specific plant products. The development of a lectin tolerance is the result of active immune mechanisms and both the development and maintenance of a lectin tolerance. Nutrition may be the source of antigens that aid in increasing the lectin tolerance of the immune system as well as in providing factors, including nutrients; they themselves might modulate immune maturation and responses and provide factors that influence intestinal microbiota (33). Allergenic proteins present in food may contain components that induce food intolerance or allergy (34). Research related to lectins in food and their interaction with the gastrointestinal tract reveal that there is a direct correlation between the evolution of blood types and food intake types (35). The development of blood groups is based on many factors such as genetic constitution, geographical and environmental factors and the adaptation to various food types from the early days of growth during evolution. Anthropologists recommended that the order of blood group development be based on the food habits of humans (28). This concept states that the first blood group evolved on earth was O: it belonged to the ancestors who hunted, made their own weapons and ate meat. The second blood group that evolved was blood group A, who comprised pure vegetarians. Blood type B was the third blood type developed, which emerged as a result of migration and further climate change. People with this blood type were habituated to a diet that included meat and plants as well as dairy products. The final blood type to evolve was type AB (36).

In this study, it was established that a high percentage of plants (55%) used in the human diet have the ability to cause agglutination of erythrocytes. Lectins from 40% of the plants tested had the ability to agglutinate blood group A, 40% had the ability to agglutinate blood group B, 50% had the ability to agglutinate blood group AB, and 50% had the ability to agglutinate blood group O. Nine plants did not show any lectin activities against any blood groups tested in our study. Some authors claim that the absence of lectin activity in these plants against blood groups has great evolutionary importance in the diversified tolerance development to these foods in the internal lining of the gastrointestinal tract from the mouth to the anus in humans. Studies have shown that lectins from the family Fabaceae usually cause agglutination. Three plant species, *Pisum sativum*, *Phaseolus vulgaris*, and *Glycine max* caused agglutination of erythrocytes from all blood groups. Lectins are found in abundance in le-
gume seeds. *Phaseolus vulgaris* is an herbaceous annual plant grown worldwide for its edible beans, popular in both dry and green bean forms. The commercial production of beans is well distributed worldwide. Haemagglutinating activity in the processed *Phaseolus vulgaris* and *Pisum sativum* that we tested showed high lectin activity. It can be concluded that at least some lectins in food products will survive one or both degradative processes to interact with cells, secretions, and microflora of the digestive tract resulting in, as yet unknown, functional consequences. Extracts from *Cucumis sativus* (Cucurbitaceae) caused agglutination of the AB and O blood groups. Lectins from *Musa paradisiaca* (Musaceae) did not agglutinate human erythrocytes. Extracts from the fruit pulp of the plant *Vitis vinifera* (Vitaceae) did not agglutinate erythrocytes; however, lectins extracted from grape skin caused agglutination of all blood types, as shown in one previous research study (35). The different agglutination abilities of one plant species occur because lectins are present in various plant parts in different concentrations. It is evident that most lectins are present in plant seeds and fruit, while lower concentrations are found in plant roots, stems and leaves. We observed that lectins from seeds (soy, beans and peas) agglutinated all erythrocyte blood types, and lectins from fruit agglutinated different blood types.

The subject of the lectin pathways and effects on organisms is very broad and deserves more discussion. Although lectins are identified as potential toxins, there are some lectins that are beneficial to the body. Lectins provide a diverse and an increasing number of applications. For example, the ability of lectins to bind selectively to carbohydrate residues of glycoproteins makes these proteins useful as differentiating markers to study cancer cells (37) and to characterize differentiation among stem cells (38). Several lectins are reported to possess different biological activities that include anti-bacterial (39), anti-fungal (40), anti-HIV (41), and immunomodulatory effects (42) as well as anti-proliferative and mitogenic stimulation for specific cell types. Lectins are also being studied as tools for drug delivery (43). With the numerous and increasing applications of lectins, it is imperative and relevant to identify novel plant sources of lectins.

**CONCLUSIONS**

The identification of the plant parts that have different concentrations of lectin is important from the aspect of human nutrition due to gastrointestinal problems caused by food containing a large amount of lectins and the ability of lectins to agglutinate human erythrocytes of different blood groups. A large number of plants used in food contain lectins and the largest concentration of lectin is usually found in the plant’s seeds. The proper differentiation and knowledge of plant species is important from the aspect of nutrition, and all present selection for a blood type diet.

**Authors’ contributions**

Nada Zubčević, Bachelor of Biology, Biochemistry and Physiology field designed experiments as well as collected and prepared blood samples and plant specimens. Dr. Sci. Damir Suljević and Muhamed Fočak, MSc. of Biochemistry and Physiology, conducted the analysis to determine the presence or absence of agglutination after mixing blood with the water extract of plants. Dr. Sci. Dunja Rukavina performed research in the laboratory, explored other authors’ research and compared their data with our own.

**Conflict of interest**

We do not have any conflicts of interest to report.

**REFERENCES**


and invasion of Shigellae to HT29 cells. PloS One. 6: e16231-e16231.


