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EFFECT OF LECTINS FROM SELECTED MALAYSIAN LOCAL FRUITS ON HUMAN BLOOD GROUP ANTIGENS

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Abstract: Many individuals nowadays enjoy eating various types of local fruits without realising the impact of the fruits on blood group antigens. The fruit lectin can cause damage to the mucosal surface in the gut when the lectin is incompatible with blood group antigens. Therefore, the present study aims to determine the *in vitro* plant lectins reaction from the extraction of *Artocarpus heterophyllus* Lam. (Jackfruit), *Artocarpus altilis* (Parkinson ex F.A.Zorn), Fosberg (Breadfruit), *Carica papaya* (Papaya), *Manilkara zapota* (L.) P. Royen (Sapota) and *Salacca zalacca* (Gaertn.) Voss (Salak) with specific human blood group antigens. The local fruit extract was tested with human blood groups A and B for hemagglutination assay. The agglutination titer of the hemagglutination assay results was then evaluated. The total of 3+ reactions for jackfruit was 11%, salak 4% and papaya 6% for both blood groups. The breadfruit shows the highest titer reaction, 19% against A and 16% against B. In comparison, 19% was against A for sapota but only 4% towards group B. The highest titer value of erythrocytes by crude lectins of selected local fruits on blood groups A and B was given by extraction of sapota while for blood group B on papaya. This discovery proves that plant lectins from five varieties of local fruits react with blood group antigens and that comparable interactions may occur at the mucosal surface of the gut in susceptible individuals, causing significant inflammation, swelling, and ulceration. The mechanism and effect of fruit lectin on human blood types should be investigated further.

Keywords: Breadfruit, sapota, hemagglutination assay, agglutination titer.

Introduction

Lectins are carbohydrate-binding proteins found in all living things. Lectins are also known as Phytohemagglutinins (PHA) in the plant kingdom (Lannoo & Van Damme, 2014). Lectins were found in almost all edible plants (Jain *et al*., 2022; Singh & Sarathi, 2012). Examples of edible plants reported by Barre *et al*. (2020) include lentils (*Lens culinaris*), peas (*Pisum sativum*), soybean (*Glycine max*), peanuts (*Arachis hypogaea*), horse gram (*Dolichos biflorus*), kidney bean (*Phaseolus vulgaris*), wheat (*Triticum aestivum*), garlic (*Allium sativum*), black elderberry (*Sambucus nigra*), banana (*Musa acuminata*). According to De Coninck and Van Damme (2021), lectins serve many different biological functions to plants, such as resisting bacterial, fungal and viral pathogens during growth. In other words, lectins act as a defence mechanism for plants. High levels of lectins exist in seeds, followed by roots, stems and leaves. The lectins often contain multiple binding sites that enable the lectin to interconnect with cells and cause agglutination (Ribeiro *et al.,* 2018). Plant lectins' structure can be divided into four major classes: merolectins, hololectins, chimerolectins, and superlectins (Mishra *et al*., 2019). A study by Lucius (2020) discusses lectins' potentially harmful and physiologically stimulating effects on human beings. People with IgG antibodies to wheat germ agglutinin were more likely to have elevated autoantibodies to a variety of tissue components (such as thyroid peroxidase, myelin basic protein, fibulin, or 21-hydroxylase) than those who did not have IgG antibodies to this lectin.

In addition, infectious or dietary antigens appear to play a role in triggering the development of IgA nephropathy. Furthermore, it also reported altered IgG glycosylation impact of dietary lectins is seen in other autoimmune conditions, such as Rheumatoid Arthritis, Sjogren's disease and Hashimoto's thyroiditis (Basset *et al*., 1998; Cordain, Toohey, Smith, & Hickey, 2000the nature of this relationship remains elusive. In the present review, we provide evidence for how the interaction of dietary lectins with enterocytes and lymphocytes may facilitate the translocation of both dietary and gut-derived pathogenic antigens to peripheral tissues, which in turn causes persistent peripheral antigenic stimulation. In genetically susceptible individuals, this antigenic stimulation may ultimately result in the expression of overt rheumatoid arthritis (RA; Yuan *et al*., 2015).

Dietary lectins act as protein antigens that are free to bind to the surface of erythrocytes or lymphocytes (Gemede & Ratta, 2014). Previous research proved that lectins have potent *in vivo* effects (Teixeira, 2012). Fruits and plant lectins showed different degrees of agglutination depending on the ABO blood group. The agglutination occurs when interacting with the specific blood group antigen (Zubcevic *et al*., 2016). In 1945, William Boyd discovered lectin in lima beans to agglutinate red cells of human blood type A but not O or B (Shah, 2017). Hamid *et al*. (2009) reported that long-term intake of plant lectins in rodent models shows epithelial cell necrosis in the stomach and intestine biopsies.

Furthermore, the lectins from ingested food can bind to the small intestine, known as absorptive microvilli. The surface of the lining part of the digestive system is also attached to blood group antigens (Raman *et al*., 2012). This interaction will cause harmful effects such as loss of gut epithelial cells, epithelium luminal membranes, stimulation of a shift in bacterial flora, and disruption of the digestive tract's immune system (Vasconcelos & Oliveira *et al*., 2004). A recent study by researchers identified strains of *Ruminococcus gnavus* able to produce the enzymes to break down mucin caps made from the blood group A antigen in the gastrointestinal tract (Wu *et al*., 2021). Although numerous studies have demonstrated the association between ABO blood types and diseases with possible mechanisms, other studies have not confirmed it, and a definitive conclusion cannot be reached because of inconsistent results (Abegaz *et al*., 2021).

Not all plant lectins were able to promote physiological reactions. Only some plant lectins can act as a chemical messenger that can bind to the sugars of cells in the gut, which are blood antigens and initiate inflammatory reactions. Plant lectins that are not efficiently degraded by digestive enzymes and are incompatible with antigens on the surface of gut epithelial cells can potentially be poisonous (Kumar *et al*., 2012). Besides that, 95% of dietary lectins will be destroyed during digestion, but the remaining 1% to 5% is absorbed into the bloodstream. It can trigger the destruction of red blood cells (Thakur *et al*., 2019). Ingestion of the lectins present in certain improperly cooked vegetables can result in acute gastrointestinal tract distress; a study by Miyake *et al*., 2007 revealed that cell surface-bound lectins potently inhibited plasma membrane repair and the exocytosis of mucus that normally accompanies the repair response.

In this study, five Malaysian local fruits are *Artocarpus heterophyllus* Lam. (Jackfruit), *Artocarpus altilis* (Parkinson ex F.A.Zorn), Fosberg (Breadfruit), *Carica papaya* (Papaya), *Manilkara zapota* (L.) P.Royen (Sapota) and *Salacca zalacca* (Gaertn.) Voss (Salak) were chosen. This study aims to identify the effects of plant lectins from selected local fruits on human blood group antigens. Furthermore, this information helps to facilitate which local fruits are suitable to be eaten based on blood type.

Materials and Methods

Blood Collection from Human Subjects

Blood samples were taken from twenty healthy subjects among UiTM Puncak Alam students aged 21 – 25 without psychiatric, neurological or somatic disorders, history of head injuries, lipid or carbohydrate metabolism illnesses with an average body mass index and not being treated with any drugs. Healthy participants do not use addictive substances, take antioxidant supplements, and eat a well-balanced diet (meat and vegetables). The blood sample was taken from venous blood and drawn by a phlebotomist, and qualified staff nurses from the Nursing Department of UiTM Puncak Alam. The blood samples were collected into an acid citrate dextrose (ACD) tube of about 5ml and were stored at 4ºC.

The experiments were conducted according to the ethical guidelines and approved by the Research Ethics Committee Faculty of Health Sciences, UiTM Puncak Alam, Selangor, with Ethical approval (600-FSK (PT. 5/2).

Local Fruit Materials

The selected local fruits were *Artocarpus heterophyllus* Lam. (Jackfruit), *Artocarpus altilis* (Parkinson ex F.A.Zorn), Fosberg (Breadfruit), *Carica papaya* (Papaya), *Manilkara zapota* (L.) P.Royen (Sapota) and *Salacca zalacca* (Gaertn.) Voss (Salak) was collected from Felda Bukit Cerakah, Kuala Selangor. Forest Research Institute Malaysia (FRIM) identified and confirmed all the local fruits. The fruits were thoroughly washed with tap water and then rinsed twice with distilled water.

Preparation of Extracts

This extraction method was applied based on the previous study method with modifications (Hou *et al*., 2010). The extraction was prepared by using a 0.9% sodium chloride solution. Four grams of each fruit were weighed accurately using an analytical balance. Then, directly grind up the fruits with an additional 8ml of 0.9% sodium chloride using a mortar and pestle and then centrifuge at 10,000 rpm for ten minutes to get the clear extract. The clear section of supernatant contained lectins.

Hemagglutination Assay

Hemagglutination assay was carried out according to (Qadir *et al*., 2013) with modification. Two drops of red cell suspension were mixed with two drops of fruit extraction fluid in a glass test tube. The mixture was centrifuged for 15 seconds at 3400 rpm. The red cell agglutination was observed within two minutes. A control sample containing two drops of red cell suspension and two drops of 0.9% sodium chloride was also observed. Agglutination of red blood cells indicates a result. Each reaction that gives results (agglutination) was then tested for agglutination titer.

Agglutination Titer

Agglutination titer was done to find the strength at which dilution cell clumping still occurs. It was performed by prepared dilution of fruits extraction (two drops of fruits extraction diluted 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and 1/128 with 0.9% sodium chloride) and mixed with two drops of red cell suspension. A control sample was also prepared by mixing the red cell suspension with 0.9% sodium chloride. Finally, centrifuge the mixture at 3400 rpm for 15 seconds.

Results

The outcome from the hemagglutination assay revealed that all selected local fruits showed agglutination reactions. This outcome articulates that the five selected local fruits affect the antigen of blood groups A and B (Table 1). Results showed that the total of 3+ reactions was 47 (100%). The sum of 3+ reactions for jackfruit was 5(11%), salak 2 (4%) and papaya 3 (6%) for both blood groups. Conversely, for breadfruit, between blood groups A and B was 9 (19%), 7 (16%), and for sapota, 9 (19%), 2 (4%). The highest titer value of erythrocytes by crude lectins of selected local fruits on blood groups A and B were shown in Table 2. The highest titer values for blood group A were given by extraction of sapota (32), while for blood group B was papaya (128).

Table 1: Distribution pattern of 3+ reactions by blood groups A and B

	Blood Group A	Blood Group B
Jackfruit	5(11%)	5(11%)
Breadfruit	9(19%)	7(16%)
Salak	2(4%)	2(4%)
Papaya	3(6%)	3(6%)
Sapota	9(19%)	2(4%)

Table 2: Highest titer value of erythrocytes by crude lectins of selected local fruits on blood groups A and B

Plant Name	Vascular Name	Family	Highest Titer Value	
			Blood Group A	Blood Group B
Jackfruit	*Artocarpus heterophyllus* Lam.	Moraceae	4	8
Salak	*Salacca zalacca* (Gaertn.) Voss	Palmae	2	2
Breadfruit	*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg	Moraceae	16	32
Papaya	*Carica papaya*	Caricaceae	8	128
Sapota	*Manilkara zapota* (L.) P. Royen	Sapotaceae	32	32

Discussion

Lectins have been known for a century. There were first discovered in castor beans by Stillmark in 1888 and in non-toxic plants in 1906 (Sharon, 2008). In 1945, a study by William Boyd from Boston University School of Medicine discovered that lectins could be blood group specific because some lectins can agglutinate the red cells of one type but not those of another. He found that lima bean lectin would agglutinate red cells of human blood type A but not those of O or B (Hamid & Masood, 2009). Although many studies on lectins were conducted, no investigation was carried out using local fruits in Malaysia against ABO blood groups. Therefore, it is important to investigate the effect of plant lectins from selected local fruits on human blood group antigens.

Jackfruit, breadfruit, papaya, sapota and salak are some of the favourite local fruits among Malaysians. The local fruits' extraction using 0.9% sodium chloride has been tested against blood groups A and B. The difference between blood groups A and B lies in a simple sugar unit that sticks out at the end of a carbohydrate chain of a glycoprotein or glycolipid. In blood type A, the determinant is acetyl galactosamine, while group B is galactose (Rose, Palcic, & Evans, 2005). Raman *et al*. (2012) did a similar study instead of using fruits found in India and tested against blood groups A, B, AB and O.

The outcomes for the hemagglutination assay revealed that all types of local fruits selected showed agglutination reactions. Surprisingly, no one from these local fruits is compatible with antigens from blood groups A and B. Similar findings from jackfruit, papaya and salak show an equal percentage of 3+ reactions for both blood groups, which were 11% for jackfruit, 4% for salak, and 6% for papaya. A slight difference of 3+ responses by breadfruits with 19% (blood group A) and 16% (blood group B) was observed. A big contrast of 3+ reactions by sapota represented between two blood groups, which were 19% for blood group A and 4% for blood group B. A recent review by Lucius, 2020 and a similar study by Raman *et al*. (2012) reported that most dietary lectins bind to the absorptive microvilli of the small intestine after ingestion findings show that the interactions between lectins and gut are varied between individuals.

Interestingly, the screening test of plant lectins effect by hemagglutination assay indicates the diversities of results. Based on that fact, the lectins were proteins, and the protein often has multiple binding sites. When lectins contain numerous binding sites, they can interconnect large numbers of cells, causing them to clump together or agglutinate. Each molecule of lectins has two or more regions, perhaps clefts or grooves, each of which fits a complementary molecule of sugar or several sugars (Mishra *et al*., 2019). Agglutination occurred, indicating a reaction between plant lectins and blood antigens.

By performing an agglutination titer, the strength of agglutination was carried out. A titer is a final concentration at which lectins are still giving precipitation reaction with the corresponding blood group (Gorakshakar & Ghosh, 2016). Even though all local fruits investigated were found to contain lectins, as shown in Table 1, their binding affinity and agglutination activities towards blood groups A and B are entirely different. Their titer values measured this great diversity among plant lectins against blood group antigens and lectins.

Based on the findings' outcome, Table 2 shows the strengths of the highest titer value reactions between blood groups A and B. Attention has been given to jackfruit, salak and papaya titration values. Their titration values contradict the hemagglutination assay results, similar to 3+ reactions. The highest titration value of these three controversial local fruits for blood group A were 4 (jackfruit), 2 (salak) and 8 (papaya). In like manner for blood group B, the greatest titration reading that was found were 8 (jackfruit), 2 (salak) and 128 (papaya).

A similar pattern of highest titer value from breadfruit and sapota for blood group A was 16 (breadfruit) and 32 (sapota). Compared to reactions in blood group B, the findings revealed similar highest titer values between breadfruit and sapota, which is 32. A recent study by Zubcevic *et al*. (2016) demonstrated that the lectins from beetroot caused blood type O agglutination, while those from apple caused blood type AB agglutination. On the other hand, lectins from cucumber caused blood type AB and O agglutination. While a study by Raman *et al*. (2012) came up with papaya and sapota, and his finding was the highest titer values for sapota 256 (blood group A), 1024 (blood group B) and papaya 256 (blood group A and B) which contradict with the current present study. It might be due to different species of fruits, geographical factors, and different hemagglutination assay methods.

The lectins will bind to specific sugar segments through hydrogen bonds and Van Der Waals interaction (Martinez *et al*., 2019)a lectin of biomedical interest, which is involved in different viral infections, including HIV and Ebola, and is able to recognize a variety of self- and non-self-glycans. The strategy employed allows not only screening of a mixture of compounds, but also obtaining valuable information on the specific sugar–protein interactions. The analysis of the data demonstrates that monosaccharides Fuc, Man, Glc, and Gal are able to bind DC-SIGN, although with decreasing affinity. Moreover, a new binding mode between Man moieties and DC-SIGN, which might have biological implications, is also detected for the first time. The combination of the 19F with standard proton saturation transfer difference (1H-STD-NMR. Lectins like to attach to carbohydrates, mainly sugars or glycoproteins. Many of these carbohydrates are found on the exterior walls or membranes of simple cells, constituting that living creature's outer markers or antigens (Hassan *et al*., 2020). Once bound to cells lining the digestive tract, the lectins can cause dramatic changes in the cellular morphology and metabolism of the stomach and small intestine and activate a cascade of signals that alters the intermediary metabolism (Vasconcelos & Oliveira, 2004).

The biological variation in the glycoconjugates that coat our cells and the protection by sialic acid molecules that are attached to the glycoprotein is a mechanism that protects healthy individuals from certain diseases and complications to organs late in life when they consume fruits containing lectins (Hamid & Masood, 2009). Sialic acids also protect healthy individuals from lectin action as they are a family of nine-carbon acidic monosaccharides that occur naturally at the end of sugar chains attached to the surfaces of cells and soluble proteins (Varki & Gagneux, 2012).

Scientific literature shows that in the human body, the highest concentration of sialic acid (as N-acetylneuraminic acid) occurs in the brain, where it participates as an integral part of the ganglioside structure synaptogenesis and neural transmission (Wang, 2009). But the sialic acid molecules can be stripped off by the enzyme neuraminidase, present in several microorganisms such as influenza virus and streptococci. Our genetic inheritances determine how and to what degree lectins can affect us (Hamid & Masood, 2009).

As highlighted, the effect of the plant lectins is scientifically proven *in vitro*. Almost every individual has antibodies to some dietary lectins in their bloodstream, and many food allergies are due to the immune system's reactions to these lectins. Research on lectins in food and their interaction with the gastrointestinal tract reveals a direct correlation between the evolution of blood types and food intake (Sravani, 2011). The critical point is that some plant lectins consumed in every fruit act as chemical messengers that can bind to the sugar of cells in the gut and the blood cells, initiating an inflammatory response (Lucius, 2020).

Based on the overall findings, the results presented the plant lectin from jackfruit, breadfruit, papaya, sapota, and salak caused a clumping process when tested with blood groups A and B lectins are found in a wide range of plants used in food, with the highest concentrations being found in the plant's seeds. Correct differentiation and understanding of plant species are critical from the nutrition standpoint and all present selection for a blood-type diet. Therefore, proper diet management in healthy individuals and versa should be carefully monitored. According to "The blood type diet" theory advocated by D'Adamo (D'adamo & Whitney, 2016), its fundamental premise is that ABO blood type is the most critical factor in determining a healthy diet. On the other hand, food beneficial for one blood type may be an 'enemy' to other blood types (Hamid & Masood, 2009).

Conclusion

In conclusion, this study articulated that the plant lectins from breadfruit, jackfruit, papaya, salak, and sapota gave an agglutination reaction when tested with blood groups A and B. Nevertheless, this study was done as a screening test. Therefore, it is recommended to explore the molecular mechanism further and identify the specific types of plant lectins present in each local fruit soon.

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