Studies on Lectin Mediated Agglutination Reaction on Red Blood Cell Surface Antigens Using Hot and Cold Water Plant Extracts

ABSTRACT

Lectin has various physiological roles in cell agglutination, based on their carbohydrate-binding properties, plant lectins are widely used for the detection, segregation, and characterization of glycoconjugates. Rhesus (Rh) factor is a protein that is inherited and found on the surface of red blood cells. If the surface protein is present, the RBC is Rh positive; otherwise, it is Rh-negative in nature. In this paper, we use agglutination reactions to investigate the effect of different cold and hot water extracted plants on RBC antigens as an alternative to commercial monoclonal antibodies. Extensive research on the sequence homology and 3-D structure of various plant lectins suggests that they have been conserved throughout evolution and may play important physiological roles that are still unknown.

Keywords: Antigen, Agglutination reaction, Plant Lectins, Rh factor, RBC antigen.

INTRODUCTION

Plant extracts contain lectins, which are carbohydrate-binding proteins found in the seeds of many plants, particularly corals and beans, as well as fungi, bacteria, and animals [1]. Aside from their hemagglutinating ability, they have been assigned a variety of functions and significance in immunohematology [2]. They are used to detect specific red cell antigens, to activate various types of lymphocytes, and to resolve poly agglutination issues [3]. Lectins are powerful tools for recognizing a diverse range of oligosaccharides, and they have been widely used in many fields of cell biology, biochemistry, and food technology [4-8]. These are glycoprotein domains with highly specific pockets for their counter sugar moieties of polysaccharides, glycolipids, glycoproteins, proteoglycans, and peptidoglycans found on the exterior walls or membranes of both vertebrate and invertebrate cells and microorganisms [9]. Lectins participate in biological recognition phenomena in cell-to-cell contact of all living organisms, such as binding of microorganisms to target tissues, protein sorting, control of morphogenesis, cellular differentiation, fertilisation, adhesion and trafficking of leukocytes, metastasis, and inhibition of natural killer cell activity against healthy cells [10-12]. To determine their suitability for consumption, different plant lectins were individually

tested for lectin binding activity on the ABO blood group system [13]. There are about 500 species of plants where the hemagglutinating lectins have been documented. Of the total number of plants available in the plant kingdom, it can be considered that lectins are present in different plants as an exception rather than the rule. The hemagglutinating activity of lectins is inhibited by simple sugars (monosaccharide), which represent the binding site for the lectin on the cell surface [14]. Certain plant extracts contain more than one form of lectin [15]. Lectins can be inactivated by procedures which denature or break down proteins such as heating, extremes of pH, and treatment with proteolytic enzymes such as papain or trypsin [16].

MATERIALS & METHODS

Materials

Leaf samples, distilled water, Eppendorf tubes, tissue grinders, centrifuge, anticoagulant, micro-pipettes, micro tips, glassware, Blood samples of blood types A+, A-, B+, O+, O-, AB+, and AB- from volunteers.

Preparation of extracts

Leaves of 35 different plants were collected from Green Fields, KLEF, Andhra Pradesh, India and made into smoothies [17]. 50ug of leaf sample, 1ml each of hot and 1ml of cold water were added and the extract was prepared using a tissue grinder. The prepared extracts were centrifuged and the supernatant was separated and stored separately.

Agglutination reactions on RBC agents

The blood samples from different individuals having blood groups A+, A-, B+, O+, O-, AB+ and, AB- were collected and stored by adding anticoagulant. The experiment was performed by adding 20ul each of blood sample and plant extract to the ELISA plates. The ELISA plates were placed in the mixer for 10 minutes and the results were obtained and tabulated.

RESULTS AND DISCUSSIONS:

Agglutination reaction

The results of the studies in table 1, were expressed as (P) if the sample shows an agglutination reaction and as (N) if the sample shows no agglutination reaction.

Table 1: Effect of plant extract on blood sample

S.No	Blood Type	A+		A-		B+		AB+		AB-		O+		O-	
	Original Name	CE	HE	CE	HE	CE	HE	CE	HE	CE	HE	CE	HE	CE	HE
1	Banana	N	P	P	N	P	N	P	P	N	N	N	P	P	N
2	Drumstick	P	P	N	N	P	N	P	P	N	P	P	N	N	N
3	Black gram	N	P	P	N	P	N	N	P	P	N	N	P	P	N
4	Henna	P	P	P	N	N	P	P	N	P	P	N	P	N	P
5	Night flowering	P	P	N	N	P	N	P	P	N	N	P	P	P	N
6	Jasmine	N	P	N	N	P	P	N	N	N	P	P	N	P	N
7	Bottle guard	P	N	P	P	N	N	P	N	N	N	P	P	N	P
8	Holy basil	P	N	P	P	P	N	P	P	P	N	N	P	N	P
9	Basil	P	P	N	N	P	P	N	P	N	P	P	N	P	N
10	Yellow cucumber	N	P	P	P	P	N	N	P	N	P	N	P	P	N
11	Madonna lily	P	N	N	N	N	P	P	N	N	P	P	P	N	P
12	Marigold leaf	N	P	P	P	P	N	P	P	N	P	N	N	P	P
13	Tomato	P	P	N	N	P	P	N	P	P	N	P	N	P	N
14	Snake gourd	P	N	P	P	N	N	P	P	N	P	P	N	P	P
15	Lesser mallow	N	P	P	N	N	P	P	P	P	P	P	N	N	P
16	Ridge gourd	P	P	N	P	N	P	P	N	P	P	N	P	P	N
17	Brinjal	P	P	N	P	N	P	N	N	N	P	P	N	P	N
18	Bitter gourd	P	N	N	P	P	N	P	P	P	N	N	P	P	P
19	Periwinkle	P	P	P	P	N	P	N	N	P	P	N	P	N	P
20	Winter jasmine	N	P	P	P	N	N	N	P	P	N	N	P	P	N
21	Aloe Vera	P	N	N	N	P	P	P	N	P	N	N	P	N	N
22	Apple of Sodom	N	P	P	N	N	P	P	N	P	P	N	N	N	P
23	Guava	P	P	N	P	N	P	N	P	P	P	N	P	P	P
24	Hibiscus	N	P	P	N	N	P	P	P	P	N	P	N	P	N
25	Pomegranate	N	P	P	P	N	P	N	P	P	N	P	P	N	N
26	Neem	P	P	N	N	P	P	N	P	N	P	N	N	P	N
27	Money plant	P	P	N	P	N	P	P	P	P	P	P	N	N	P
28	Soap nut	P	N	N	P	P	N	P	P	N	N	P	P	P	P
29	Rudraksha	P	N	N	N	P	P	N	P	P	N	P	N	P	P
30	Jackfruit	N	P	P	N	N	P	P	N	P	N	P	N	P	P

31	Canon ball flower	N	P	N	P	P	N	N	P	N	N	P	N	P	N
32	Black plum	N	P	P	N	N	P	P	N	P	N	P	N	P	N
33	Mango	P	P	N	N	N	P	P	N	P	N	P	P	N	P
34	Custard apple	N	P	P	N	N	P	P	P	N	P	P	N	P	P
35	Butterfly pea	N	N	P	P	N	N	P	P	N	N	P	P	N	P

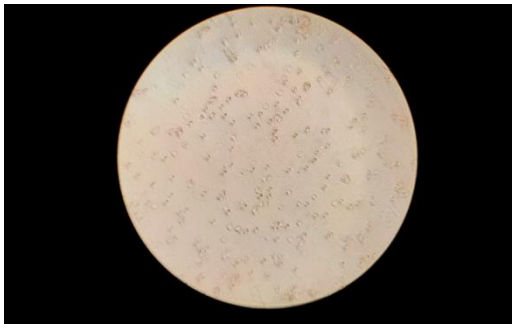

Plate I: Henna (*Lawsonia inermis*)

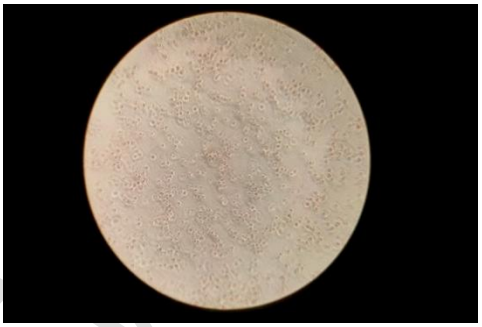

Plate II: Neem (*Azadirachta indica*)

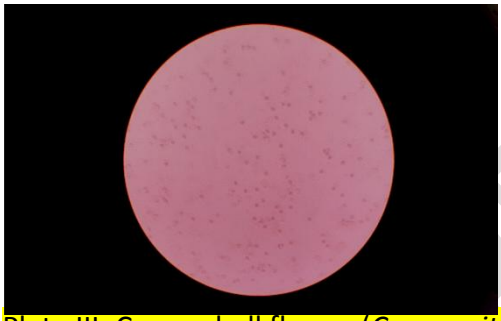

Plate III: Cannonball flower (*Couroupita guianensis*)

Plate IV: Soapnut (*Sapindus trifoliatus*)

Blood cross-matching was done to verify our results. Plate I shows, Henna (*Lawsonia inermis*) for B+ve blood sample with a cold water sample and O+ve serum sample, are compatible. Plate II shows, Neem (*Azadirachta indica*) for AB-ve blood sample with a cold water sample and O+ve serum sample, they are compatible. Plate III shows, Cannonball flower (*Couroupita guianensis*) for the B+ve blood sample and the O+ve serum sample, they are compatible. Plate IV shows, Soapnut (*Sapindus trifoliatus*) for the A-ve blood sample with a cold water sample and O+ve serum sample, are incompatible. In our experiments about four plants were not shown any lectin activities against any one of the blood groups tested. Authors opined that these plants have long standing relationship with human blood cells and during the evolution, body weight be slowly energized due to the above foods.

CONCLUSION

Most of the plants investigated and their seeds, leaves, and other parts are being using as ingredients for making curries, pickles, main components of folklore homemade medicine, cocktail homeopathic drugs designed for oral routes only. Author assuming that, this report showing a direct significant correlation with no lectin activity in these plants against all blood groups tested and has great evolutionary significance in the diversified tolerance development to this food. It is concluded that most of the plant varieties have shown a positive result for the B+ blood group (31 positive results). Followed by AB+ blood group (27 positive results), 0+ and 0- blood groups (26 positive results), AB- blood group (23 positive results), A- blood group (20 positive results), and A+ blood group (16 positive results).

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