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**RESEARCH ARTICLE**

# Biological Activity of Purified *Momardica Charantia* Lectin

AJAY PRATAP SINGH* and K. D. SAXENA

Department of Chemistry, Bareilly College, Bareilly-243001(U.P), India
*ajayojas@yahoo.co.in*

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**Abstract**: *Momardica Charantia* L. Var. Jhalri (MCJ) is the member of lectins from family Cucurbitaceae. Haemagglutination of purified MCJ lectin was carried out by titration assay with human and animals *viz.* rabbit, sheep, goat, pig, buffalo and dog erythrocytes. The lectin showed positive haemagglutination test for trypsin treated and non-treated erythrocytes of human blood group 'O' and rabbit both. Trypsin treated erythrocytes of human blood group 'O' showed the enhanced agglutination while that of rabbit, reduced agglutination. The haemagglutination units(HUs) for untreated and trypsin treated erythrocytes in case of human blood group 'O' were found to be 0.043 µg/mL and 0.0107 µg/mL while in case of rabbit erythrocytes these were 0.0215 µg/mL and 0.175 µg/mL respectively.

**Keywords:** Lectin, Haemagglutination, Erythrocytes, Trypsin, Titration

## Introduction

Plant lectins are a class of proteins of non-immune origin that possess at least one non-catalytic domain that specifically and reversibly bind to mono and oligosaccharides[1-2]. Lectins are widely distributed in nature. Plant lectins have been isolated from all parts of plant but largely from seeds. Besides plants they have been found in bacteria, fungi, snails and even mammals. These proteins can agglutinate cells and combine with glycocomponents of the cell surface causing a number of biological properties[3]. The biological activity of lectins was first recognized by their ability to agglutinate red blood cells[4]. Agglutination reaction is a kind of antigen-antibody interaction where there is a specific antibody to an antigen present on surface of cells. The agglutination of erythrocytes (antigen) by an antibody (lectin in this case) is termed as haemagglutination. Lectins are multivalent in nature and therefore they may be able to form bridges between different carbohydrate chains on the surface of cells. This causes RBC's to come closer resulting in the formation of visible clumps *i.e.* agglutination. Because of this activity of lectins they are also known as phytohaemagglutinins.

Agglutination is a complex process influenced by the nature of the lectin, the number and structure of surface receptors and cell membrane factors[5-6] and temperature[7]. In addition to possible effects on the mobility of membrane receptors the temperature determines the extent to which cells can internalize lectin[8-9]. MCJ lectin was isolated from *Momardica charantia* L. var. Jhalri and purified by ion exchange chromatography[10]. In the present work the haemagglutination was carried out at room temperature by titration assay[11].

## Experimental

Phosphate buffered saline (PBS) pH 7.4 was purchased from Bangalore Genei Pvt. Ltd., Alsever's solution (Anticoagulant) was prepared by mixing 2.05 g dextrose (s.d. fine-chem Pvt.Ltd.), 0.80 g sodium citrate (CDH Pvt. Ltd)., 0.42 g sodium chloride (Qualigens Pvt. Ltd.) and 100 mL distilled water at pH 6.1 with 10%v/v citric acid. The human erythrocytes A, B, AB and O were obtained from healthy blood donors. The bloods were also provided by Gangasheel blood bank, Bareilly. The animal erythrocytes of rabbit, sheep, goat, pig, buffalo and dog were used. For this purpose the bloods of these animals were collected from Indian Veterinary Research Institute, Izzatnagar and Bareilly. Trypsine (Biogenic, USA) was a kind gift from Dr. Praveen Singh IVRI Bareilly. All other reagents and chemicals used were of highest analytical grade and obtained from local sources.

### *Preparation of erythrocytes*

Fresh human and animal bloods were collected into one half of its volume of Alsever's solution. The erythrocytes in Alsever's solution were centrifuged (Remi T8C, Bombay) at 800 g for 10 min. The supernatant and 'buffy coat' of white cells, which formed a layer on top of the packed erythrocytes, were removed using a micropipette. The cells of each type blood were resuspended in 2 volumes of PBS (pH 7.4) and were washed. After washing each of the cells was diluted to 2% v/v in PBS for haemagglutination assay.

### *Preparation of trypsinised erythrocytes*

Washed erythrocytes were suspended in PBS at a concentration of 4% v/v. One volume of trypsin solution was added to 100 volume of the diluted cell suspension and incubated at 37 ˚C for 1 h. After centrifugation the trypsinised cell were washed in PBS. The washed cells were resuspended at a concentration of 2% v/v in PBS for agglutination assay.

### *Two fold serial dilution of MCJ lectin solution (Antibody)*

The twofold serial dilution[12] of MCJ lectin was performed in microtitre plate (96 wells U-Bottom, Axygen Scientific Pvt. Ltd. Delhi). The guiding principle of this dilution is the fact that each well/tube contains half the antibody and half diluent (PBS, pH 7.4).

### *Titration of MCJ lectin solution (Antibody) against erythrocytes (Antigen)*

The titration was performed at 32 ˚C. For this purpose, 100 µL of 2% (v/v) suspension of $O^+$ type human RBC's in PBS were added to each well of row A and B [Figure 1]. 100 µL of 2% (v/v) suspension of $O^+$ type trypsin treated RBC's were added to each well of row C and D. Similarly, 100 µL of 2% (v/v) suspension of rabbit RBC's were added to each well of rows E and F and that of trypsin treated in wells of rows G and H. In two other microtitre plates, using the same procedure, the titration were carried out for MCJ lectin against untreated and trypsin treated erythrocytes of human blood groups A, B, AB and that of sheep, goat, pig, dog and buffalo in the wells of rows A, B, C, D, E, F, G and H respectively. Each microtitre plate was carefully covered with aluminium foil and left for 1 h at present room temperature (32 ˚C).

Agglutination of erythrocytes by lectin (antibody) was indicated by a complete carpet of cells covering the bottom of the well in microtitre plate while non-agglutinated cells formed a compact button or ring at the center of the curved well[13]. The end point of titration was taken as an even carpet of cells with a ring at the edge. The titre was recorded as the dilution of the lectin at the end point or the reciprocal of this dilution[14]. A haemagglutination unit (H.U.) was defined as the minimum amount of the lectin capable of inducing agglutination[15].

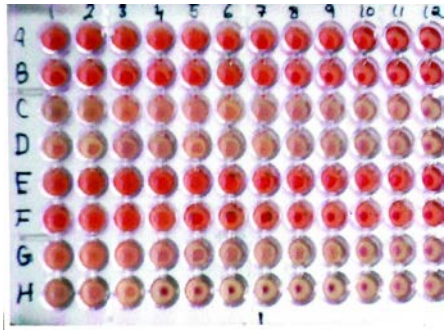

**Figure 1**. Representation of haemagglutination assay of erythrocytes of human blood group 'O' and rabbit, ROWS **A** and **B**: Untreated erythrocytes of human blood group 'O'. ROWS **C** and **D:** Trypsin treated erythrocytes of human blood group'O'. ROWS **E** and **F**: Untreated erythrocytes of rabbit. ROWS **G** and **H**: Trypsin treated erythrocytes of rabbit

## Results and Discussion

Haemagglutination assay of lectin was performed with human and animal erythrocytes. RBC's have been widely used in agglutination tests because they are readily obtained and easily visualized due to their red colour. Their surfaces carry a range of different of types carbohydrates units. The negative charge of human erythrocytes is largely due to the sialic residues[16]. The repulsive force due to this charge makes them stable. It was observed from haemagglutination assay that MCJ lectin was successful in agglutinating both untreated and trypsin treated erythrocytes of human blood group 'O' and that of rabbit among the animals (Table 1). MCJ lectin showed no agglutination for untreated and trypsin treated erythrocytes of human blood groups A, B and AB and that of other animals *viz*. sheep, goat, pig, dog and buffalo (Table 2). The trypsin treated erythrocytes of human blood group 'O' showed enhanced agglutination. Probably it is because that trypsinisation of RBC's removes the sialoglycopeptide of the cells. Thus the negative charge on the surface of the cells is demolished to a large extent, which causes a decrease in repulsive force between the cells and hence increases in agglutination. On the other hand the erythrocytes of rabbit unexpectedly showed the reduced agglutination than their untreated erythrocytes. The reason for this phenomenon could be not understood. The titter values (Table 3) of untreated and trypsin treated erythrocytes in case of human blood group 'O' were '4096' and '16384' while in case of rabbit these were '8192' and '1024' .

**Table 1.** Experimental results for the agglutination of human erythrocytes by MCJ lectin

| S. No. | Human erythrocytes | Treatment of erythrocytes | Agglutination |
|---|---|---|---|
| 1. | A | Untreated | - |
| | | Trypsin treated | - |
| 2. | B | Untreated | - |
| | | Trypsin treated | - |
| 3. | AB | Untreated | - |
| | | Trypsin treated | - |
| 4. | O | Untreated | + + |
| | | Trypsin treated | + + + |

*Note: (-): No agglutination. (++): Normal agglutination. (+++): Enhanced agglutination*

**Table 2.** Experimental results for the agglutination of animal erythrocytes by MCJ lectin

| S. No. | Animal erythrocytes | Treatment of erythrocytes | Agglutination |
|---|---|---|---|
| 1. | Rabbit | Untreated | + + |
| | | Trypsin treated | + |
| 2. | Sheep | Untreated | - |
| | | Trypsin treated | - |
| 3. | Goat | Untreated | - |
| | | Trypsin treated | - |
| 4. | Pig | Untreated | - |
| | | Trypsin treated | - |
| 5. | Dog | Untreated | - |
| | | Trypsin treated | - |
| 6. | Buffalo | Untreated | - |
| | | Trypsin treated | - |

*Note: (-): No agglutination. (+): Reduced agglutination. (++): Normal agglutination*

**Table 3.** Experimental data for haemagglutination assay of MCJ lectin against human and animal erythrocytes

| S. No. | Type of erythrocytes | Treatment of erythrocytes | Titre value | Haemagglutination unit (H.U) µg/mL | Agglutination zones in terms of titre |
|---|---|---|---|---|---|
| 1 | Human blood group O | Untreated | 4096 | 0.043 | (1:4) to (1:4096) |
| | | Trypsin treated | 16384 | 0.0107 | (1:2) to (1:16384) |
| 2 | Rabbit | Untreated | 8192 | 0.0215 | (1:8) to (1:8192) |
| | | Trypsin treated | 1024 | 0.175 | (1:4) to (1:1024) |

The haemagglutination units *i.e*. the minimum concentration of antibody (MCJ lectin) at the titre value to show visible agglutination for untreated and trypsin treated erythrocytes in case of human blood group 'O' were found to be 0.043 µg/mL and 0.0107 µg/mL while in case of rabbit erythrocytes these were 0.0215 µg/mL and 0.175 µg/mL respectively.

Agglutination zones (regions with visible agglutination) for untreated and trypsin treated erythrocytes in case of human blood group 'O' were from dilution (1:4) to dilution (1: 4096) and from dilution (1:2) to dilution (1: 16384) respectively. While in case of rabbit these zones were from dilution (1:8) to dilution (1: 8192) and from dilution (1:4) to dilution (1:1024) respectively.

## Conclusion

The experimental study preludes that MCJ lectin from cucurbitaceae family potentially agglutinates both trypsinised and non-trypsinised specific erythrocytes of human and that of rabbit. Therefore MCJ lectin is a type of phytohaemagglutinin. Furthermore haemagglutination activity of MCJ lectin is affected by trypsinisation of erythrocytes.

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