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IMMUNOMODULATORY ROLE OF LECTIN FROM *PHASEOLUS VULGARIS* (RAJMA BEANS/ RED KIDNEY BEANS)

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ABSTRACT

The seed of rajma beans (red kidney beans) were taken and extract was prepared. This extract was used and the amount of protein present in the sample was estimated by protein estimation by Bradford reagent. Hemagglutination test was performed on human blood [all four blood groups A, B, AB and O and blood group B showed highest agglutination. Hemagglutination inhibition was done using the extract and dextrose, maltose, arabinose and lactose was used as substrates. The physicochemical properties of the extract at different pH and temperature were evaluated. Human lymphocyte was isolated and cultured. The extract was added to the cell culture at different concentrations and incubated for 24 hours. Finally the extract showed immunosuppressive activity on the human lymphocyte. The extract was further added on cultured HEL 92.1.7 and observed for cytotoxicity. The extract showed greater cytotoxicity towards HEL 92.1.7 cell line compared to human lymphocyte cell culture.

KEYWORDS: ***Phaseolus vulgaris*, immunomodulation, lectin, HEL 92.1.7, human lymphocyte**

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INTRODUCTION

Lectins are carbohydrate binding proteins present in most of the plants and in some animals[1]. The word Lectins is derived from Latin-'Legree' = to pick or select. Lectins are mainly made-up of carbohydrate binding proteins or glycoproteins of non-immune origin which binds the cells or precipitates, glycoconjugates or sometimes they are found to be showing both properties i.e precipitation as well as glycol-conjugation[2]. Structurally, these lectins have a diverse class of proteins, which have the ability to bind carbohydrates with considerable specificity[3]. The binding of Lectins is reversible and non covalent with simple or complex carbohydrate conjugates, whether free in solution or on cell surfaces. Lectins do not cause any antigenic stimulation within the immune system but they have the basic capacity to bind analogously to an antibody[1]. They possess the ability to aggregate immunoglobulin's, to trigger the alternative complement pathway, to inhibit fungal growth and also to induce histamine release from basophilic and mast cells. The specificity of lectins is generally based on hapten inhibition test in which various sugars will be tested for their capacity to inhibit hemagglutination of erythrocytes. All lectin molecules posses two or more carbohydrate binding sites, a property which is essential for their ability to agglutinate cells or to react with complex carbohydrates. Lectins were identified almost a century ago. Initially they were discovered in castor bean by a scientist in the year of 1888 named as Stillmark[4].Boyd, in 1945 found that some of the lectins were blood type specific. Lectins are essential and ubiquitous plant constituents. Over 70 different seed Lectins have been identified in various legumes. Among other possible functions, they are responsible for innate immunity and defense mechanisms in plants and interactions with symbionts. The best-characterized family of plant lectins is the Leguminosae. This family includes lectins such as ConA, soybean agglutinin, and lentil lectin[5]. The main objective of the research was to isolate and study the lectin found in Red kidney bean and to observe if it has any role in immunomodulation,either proliferative or suppressive on human lymphocyte and human erythroleukemia cell line (HEL 92.1.7 cell lines)in in-vitro condition

MATERIALS AND METHODS

Material

Phaseolus vulgaris [red kidney bean],also known as 'rajma' was obtained from the local market of Bangalore, Karnataka, India. Chemicals used include MTT dye, LSM(Hisep$^{TM)}$, HEPES, PBS, RPMI1640, PHA, Streptomycin, SodiumAzide, Sodium Hydroxide, Dextrose, Lactose, β-Mercaptoethanol, Ammonium Persulfate, Ethanol [Jiangsu Huoxi international Trade co. Ltd.]Sucrose, HCl, Maltose, Methanol, Potassium Chloride from Rankem and Ammonium sulphate [NICE chemical Pvt. Ltd.] bought from local suppliers. Human blood of different blood groups [A,B,AB,O] were obtained from volunteers. Cancer Cell Line Hel92.1.7 was provided by Skanda Life Sciences Pvt Ltd, Bangalore.

(ii). Isolation and extraction of red kidney bean lectin

Soxhlet extractions [6] with methanol were carried out. Twenty (20) g of each sample was weighed into 300 ml of the methanol & extraction for 36 hours. After extraction, the concentrated residues weighed before and after to determine the yield of the active fractions. The yield was around 12.36%.

(iii) Thin-Layer Chromatography (TLC) for extracts

Thin-layer chromatography (TLC)[7] is a chromatographic technique that is useful for separating organic compounds. Because of the simplicity and rapidity of TLC, it is often used to monitor the progress of organic reactions and to check the purity of products

(iv) Estimation of protein by Bradford reagent

The standard protocol can be performed in three different formats, 5 ml or 1 ml cuvette assay and a 250 µl micro plate assay. The 1x dye reagent was removed from 4°C storage and left to warm to an ambient temperature. For the diluents, the same buffer is used as in the samples. The sample was assayed in triplicate. Pipette each standard and unknown sample solution into micro plate wells and added the 1x dye reagent to each well, mixed it, and incubated it for 5 minutes. Absorbance was measured at 590nm in Tecan plate reader[8].

(v) Preparation of erythrocyte suspension

Self donated Human blood in EDTA was obtained. Blood from chicken was collected from slaughter house. Blood samples from humans and animals were centrifuged (10,000 rpm, 10 minutes). The serums over the packed erythrocytes were removed by aspiration. The cells were washed thrice with saline 0.9% (w/v). Finally, a 5% (v/v) suspension was made in PBS for use in hemagglutination assay.

(vi) Hemagglutination Assay

The hemagglutination assay[9, 10] was performed in 96 well polystyrene U bottomed microtitre plates. To serially diluted agglutinin solution (100µl) in PBS (0.1 M, pH 7.2) 100 µL (equal volume) of 5% suspension of washed erythrocytes was added and the plate after gentle shaking was kept at 37°c for 1 hr. Hemagglutinin was examined visually and reciprocal of the maximum dilution of the agglutinin solution showing hemagglutination was recorded as titer. Control was prepared using only PBS and erythrocytes suspension. Experiment was conducted in triplets and mean value was taken.

(vii) Study of chemical components of extract by HPLC

High-performance liquid chromatography (formerly referred to as high-pressure liquid chromatography), HPLC, is a chromatographic technique used to separate the components in a mixture, to identify each component, and to quantify each component. The sample prepared was sent to Skanda Biotech Pvt Limited, Bangalore, India for HPLC analysis.

(viii) Study of Physicochemical properties of the lectin

pH stability

The stability of lectin in different pH was found out. For this PBS buffer of different pH ranging from pH 1 to pH 14 was prepared. 100µl of agglutinin sample was serially diluted in PBS having different pH and hemagglutination was performed as described earlier[11].

Thermal stability

The agglutinin in PBS (pH 7.2) was incubated separately in water bath from 10°c-100°c for 1hr. At each specified temperature, aliquots (100µl) was withdrawn, cooled and hemagglutination was performed as described earlier [12].

(ix) Hemagglutination inhibition assay

The hemagglutination inhibition-inhibition assay[9] was performed as follows in the same type of assay plate as used in the hemagglutination study. All inhibitors (Dextrose, Lactose, and Maltose& Arabinose) were dissolved in 0.9% saline at a concentration range of 0.5mg/ml t0 10 mg/ml. To serially diluted agglutinin solution (100µl) in PBS (0.1 M, pH 7.2), equal volume (100 µl) of inhibitor solution was added and the plates were left for incubation for 1 hr at room temperature.100 µl of human erythrocytes (5% v/v) suspension were added to each well and allowed to incubate for 1 hr at room temperature, the degree of hemagglutination was examined and the maximum dilution of the agglutinin solution showing inhibition was recorded. The controls were set up with PBS, sugar and erythrocytes. Experiment was conducted in triplets and mean value was taken.

(x)Isolation of human peripheral blood mononuclear cells (Lymphocytes)[13]

Blood from healthy individual was taken in EDTA layered on LSM solution and centrifuged at 1800 rpm for 30 minutes at room temperature. The PBMC were collected from the interface, washed thrice with RPMI-1640, depleted of contaminating erythrocytes. After washing, the cells were suspended in RPMI-1640 supplemented with fetal bovine serum (10%), HEPES (25Mm), penicillin-streptomycin (100µg/ml) and 0.5% fungizone[14]. Cellular viability in all experiments was assayed by the trypan blue exclusion test. The percentage of cells that excluded the dye, representing the viable population, was enumerated in a haemocytometer[15].

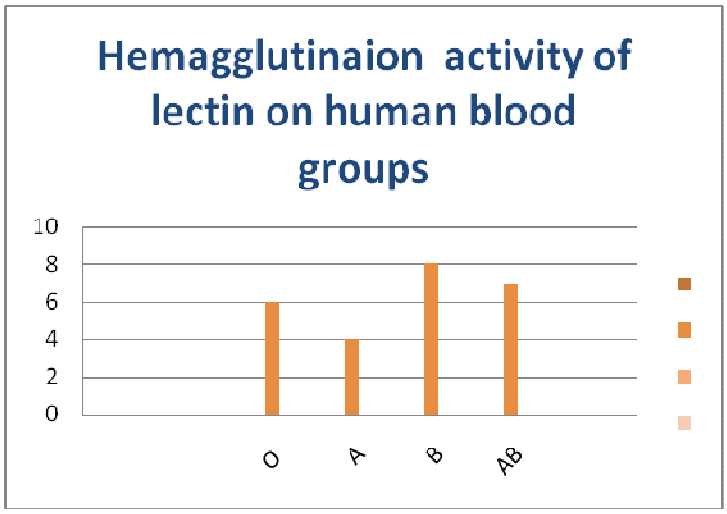
(xi) Study of immunomodulatory role of lectin on Human erythroLeukemia cells (HEL 92.1.7 cell lines) and on normal human lymphocyte

70-80% confluent cell line (HEL 92.1.7) was trypsinized and were checked for the viability[16]. For this the cells were centrifuged after quick trypsinization. Cells were washed and resuspended in media and were seeded 50,000 cells / well of HEL 92.1.7in a 96 well plate and incubated for 24 hrs at 37^{o}C, 5 % CO_2 incubator. Compounds were tested from 10, 50, and 100µg/well in RPMI media without FBS &were incubated for 24 hr. After incubation with compounds, the media was removed from the wells and fresh plain media [100µl] was added. After that 100µl/well (50 µg /well) of the MTT (5 mg/10ml of MTT 1X PBS was added to each well. The solution was filtered through a 0.2 µ m filter and stored at 2–8 °C for frequent use or frozen for extended periods. Working solution was added and incubated for 3-4 hours. After incubation with MTT reagent, the media is removed from the wells and add 100 µl of DMSO was added per well to rapidly solubilise the formazan**.** Absorbance was measured at 590 nm. For comparative analysis with the cancer cell line the same experiment was carried out with human lymphocytes as above. Experiment was conducted in triplets, mean value of the three was taken.

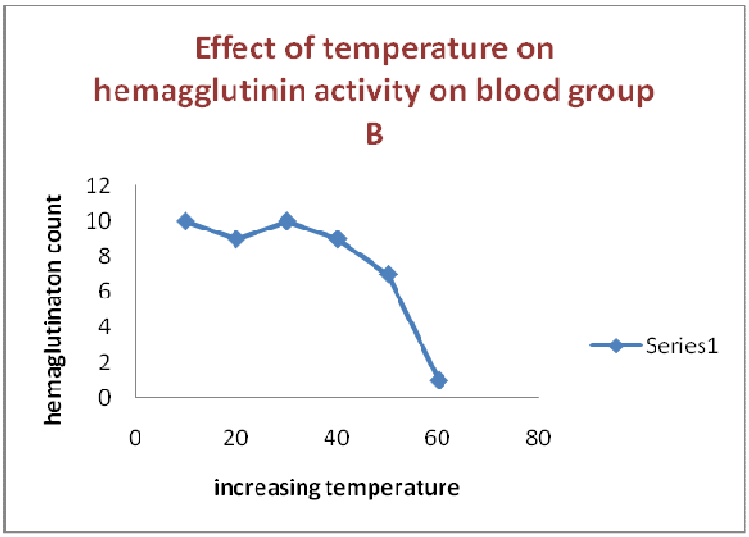
RESULTS

The Seed extract of red kidney beans had shown maximum agglutination activity with RBC of Blood Group 'B'. The decreasing amount of agglutination pattern was found to be as B>AB>O>A (Graph 1).The seed lectin was found to be active and stable between the pH range of 1 to 13 and maximum activity was found to be in between pH 2 to pH 12 (Graph 2).The seed lectin was found to be active up to 50°c temperature and maximum activity was found to be in between 10°c and 30°c (Graph 3).Partial hemagglutination inhibition with arabinose (6mg/ml) was observed with seed the extract where as inhibition was not observed in case of Maltose. Agglutination inhibition was found less for dextrose and lactose (Table 1,2,3,4). In kidney bean the protein concentration was estimated to be 108 µg/ml. HPLC analysis of sample showed peak against lectin along with few other minor impurities (Fig 1). This peak matches to TLC band (lectin) (Fig 2).The cytotoxic assay (MTT) has demonstrated that *Phaseolus vulgaris* crude extract exhibits considerable anti proliferative activity against cancer cell line HEL 92.1.7 while it has shown comparatively lower anti proliferative activity towards normal human cells (Graph 4, Table 5).

Graph 1
Showing hemagglutination activity of lectin isolated from Kidney bean on different blood groups.



Graph 2
Effect of temperature on hemagglutination activity of crude lectin extract on blood group B.



Graph 3
Showing effect of pH on hemagglutination activity (Blood Group B) of lectin isolated from Kidney Bean.

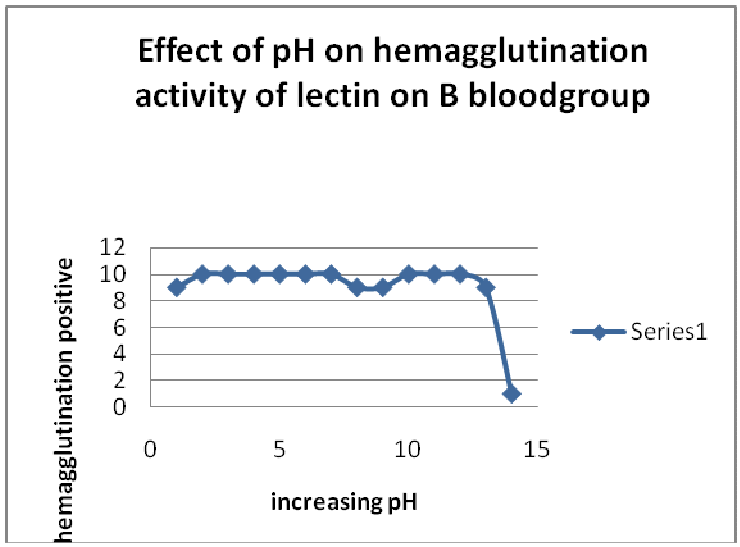


Table 1

Showing hemagglutination inhibition (Blood group B) of lectin isolated from Kidney bean on Dextrose. *Results on the basis of three replicates/treatment

Dilution→ Dextrose(mg/ml)↓	0:0	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
0.5	-	+	+	+	+	+	+	+	+	-
1.0	-	-	-	-	+	+	+	+	+	-
2.0	-	+	+	+	+	+	+	+	+	-
3.0	-	+	+	+	+	+	+	+	+	-
4.0	-	+	+	+	+	+	+	+	+	-
5.0	-	+	+	+	+	+	+	+	+	-
6.0	-	+	+	+	+	+	+	+	-	-
7.0	-	+	+	+	+	+	+	+	-	-
8.0	-	+	+	+	+	+	+	+	-	-
9.0	-	+	+	+	+	+	+	+	-	-
10.0	-	+	+	+	+	+	+	+	-	-

Table 2

Showing hemagglutination inhibition (Blood group B) of lectin isolated from Kidney bean on Lactose. *Results on the basis of three replicates/treatment.

Dilution→ Lactose(mg/ml)↓	0:0	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
0.5	-	+	+	+	+	+	+	+	+	+
1.0	-	-	-	-	+	+	+	+	+	+
2.0	-	+	+	+	+	+	+	+	+	+
3.0	-	+	+	+	+	+	+	+	+	-
4.0	-	+	+	+	+	+	+	+	+	-
5.0	-	+	+	+	+	+	+	+	+	-
6.0	-	+	+	+	+	+	+	+	+	+
7.0	-	+	+	+	+	+	+	+	+	+
8.0	-	+	+	+	+	+	+	+	+	+
9.0	-	+	+	+	+	+	+	+	+	+
10.0	-	+	+	+	+	+	+	+	+	+

Table 3

Showing hemagglutination inhibition (Blood group B) of lectin isolated from Kidney bean on Maltose. *Results on the basis of three replicates/treatment

Dilution→ Maltose(mg/ml)↓	0:0	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
0.5	-	+	+	+	+	+	+	+	+	+
1.0	-	-	-	-	+	+	+	+	+	+
2.0	-	+	+	+	+	+	+	+	+	+
3.0	-	+	+	+	+	+	+	+	+	+
4.0	-	+	+	+	+	+	+	+	+	+
5.0	-	+	+	+	+	+	+	+	+	+
6.0	-	+	+	+	+	+	+	+	+	+
7.0	-	+	+	+	+	+	+	+	+	+
8.0	-	+	+	+	+	+	+	+	+	+
9.0	-	+	+	+	+	+	+	+	+	+
10.0	-	+	+	+	+	+	+	+	+	+

Table 4

Showing hemagglutination inhibition (Blood group B) of lectin isolated from Kidney bean on Arabinose. *Results on the basis of three replicates/treatment

Dilution→ Arabinose(mg/ml)↓	0:0	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
0.5	-	+	+	+	+	+	+	+	+	-
1.0	-	-	-	-	+	+	+	+	-	-
2.0	-	+	+	+	+	+	+	+	-	-
3.0	-	+	+	+	+	+	+	+	-	-
4.0	-	+	+	+	+	+	+	+	-	-
5.0	-	+	+	+	+	+	+	+	-	-
6.0	-	+	+	+	+	+	+	+	-	-
7.0	-	+	+	+	+	+	+	-	-	-
8.0	-	+	+	+	+	+	+	-	-	-
9.0	-	+	+	+	+	+	+	-	-	-
10.0	-	+	+	+	+	+	+	-	-	-

Table 5

Cytotoxicity assay using HEL 92.1.7 & Human lymphocyte. *Results on the basis of three replicates/treatment. [Name of Cell Line: HEL 92.1.7, Passage No: 19 & Human lymphocytes. No of Cells Seeded: 50,000 cells/well (Both cells), Incubation Time: 24 hours, Vehicle Control: 1% DMSO used to solubilise Kidney bean extracts]

Cytotoxicity assay (MTT) using HEL 92.1.7 & Human Lymphocytes				
Cell line/ Cell culture	**Test material**	**Conc. □g/ml**	**Absorbance at 590***	**% inhibition***
HEL 92.1.7	Control	--	0.61	--
	Vehicle Control	--	0.58	--
	Kidney bean extract	10	0.50	13.79
		50	0.39	32.75
		100	0.29	50.01
Human Lymphocyte	Vehicle control	--	0.62	--
	Kidney bean extract	10	0.60	3.22
		50	0.56	9.67
		100	0.52	16.12

Graph 3

Comparative analysis of cytotoxicity in HEL 92.1.7 cell line and human lymphocyte using 96 well plate, each well containing 50,000 cells using tecan plate reader. Results on the basis of three replicates/treatment

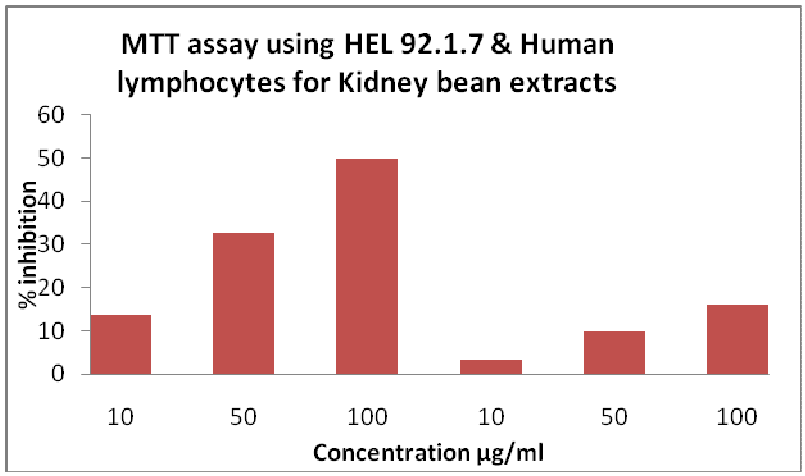


Figure 1

HPLC study result. The HPLC peak has desired compound along with the merged impurities

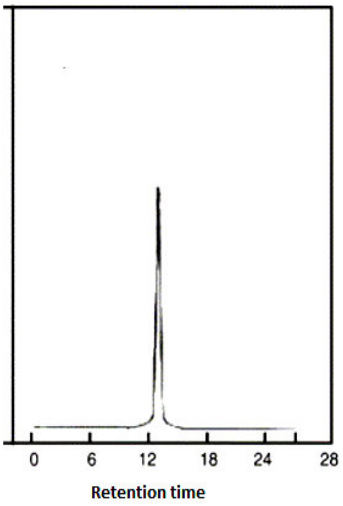


Figure 2
TLC of Red Kidney Bean Extracts

DISCUSSION

Natural products play an important role in the field of new drugs research and development. Knowledge of plant biologically active compounds and their mechanism (s) of action are desirable, not only for the discovery of novel therapeutic agents that would validate folkloric remedies, but also for the design of new active molecules or modification of current drugs against diverse maladies. One important application area is the immunotherapy; plant products have been shown to modulate the immune system. This study provides compelling evidence for an immunosuppressive effect of red kidney bean extract. The crude extract prepared contained lectin was proved by its display of hemagglutination activity shown towards different blood groups. The extract prepared showed maximum hemagglutination activity toward blood group B positive (B>AB>O>A) (graph1).The extract prepared containing lectin proved to be thermostable with maximum activity between 10°c and 30°c and stable up to 50°c (graph2). Hemagglutination inhibition study with simple sugars was done, partial inhibition with Arabinose (6mg/ml) was observed with the Seed sample (table4). No inhibition was observed in Maltose (table 3).This indicates that the lectin is not specific for simple sugars and is complex in nature. HPLC data of the extract supplied shows peak for lectin but also indicates the presence of other impurities in the sample (fig1).The peak corresponds with the TLC data of lectin (fig2).The cytotoxicity of the extract on HEL 92.1.7 cell line was examined where the extract with different concentration showed increased cytotoxicity and proved to play an immunosuppressive role (table 5).In a comparative study, 10µl,50µl and 100µl of the extract was added to both HEL 92.1.7 cell line culture and normal human lymphocyte culture and incubated, cell viability was observed by MTT assay. Here at 100µl the extract showed 50% inhibition of tumour growth when compared to the untreated control. In case of normal lymphocyte at 100 µl, 16.12% inhibition was observed. This comparative study was done using 96 well plate and Tecan plate reader using MTT assay. The study showed the extract containing the lectin is a more effective cytotoxic agent towards the cancer cell line than normal lymphocyte cells.

CONCLUSION

This investigation reveals that crude extract of red kidney bean [rajma] showed cytotoxic activity towards both human lymphocyte cells and tumor cells HEL 92.1.7 cell line. The experimental results demonstrated that *Phaseolus vulgaris* lectin crude extract has a remarkable immunomodulatory effect on cultured lymphocyte cells, which suggested that it could be useful for further study as an immunomodulating agent. Further investigation of its immunomodulatory properties on different tumor cells and on its chemical components is needed.

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CONFLICT OF INTEREST
None.

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