ISOLATION AND CHARACTERIZATION OF LECTINS

FROM *RICINUS COMMUNIS*

THESIS SUBMITTED TO

NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA

FOR THE PARTIAL FULFILMENT

OF THE MASTER OF SCIENCE DEGREE IN LIFE SCIENCE

Submitted by
ARCHANA BHOI
ROLL NO – 410LS2042

Under the guidance of
Dr. SUJIT KUMAR BHUTIA
ASSISTANT PROFESSOR

DEPARTMENT OF LIFE SCIENCE
NATIONAL INSTITUTE OF TECHNOLOGY
ROURKELA-769008, ODISHA

2011-2012

DEPARTMENT OF LIFE SCIENCE
NATIONAL INSTITUTE OF TECHNOLOGY,
ROURKELA-769008

Dr. Sujit Kumar Bhutia

Assistant Professor.

Ref. No.

Date: .....

CERTIFICATE

This is to certify that the thesis entitled "**Isolation and Characterization of Lectins from *Ricinus communis***" which is being submitted by **Miss.Archana Bhoi**, Roll No. **410LS2042**, for the award of the degree of Master of Science from National Institute of Technology, Rourkela, is a record of bonafide research work, carried out by her under my supervision. The results embodied in this thesis are new and have not been submitted to any other university or institution for the award of any degree or diploma.

Dr. Sujit Kumar Bhutia

Assistant Professor

Department of Life Science

National Institute of Technology

Rourkela – 769008, Odisha, India.

Phone no: 91-661-2462686

Email:sujitb@nitrkl.ac.in

ACKNOWLEDGEMENT

This project is by far the most significant accomplishment in my life and it would not have been impossible without people who supported me and believed in my calibre.

I would like to extend my gratitude and sincere thanks to my honourable supervisor **Dr. Sujit Kumar Bhutia,** Assistant Professor, Department of Life Science. He is not only a great lecturer with deep vision but also most importantly a kind person. I sincerely thank for his exemplary guidance and encouragement. His trust and support inspired me in the most important moments of making right decisions and I am glad to work under his supervision.

I express my sincere thanks to Head of the Dept. Dr. S.K Patra, and all other faculties of Department of Life Science , NIT Rourkela for showing sustained interest and providing help throughout the period of my work.

I express my heartfelt thanks to PhD scholars for their active cooperation and sincere help.

I am genuinely appreciative of all my batchmates for their suggestions and moral support during my work.

Last, but not the least, I would thank the Almighty and my parents, whose dedicated and untiring efforts towards me has brought me at this stage of my life.

Archana Bhoi

410LS2042

DECLARATION

I do hereby declare that the Project Work entitled "**Isolation and Characterization of Lectins from *Ricinus communis*"**, submitted to the Department of Life Science, National Institute of Technology, Rourkela is a faithful record of bonafide and original research work carried out by me under the guidance and supervision of Dr. Sujit Kumar Bhutia, Asst. Professor, Department of Life Science, National Institute of Technology, Rourkela, Odisha.

Date:

Place: **Archana Bhoi**

CONTENTS

SL.NO	TOPIC	PAGE NO.
1	ABSTRACT	I
2	INTRODUCTION	1
3	REVIEW OF LITERATURE	3
4	MATERIALS AND METHODS	6
5	RESULT	12
6	DISCUSSION	16
7	CONCLUSION	17
8	REFERENCES	18

ABSTRACT

Lectins are proteins which are well distributed in nature. They are found in both plants and animals but plants are the richest source. On the surface of the cell lectins are attached with their sugar binding site. Lectins are found to be toxic, as it is poisonous it results in the death of cells both *in vivo* and *in vitro*. Leguminous plants serve as the main source of lectin, most of the lectins are derieved from seed, present in cytoplasm of the seed. Ricin is the toxic lectin which is derived from the castor bean plant *Ricinus communis* belong to the family of Euphorbiaceae. The toxicity of Ricin is bound with their two chain A and B chain. The A and B chains of ricin, more toxic to mammalian cells. Lectins exhibit a promising diagnostic and therapeutic approach for treating cancer. The study of this project encompasses the purification of ricin through affinity chromatography, characterization of ricin through haemagglutination assay and SDS-PAGE. The cytotoxicity on cancer cells is yet to be explored in order to showcase its anti-cancer property.

INTRODUCTION

Lectins are proteins with carbohydrate binding pockets. These proteins form reversible complexes with mono- or oligosaccharide structures. Lectins are well distributed in nature found in both plants and animals.Although lectins are present in almost all organisms, plants are the richest source of lectin. Lectin is nonimmune in origin and bind to carbohydrates reversibly without modifying them (Goldstein *et al.,* 1980). Lectins are found in many plant species and are differ from one another on the basis of their structure and specific activities.

In plants lectins may have many physiological role also (Van Damme *et al.,* 1998). In leguminous plants lectins may involved in the establishment of symbiosis with bacteria *Rhizobia* . In some plants lectin protects the plants from various types of infections by acting as a defence proteins (Cavada *et al.,* 1993) .

In many cases the cellular ligands for lectins are the carbohydrate chains of glycoproteins and glycolipids. Binding of lectins to this cell surface glycoproteins and glycolipids result in the formation of cross-linked complexes which are often associated with the biological responses of cells (Nicolson, 1976).

Lectin is very poisonous in nature.It cannot be degraded by digestive enzymes ,so intake of food that contains lectin causes many health disorders like vomiting, diarrhea etc(Vasconcelos IM, *et at.*,2004). Particularly the epithelial cell microvilli are affected by lectin exposure and initiates disruption and shedding of these membrane rich surface projections (Hart CA *et al.,* 1988).

Because of their varying carbohydrate-binding specificities lectins are classified into different types according to the small carbohydrate haptens they recognize, such as mannose-binding lectins , galactose-binding lectins or GlcNAc-binding lectins.

Leguminosae is the best-characterized family of plant lectins . Other families of plant lectins are the Gramineae , Solanaceae, Euphorbiaceae etc. These lectins differ considerably in terms of primary/secondary/tertiary structure.

Lectins have the ability to distinguish carbohydrate determinants in human blood cells, so blood typing can be performed by lectins . Lectins are useful in immunological studies, because at low concentrations, some lectins are mitogenic to peripheral blood lymphocytes. However, some lectins, such as ConA and Ricin are extremely cytotoxic to cells at higher concentrations and may be used for cancer treatment.

Ricin and RCA are lactose-specific lectins, possessing sugar binding sites. These are highly toxic proteins found in the castor seed (*Ricinus communis*) (Lord *et al.*, 1994). It was first described by H. Stillmark in the late nineteenth century.

Ricin is a polypeptide, it belongs to the Type 2 ribosome-inactivating proteins (RIPs).It is a potent cytotoxin. It has two glycoprotein chains, A-chain and B-chain, which are covalently bound by disulfide bridges. The A-chain is an N-glycosidase enzyme and it is the active toxic component, that can enzymatically inactivate a large number of ribosomes; while the B chain is necessary for binding of this toxin to the cell surface and there by B-chain helps A-chain to enter into the cell.

Ricinus communis agglutin(RCA) is a strong haemagglutinin but a weak cytotoxin (Nicolson *et al*.,1974). RCA is a tetramer having two ricin-like heterodimers, each of which consists of an A and a B chain.

REVIEW OF LITERATURE

Lectin: Ricin

Lectins are the most violent toxins of the earth. These are present almost in all the organisms starting from bacteria to many higher plants and animals. Lectins defined as proteins which interact non-covalently with carbohydrate moieties, showing high affinity and specificity for their ligands. Many plant seeds are the richest source of lectins and large number of plant lectins have been isolated at present with established biochemical characteristics . Recently by x-ray diffraction a number of three-dimensional structures of plant lectins have been observed (Bourne *et al*., 1990).

Among the various toxic plant lectins Ricin and Ricinus communis agglutinin (RCA) are the two most poisonous lectins on earth.These two lectins are found in the endosperm of Castor seed *(Ricinus communis).*

Plant description:

The castor oil plant, *Ricinus communis* is an herbaceous plant belongs to the family *Euphorbiaceae* .Castor is widely distributed in the tropics . It is a monoecious flowering plant. It's fruit is a capsule containing the highly toxic castor bean which contains oil and lectins in it.

Figure1: Castor plant

Figure2: Castor seed

Ricin Location:

Ricin and RCA are stored in protein bodies in endosperm cells *of* maturing *Ricinus* seeds (Lord et al., 1994). Structurally these lectins differ in seeds of different origin and even within the same seed . Seeds within the lower portion of the raceme are more toxic.

Structure:

The castor bean contains two potent toxins Ricin and Ricinus communis agglutinin (RCA). These are glycoproteins and present in the protein bodies of the endosperm tissue(Tully *et al.*,1976).

Ricin is a heterodimeric protein , it composed of two subunits A-chain and B-chain linked by a single disulfide linkage. A-chain is the actual toxic component and the B-chain only helps in entry of the toxin to the cell by interacting with the cell surface.The A-chain is composed of 267 amino acid residues and B-chain consists of 262 amino acid residues.The active A-chain of ricin is approximately 30% helical and contains 7 alpha helices and also it contains about 15% beta structure that made up of 5 standard beta sheet.

RCA is structurally similar to ricin , however, RCA is composed of two A-chains and two B-chains and RCA is less toxic then ricin (Saltvedt,1976).

Ricin- A Chain:

The A chain of ricin is a ribosome-inactivating protein (Lord *et al.*, 1994).Its molecular weight is 32 kDa .This subunit permanently prevents protein synthesis by depurinating the 28S rRNA of ribosome. The A and B chains together are highly toxic and are referred to as heterodimeric toxins.

Ricin -B Chain:

B chain is the lectin portion of ricin.B-chain binds to the cell surface by attaching to the glycoproteins and glycolipids present in the membrane (Lord *et al*., 1994). The B chain binds more strongly to complex galatosides than to simple sugars. The B chain has four disulfide bonds that provide N-acetylgalactosaminebinding activity. This four disulfide bonds form subdomains that may represent a conserved 40 residue peptide which is repeated four times through gene duplication (Robertus *et al.*, 1985). B chain has the ability to bind up to 108 ricin molecules to an individual cell by hydrogen bonds (Robertus, 1991).

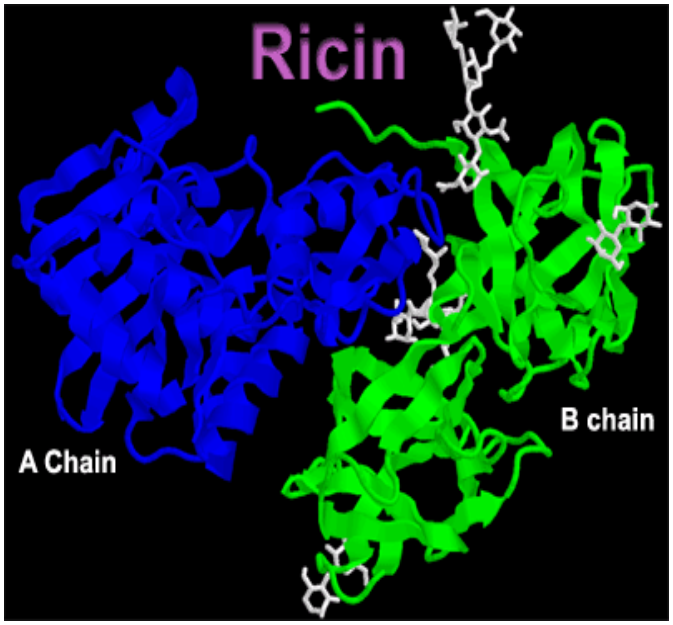

Figure3: Structure of Ricin

Toxicity and mode of action:

Ricin is a highly toxic protein. It works by getting inside the cells of a person's body and preventing the cells from making the proteins they need and without the proteins, cells die. In a rabbits body ricin has a LD50 of 1 ppm (Khvostova, 1986).

Ricin is a cytotoxin, it consists of an enzymic polypeptide the A-chain that catalyzes the N-glycosidic cleavage of a specific adenine residue from 28S ribosomal RNA. So, the ribosome with depurinated RNA is not able to synthesize the protein.The A-chain cannot enter the cell alone so,the B-chain helps the A-chain to get enter into the cell (Robertus, 1991).

Ricinus communis agglutinin(RCA) is a another lectin found in Castor seeds. It consists of four subunits, two A-chains and two B-chains.RCA has high affinity and great specificity for glycoproteins and glycolipids present on the cellwall (Nicolson and Blaustein, 1972) .It is a strong agglutinin but a poor cytotoxin.

MATERIALS AND METHODS

Chemicals:

Sodium hydroxide (NaOH), Sodium carbonate (Na_2CO_3), Glycine,Cuppersulphate($CuSO_4$) , Potassium sodium tartarate ($KN_aC_4H_4O_6$) were purchased from SRL, Sisco Research laboratories Pvt. Ltd., Mumbai. Acrylamide, bisacrylamide, Ammonium persulphate (APS), Sodium dodecyl sulphate (SDS), N,N,N',N'-tetramethylenediamine (TEMED), Bovine serum albumin(BSA), Tris were purchased from Sigma Aldrich, USA. Folin-Ciocalteau phenol reagent, Potassium Dihydrogen Phosphate (KH_2PO_4), Potassium hydrogen phosphate (K_2HPO_4) were purchased from S.D. fine chem. Ltd., Mumbai. Acetic acid, Bromophenol blue, agarose were purchased from Himedia, Mumbai. Glycerol was purchased from Rankem Pvt Ltd. Ethanol purchased from Trimurty Chemicals, India. Pre stained molecular weight marker was purchased from Bio-Rad, India. Methanol, Silver nitrate, Sodium thiosulphate were purchased from Nice chemicals Pvt.Ltd. India.

Sample Collection:

The Castor Seeds (*Ricinus communis*) were collected for isolation and purification of Lectins from the Department of Biotechnology , Indian Institute of Technology, Kharagpur and blood sample was collected from CWS Hospital , Rourkela.

Seed Coat Removal:

Castor seeds were taken and grinded in a mixer for removal of seed coats and 45gms of uncoated seed were taken for the study. The uncoated seeds were deeped in PBS of 100 ml for one day. Then the seeds were grinded with PBS and the pastes were collected in 50 ml Centrifuge tubes and the weights were made equal by measuring the weights by the electronic weighing balance. Then the samples were centrifuged by the eppendorf centrifuge with 7500rpm, at 4^0 c for 20 mins. The supernatant were taken after centrifuge and measured by a measuring cylinder. Some supernatant were stored in an eppendorf tube as crude at 4^0 C and the remaining were taken for salting out process.

Salting Out:

Salting out is a process in which separation of proteins takes place as they are less soluble at high salt concentrations. The concentration of salt requires for precipitation of the protein out of the solution is varies greatly in different proteins. It is also used to concentrate dilute solutions of proteins. Ammonium sulphate salt was taken in the salting out process. 40ml of crude were taken for 30% cut off in the salting out process. According to the salt chart 6.56 gm of ammonium sulphate were added to the crude by pinch wise and continues stirring was done by magnetic stirrer.

Figure 4: Salting out with Magnetic Stirrer

Then the sample was stored for overnight at 4^0 C and in the next day the sample was taken for centrifugation , then supernatant and pellet was collected. The amount of supernatant was measured by a measuring cylinder and taken for 90% cut off. 38ml of supernatant was taken and 15.276 gm of ammonium sulphate salt was added in pinch wise and continuous stirring was done by magnetic stirrer.

Preparation of Lactamyl Sepharose 4B affinity matrix:

4gm of lactamyl sepharose 4B matrix was washed with 6ml distilled water and mixed with 2.6ml of 2N NaOH and 0.66ml epichlorohydrin were added so that the final concentration of the various components were 30% v/v sepharose, 5% epichlorohydrin, 0.4 M NaOH. It was cover with aluminum foil and incubated at 40^0 C for 2h with shaking. It was then transferred to a glass filter funnel and the gel was washed with 500 ml of distilled water.

Preparation of Amino Sepharose 4B:

Epoxy activated sepharose 4B was suspended in 1.5 volume of concentrated ammonia solution i.e. 6 ml. The suspension was incubated at 40^0 C for one and half hour. It was then again transferred to a glass filter funnel and the gel was washed with distilled water.

Coupling of Lactose with amino sepharose 4B:

4 gms of suction dried amino sepharose 4B was suspended in 3ml of 0.2M K_2HPO_4 buffer, which was contain 51mg $NaCNBH_3$ and 104 mg of lactose. The Suspension was incubated at room temperature for 10 days with occasionally shaking. The free amino groups which remained in the gel were acetylated by adding 2 ml of acetic anhydride. The suspension was incubated in the room temperature for 1 hour. The lactamyl sepharose 4B thus obtained was subsequently washed with distilled water, 0.1 M NaOH, distilled water and 10 mM PBS subsequently. It was stored in distilled water with traces of sodium azide at 4^0 C.

Affinity chromatography:

The lactamyl sepharose column washed by PBS solution (pH7.2) and O.D of the washed PBS was measured at 280nm. When the OD value decreases and tend to zero then the protein sample of 90% cut off was passed through lactamyl sepharose beads and the eluted sample was collected and its O.D was measure at 280nm.

Lactatamyl sepharose beads were again washed with PBS solution (pH7.2) and the O.D of the washed PBS was measured at 280nm. When the OD value decreases and tends to zero then 20ml lactose solution was loaded on lactose sepharose beads and O.D of the eluent was measured at 280nm. The eluent was collected for dialysis in PBS (pH 7.2) and stored at 4^0c for 1day.

Dialysis:

Dialysis was done against PBS at 4^0C of 30%cut off, 90% cut off and 90% Affinity samples.

Figure 5 : Dialysis of sample with PBS

Determination of concentration of protein:

The concentration of crude, 30% cut , 90% cut, and 90% affinity were measured by Lowry's Method.

Lowry's Method:

Reagent A=Sodium hydroxide(0.5%)

Sodium carbonate(2%) make it upto 1 litre

Reagent B1=1% Copper sulphate

Reagent B2=2% Sodium potassium tartarate

Reagent C=A:B1:B2=100:1:1

BSA Standard=1mg/ml

Folinciocalteau's reagent=1N (5 ml solution +5 ml distill water)

Different concentration of BSA solution from stock solution were taken and distilled water was added to it and made up to 2ml. Ricin protein taken unknown quantity dissolved in 1ml distilled water and added reagent C of 5 ml and protein of 0.5ml. Mixed properly and incubate for 10 mins. Then 0.5 ml of Folin reagent was added and incubated for 30min. was taken OD at 750nm.

Preparation of Human Erythrocyte:

Healthy human venous blood was collected by a syringe and poured into a 15 ml tube to which the anticoagulant EDTA was previously added.

Haemagglutination Assay:

1ml blood sample was centrifuged in 2ml microtube at 1000 rpm for 5min at room temperature by eppendorf mini spin. Then the pellet was collected and to it 10ml of PBS was added . The mixture of blood and PBS was centrifuged at 1000 rpm for 5min at room temperature. After centrifugation the pellets was collected and from this 100µl of pellet was taken and added to10ml of PBS solution (pH 7.2). The Haemagglutination activity of castor lectin was detected when blood erythrocytes were added to it. The assay was carried out in a 96 well round bottom micro-titre plate. The first well of each row was served as positive control to which 100µl of normalized sample and 100µl of blood was added and the last well served as negative control since it contained 100µl of blood and 100µl of PBS solution. Between the positive and negative control each well contains blood, PBS and lectins. First of all 100µl PBS was added to all the wells. Then 100µl of normalized crude was poured to the first well and it was serially diluted till the negative control. Similar procedure was followed for the other samples. Finally 100µl of processed blood sample was poured to each well. After that the plate was placed in a plane surface without disturbing it. After 30mins the haemagglutination assay result was observed.

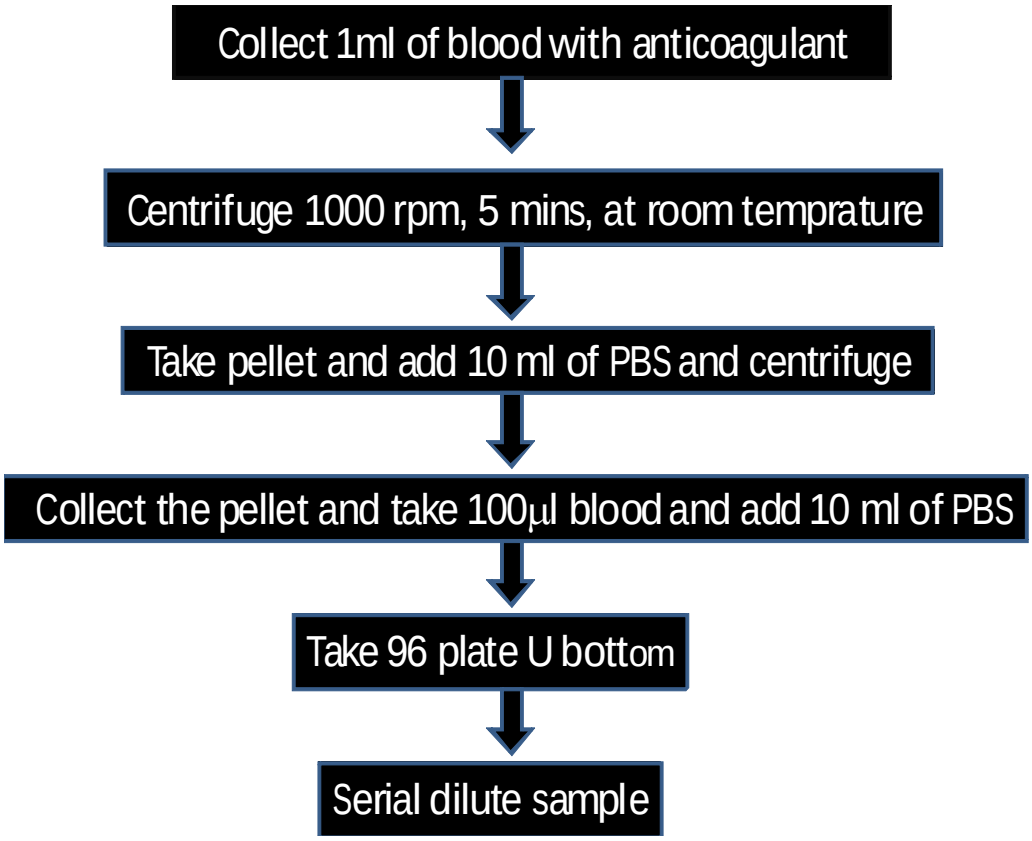

Figure 6: Protocol for Haemagglutination assay

SDS-PAGE:

The molecular mass of the subunits of the lectins was estimated by SDS-PAGE. The poly-acrylamide gel electrophoresis was done according to the protocol given in the Book "Molecular cloning" by Sambrook & Russell on a 12% gel. For the native 12% polyacrylamide was employed and SDS along with β-mercapto ethanol was not added. The mixture of 10µl of sample, 10µl of sample loading buffer and 5µl of Coomassie Brilliant Blue were added to the well. In my experiment crude, 90% and 90% affinity was added with sample loading buffer and Coomassie Brilliant Blue. The gel was again stained with Silver salts. Silver nitrate was used in the preparation of silver staining.

RESULT

Purification of Ricin:

- The solutions of eluted protein had bound on Lactose sugar of lactamyl beads.
- Lactose was removed from the protein by the method of dialysis in PBS solution (pH-7.2) for 1day.

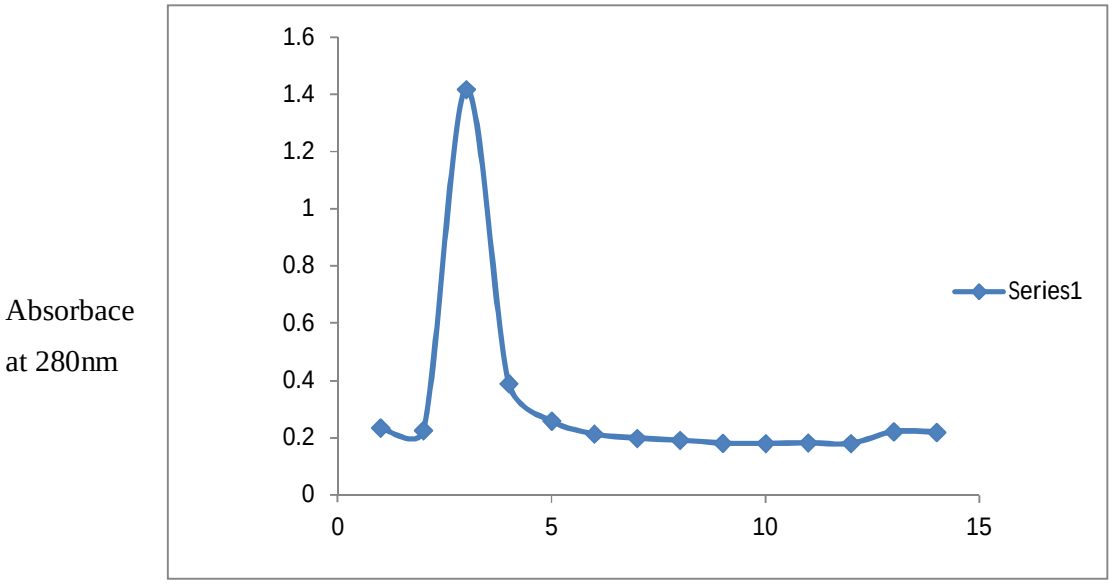

Figure7: Lactose elution graph

Estimation of protein concentration by taking OD at 280nm:

Table 1: Total protein estimation

Sample	Volume	OD(280nm)	Concentration (mg/ml)	Total protein concentration
Crude	40ml	0.771	46.26	1850.4
30%	38ml	0.641	38.46	1461.48
90%	35ml	0.497	3.18	111.328
Affinity	33ml	0.430	1.75	56.76

Haemagglutination Result:

- Agglutination activity of normalized sample of ricin was tested with human RBC.
- The haemagglutination assay is used to determine titre value of proteins based on their ability to attach to molecules present on the surface of red blood cells.

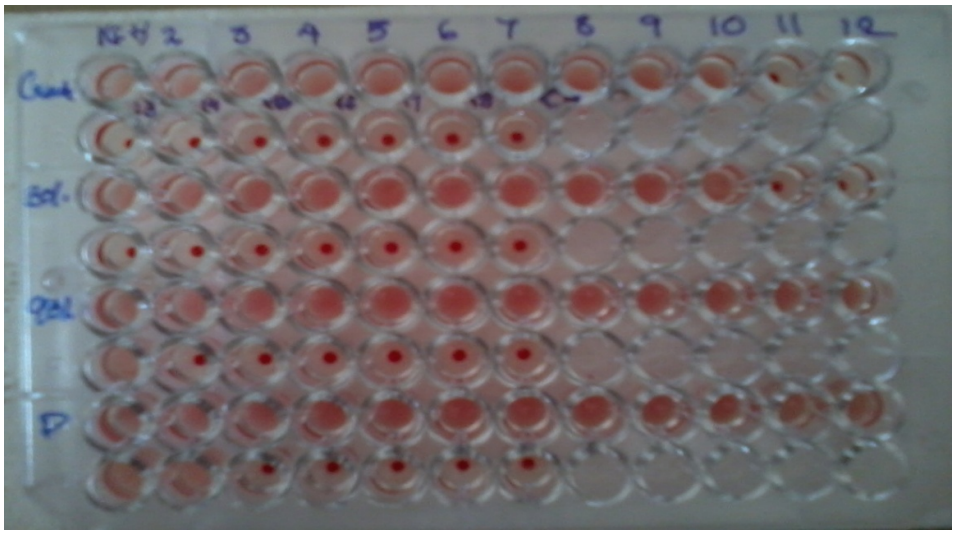

Figure 8: Haemagglutination assay

Table 2: Haemagglutination result

SAMPLE	HA VALUE
Crude	$1:2^{10}$
30%	$1:2^{10}$
90%	$1:2^{13}$
Affinity	$1:2^{14}$

1) Crude HA=$1:2^{10}$,means that the protein was titered by Hemagglutination Assay (HA), and the endpoint for the assay was a dilution of $1:2^{10}$, that is 1: 1024.
2) 30% HA=$1:2^{10}$ means that the protein was tittered by Hemagglutination Assay (HA), and the endpoint for the assay was a dilution of $1:2^{10}$, that is 1: 1024.
3) 90% HA=1: 2^{13} means that the protein was tittered by Hemagglutination Assay (HA), and the endpoint for the assay was a dilution of $1:2^{10}$, that is 1:8190
4) Affinity HA =1: 2^{14} means that the protein was tittered by Hemagglutination Assay (HA), and the endpoint for the assay was a dilution of $1:2^{10}$, that is 1:16,384

So, from the haemagglutination result it is found that affinity value is higher which indicates the presence of high concentration of protein.

SDS-PAGE Result

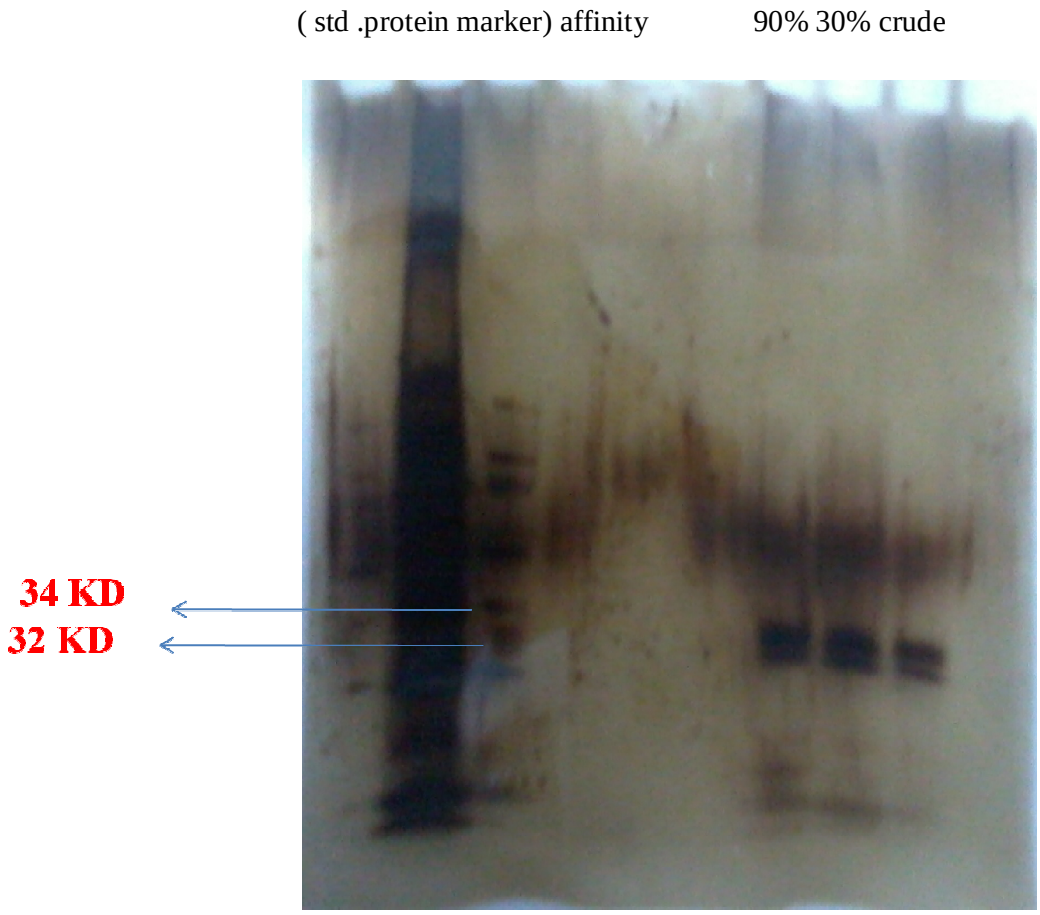

Figure 9:SDS-PAGE

Two chain of ricin was identified, A chain with its molecular weight 32 kD and B chain with its molecular weight 34 kD.

DISCUSSION

The haemagglutination assay is used to determine titre value of proteins based on their ability to attach to molecules present on the surface of red blood cells. The red blood cells are agglutinated by protein suspension which ensures there is no settling of RBCs out of suspension. Serial dilution of a protein is performed in a 96-well plate and with consistent addition of red blood cells, an estimation of the amount of protein in crude, 30 %, 90 % and purified protein present is estimated.

Positive controls are seen with a uniform film with indistinctive shape covering the bottom of the tube. Negative control is seen perfectly outlined with round "button" of cells settled at the bottom of the tube. Irregular clumps of cells are specified for the intermediately positive results and are seen at the bottom of the tube.

The highest dilution of protein suspension that produces a positive result is termed as the end point . HA (haemagglutination assay) is where the protein is mixed with diluted red blood cells, the protein forms a network (lattice formation) with the red blood cells. The red blood cells spread out as these "lattice" formations settle to the bottom of the tubes. If the protein is absent, then the red blood cells are unable to form the lattice, and instead they settle down at the bottom of the tube as a condensed button; interpreting the immunological property of ricin, which is a weak agglutinin.

SDS PAGE : The purpose of SDS-PAGE is to separate proteins according to their size. The molecular weight of desired protein after affinity was determined with respect to the corresponding molecular weight of standard protein marker. We found out that Ricin comprises of two chains A chain : 32 kD and B chain : 34 kD.

CONCLUSION

SDS-PAGE enhanced our measurement of protein of interest, Ricin . Significantly, which are of 32kD and 34 kD, characterized as A-chain and B-chain .

Ricin is a weak agglutinin. As it is toxic, its cytotoxicity on normal cells and cancer cells should be further explored to quote its anti-cancer property. Further researches are required for its application in different cancer cell lines to see whether it has inhibitory action on proliferation.

REFERENCES

1) Bourne, Y.; Abergel, C.; Cambillau, C.; Frey, M.; Rougé, P. & Fontecilla-Camps, J-C. (1990a), X-ray crystal structure determination and refinement at 1.9 Ao resolution of isolectin from the seeds of *Lathyrus ochrus* I. *J. Mol. Biol.*, 214: 571-584

2) Cavada, S.B.; Moreira, R.A.; Oliveira, J.T.A. & Granjeiro, T.B. (1993). Primary structures and functions of plan lectins. *R. Bras. Fisiol. Veg*. 5 (2): 193-201

3) Diaz, C.L.; Melchers, L.S.; Hooykaass, P.J.J.; Lugtenberg, B.J.J. & Kijne, J.W. (1989), Root lectin as a determinant of host-plant specificity in the *Rhizibium*-legume symbiosis. *Nature* 338: 579-581.

4) Goldstein, I.j. & Hayes, C.E., 1978. The lectins: carbohydrate binding proteins of plant and animals.Adv. Carbohydr. Chem. Biochem., 35:127.340.

5) Hart CA, Batt RM, Saunders JR, Getty B (1988) Lectin-induced damage to the enterocyte brush border. An electron-microscopic study in rabbits. Scand J Gastroenterol 23: 1153–1159.

6) Khvostova, I.V. 1986. Ricin: The Toxic Protein of Seeds. In Castor. Ed. V.A. Moshkin. Amerind Publ. Co. New Delhi, pp. 85-92.

7) Lord, Michael J., L. M. Roberts, and J. D. Robertus.1994. Ricin: structure,mode of action, and some current applications. FASEB J. 8: 201-208.

8) Nicolson, G.; Blaustein, J.; Etzler, M. Characterization of two plant lectins from *Ricinus communis* and their quantitative interaction with a murine lymphoma. *Biochemistry* 1974, *13*, 196–204.

9) Nicolson.G.L. (1976) Transmembrane control of the receptors on normal and tumor cells. I. Cytoplasmic influence over cell surface components. *Biochim. Biophys Acta,* 457, 57-108.

10) Phillips, Roger; Martyn Rix (1999). *Annuals and Biennials*. London: Macmillan. p. 106. ISBN 0333748891.

11) Robertus, J.D. 1991. The Structure and action of ricin, a cytotoxic Nglycosidase. Sem. in Cell Biol. 2:47-58.

12) Saltvedt, E. (1976) Biochim. Biophys. Acta 451, 536-548.

13) Tully, R. E. & Beevers, H. (1976) Plant Physiol. 58, 710-716.

14) Van Damme, E.j.M, Brike,F.,Winter, H.C.,Van Leuven, F., Goldstein,I.J and Peumans, 1 W.J. 1996.Molecular cloning of 2 different mannose- binding lectins from tulips bulbs. European journal of Biochemistry 236,419-427.

15)Van Damme, E.J.M., Peumans, W.J., Barre, A. & Rougé, P. (1998), Plant lectins: A composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles. *Critical Reviews in Plant Science* 17(6):575- 692.
16)Vasconcelos IM, Oliveira JT (2004) Antinutritional properties of plant lectins. Toxicon 44: 385–403.