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Original Article

Biological Properties of Hemolytic lectin from *Acacia Melanoxylon*

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Abstract

The lectin of seed of *Acacia melanoxylon* was isolated by gel filtration on Sephadex$_{G-75}$. The molecular mass of the lectin was approximately 205kDa. Hemolytic activity of lectin increased upon addition of trypsin and calcium ions. The lectin specifically inhibited by N-acetyl D galactosamine, N-acetyl galactopyrenosamine and mannose. The hemolytic activity was stable up to 100^{0}C and optimal at p^H 6-10. The lectin was exhibited a strong cytotoxic effect in a brine shrimp and insecticidal activities on mosquito larva. It also exerted antibacterial activity against *Klebsilla pneumonia* and *Escherichia coli*.
Keywords: Acacia melanoxylan, SephadexG$_{-75,}$ Trypsin, N-Acetyl D galactosamine, Brine shrimp.

Introduction

Lectins are proteins that recognize and bind to specific carbohydrate moieties of glycoproteins and glycolipids and they are widely distributed in microorganisms, plants and animals (Kilpatrick, 2000). Plant lectins are usually localized in the cotyloden and seed embryos, (Sharon and Lis,1987). They are also found in plant tissues such as leaves, bark and root although in very small amounts were they play important roles in their growth processes (Wittsumannaul *et al*.,1998). Plant lectins are classified based on their plant family. Lectins are also involved in processes such as non-self recognition, inflammation, cell-cell or cell-extracellural matrix interactions, fertilization, development and regeneration (Saito *et al*., 1997). Lastly they may also be involved in signal transduction the organization and transport (sugar) of macromolecules or multi-enzyme complesces and even in promoting the transport of calcium or sugars. Earlier studies of isolation and characterization of lectin from *Bauhinia purpurea*. *Bauhinia purpurea* seeds are contains a typical legumel ectin that was purified by affinity chromatography on immobilized N-acetyl-*D*- galactosamine (Young *et al*.,1985). The isolation and characterization of novel lectins reveal properties which are of practical importance for different areas of biological research. Therefore, the present study was biological potential of hemolytic lectin isolated and identified from *Acacia melanoxylon* seeds.

Materials and Method

Collection of samples

Plant materials of *Acacia melanoxlan* seeds were collected from Pioneer Kumaraswamy college, Campus, Nagercoil, Tamil Nadu, India, and identified and confirmed with Botanical survey of India, Coimbatore. .

Blood Samples

Human erythrocytes were obtained from Sivanthe Athetanar blood bank, Nagercoil, Tamil Nadu, India, and animal erythrocytes were supplied by the veterinary Fuculty's hematology laboratory.

Desalting of the Sample

The dialysis membrance (Sigma) cut into small pieces using sharp scissors with molecular cut of 12 kDa. It was washed 3-4 hrs running water. Then it was boil for 20 min in 0.1 M EDTA and 2w /v sodium bicarbonate and again washed in distilled water one end of the tube was sealed with thread. Then the content was assuaged overnight is assay buffer and stored at 4^{0}C.

Purification of the samples

The sample was dialysed in Tris buffer saline, p^H 7.6 above the same buffer overnight at 4^{0}C. Purification of the lectin was performed by gel filtration chromatography. The column was washed with the same buffer until the optical density was zero. The dialysed sample was applied on the Sephadex G_{75} matrix. The sample was equlibrated with the same buffer. The fraction was carried out at a flow rate of 0.2ml / min^{-1} and the presence of protein in each fraction was monitored at 280nm.

SDS-PAGE

Sodium-dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out

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by the method of Laemmli, (1970). Electrophoresis was performed in reducing and non-reducing condition using 12% polyacrylamide as sepa rating gel and 6% polyacrylamide as stacking gel. The molecular mass of the protein was determined using high and low molecular weight markers (Sigma). Silver staining method was used for detection of the band.

Protein Estimation

Lowry's method (1951) was used in determing the protein content of the crude extract and the fraction obtained from gel fraction with Bovine serum albumin as standard.

Hemolytic Assay

The hemolytic activity of the sample was determined by serial two-fold dilution as described by Kuku and Ereton, (2004). The sample was serially diluted with the same. Tris buffer p^H 7-6 was mixed with 25µl of 2% rabbit erythrocyte suspension. The plate was incubated at room temperature for 1hr. Negative result was indicated by the formation of a distinct button at bottom of the well. The hemolytic unit is rerfered to as the minimum protein concentration (mg/ml) required for positive heamagglutinin.

Inhibition of Agglutiniin

For the inhibition test, 25µl of serial two fold dilution of the various sugars or glycoprotein's are first added to each well of 96-well microtitreplate. An equal volume of sample was added to each well, and this was gently shake and incubated for 1 hr at room temperature. Finally 25µl of 2% rabbit erythrocyle suspension was added. After 1 hr the results were expressed as the minimal sugar or glycoprotein concentration required to inhibit heamagglutinin (Vazquez *et al*., 1997).

Thermostability of the Sample

To examine the thermal stability of the protein, the sample was incubated for 20 min at 30-100^{0}C was and cooled down for 10min on ice the hemolytic assay is used in this sample.

Optimum p^H of the sample

To find out effect of p^H in the hemolytic assay the following buffer solutions were used. Acetic buffer p^H 3 -5, Tris buffer p^H7-10 sample was dialysed with each buffer and the hemolytic activity was evaluated.

Effect of metal ions of the sample

The sample was dialyzed with TBS containing $CaCl_2$, $MgCl_2$, $MnCl_2$ and EDTA at overnight. The previous determined hemolylic activity was used in this sample.

Seed germination activity

Black grams were collected from, Tamil Nadu Agricultural university, Coimbatore. Some seeds were incubated in the sample for 2 hrs at room temperature. Then it was tied in a piece of cloth and was kept in wet places. After 48hrs the result was noted. The control used distilled water.

Cytotoxic lethality assay

48 hrs cultured naulii were collected from Brine shrimp hatching tank 10 naupli were dispensed to each individual well of the sample. The setup was incubated in a dark place for 48 hrs. Percentage of dead shrimps was counted under the microscope. Normal saline was served as negative central. The sample was used as positive control.

Antimicrobial activity

Antimicrobial activity was described by Olafsen *et al*.,(1992). Petri dishes with nutrient agar were inoculated with following microorganisms *Klebsilla pneumoniae, Protus vulgaris, Vibrio parahemolyticus, Escherichia coli, Staphyllococus*, and *Pseudomonas aeruginosa*. Sample was loaded in the center of well. Bacterial colonies were allowed to grow overnight at 37^{0}C. Then the inhibition zone around the well was measured.

Blood agar test

1% of agarose was slowly heated until it completely got dissolved in distilled water. After cooling the blood was added and this was poured into clean petri dishes. A well was made at the center of petri dish. 100µl of sample was added at the center well. It was incubated in wet places. After 24hrs the result was noted.

Insecticidal activity

Mosquito larvae were placed in vials containing distilled water with different concentration of lectin. Insecticidal activity was measured in terms of the mortality of larvae in 12hr interval for 24hrs.

Results and Discussion

In this study, the hemolytic lectin was isolated from *Acacia melanoxylon* seeds, which

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reacted w ith m any er ythrocytes. H orejsi *et al*., (1980) showed that the purified *Erythrina indica* lectin a gglutinated in h uman group O^+ erythrocytes. *Acacia melanoxylon* hem olytic activity was se en i n a ll a nimals and h uman erythrocytes. The str ongest hemolytic t itre w as seen in human A^+ and rabbit erythrocytes and the weakest w as in hen erythrocytes. Trypsin i s on e of t he proteolytic enz ymes that ac ts s pecifically on the peptide bonds of basic amino acid (Murrey *et al*.,1990) and t his is ad ded to inc rease the suscepcibility of e rythrocytes to a gglutination without affec ting t he t otal num ber of le ctin binding site s (Lis and S haron,1986). Aca cia melanoxylon showed hemolytic ac tivity t owards papain, try psin and untreated e rythrocytes. The trypsin t reated er ythrocytes were high he molytic activity b ut p apain and u ntreated er ythrocytes was low (Table- 1). Multiple binding sites of the lectin such that it can interact with a wide variety of sugars or it is p ossible t hat the b inding site is flexible on t he si ze and shape o f sugar residue (Lacsammona and Mer ca,1994). A number of monosaccharides, di saccharides and glycoproteins w ere tested for their ability to inhibit th e hemolytic re action between the erythrocytes an d the l ectin. Th e hemolytic activity of th is l ectin was co mpletely N. acetyl galactosamine, N a cety, galactopyrenosamine and m annose respectively. The heamagglutination a ctivity of t he seed *Psophoocrpus palustris* e xtract w as i nhibited by fructose, maltose, ma nnose and ga lactose while xylose, lactese and glucose (Kuku Adenika *et al*., 2005).

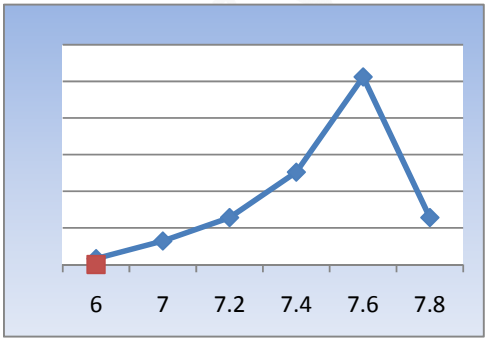

Fig.1. Effect of pH on *Acacia melanoxylon*

The hemolytic a ctivity o f *Acacia melanoxylon* was independent of the di valent cations. S ignificant hemolytic activity of Acacia melanoxylon was obser ved betw een P ^{H}6-10 (fig.1) The requirement for m etal is a genera l physic-chemical property of most legume lectins (Goldstein a nd P oretz,1986; S haron and Lis, 1998).

Table-1: Effe ct of hemolytic ac tivity in Acacia melanoxylon using pretreated rabbit erythrocytes.

Sample	Titre value
Untreated erythrocytes	1:256
Trypsinized erythrocytes	1:024
Papanized erythrocytes	1:512

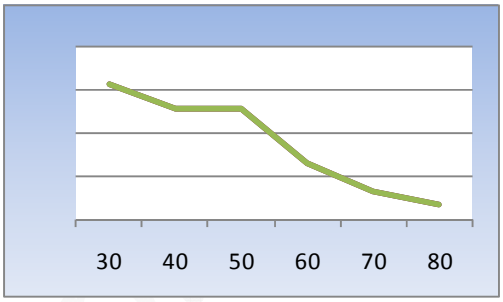

Fig.2: Effect of te mperature on *Acacia melanoxylon*

The t emperature effect on Acacia melanoxylon h emolytic activity w as determined by incubated at d ifferent te mperature r anging between 30 - 10 0^0C. T he hem olytic ac tivity reduce 50^0c but the hemolytic activity completed disstroied in 10 0^0C (f ig.2). The heamagglutination ac tivity of *Psophocorpus palustrie* l ectin declined when the lec tin was heated a bove 50^0C, activity w as reduced to half at 60^0c a nd was completely lo st a t 70^0C (K uku Adenike,2005). *Bauhinia purpurea* seeds contain a typical legume lectin that is purified by affinity chromatography on immobilized N -acetyl – D galactosamine (Young *et al*., 198 5). The molecular weight of the native lectin is similar to those t o *Trihosanthes anguinea* seeds, 45.000 daltons (Anuradha a nd B hide,1999). *Acacia melanoxylon* seed lectin purified on Sephadox G-75. The purified firs t F1 a sma ll peak that eluted with t he eq uilibrating b uffer ha d no hemdytic activity. The second broad peak F_2 had hemolytic a ctivity. The apparent m olecular weight o f A cacia m elanoxylon was de termined by SDS-PAGE. The lec tin ap pears to be composed of polypeptide c hains o f a pproximate molecular weig ht of 205kDa Ge nerally mos t of the seed ger mination inh ibitors are secondary metabolites (P utnam,1988). In this ex periment the results d id n ot show seed ger mination of *Acacia melanoxylon*. The control w as w ell growthed b ut t he *Acacia melanoxylon* tr eated

black grams were not grow n and t he seeds w ere squenge and t he size w as decr eased. Antibacterial pro perties of *Acacia melanoxylon* indicated tha t aga inst some bacterials *Klebsilla pneumoniae, Proteus vulgaris, Vibrio parahemolyticus, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococous*. T he result was shown in Ta ble - 2 t he zone of inhibition high in *Klebsilla pneumoniae* and *Escherichia coli*. Lectins in hi gher pla nts defend ag ainst pathogenic bacteria and fungi by recognizing and immobilizing the infecting micr oorganisns via binding, t hereby preventing t heir su bsequent growth and multiplication (Etzler, 1986).

Table-2: A ntimicrobioal activity of *Acacia melanoxylon* a gainst ba cterial sp ecies tested by disc diffusion method.

Bacteria	Zone of inhibition (mm)	
	15 µl	30 µl
Pseudomonas	9±0.8	15.3 ± 0.9
Proteus vulgaris	8.3 ±0.47	13.6 ± 0.9
Klebsilla pneumoniae	9 ± 0.8	16 ± 1.63
Escherichiacoli	12.6 ± 0.47	19 ±1.24
Vibrio parahaemolyticus	7±0.7	12.5±0.8

Sabodh k umar sa rkar *et al*., (20 07) reported the *Cucurbita maxima* ha d st rong cytotoxic acti vity. Th e p resent st udy o f b rine shrimp and mosquito larvae lethality bioassay of *Acacia melanoxylon* lec tin show ed negative results were obse rved. H emolytic a ctivity being indicative of cytotoxicity m akes the lectin of *Acacia melanoxylon* wor th whil e for further studies o n their an titumour/anti-neopastic activities, w hich mi ght ultimately le ad t o th e detection of anticancer compounds.

Conclusion

An Intere sting finding in the studies describing the lectin is a non blood group specific lectin s ince it hem olytic a ctivity in a ll h uman blood types a nd an imal erythrocytes. When blood is ad ded to the agar te st, t he reaction t hat was brought to t he b lood c ells was ly sis wh ich will help as a m edicine to prevent heartattacks. It can also be used a kit of mosquito killing sprayer through th e t echnique c ytotoxic lethality mosquito larva in fut ure. The antimicrobial activity of *Acacia melanoxylan* l ectin was potential ap plicators in t he treatment and prevention of disease cau sing bacteria *Klebsilla pneumonia* and *Escherichia coli*. These properties are he lpful i n the bi omedical science and further s tudies m ust b e do ne for furt her researches with other biological properties.

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