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Biochemical characterisation of lectin from Indian hyacinth plant bulbs with potential inhibitory action against human cancer cells

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ABSTRACT

This work describes purification and characterisation of a monocot mannose-specific lectin from Hyacinth bulbs. The purified lectin has a molecular mass of ~30 kDa in reducing as well as in non-reducing SDS-PAGE. In hydrodynamic studies by Dynamic Light Scattering (DLS) showed that purified lectin was monomeric in nature with a molecular size of 2.38 ± 0.03 nm. Agglutination activity of purified lectin was confirmed by rabbit erythrocytes and its agglutination activity was inhibited by D-mannose and a glycoprotein (ovalbumin). Glycoprotein nature of purified lectin was confirmed by Periodic Acid Schiff's (PAS) stain. Purified lectin showed moderate pH and thermal stability by retaining hemagglutination activity from pH 6–8 and temperature up to 60 °C. It also suppressed the growth of human colon cancer cells (Caco-2) and cervical cancer cells (HeLa) with IC_{50} values of 127 μg/mL and 158 μg/mL respectively, after 24-h treatment. Morphological studies of treated cells (Caco-2 and HeLa) with hyacinth lectin by AO/EB dual staining indicated that purified lectin is capable of inducing apoptosis.

1. Introduction

Lectins are non-immune origin glycoproteins having tremendous applications in analytical biochemistry as well as in pharmacology [1]. Initially, lectins were characterised as hemagglutinins due to their ability to agglutinate either human or rabbit erythrocytes [2]. They are found in all living organisms (bacteria, fungus, plant, and animal) [3] and are mainly involved in carbohydrate recognition with various applications in immunology, glycoproteins purification, virology, cytochemistry and cell biology [2,4–7]. In plants, they are expressed in all parts and are extracted from seeds, bulbs, leaves, fruits, roots, and flowers [8–11]. Due to their diverse role in plants such as storage of proteins [12], protection from insects [13], cell wall propagation [14], a carrier for sugar moieties [15] and mitogenic stimulation [16], lectins have been explored more in detail over the last couple of years. Besides their biological activities plant lectins are also used for diagnostics purpose of various diseases, and widely used in drug delivery systems [17]. Several lectins also act as antiviral agents such as concavalin A, musa acuminate lectin, narcissus pseudonarcissusu lectin and many more [18,19]. Mannose specific lectin isolated from plant showed anti-corona viral activity which is an acute respiratory syndrome caused by coronavirus [20].

Identification and biochemical characterisation of new lectins will certainly help in the development of new tools for disease control or to understand disease biology. Lectins have been characterised from various plant families like Solanaceae, Rosaceae, Malavaceae and Leguminceae [21]. However, lectin from Liliaceae, Asparagaceae, Iridiaceae and other plant families have not been studied much in details and therefore it is necessary to characterise new lectins from these classes of plants [22]. Hyacinth lily is a bulbous monocotyledon plant that belongs to the family of Asparagaceae and subfamily Scilloideae. In literature some reports on few small molecules like carbohydrate, alkaloids from this plant exist, however the functions of these molecules are not clear and there is strong evidence that these molecules may play significant role in plant defence system.

In this study we have made attempts to purify hyacinth lectin with significant application in disease biology. Many reports about lectins studies have been shown that lectins bind to specific carbohydrates that make it to distinguish between normal and malignant cells [23,24]. It was reported that lectins such as ricin, mistle-

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toe, many more can be inhibiting the growth of malignant cells in dosage dependent manner [25,26]. Therefore, we have investigated anti-proliferative activity and apoptosis induced activity of hyacinth lectin on human cancer cells, colon cancer cell line (Caco-2) and cervical cancer cell line (HeLa).

2. Materials and methods

2.1. Extraction of plant material

Hyacinth plant bulbs were collected from New-Delhi, India during the months of Oct-Dec. The bulbs were washed with Milli Q water; air dried and kept in −80 °C until purification was carried out. Bulbs of hyacinth were cut into small pieces and pulverized in presence of liquid nitrogen in a ventilated hood. The pulverized plant tissues were grinded and homogenized for 24 h at 4 °C in 300 mL of extraction buffer (50 mM Tris buffer; pH 7.2) containing 2.5% of phenylmethylsulfonyl fluoride (PMSF). The homogenate obtained was centrifuged at 8000 rpm for 30 min at 4 °C; supernatant was further processed for protein purification.

2.2. Fractionation of protein by ammonium sulphate precipitation $(NH_4)_2$ SO_4

The supernatant was subjected to fractionation by ammonium sulphate in order to remove non-proteinaceous contaminants and non-desired proteins. The hyacinth crude sample was first processed by 20% saturated ammonium sulphate precipitation. The precipitate solution was centrifuged at 8000 rpm for 30 min at 4 °C. The resultant supernatant was further processed with 80% saturated ammonium sulphate precipitation. After centrifugation at 8000 rpm for 30 min at 40 °C, precipitate was then re-suspended in 50 mM Tris buffer (pH 7.2) and dialyzed to remove $\mathbf{(NH_4)_2\ SO_4}$ against 50 mM Tris buffer for 24 h at 4 °C under constant stirring on a magnetic stirrer. Dialyzed sample was then analyzed on Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE).

2.3. Ion exchange chromatography

Ion exchange chromatography was carried out using diethylaminoethyl (DEAE)- Sepharose Fastflow resin (GE Healthcare). Activated resin was loaded on polypropylene column of dimension 40 × 1.5 cm with an excess of Tris buffer pH 7.2. Dialyzed protein sample 50 mg/mL concentration was loaded on the column which was pre-equilibrated with equilibration buffer (50 mM tris buffer, pH 7.2). Unbound protein was washed with wash buffer (50 mM tris buffer, pH 7.2). Bound protein was then eluted with elution buffer (50 mM Tris buffer) containing increasing concentration of NaCl in the concentration range of 100, 200, 300 mM, and 1 M with an elution rate of 500 μL/min. All fractions were collected and individually evaluated for hemagglutination activity and protein content by Bradford protein assay [27]. The fraction that displayed hemagglutination activity was subjected to gel filtration chromatography (GFC).

2.4. Gel-filtration chromatography

GFC was carried out using Sephadex G-75 (GE healthcare). Activated G-75 swelled beads were loaded on a polypropylene column with a dimension 150 × 1.6 cm. 100 mM DEAE fraction was loaded in Sephadex G-75 column and eluted with an excess of 50 mM Tris buffer, pH 7.2 with at flow rate of 250 μL/min.

2.5. SDS-PAGE and silver staining

The average molecular weight of the purified protein from Hyacinth bulb was determined by SDS-PAGE. 10 μL of purified lectin was loaded on SDS-PAGE (12.5%) with standard broad range protein ladder (Bio-Rad) as a marker. Coomassie brilliant blue G-250 staining was used to visualize protein bands on the gel. Further, purity of the purified lectin was determined by silver staining [28].

2.6. Hemagglutination assay

Hemagglutination assay of purified lectin was performed by using rabbit and human (A, B and O) erythrocytes both in 96-well microtiter U-plate. The purified hyacinth lectin solution of 100 μL (1 mg/mL) was added to the first well and from that 50 μL was serially diluted into the consecutive wells with phosphate buffer saline (PBS), pH 7.2 containing 2% rabbit red blood cell suspension. The plate was incubated for 30 min at 37 °C and hemagglutination activity was observed by the presence of agglutinated red blood cell in the well relative to negative control. One hemagglutination titer was defined as the reciprocal of the maximum dilution of sample inducing complete agglutination of RBC [29].

2.7. Inhibition of hemagglutination in presence of different sugars

The inhibition of hemagglutination by sugars was examined by 50 μL of 50 mM monosaccharides (Glucose, fructose, arabinose, mannose, galactose), disaccharides (Cellobiose, lactose, sucrose,) and glycoprotein (Ovalbumin). The sugar solutions were placed in well and serially diluted with PBS (pH 7.2) in a microtiter U-plate followed by addition of 50 μL of 1 mg/mL protein solution into each well. Plates were then incubated for 30 min at 37 °C. After incubation 2% rabbit erythrocytes were added to each well and further incubated for 30 min at 37 °C. Plates were subsequently observed for hemagglutination activity and hemagglutination inhibition titers were observed according to Benevidies et al. [30].

2.8. Total carbohydrate estimation and glycosylation observation using periodic acid Schiff's (PAS) stain

Total carbohydrate was estimated as described by Hedge et al. [31]. 1 mg of lyophilized hyacinth protein was hydrolyzed using 250 μL of 2N HCl in boiling water bath for 3 h. Excess of HCl was neutralized by addition of Na_2CO_3 and diluted to 1 mL. 50 μL of protein hydrolysate was used for total carbohydrate estimation using anthrone reagent. Standard graph was prepared using 1 mg/mL glucose stock solution as standard.

Glycosylation of protein was detected according to the method described by Zacharius et al. Briefly, protein band on 12.5% SDS-PAGE was fixed in 12% tri-chloro acetic acid for 30 min and washed 2–3 times with water for 10 min. After washing, gel was placed in 1% periodic acid solution for 1 h. Subsequently, gel was washed thoroughly with distilled water for 90 min. The gel was then immersed in Fuchsin sulphate reagent (Schiff's) for 1 h in dark followed by washing with freshly prepared sodium meta bisulphite for 30 min. The gel was then kept in 7% acetic acid for 1 h and dried for development of glycosylated protein bands.

2.9. Effect of temperature, pH and denaturant on stability of lectin

To investigate the thermal stability of purified hyacinth lectin, 1 mg/mL of purified lectin was dissolved in 50 mM PBS (pH 7.2). Thermal stability was examined by incubating the lectin at temperatures ranging from 30 °C to 80 °C for 1 h and samples were set aside to cool down to room temperature. Hemagglutination activ-

ity was carried out by adding 2% rabbit erythrocytes and further incubated for 30 min at 37 °C.

To investigate the pH stability of purified hyacinth lectin, 1 mg/mL of protein was dissolved in different buffer solutions; phosphate buffer (pH 4 and 6), Tris-HCl buffer (pH 8) and glycine NaOH buffer (pH10 and12) and incubated for 24 h at 37 °C. Hemagglutination activity was carried out by adding 2% rabbit erythrocytes and further incubated for 30 min at 37 °C.

Purified hyacinth lectin (1 mg/mL) in PBS (pH 7.2) was incubated with different concentrations of denaturant (Urea 1 M, 2 M, 3 M and 4 M) in microtiter plate for 0, 2, 4 and 24 h. Hemagglutination activity was carried out by adding 2% rabbit erythrocytes and further incubated for 30 min at 37 °C.

2.10. Protein size estimation by dynamic light scattering measurements

To measure the hydrodynamic radius of purified protein, Dynamic light scattering measurements were performed. Hyacinth lectin was dissolved in millipore water at a concentration of 1 mg/mL. The samples were mixed thoroughly using a vortex mixer. The prepared protein solutions were filtered through 0.22 μm millipore syringe filter followed by centrifuge for removal of dust from the samples. Only the supernatant was considered for all the DLS measurements. Dynamic light scattering measurements were performed using Photocor Complex (Photocor, Russia) equipped with a 25 mW diode LASER operating at a wavelength of 654 nm, a multiple tau correlator card and an Avalanche photo diode as a detector. This measures the normalized intensity – intensity autocorrelation function (ICF), $g^2(q, t)$, was at scattering vector, q given by,

$$g^2(q,t) = \frac{\langle I^*(q,0)\, I(q,t)\rangle}{\langle I(q,0)\rangle^2}$$

Where the scattering vector, $q = \left(4\pi n/\lambda\right) sin\left(\theta/2\right)$, with, λ, θ, and n are the wavelength of incident light, scattering angle and solution refractive index respectively.

The measured normalized ICF, $g^2(q, t)$ is related to the normalized field – field autocorrelation function, $g^1(q, t)$, by the Siegert's relation given by;

$$g^2(q,t) = 1 + \beta |g^1(q,t)|^2$$

where, 'β' is the instrumental constant. From the field correlation function, information about decay constant, Γ, is extracted which is related to translational diffusion coefficient, D, by the relation, $\Gamma = Dq^2$.

From the measured diffusion coefficient, the protein size estimated using the Stokes – Einstein relation given by,

$$D = k_B T/6\pi\eta r_H$$

where, r_H is the hydrodynamic radius of the proteins, k_B is the Boltzmann constant, T is the absolute temperature, and η is the solution viscosity.

2.11. Cell cultures

The human cancer cell lines Caco-2 and HeLa were obtained from NCCS, Pune (India). Cells were propagated and maintained in culture Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum, 1% glutamine, 1% (v/v) penicillin (10,000 IU/mL) and streptomycin (100 mg/L) in a humidified 5% CO_2 incubator at 37 °C.

2.12. Cell growth inhibition assay by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT)

In vitro inhibition effects of hyacinth lectin on human cancer cell lines Caco-2 and HeLa were demonstrated using MTT assay. The cells in logarithmic growth phase were digested with 1% trypsin and adjusted to 5×10^4 cells/mL using DMEM complete medium. 150 μL of the cells sample were pipetted into each well of 96-well plates and cultured for 24 h at 37 °C in 5% CO_2. Then cells were cultured with different concentrations of hyacinth lectin (varying from 23 to 185 μg/mL) and incubated for 24 h at 37 °C in 5% CO_2. After incubation, the culture medium was removed and adhered cells were washed with PBS. Subsequently, 100 μL of MTT reagent diluted in culture medium to the final concentration of 0.5 mg/mL was added and incubated for 4 h. MTT reagent was then removed and 150 μL of Dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The absorbance of the solution was recorded at 570 nm and cell viability was determined for each assay including control wells that did not contain lectin. All measurements were performed in triplicates. The inhibition rate was calculated according to the formula as following:

$$\textit{Cell growth inhibition rate}\,(\%) = \frac{A570\ of\ control - A570\ of\ sample}{A570\ of\ control} \times 100$$

$$\textit{Cell viability}\,(\%) = \frac{A570\ of\ control}{A570\ of\ control} \times 100$$

2.13. Cell morphological changes by acridine orange/ethidium bromide (AO/EB) dual staining

Caco-2 and HeLa cells (5×10^4 cells/well) were seeded into 24-well culture plate and incubated in presence of different concentrations of lectin varying from 23 μg/mL to 185 μg/mL for 24 h. Two controls, negative control that did not contain lectin and positive control that contained doxorubicin were also included in the experimental set. After incubation, media was removed and cells were washed with PBS. Cells were then fixed with 4% paraformaldehyde for 15 min and washed twice with PBS followed by staining with 50 μM AO/EB dye solution for 10 min at room temperature in dark and washed twice with PBS. Cellular morphology was observed under an inverted fluorescence microscope.

3. Results

3.1. Purification of hyacinth lectin

Lectin was purified using ammonium sulphate precipitation method [32] followed by anion exchange and gel filtration chromatography. Hemagglutination activity showed in 80% of saturated ammonium sulphate precipitation towards rabbit blood RBC was taken for the lectin purification. The DEAE (anion exchange) elution profile of 80% saturated ammonium sulphate precipitate of hyacinth bulbs showed four peaks (I–IV; Fig. 1A) with hemagglutination activity being observed only in the elute of the 1st peak (Fig. 1A). On resolving the elute obtained in 1 st peak by SDS-PAGE we obtained two protein bands with molecular masses of approximately 30 kDa and 60 kDa (Fig. 1B). For further purification of the sample 1 st peak eluate was concentrated and loaded on Sephadex G-75 column with a fixed fraction collection volume of 1 mL. The elution profile showed two distinct peaks (I and II; Fig. 1C). We further tested the eluate peaks for hemagglutination activity and observed that only peak II showed hemagglutination

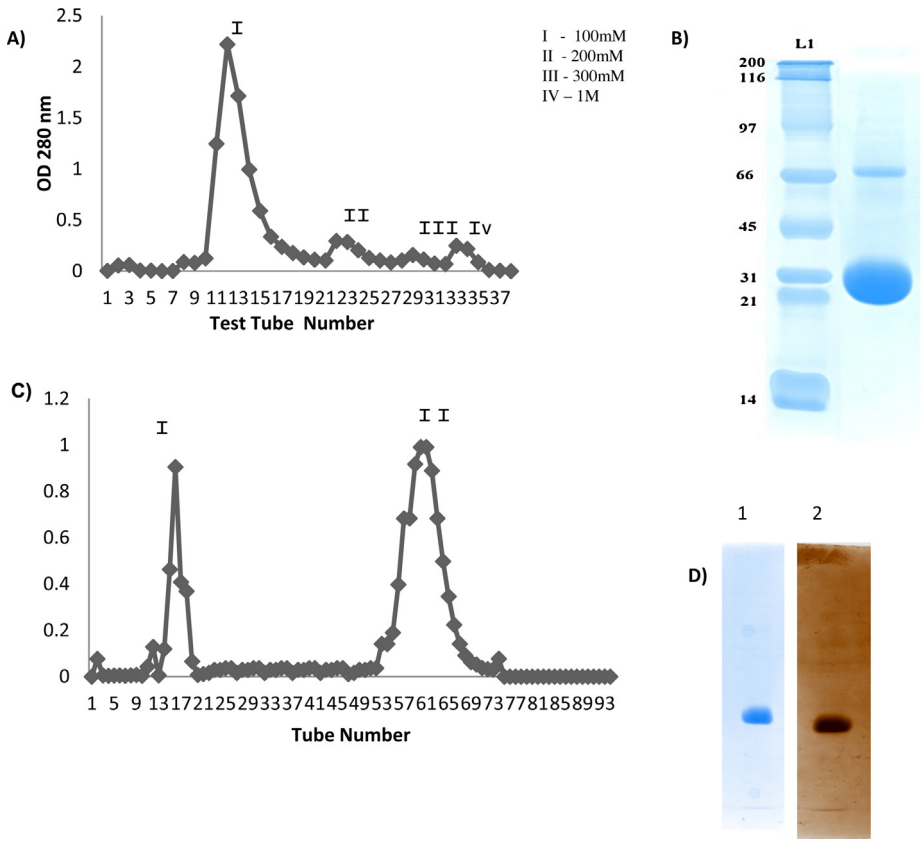

Fig. 1. Purification of protein using DEAE-Sepharose. (A) Chromatogram showing peaks for; (I) Elution with 100 mM NaCl, (II) 200 mM NaCl, (III) 300 mM NaCl and 1 M (IV). (B) SDS-PAGE analysis of I peaks obtained from chromatographic procedure showing; Commercial marker (L1) and Elution with 100 mM NaCl (L2). (C) Gel filtration chromatography using G-75, chromatogram showing two peaks, (D) SDS-PAGE analysis of peak (II) shows 30 kDa of protein (1)silver staining of polyacrylamide gel (2).

Table 1
% Recovery of lectin at different purification steps.

Procedure/step	Fraction volume (mL)	Protein (mg/mL)	Total protein (mg)	Recovery (%)	Hemagglutination unit	Total hemagglutination activity	Specific activity	Fold purification
Crude	180	06.48	1167.12	100.00	1	180	0.15	1
$(NH_4)_2SO_4$ precipitation	50	17.80	0890.15	076.26	4	200	0.22	1.45
Ion exchange	50	06.00	300.01	033.70	4	200	0.66	4.28
Gel filtration	15	04.33	065.00	021.66	16	240	3.69	25.71

activity. We further performed SDS-PAGE in a non-reducing (without treatment with β-mercaptoethanol) and reducing (treatment with β-mercaptoethanol) conditions with peak II eluate. Staining with Coomassie brilliant blue as well as silver staining of gel showed that hyacinth lectin is a monomeric protein with the molecular mass of ~30 kDa (Fig. 1D). The data obtained during the extraction and purification process are shown in Table 1.

3.2. Hemagglutination and hemagglutination inhibition assays

Purified lectin showed hemagglutination activity with rabbit erythrocytes only (Fig. 2A) whereas in case of human (A, B and O) erythrocytes purified lectin was not shown any agglutination activity. Titer value of hemagglutination was observed at minimum concentration up to 0.06 mg/mL of hyacinth lectin. Hemagglutination assay of hyacinth lectin in presence of carbohydrates showed that lectin is highly specific for mannose as inhibition was not observed in case of any other monosaccharides or disaccharides tested (Table 2). Hemagglutination activity of hyacinth lectin was also inhibited by ovalbumin which is a glycoprotein containing mannose as glycan moieties.

Table 2
Minimum Inhibitory Concentration (MIC) values of different sugars.

Sugars	Minimum inhibitory concentration (mM)
Mannose	3.00
Glucose	No inhibition
Fructose	No inhibition
Arabinose	No inhibition
Galactose	No inhibition
Lactose	No inhibition
Sucrose	No inhibition
Cellobiose	No inhibition
Ovalbumin	5.00

3.3. Carbohydrate content and glycosylation nature of hyacinth lectin

Carbohydrate content and glycosylation nature of Hyacinth bulb lectin was determined by anthrone assay and PAS staining respec-

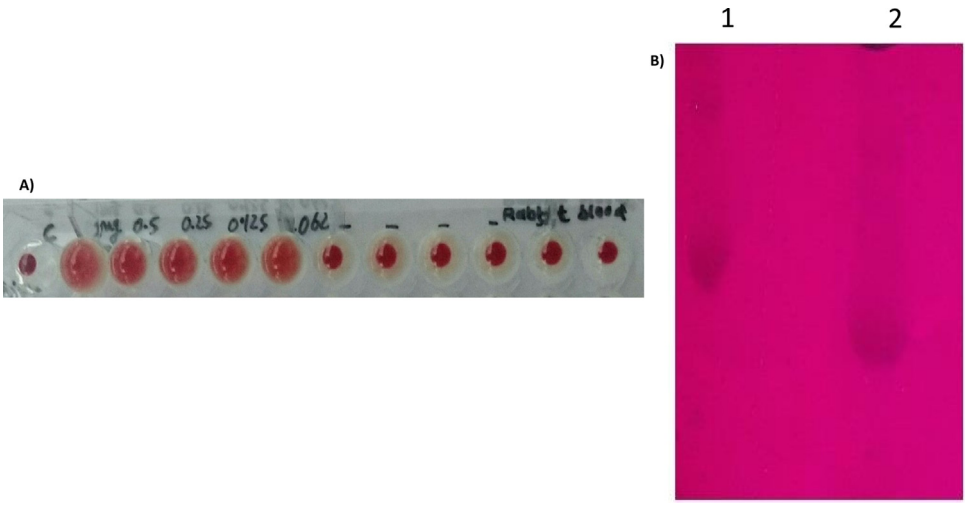

Fig. 2. (A) Hemagglutination activity of purified lectin with rabbit blood cells. (B) Periodic acid–Schiff (**PAS**) staining of ovalbumin as positive control (1)and purified lectin (2).

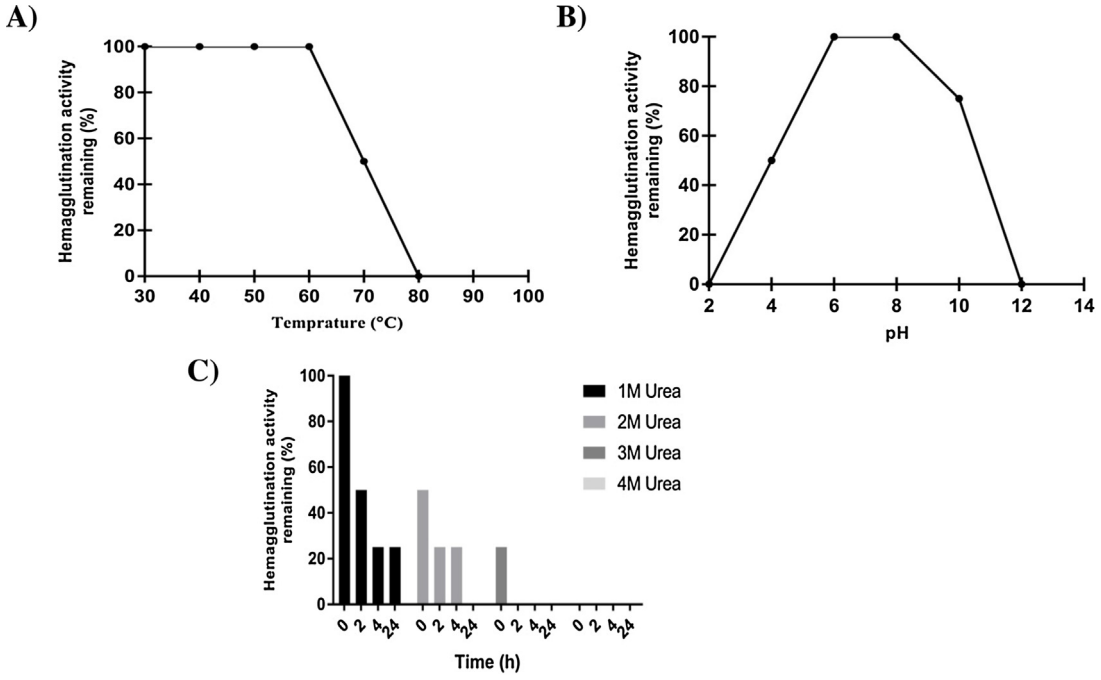

Fig. 3. Biophysical properties of hyacinth lectin. (A) Themal stability of lectin activity. (B) Effect of pH on lectin activity. (C) Impact of urea on lectin activity.

tively. The carbohydrate content of purified lectin was found to be 5.2% and according to literature carbohydrate content of lectin vary from 1.5 to 16% [33,34]. PAS stain result indicated that hyacinth lectin was glycoprotein in nature (Fig. 2B).

3.4. Hyacinth lectin stability at different temperatures, pH and denaturant

Hemagglutination activity of hyacinth lectin was completely retained in temperature range of 30 °C to 60 °C. Lectin activity decreased gradually at temperatures beyond 60 °C with no activity exhibited at 80 °C (Fig. 3A).

In case of variable pH range hyacinth lectin showed moderate stability. At pH 6–8, complete hemagglutination activity was retained whereas at low pH 4 hemagglutination activity was decreased to half and at pH 2 it is totally inactivated. Moreover hyacinth lectin showed residual activity even at pH 10 but has complete loss of activity at pH 12. It is important to note that hyacinth lectin maintained its hemagglutination activity from pH 4–10 even after 24 h of incubation. (Fig. 3B).

In case of 1 M urea hyacinth lectin retained hemagglutination activity 100% at zero h and almost half after 2 h of incubation while after 4 h and 24 h hemagglutination activity was retained up to 20%. Where as in case of 2 M urea lectin retained its hemagglutination activity up to 50% at zero h while after 2 h and 4 h hemagglutination activity was retained up to 20% and after 24 h lectin was denatured completely no activity was observed. In case of 3 M urea only at zero h of incubation lectin activity was observed up to 20% after that lectin was denatured completely no activity was observed while in case of 4 M of urea no activity was observed in any incubation time (Fig. 3C)

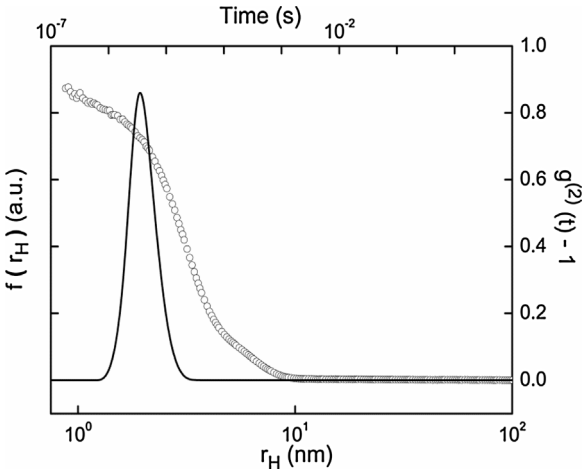

Fig. 4. The above figure is shows auto-correlation and the corresponding hydrodynamic radius distribution function for purified lectin as measured by dynamic light scattering. The left axis of the figure corresponds to the hydrodynamic radius, where as the right axis corresponds to the auto correlation function.

3.5. Molecular size determination of hyacinth lectin

The autocorrelation function shown in (Fig. 4) was fitted using DynaLS software to determine decay constant, Γ of the particles. The diffusion constant of protein molecules is related to decay constant by the relation $\Gamma = Dq\hat{2}$. From the translational diffusion coefficient the hydrodynamic size, rH of purified hyacinth lectin was estimated to be, 2.38 ± 0.03 nm using the equation D = (k_B T)/(6πhr_H). The reported, rH, values are average of multiple runs carried out on the protein samples. All the measurements were carried out at a constant temperature of 25.0 ± 0.1 °C.

3.6. Effect of hyacinth lectin on Caco-2 and HeLa cells proliferation

The anti-proliferative activity of hyacinth lectin was checked by MTT assay [35,36]. Human cancer cells Caco-2 and HeLa when treated with different concentrations of hyacinth lectin ranging from 23 μg/mL to 185 μg/mL for 24 h, showed significant change in cell viability of both the cells when compared to untreated cells with IC_{50} of 127 ± 5 μg/mL and 158 ± 5 μg/mL respectively (Fig. 5a). Anti-proliferative activity of various plant lectins against different cancer cell lines have been compared with hyacinth lectin in Table 3.

3.7. Hyacinth lectin promotes apoptosis in Caco-2 and HeLa cells (observation of morphological changes by AO/EB staining)

Morphological changes of human cancer cells Caco-2 and HeLa with dual stain AO/EB clearly indicated that hyacinth lectin induce apoptosis in cancer cells. Treatment of cells with lower concentration (46 μg/mL) of hyacinth lectin induced early apoptosis that produced apoptotic bodies. These apoptotic bodies absorbed acridine orange and were observed as doted green colour (Fig. 5B2 & C2). We have observed orange colour stained cells which could be cells which were in late apoptosis after treatment with slightly higher concentration of lectin (69 μg/mL) (Fig. 5B3 & C3) [37]. Further, with higher concentration of hyacinth lectin (92 μg/mL) the cells were dead (due to loss of their membrane integrity) which incorporated only ethidium bromide stain and are observed as red (Fig. 5B4 & C4), whereas control cells without lectin showed uni-

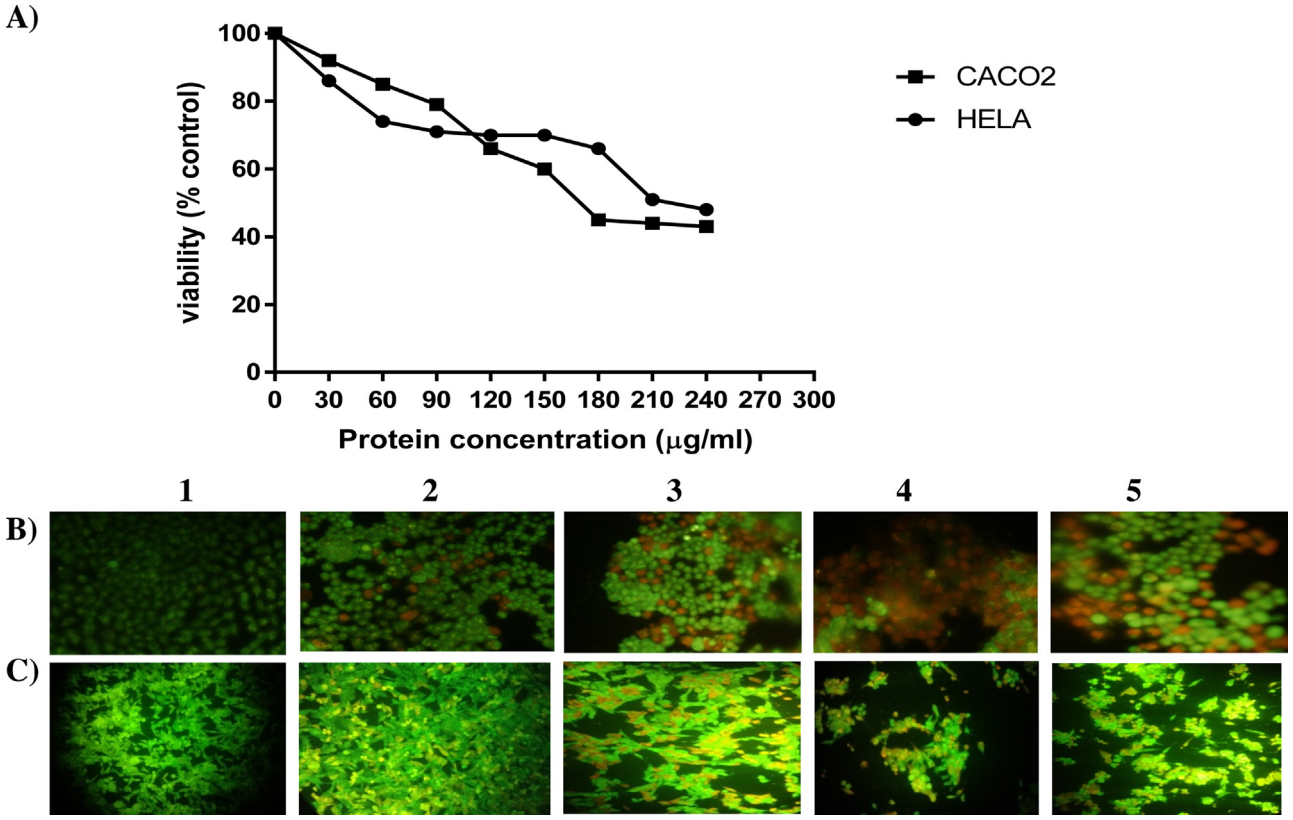

Fig. 5. (A) Cell viability of Caco-2 and HeLa Cell lines treated with varying concentrations (23–185 μg/mL)of Hyacinth lectin. (B) and (C) Morphological observation of treated human colon cancer cell line (CaCo2) and human cervical cancer cell line (HeLa) with different lectin concentrations (1) Control (2) 46 μg/mL (3) 69 μg/mL and (4) 92 μg/mL (5) Positive control treated with Doxorubicin further stained with AO/EB.

Table 3
Reported plant lectins with their IC50 values.

Sr. no.	Plant source	Cell line	IC_{50} (μg/mL)	Exposure time (h)	Reference
1.	*Caladium bicolor lectin*	SW-620	100	48	[43]
		Colo-205	100	48	
2.	*Dioscorea bulbifera lectin*	HT29	110	72	[44]
3.	*Arisaema utile lectin*	SW 620	9.8	72	[45]
		HepG2	40	72	
		SW-620	38	72	
4.	*Allium chinense lectin*	HCT-15	42	72	[46]
		SK-N-SH	43	72	
		IMR-32	49	72	
		Colo-205	50	72	
		HT-29	89	72	
		HEP-3B	60	48	
5.	*Pouteria torta lectin*	Hela	5	24	[36]
6.	*Bauhinia ungulate lectin*	HT-29	6	24	[47]
		HEP-2	6.8	24	
		HT29	130	24	
7.	*Lotus corniculatus lectin*	THP-1	39	24	[48]
8.	*Phaseolus vulgaris lectin*	HOP62	50	24	[49]
		HCT116	60	24	
		HONE-1	1000	24	
9.	*Hyacinth lectin*	HELA	158	24	–
		CACO-2	127	24	

formly green colour. In case of HeLa cells after treatment with lectin with higher concentration the dead cells were detached during the staining processe.

4. Discussion and conclusion

In the present study, lectin was successfully purified from hyacinth lily bulbs which belong to the family *Asparagaceae*. This is the first report on lectin from hyacinth plant. Lectin was purified using a combination of an ion exchange chromatography DEAE and gel filtration chromatography sephadex G-75. Purified hyacinth lectin showed hemagglutination activity towards rabbit erythrocytes with titter value of hemagglutination observed up to 0.06 mg/mL. Purified lectin was found to be a glycoprotein as determined by PAS staining whereas carbohydrate content of lectin was observed up to 5.2%. Similar carbohydrate contents of lectin have been reported in case of lectin purified from *Artocarpus integerifolia* and *Clathrotropis nitida* [34] Hemagglutination activity of hyacinth lectin was inhibited by mannose and ovalbumin which suggested that its manose binding lectin which is one of the significant properties of lectin purified from monocotyledon plants. Hyacinth lectin showed moderate temperature and pH stability. It is stable up to 60 °C and started to lose hemagglutination activity after heating above 60 °C. It maintained 50% hemagglutination activity upto 70 °C while above 70 °C it did not showed any activity. Similar pattern of stability of lectin was shown in case of *Colocasia esculenta, Crinum latifolium* [38]. Hyacinth lectin is stable over wide a range of pH from 4 to 12. Hemagglutination activity of hyacinth lectin is maximum in the range of pH 6–8 while in lower pH 4 its hemagglutination activity decreased upto 50% in higher pH 10 it is reduced upto 70% and above pH 10 it did not showed any hemagglutination activity. Dynamic scattering experiment showed that hyacinth lectin was monomeric in nature with no aggregation in solution. The hydrodynamic redii of hyacinth lectin showed 2.38 ± 0.03 nm which corresponds to a molecular weight of ~30 kDa [39]. Antiproliferative and apoptosis activity have been shown in case of various lectins isolated from plant bulbs such as *Sauromatum venosum, Arisaema jacquemontii* [40,41] We have demonstrated dose dependent antiproliferative nature of hyacinth lectin against human cancer cell lines Caco-2 and HeLa. Among the two cell lines we observe higher sensitivity of lectin mediated apoptotic response in Caco-2 cells with an IC_{50} value of 127 ± 5 μg/mL. This is the first report from hyacinth lectin on antiproliferative activity although other lectins from different plant sources have been reported for their anti-proliferative activity [42].

To demonstrate apoptotic cell death induced by hyacinth lectin, we treated Caco-2 and HeLa tumor cell lines with hyacinth lectin. Apoptotic activity was checked by Acridine orange/ethidium bromide dual staining and morphological changes were observed using fluorescence microscopy. We know that live cells absorb acridine orange and appear bright green whereas apoptosized cell shows orange colour. Upon treatment with hyacinth lectin we observe an increase in orange fluorescence in both Caco-2 and HeLa cells in higher concentrations indicating induction of apoptosis by hyacinth lectin.

The present study describes the purification and characterisation of lectin from Indian hyacinth plant bulbs having potential biological function in cancer biology.

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