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Purification and Characterization of a Lectin from *Arisaema tortuosum* Schott Having *in-vitro* Anticancer Activity against Human Cancer Cell Lines

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A lectin with *in-vitro* anticancer activity against established human cancer cell lines has been purified by affinity chromatography on asialofetuin-linked amino activated silica beads from the tubers of *Arisaema tortuosum*, popularly known as Himalayan Cobra lily, a monocot plant from the family Araceae. The bound *Arisaema tortuosum* lectin (ATL) was eluted with glycine-HCl buffer, pH 2.5. ATL was effectively inhibited by asialofetuin, a complex desialylated serum glycoprotein as well as by N-acetyl-D-lactosamine, a disaccharide. It gave a single band corresponding to a subunit molecular weight of 13.5 kDa in SDS-PAGE, pH 8.8 both under reducing and non-reducing conditions. When subjected to gel-filtration on Biogel P-200, it was found to have a molecular weight of 54 kDa, suggesting a homotetramer structure, in which individual polypeptides are not bound to each other with disulfide bonds. ATL is a glycoprotein with 0.9% carbohydrate content, stable up to 55°C and at pH 2 to 10. The lectin had no requirement for divalent metal ions i.e. Ca^{2+} and Mn^{2+} for its activity. However, as reported for other monocot lectins, ATL gave multiple bands in isoelectric focusing and Native PAGE, pH 8.3. The lectin was found to inhibit *in vitro* proliferation of human cancer cell lines HT29, SiHa and OVCAR-5.

Keywords: Arisaema tubers, Asialofetuin, Human cancer cell lines, Purification

Introduction

Lectins are (glyco)proteins of non-immune origin that agglutinate cells/ and or precipitate complex carbohydrates (Goldstein *et al*., 1980). It is their unique ability to recognize and bind reversibly to specific carbohydrate ligands without any chemical modification that distinguishes lectins from other carbohydrate-binding proteins and enzymes and makes them invaluable tools in biomedical and glycoconjugate research.

Lectins are ubiquitous in biosphere and have traditionally been found in the dicotyledons, especially in seeds (Etzler, 1986; Sharon and Lis, 1990). However, during the last decade, they have also been isolated and characterized from monocotyledonous families such as alliaceae (Van Damme *et al*., 1993), amaryllidaceae (Van Damme *et al*., 1987; Kaku *et al*., 1990), Araceae (Van Damme *et al*., 1995; Mo *et al*., 1999), liliaceae (Van Damme *et al*., 1996) and Orchidaceae (Van Damme *et al*., 1994). Interestingly, lectins from these families belong to a single monocot mannose-binding lectin superfamily as revealed by their molecular structure, sequence homologies and exclusive specificity for mannose (Annick Barre *et al*., 1996).

A few lectins are being investigated for their use in cancer research and therapy. Preliminary findings suggest that some lectins but not all can detect alterations of malignant cells as well as reduce the cancer cell tumorigenicity and thus may be helpful for prognosis of the immune status of the patients (Gabius, 1987). Lectin from *Viscum album* (mistletoe) for instance is known to increase the reactivity of the lymphocytes of tumor-bearing mice to the mitogens *in vitro*, thus indicating its immune stimulating effects for the cancer-immunosuppressed lymphocytes. It also inhibits the protein synthesis in various malignant cell lines (Zarkovic *et al*., 2001). Similarly, because of the cytostatic/apoptotic and immunomodulatory effects of the mistletoe lectin, the extracts are often applied in the treatment of tumor bearing patients (Hajto *et al*., 1999). Another lectin from *Agaricus bisporus* reversibly inhibits

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proliferation of colonic cancer cell lines without causing cytotoxicity (Parslew *et al*., 1999).

Since the isolation of first mannose specific lectin from bulbs of *Galanthus nivalis* (snowdrop), many mannose binding monocot lectins have been reported. A few studies have however revealed that monocot mannose binding lectins also have specificity for some complex glycoproteins (Shangary *et al*., 1995) and not for mannose. We here by report purification and characterization of another non-mannose binding monocot araceous lectin having anticancer activity against some human cancer cell lines from a wild Himalayan cobra lily, *Arisaema tortuosum* having complex specificity towards a serum glycoprotein asialofetuin and N-acetyl-D-lactosamine (LacNAc), a disaccharide.

Material and Methods

Materials

Chemicals and reagents Fetal calf serum from Sera Lab (GB) and RPMI1640 from GIBCO-BRL (New York, USA) were procured and stored at 4°C. Carbohydrates, Bovine serum albumin, Freund's complete adjuvant, sodium azide, Adriamycin, 5-fluorouracil and other general chemicals were obtained from Sigma Chemical Co. (St. Louis, USA). Standard molecular weight markers, gel filtration markers and ampholine of pH range 3.0-10.0 were procured from Amersham Pharmacia (New Jersey, USA). Amino activated silica beads used were from Clifmar, UK. Source of various cell lines was NCCS Pune.

Plant material *Arisaema tortuosum* tubers were collected from Dalhousie and adjoining areas, Himachal Pradesh (India) by organizing field trips to that area in the month of September.

Isolation and purification of *Arisaema tortuosum* lectin (ATL) Solid tubers of *Arisaema tortuosum* weighing 100g were chopped, cut into small pieces, immersed in 0.01 M phosphate-buffered saline (PBS), pH 7.2, and homogenized in waring blender in a 1 : 5 (w/v) ratio of PBS. The lectin was purified on asialofetuin linked amino activated silica beads as described earlier (Shangary *et al*., 1995).

Carbohydrate-binding specificity To determine the sugar binding specificity of the lectin, 39 different sugars including pentoses, hexoses, deoxysugars, amino sugars, glycosides, sugar alcohol, sialic acid, disaccharides, trisaccharides, polysaccharides and glycoproteins were tested for their ability to inhibit lectin induced hemagglutination. Lectin concentration, just one step upstream the end point of hemagglutination titre was chosen for the hemagglutination inhibition assay. A volume of 30 mL of the test lectin with an equal volume of 100 mM sugar or 2 mg mL^{-1} of glycoprotein asialofetuin was incubated at 37°C for one hour. 60 mL of 2% (v/v) rabbit erythrocytes suspension was added to each well of the 96 well microtitre plate and was incubated for half an hour at 37°C (Kaur *et al*., 2002).

Chromatographic and electrophoretic analysis

Gel filtration chromatography Affinity purified lectin was subjected to gel exclusion chromatography for the estimation of native molecular mass on a calibrated Biogel P-200 column using PBS of pH 7.2, according to the method of Whitakar (1963) and Andrews (1964).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) Affinity purified lectin preparation was subjected to SDS-PAGE, pH 8.3, using 11% (w/v) acrylamide slab gel for subunit molecular mass determination as described by Laemmli, (1970). The sample was heated in the presence of 2-mercaptoethanol for 5 minutes in boiling water bath. The gel was stained with Coomassie brilliant blue. Destained gel was scanned using CCD camera (Ultra Lum, Inc. USA). The molecular weights of the standard marker proteins were matched with the sample protein by the software (Gel Pro Analyser 3.1, USA) to determine the subunit molecular weight of ATL.

Native polyacrylamide gel electrophoresis (Native PAGE) Electrophoretic analysis under acidic and basic conditions was performed to test the homogeneity of affinity purified lectin preparation. PAGE of non-denatured purified lectin at pH 8.3 was performed by the method of Davis (1964) and Bryan (1977), and at pH 4.5 according to Reisfeld *et al*. (1962).

Isoelectric focusing The isolelectric point of affinity-purified lectin was determined using carrier ampholine of pH range from 3.5-10.0 (Amersham Pharmacia) in 7 % slab gel (Robertson *et al*., 1987).

Biochemical and biological characterization

Metal ion requirement Demetallization of purified lectin was performed by the method of Paulova (1971). The activity in normal and demetallized samples was compared by the hemagglutination assay.

Thermal stability 1 mg of affinity-purified lectin was incubated in water bath at 20°C for 15 min, with 5°C increase at each step up to 100°C. After each step of incubation, hemagglutination assay was done to study the effect of temperature.

pH Stability The pH stability of the lectin was determined by extensive dialysis of the lectin (1 mg/mL) against buffers of different pH values ranging from pH 1.5-13.0. The pH of the lectin solution was adjusted to pH 7.0 by the addition of 0.1 N HCl or 0.1 N NaOH before hemagglutination activity was determined.

Protein and neutral sugar analysis Protein concentration was determined following the protocol of Lowry (1951) using bovine serum albumin (BSA) as standard. Total neutral carbohydrate content was estimated by the method of Spiro (1966) using D-glucose as standard.

Hemagglutination assay Hemagglutination assay was carried out using a battery of erythrocytes from human ABO blood groups, rabbit, rat, guinea pig, sheep and goat. Two-fold serial dilution of affinity-purified lectin was made by taking 30 μL of each fraction and mixing with an equal volume of 2% rabbit erythrocyte suspension (3.5×10^8 cells/mL) in PBS. The plate was incubated at

37°C for 30 min and the observations were recorded as titre. Neuraminidase treatment was performed using 1 mL of 2% rabbit erythrocytes suspension in normal saline, pH 5.0, containing 0.125 units of neuraminidase (Sigma) at 37°C for 20 min. The cells were finally suspended in 1 mL of PBS after three washings in the same buffer and used for the hemagglutination assay.

Serological studies Antiserum against Purified ATL was raised by immunizing healthy rabbits with one mg/ml of the lectin and 1 mL of Freund's complete adjuvant (Sigma). Three doses were given at one-week interval each. After a weak of the last dose, the blood was collected by puncturing the ear pinna vein of the animal. Antiserum separated was preserved at –20°C in aliquots containing 0.01% sodium azide. Double immunodiffusion (Ouchterlony) was performed to study serological cross-reactions. 4 mL of hot molten agar (Difco, Detroit, USA) at a concentration of 1.25% in 0.01 M PBS, pH 7.2, was poured in glass Petri plates (50 mm outer diameter × 17 mm height). After solidification, six peripheral wells and one central well were punched with a brass template. Antisera raised against ATL were loaded in central well (A) while affinity purified araceous lectins namely *Arisaema tortuosum, Sauromatum guttatum* and *Gonatanthus pumilus* were added in the peripheral wells 1-3 respectively. *Amaryllidaceae* lectins *Galanthus nivalis, Crinum lattifolium and Zephyranthes candida* were added in the peripheral wells 4-6 respectively. The dishes were incubated at 37°C in humidified chamber for 48 h till the precipitin bands appeared. The precipitin lines were stained with 0.01% amido black for 30 min and destained with 7% glacial acetic acid to visualize the stained precipitation lines against the clear background.

In-vitro anticancer activity against human cancer cell lines *In vitro* anticancer potential of ATL against human cancer cell lines such as HT-29 (Colon), SiHa (Cervix), OVCAR-5 (Ovary), SNB-78 (CNS) and PC-3 (Prostate) was tested according to the method of Monks *et al* (1991). Different human cancer cell lines growing in RPMI 1640 medium with 10 % FCS at 37°C, 5 % CO_2 in air and 90 % relative humidity in CO_2 incubator (Heraeus, *Heracell*) were harvested at a sub confluent stage by trypsin treatment. These were suspended as single cells in the above mentioned medium. 100 µL of the cell suspension of each cell line was added to the wells of the 96 well plate and incubated for 24 hours in CO_2 incubator. Subsequently, 100 µL of lectin solution, (100 µg/ml) prepared in RPMI 1640 medium with 10% FCS, was added and the cultures were incubated for 48 hours. After 48 hours, adherent cell cultures were fixed *in situ* by adding 50 µL of 50 % (v/v) trichloroacetic acid (final concentration 10 % TCA) and incubated for one hour at 4°C. The supernatant was discarded and the plates were washed five times with deionized water and dried. 100 µL of sulforhodamine B (SRB, 0.4% w/v in acetic acid) was added to each well and the cultures were incubated for 10 minutes at room temperature. The unbound SRB was removed by washing with 1 % acetic acid and the plates were air-dried. The dye bound to basic amino acids of the cell membrane was solubilized with Tris buffer (10 mM, pH 10.5) and the absorption was measured at 540 nm using ELISA reader to determine the relative cell growth viability in the treated and untreated cells. The anticancer drugs adriamycin (1×10^{-5} M) and 5-fluorouracil (2×10^{-5} M) were used as positive controls.

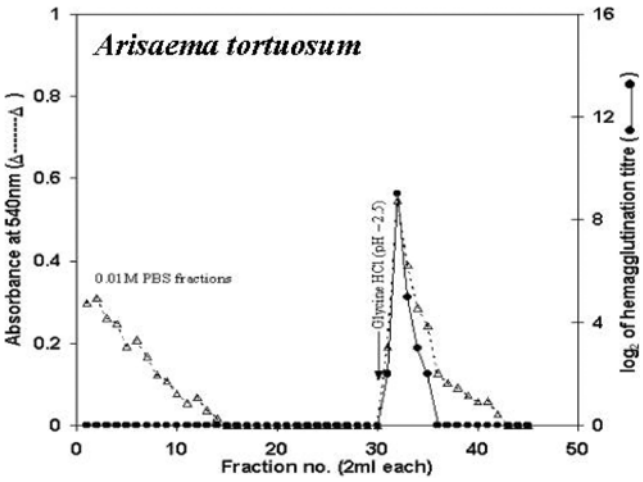

Fig. 1. Affinity purification of ATL from crude extracts of their tubers on asialofetuin linked amino-activated silica beads (0.8 × 6.0 cm). Dialyzed crude extracts of ATL (26 mg) was applied to the column, pre-equilibrated with 0.01 M PBS, pH 7.2. Bound lectin was eluted with Glycine-HCl, pH 2.5; Flow rate, 40 ml/h; △-----△ Absorbance at 540 nm of Lowry's reaction (Lowry *et al.*, 1951) ●-● Log_2 of hemagglutination titre against rabbit erythrocytes.

Results and Discusion

Lectin purification A plant lectin has been purified by affinity chromatography from tubers of wild Himalayan Cobra Lily, *Arisaema tortuosum* on asialofetuin-linked amino-activated silica beads. The unbound protein fractions in 0.01 M PBS, pH 7.2, were devoid of hemagglutination activity, indicating that the lectin was fully adsorbed to the matrix. The bound lectin was eluted as a single peak after desorption with 0.1 M Glycine-HCl, pH 2.5 (Fig. 1). It is noteworthy that single step affinity purification exploiting sugar specificity and the use of amino activated silica beads could recover approximately 65% of the lectin activity from the crude sample. It is also noteworthy that lectin is about 40% of the total tuber protein and can probably serve as major tuber storage protein. This finding is in accordance with Van Damme *et al.* (1995).

Sugar specificity Out of the 39 carbohydrates tested, N-acetyl-D-lactosamine (LacNAc) and asialofetuin inhibited lectin-induced haemagglutination. Earlier reports on monocot lectins from the family araceae (Shangary *et al.*, 1995) established their inhibition by asialofetuin only. However, in the present study, *Arisaema tortuosum,* that is also a member of family Araceae has shown specificity towards LacNAc also. It is important to note that LacNAc is one of the most important cancer markers studied so far (Ito *et al.*, 1996). In this regard the ATL having specificity for this disaccharide may serve as an excellent marker for the detection of various types of cancers. Minimal inhibitory sugar concentration with

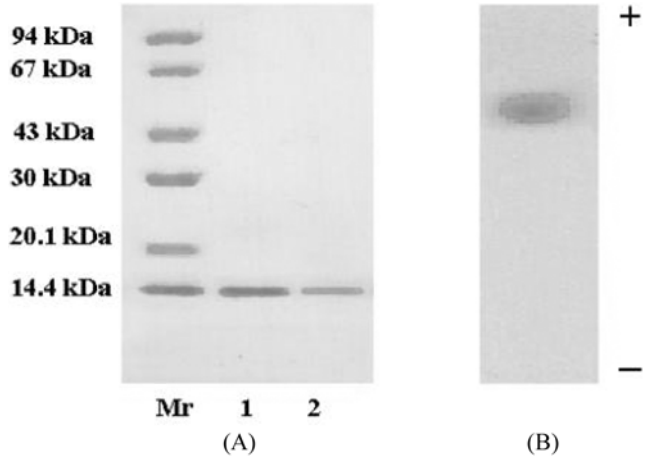

Fig. 2. (A) SDS-PAGE, pH 8.3 purified ATL using 11% gel with (lanes 1) and without (lanes 2) 2% 2-mercaptoethanol (running time 3 h at a constant 100 V). The amount of purified lectin loaded is 20 µg. Lane Mr, Molecular weight markers (from top to bottom): Phosphorylase b (94 kDa); Albumin bovine (67 kDa); Ovalbumin (45 kDa); Carbonic anhydrase (30 kDa); Trypsin inhibitor (20.1 kDa); and a-lactalbumin (14.4 kDa). The gels were stained with Coomassie brilliant blue. (B) Discontinuous-PAGE, pH 4.5, using 7.5% gel (running time 6 h at a constant 100 V); Protein loaded, 20 µg each; Lane 1 for ATL.

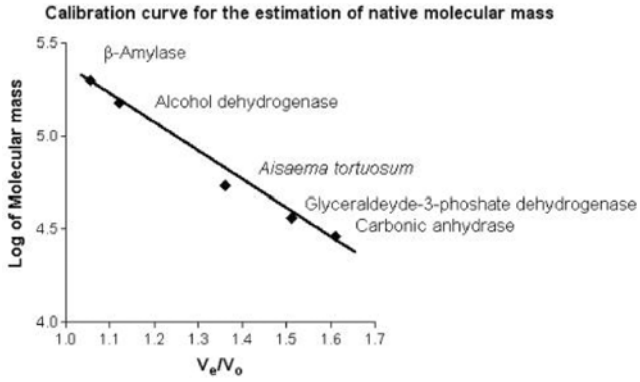

Fig. 3. Standard curve representing native molecular weight of ATL using Bio-gel P-200 superfine gel filtration column along with using standard markers β-Amylase, Alcohol dehydrogenase, Glyceraldehyde-3-phoshate dehydrogenase and Carbonic anhydrase.

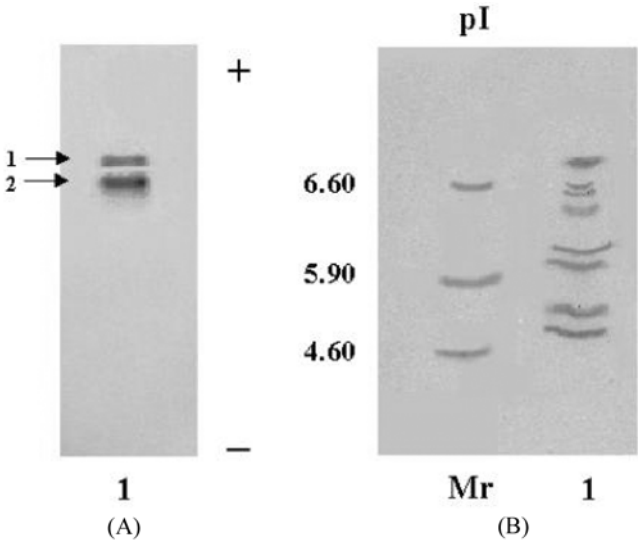

Fig. 4. (A) Discontinuous-PAGE, pH 8.3, using 10% gel (running time 6 h at a constant 100 V); Amount of protein loaded, 30 mg each; Lane 1 for ATL. (B) Isoelectric focussing of non-denatured lectin on 7% polyacrylamide gel using carrier ampholine of pH range 3.5-10.0 (running time 12 h at a constant 200 V); Protein loaded, 30 mg; Lane Mr, position of pI marker proteins; (1) Carbonic anhydrase I, human erythrocytes (pI 6.6); (2) Carbonic anhydrase II, bovine erythrocytes (pI 5.9); (3) Trypsin inhibitor, soybean (pI 4.6); Lane for ATL.

LacNAc was 12.5 mM, while asialofetuin was reactive at 125 µg/mL. Inhibition of hemagglutination with asialofetuin and not with fetuin may suggest that sialic acid hinders the binding of the lectin to the recognition sites on fetuin. A perusal of the structure of asialofetuin reveales that it consists of 80% Asn-linked oligosaccharides terminating in LacNAc (Gal-β-1, 4 GlcNAc) and 20% Ser/Thr-linked oligosaccharides having T-Disaccharide (Gal-β-1, 3-GalNAc) (Green *et al*., 1988). These findings suggest that ATL could bind to LacNAc but not to T-Disaccharide, indicating that the reactivity of araceous lectins towards asialofetuin was due to LacNAc component of the complex molecule. Furthermore, C-2 equatorial acetamido group of LacNAc seems to play a crucial role in lectin binding, as ATL was non-reactive towards lactose and maltose which lack C-2 equatorial acetamido group.

Chromatographic and electrophoretic analysis The homogeneity of the purified ATL preparation was tested by various parameters. In SDS-PAGE, pH 8.3, under both reducing and non-reducing conditions, ATL migrated as a single band of 13.5 kDa (Fig. 2A). Affinity purified lectin also gave a single band in native PAGE, pH 4.5 (Fig. 2B). The native molecular mass of ATL as determined by gel filtration chromatography on calibrated Biogel P-200 column, was 54 kDa (Fig. 3). The results of SDS-PAGE under reducing and non-reducing conditions and gel filtration chromatography revealed that the lectin exists as a homotetramer of four identical subunits which are not held together by disulphide linkages as reported earlier (Van Damme *et al*., 1991; Singh *et al*., 2004). However, ATL gave two bands when subjected to native PAGE at pH 8.3 (Fig. 4A). These results indicate that most likely there is presence of charged isomers in the affinity purified ATL. Similarly, when analyzed by isoelectric focusing, ATL yielded a complex mixture of isolectins, mostly in acidic range. The multiple bands obtained in isoelectric focusing (Fig. 4B) indicate charge heterogeneity as reported in most of the lectins. These findings for ATL corroborate with earlier observations on various monocot as well as dicot lectins (Van Damme *et al*., 1988; Chandra *et al*.,

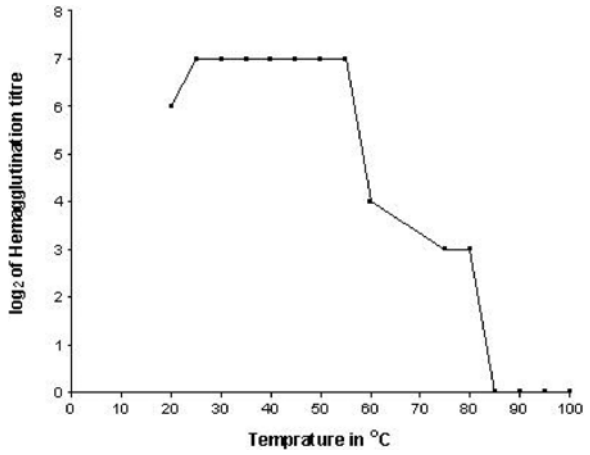

Fig. 5. Effect of temperature on lectin activity of ATL. Hemagglutination titre represents the activity left after treatment at various temperatures ranging between 20° to 100°C for 15 min.

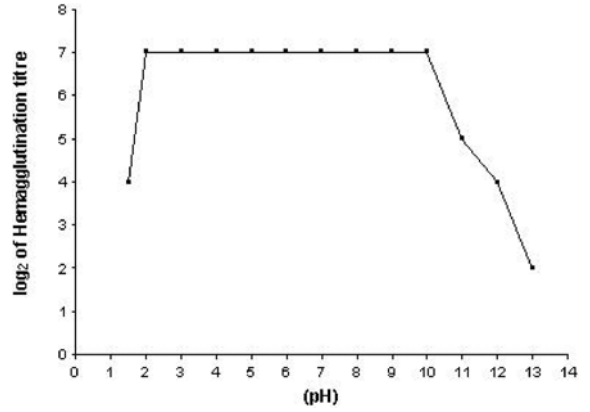

Fig. 6. Effect of pH on lectin activity of ATL. Hemagglutination titre represents the activity left after extensive dialysis of ATL at various pH ranging between 1.5 to 13.

2001; Pang *et al*., 2003). The micro heterogeneity seen may stem from few altered amino acids in lectins (Van Damme *et al*., 1992) or it may be due to variations in oligosaccharide chains (Hayes and Goldstein, 1974).

Biochemical and biological characterization Affinity purified ATL is a glycoprotein having 0.9 % carbohydrate content. Like other araceous lectins, ATL does not require metal ions for its activity, as there was no affect of demetallization on hemagglutination activity. This finding is in consonance with reports on araceous lectins from our laboratory (Singh *et al*., 2004). The hemagglutination activity of the lectin declined after incubation at 55°C and was lost by 50% when the temperature was raised to 60°C. As reported for other araceous lectins (Shangary *et al*., 1995) as much as 25% of the remaining activity was found intact even after incubating the purified lectin in a water bath at 80°C for 15 min (Fig. 5). ATL is quite stable within the pH range from 2.0 to 10.0 (Fig. 6). Beyond pH 10.0, the lectin showed significant

Table 1. Reactivity of ATL towards different types of erythrocytes

Types of cells	Hemagglutination titre of purified lectin	
	Untreated	Neuraminidase-treated
Erythrocytes		
Rabbit	256	256
Rat	128	64
Goat	64	64
Sheep	128	128
Guinea pig	256	256
Human A	NA	NA
Human B	NA	NA
Human AB	NA	NA
Human O	NA	NA

NA = No agglutination.
Hemagglutination Titre: Reciprocal of the minimum dilution of lectin concentration at which there is visible agglutination of erythrocytes.

loss in its hemagglutination activity. As the lectin was quite stable at as a low pH as 2.0, the elution pH 2.5 proved very handy for the elution of lectin without affecting its activity. ATL readily agglutinated rabbit, rat, goat, sheep and guinea pig erythrocytes and was unable to agglutinate human ABO blood group erythrocytes even after neuraminidase treatment (Table 1). Apparently, specific ATL receptors are present on all the other red blood cells while human blood is devoid of them. Denaturants, like urea, thiourea and guanidine-HCl, had significant adverse effect on the lectin activity. The activity declined down to 50% at 3.0 M concentration of urea and guanidine-HCl while in the case of thiourea, 50% decrease in the activity was observed at 3.5 M. The decrease in lectin activity may be due to disruption of hydrogen bonds and hydrophobic interactions among the chains of polypeptides of the lectin. In Ouchterlony's double immunodiffusion, ATL gave lines of identity with members of the family araceae (Fig. 7) while no precipitin lines were observed with the members of the family Amryllidaceae. The non-reactivity of anti-ATL antisera towards mannose binding lectins from the family Amaryllidaceae may indicate a completely different nature of antigens present on lectins from two taxonomically related monocot families.

In-vitro anticancer activity against human cancer cell lines Finally, we tested whether ATL has any *in vitro* anti-cancer potential towards established cancer cell lines. Five human cancer cell lines namely HT-29 (Colon), SiHa (Cervix), OVCAR-5 (Ovary), SNB-78 (CNS) and PC-3 (Prostate) were subjected to antiproliferative test (Monks *et al*., 1991). ATL showed fairly good anti-cancer activity against 3 out of five cell lines tested. As much as 56% inhibition was recorded in case of OVCAR-5 while for SiHa and Ht-29 it was found 49% and 45% respectively. However, no significant antiproliferative activity was observed with SNB-78 and PC-3 cancer cell lines

Fig. 7. Double immunodiffusion. ATL antiserum was loaded in the central well (A), 15 mg of the purified *Arisaema tortuosum* lectin was loaded in the peripheral (well 1) and the same amount of purified lectin from *Sauromatum guttatum (well 2), Gonatanthus pumilus* (well 3), *Galanthus nivalis* (well 4), *Crinum lattifolium* (well 5) and *Zephyranthes candida* (well 6) was loaded in (well 3) was loaded in peripheral wells

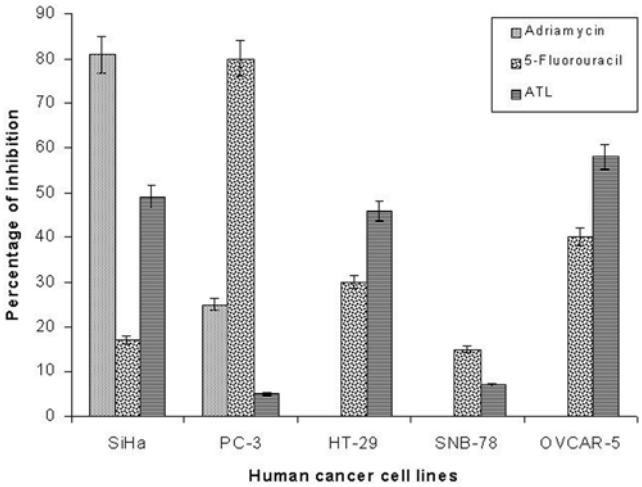

Fig. 8. The *in vitro* anti-proliferative effect of *Arisaema tortuosum* lectin. The inhibitory activity of ATL was tested on Human cancer cell lines SiHa (Cervix), PC-3 (Prostate), HT-29 (Colon), SNB-78 (CNS) and OVCAR-5 (Ovary). Anticancer drugs Adriamycin and 5-Fluorouracil were used as positive controls. The cells (1×10^4 /well) were cultured with ATL. In the control wells cells were culture with medium alone (no lectins). The growth/inhibition in the cells was measured by SRB dye staining assay. (Data represents means ± SD).

(Fig. 8). In the present study, ATL has been found specific towards LacNAc, which has been reported as a very important cancer cell marker (Ito *et al*., 1996). It is possible that as yet in some unknown way the lectin might hinder the signaling pathway of the cancer cell. At present it is very difficult to say why ATL failed to restrict the growth of SNB-78 and PC-3 cancer cell lines incorporated in the present study but significantly retarded the proliferation of HT-29, SiHa and OVCAR-5 cells. The possible answer to this question may be the difference in the signaling pathway of various cancer cell lines. Thus, keeping in view the biological properties and sugar specificities of ATL it may prove to be useful tool in cancer research, immunomodulation, glycobiology and as a chemotaxonomic marker for comparative evolutionary studies of monocot lectins.

In conclusion, it was a single step isolation and characterization of *Arisaema tortuosum* lectin with some important biological properties. Further research is warranted to clarify the molecular basis of inhibitory potential of the lectin on tumor cell differentiation and proliferation.

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References

Andrews, P. (1964) Estimation of the molecular weights of proteins by Sephadex gel-filtration. *Biochem. J*. **91**, 222-233.

Annick, B., Van Damme, E. J. M., Peumans, W. J. and Rouge, P. (1996) Structure-function relationship of monocot mannose-binding lectins. *Plant Physiol*. **112**, 1531-1540.

Bryan, J. K. (1977) Molecular weights of proteins multimers from polyacrylamide gel electrophoresis. *Annals Biochem*. **78**, 513-519.

Chandra, N. R., Prabu, M. M., Suguna, K. and Vijayan, M. (2001) Structural similarity and functional diversity in proteins containing the legume lectin fold. *Protein Engineering* **11**, 857-866.

Davis, B. J. (1964) Disc electrophoresis. II. Methods and applications to human serum proteins. *Ann. New York Acad. Sci*. **121**, 404-427.

Etzler, M. E. (1986) Distribution and function of plant lectins; in *The Lectins- Properties, Functions and Applications in Biology and Medicine*, Liener, I. E., N., Sharon, I. and Goldstein, J. (Eds.), pp. 371-435, Acadamic Press, Orlando, USA.

Gabius, H. J. (1987) Endogenous lectins in tumors and the immune system. *Cancer Investigation*. **5**, 39-46.

Goldstein, I. J., Hughes, R. C., Monsigny, M., Osawa, T. and Sharon, N. (1980) What should be called a lectin? *Nature* **285**, 66.

Green, E. D., Adelt, G., Baenziger, J. U., Wilson, S. and Van Halbeek, H. (1988) The asparagine-linked oligosaccharides on bovine fetuin. Structural analysis of N-glycanase-released oligosaccharides by 500-megahertz 1H NMR spectroscopy. *J. Biol. Chem*. **263**, 18253-18268.

Hajto, T., Hostanska, K. and Saller, R. (1999) Mistletoe therapy from the pharmacologic perspective. *Forsch Komplementarmed*. **6**, 186-194.

Hayes, C. E. and Goldstein, I. J. (1974) An a-D-Galactosyl-binding lectin from Banderiaea simplicifolia seeds. *J. Biol.*

Chem. **249**, 1904-1914.

Ito, N., Imai, S., Haga, S., Nagaike, C., Morimura, Y. and Hatake, K. (1996) Localization of binding sites of *Ulex europaeus* I, *Helix pomatia* and *Griffonia simplicifolia* I-B4 lectins and analysis of their backbone structures by several glycosidases and poly-N-acetyllactosamine-specific lectins in human breast carcinoma. *Histochem. Cell Biol.* **106**, 331-339.

Kaku, H., Van Damme, E. J. M., Peumans, W. J. and Goldstein, I. J. (1990) Carbohydrate-binding specificity of the daffodil (*Narcissus pseudonarcissus*) and amaryllis (*Hippeastrum hybr.*) bulb lectins. *Arch. Biochem. Biophys*. **279**, 298-304.

Kaur, N., Singh, J. and Kamboj, S. S. (2002) Affinity purification and characterization of a seed lectin from *Crotalaria medicaginea*. *Indian J. Biochem. Biophys.* **39**, 49-54.

Laemmli, U. K. (1970) Cleavage of structural prooteins during the assembly of the head of bacteriophage T4. *Nature* **277**, 680-685.

Lowry, O. H., Rosebrough, N. J., Farr, A. R. and Randall, R. J. (1951) Protein measurements with folin-phenol reagent. *J. Biol. Chem*. **193**, 265-275.

Mo, H., Rice, K. G., Evers, D. L., Winter, H. C., Peumans, W. J., Van Damme, E. J. M. and Goldstein, I. J. (1999) *Xanthosoma sagittifolium* tubers contain a lectin with two different types of carbohydrate-binding sites. *J. Biol. Chem*. **274**, 33300-33305.

Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paul, K., Vistica, D., Hose, C., Langley, J., Cronise, P., Wolff, A. V., Goodrich, M. G., Campbell, H., Mayo, J. and Boyd, M. (1991) Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J. Nat. Can. Inst.* **83**, 757-766.

Pang, Y., Shen, G. A., Liao, Z. H., Yao, J. H., Fei, J., Sun, X. F. and Tang, K. X. (2003) Molecular cloning and characterization of a novel lectin gene from *Zephyranthes candida*. *DNA Sequence* **14**, 163-167.

Parslew, R., Jones, K. T., Rhodes, J. M. and Sharpe, G. R. (1999) The antiproliferative effect of lectin from the edible mushroom (*Agaricus bisporus*) on human keratinocytes: preliminary studies on its use in psoriasis. *Brit. J. Dermat.* **140**, 56-60.

Paulova, M., Entlicher, G., Ticha, M., Kostir, J. V. and Kocourek, J. (1971) Studies of phytohemagglutinins. VII. Effect of Mn^{2+} and Ca^{2+} on hemagglutinin of phytohemagglutinin of *Pisum sativum* L. *Biochim. Biophys. Acta* **237**, 513-518.

Reisfeld, R. A., Lewis, O. J. and Williams, D. E. (1962) Disc electrophoresis of basic proteins and peptides on polyacrylamide gels. *Nature* **145**, 281-283.

Robertson, E. F., Dannelly, H. K., Malloy, P. J. and Reeve, H. C. (1987) Rapid isoelectric focussing in a vertical polyacrylamide minigel system. *Annals Biochem*. **167**, 290-294.

Shangary, S., Singh, J., Kamboj, S. S., Kamboj, K. K. and Sandhu, R. S. (1995) Purification and properties of four monocot lectins from the family Araceae. *Phytochemistry* **40**, 449-455.

Sharon, N. and Lis, H. (1990) Legume lectins-a large family of homologous proteins. *FASEB J.* **4**, 3198-3208.

Singh, J., Singh, J. and Kamboj, S. S. (2004) A novel mitogenic and antiproliferative lectin from a wild cobra lily, *Arisaema favum*. *Biochem. Biophys. Res. Commun*. **318**, 1057-1065.

Spiro, R. G. (1966) Analysis of sugars found in glycoproteins. *Meth. Enzymol*. **8**, 3-26.

Van Damme, E. J. M., Allen, A. K. and Peumans, W. J. (1987) Isolation and characterization of a lectin with exclusive specificity towards mannose from snowdrop (*Galanthus nivalis*) bulbs. *FEBS Lett*. **215**, 140-144.

Van Damme, E. J. M., Goossens, K., Smeets, K., Van Leuven, F., Verhaert, P. and Peumans, W. J. (1995) The major tuber storage protein of araceae species is a lectin. Characterization and molecular cloning of the lectin from *Arum maculatum* L. *Plant Physiol*. **107**, 1147-1158.

Van Damme, E. J. M., Brike, F., Winter, H. C., Van Leuven, F., Goldstein, I. J. and Peumans W. J. (1996) Molecular cloning of two different mannose-binding lectins from tulip bulbs. *Eur. J Biochem*. **236**, 419-427.

Van Damme, E. J. M., Allen, A. K. and Peumans, W. J. (1988) Related mannose-specific lectins from different species of the family Amaryllidaceae. *Physiol. Planta* **73**, 52-57.

Van Damme, E. J. M., Goldstein, I. J., Vercammen, G., Vuylsteke, J. and Peumans, W. J. (1992) Lectins of members of the Amaryllidaceae are encoded by multigene families which show extensive homologies. *Physiol. Planta* **86**, 245-252.

Van Damme, E. J. M., Goldstein, I. J. and Peumans, W. J. (1991) A comparative study of related mannose-binding lectins from the Amaryllidaceae and Alliaceae. *Phytochemistry* **30**, 509-514.

Van Damme, E. J. M., Smeets, K., Torrekens, S., Van Leuven, F. and Peumans, W. J. (1993) The mannose-specific lectins from ramsons (*Allium ursinum* L.) are encoded by three sets of genes. *Eur. J Biochem*. **217**, 123-129.

Van Damme, E. J. M., Smeets, K., Torrekens, S., Van Leuven, F. and Peumans, W. J. (1994) Characterization and molecular cloning of mannose-binding lectins from the Orchidaceae species *Listera ovata*, *Epipactis helleborine* and *Cymbidium* hybrid. *Eur. J Biochem*. **221**, 769-777.

Whitakar, J. R. (1963) Determination of molecular weights of proteins by gel filtration on Sephadex. *Annals Chem*. **35**, 1950-1953.

Zarkovic, N., Vukovic, T., Loncaric, I., Miletic, M., Zarkovic, K., Borovic, S., Cipak, A., Sabolovic, S., Konitzer, M. and Mang, S. (2001) An overview on anticancer activities of the *Viscum album* extract Isorel. *Cancer Biother. Radiopharma*. **16**, 55-62.