Conclusions
CHAPTER 2

- A galactose specific lectin has been purified from Dolichos lablab seeds by affinity chromatography on Sepharose-galactose gel, in the presence of 1.5 M ammonium sulphate.

- The activity of the lectin was inhibited by galactose at concentrations of 2.8 mM and above.

- From 100 g of seeds 175 mg of affinity purified lectin could be obtained.

- Purified lectin is a glycoprotein containing 5% carbohydrate and binds to Con A-Sepharose gel.

- It was found to be homogenous by native gel electrophoresis and exhibits a native molecular mass of 120 ± 5 kDa in gel filtration. In SDS-PAGE it dissociates into two bands with molecular masses of 31 kDa and 29 kDa.

- Both the bands stained positive for carbohydrate (PAS staining).

- Partial amino terminal sequence analysis of the two subunits revealed that they are identical suggesting the oligomeric nature of the lectin.

- Antibodies raised to the purified lectin showed specific reactivity with the protein in immunodiffusion and Western blot analysis. The lectin was also found to cross-react with the antibody raised to the well characterized glucose/mannose specific lectin from the same seeds and vice-versa.

- Purified lectin exhibited its activity upto 40 °C. Beyond this temperature its activity reduced considerably.
• The amino acid composition of this lectin shows high concentrations of acidic and hydrophobic amino acids. Cysteine and methionine could not be detected.
• Modification of tryptophan and arginine residues did not alter the agglutination property and the binding ability of the lectin to the affinity gel.
• Modification of the lysine residues resulted in decreased binding on the affinity gel by 31 % and its agglutinating activity by 43 %.
• Modification of tyrosine residues led to the acetylation of all the tyrosine residues. There was a decrease in biological activity only by 20 %.
• Modification of histidine residues showed 75 % loss in hcmagglutinating activity and about 70 % loss in its binding ability to the affinity gel. Reversal of histidine modification regained 5 out of 15 histidine residues. Histidine modification performed in presence of the inhibitory sugar showed protection against modification, suggesting the possible involvement of histidine residues in the biological activity of the lectin.
• However, modification of the amino acids did not alter the immunological property of the lectin, indicating that the loss of activity is not due to alterations in the gross changes in the overall structure of the protein.
• In vitro translation of the lablab bean mRNA yielded a single polypeptide precursor of 33 kDa that was recognized by the seed lectin antibody.

CHAPTER 3
• [3-N-acetyl hexosaminidase was purified from the seeds and its native molecular mass by gel filtration was calculated to be 104 kDa ± 5 kDa. The enzyme contains 4 % carbohydrate. In SDS-PAGE the enzyme dissociated into three sub-units of 32
kDa, 30 kDa and 29 kDa. One of the three sub-units cross-reacted with the human placental p-N-acetyl hexosaminidase antisera.

- The enzyme was fully active at 4 °C and up to 40 °C. Beyond 40 °C, the stability of the purified enzyme decreased.

- Intact, protein bodies have been isolated from the seeds successfully by sucrose density gradient centrifugation.

- Protein bodies were lysed and the supernatant obtained contained both the glucose/mannose specific lectin and the galactose specific lectin. It also showed the presence of enzymes like α-mannosidase and P-N-acetyl hexosaminidase.

- The pellet was processed by sucrose density gradient centrifugation to prepare the protein body membranes. The lectins were able to bind to these protein body membranes at pH 5.0 and can be desorbed at pH 8.0.

- The P-N-acetyl hexosaminidase enzyme also bound to the protein body membranes at pH 5.0 and was desorbed with a change in pH to 8.0.

- Modification of the histidine, lysine and arginine residues in the galactose specific lectin decreased the binding abilities of lectin to the protein body membranes to 45%, 40% and 75%, respectively.

- An endogenous lectin receptor has been detected in the acidic glutelin fraction of proteins obtained from the seed extract. Preliminary studies revealed that this receptor is a component of the protein body membrane fraction as determined by the cross-linking studies.
CHAPTER 4

• Neither of the lectins under study exhibited antifungal activity when tested with *Trichoderma viridae, Aspergillus niger* and *Aspergillus flavus.*

• Galactose specific lectin obtained from the seeds of the *Dolichos lablab* did not exhibit any mitogenic activity to the human peripheral blood lymphocytes.

• In contrast, the glucose / mannose specific lectin from the same seeds exhibits mitogenic stimulation to the human peripheral blood lymphocytes.

• Estimation of glucose levels during the course of mitogenesis reveal that the cells incubated with galactose specific lectin consume a negligible amount when compared to the cells incubated with glucose/mannose specific lectin.

• FITC labeled galactose specific lectin binds more to the human peripheral multiple myeloma cells than to the normal human peripheral lymphocytes, thus suggesting that this lectin might act as tracers in detection of multiple myeloma cells.

• α-Mannosidase and p-N acetyl hexosaminidase lysosomal enzymes present in the cell secretions of MPR (-) cells interacted well with the lectins. The α-mannosidase enzyme was recognized by glucose/mannose lectin and p-N acetyl hexosaminidase enzyme could be recognized by the galactose lectin. These enzymes were also recognized by Con-A biotin blotting suggesting them to be glycoproteins.

• α-Mannosidase and (3-N-acetyl hexosaminidase enzymes present in the normal human serum interacted only with the galactose specific lectin. In Western blot analysis of the lectin affigel eluates, a single protein band of (5-N acetyl hexosaminidase (66 kDa) was detected with human placental (3-N acetyl hexosaminidase antisera.
CHAPTER 5

• An RT-PCR approach was used to amplify the galactose specific lectin gene in two cDNA fragments that were cloned and sequenced. The sequence of the fragments showed overlapping residues.

• Since a partial sequence of 208 amino acids obtained here was homologous but different from the glucose/mannose specific lectin, it is logical to conclude that this represents the sequence of the galactose specific lectin.

• When the sequence of the galactose specific lectin was aligned with other legume lectins, it showed considerable homology.

• In the preliminary Northern blot, there were two mRNA transcripts detectable of the sizes of 3.7 Kb and 1.9 Kb corresponding possibly to the galactose specific lectin and the glucose/mannose specific lectin, respectively.

CHAPTER 6

• Preliminary experiments on the crystallization of the galactose specific lectin revealed that the lectin can be obtained as stable crystals.