RESULTS

3. RESULTS

3.1 ISOLATION AND PURIFICATION OF LECTINS.

Crude extracts of *Artocarpus integer* and *Artocarpus heterophyllus* seeds were subjected to double immobilised galactose-Sepharose affinity chromatography as described in Section 2.5.4 of the Materials and Methods. Unbound effluent from the galactose columns was rechromatographed on an immobilised mannose-Sepharose affinity column and eluted with 0.8 M D-mannose in PBS. The total protein content and percentage recovery of mannose- and galactose-reactive lectins from crude seed extracts of *Artocarpus integer* and *Artocarpus heterophyllus* are summarised in table 5. It is apparent that substantially more mannose-reactive lectins was obtained from the crude extracts of *Artocarpus integer* seeds (30.1 %) than the crude seed extracts of *Artocarpus heterophyllus* (2.4 %). Fig. 7 demonstrates typical elution profiles of mannose-Sepharose affinity chromatography of crude seed extracts of *Artocarpus integer* and *Artocarpus heterophyllus*.

3.2 STRUCTURAL STUDY

3.2.1 Gel Filtration Chromatography on Sephadex G-100

The purified mannose-reactive lectin eluted from the affinity column was subjected to Sephadex G-100 gel filtration chromatography. When elution was performed with 0.1 M glacial acetic acid, the sample was resolved as a single peak (Fig. 8), which corresponds to a molecular weight of 64,000 as estimated using the protein markers calibration curve (see Fig. 6 of the Materials and Methods section).

Table 5. Total protein content and percentage recovery of mannose- and galactose-reactive lectins.

	Total protein/g seed (mg)	% Recovery of protein
Champedak (*A. integer*)		
Crude seed extract	50.11	-
Lectin-C (Galactose-binding)	28.42	56.72
Lectin-M (Mannose-binding)	15.07	30.07
Jackfruit (*A. heterophyllus*)		
Crude seed extract	32.89	-
Jacalin (Galactose-binding)	18.22	55.40
Artocarpin (Mannose-binding)	0.79	2.40

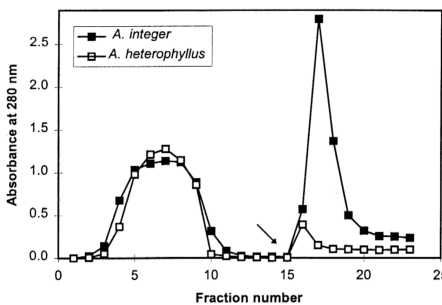


Figure 7. Elution Profiles of Lectins Isolated from Mannose-Sepharose Affinity Chromatography.

Crude seed extracts of *Artocarpus heterophyllus* and *Artocarpus integer* were separately subjected to double immobilised galactose-Sepharose affinity chromatography as described in the Materials and Methods section. Their effluents were subsequently applied into 5 ml mannose-Sepharose columns and extensively washed with PBS. The mannose-binding lectins were eluted with 0.8 M mannose in PBS. Arrow indicates start of the mannose elution.

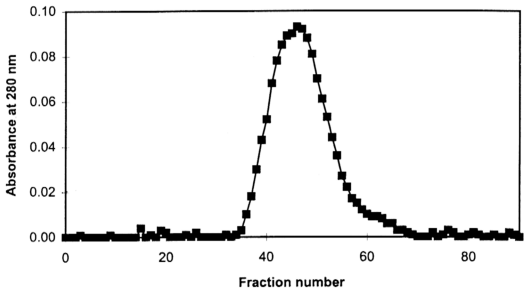


Figure 8. Gel Filtration of Mannose-Binding Lectin.

Mannose-binding lectin isolated from affinity column was applied into a 1.5 x 100 cm Sephadex G-100 gel filtration column. Elution was carried out with 0.1 M glacial acetic acid at a flow rate of 20 ml/hr. Fractions of 2 ml were collected. The sample was resolved into a single 64 kDa elution peak.

3.2.2 SDS-Polyacrylamide Gel Electrophoresis of the Purified Lectins

Peak fractions from the gel filtration chromatography were pooled and subjected to SDS-PAGE analysis under reducing and non-reducing conditions using a precast Tris-Tricine gel (16.5 %). Fig. 9 demonstrates the SDS-PAGE patterns of crude and purified lectin preparations. The 64 kDa champedak lectin-M preparation was resolved into two sharp bands with M_r of 16,800 and 31,400. However, only the 16.8 kDa band was detected when analysis was performed in the presence of a reducing agent.

3.3 BINDING SPECIFICITY STUDY

3.3.1 Determination of Specificity for Binding to Glycoproteins

Previous studies have shown that the galactose-binding lectin-C from *Artocarpus integer* selectively precipitated IgA1 and not any other isotypes of human immunoglobulin (*Hashim et al.*, 1991). In this initial study, an attempt was made to compare the reactivity of lectin-M with lectin-C by allowing both of them to interact adjacently with normal human serum using simple double diffusion. Both lectins produced precipitin lines (Fig. 10a) and these were noted to cross-over, in a reaction of non-identity.

The interaction of champedak lectin-M with various isotypes of human immunoglobulin was also investigated using the double diffusion method. Clear precipitin lines were formed from the interaction of champedak lectin-M with IgE and IgM at 2 mg/ml concentration, but not with IgA1, IgA2, IgD and IgG (Fig. 10b). When the lectin was subjected to double diffusion reactions with glycoproteins having variously defined structures of *N*-linked oligosaccharides moieties, precipitin arcs

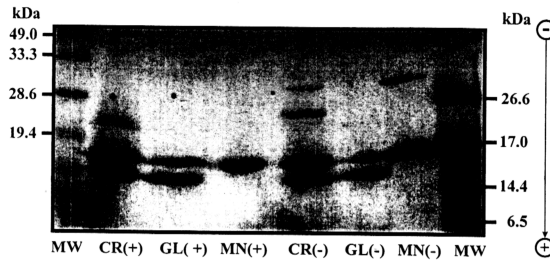


Figure 9. SDS-Polyacrylamide Gel Electrophoresis of Champedak Crude and Purified Lectins.

The crude and purified lectins were subjected to electrophoresis in a 16.5 % (w/v) Tris-Tricine precast gel. CR refers to champedak crude seed extract. MN and GL represent its mannose- and galactose-binding lectin, respectively. (+) and (-) indicate reducing and non-reducing conditions, respectively. Gel was calibrated with two sets of molecular weight (MW) markers, with set 1 consisting of prestained proteins - ovalbumin (49,000), carbonic anhydrase (33,300), soybean trypsin inhibitor (28,600) and lysozyme (19,400), and set 2 containing triosephosphate isomerase (26,600), myoglobin (17,000), α-lactalbumin (14,400) and aprotinin (6,500). M_r of lectins were estimated based on the graph of log M_r of standards vs. R_f (data not shown). Arrow indicates direction of migration of samples.

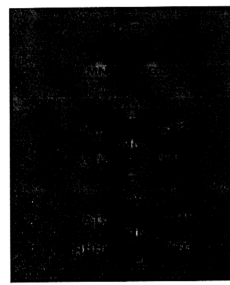


Figure 10. Interactions of Lectins with Normal Human Serum, Immunoglobulins and Glycoproteins.

Double diffusion demonstrating differences in precipitating reaction between mannose-binding lectin and glycoproteins. Experiments were carried out by loading 15 ul of each sample into the wells on 1 % (w/v) agarose gel.

Panel a: MN, mannose-binding champedak lectin (2 mg/ml); GL, galactose-binding champedak lectin (2 mg/ml); NHS, normal human serum (undiluted).

Panel b: A1, IgA1; A2, IgA2; D, IgD; E, IgE; G, IgG; M, IgM; MN, mannose-binding champedak lectin (all at 2 mg/ml).

Panel c, OV, ovalbumin; OR, orosomucoid (α_1-acid glycoprotein); TG, porcine thyroglobulin; TF, transferrin; HRP, horseradish peroxidase; AT, α_1-antitrypsin; MN, mannose-binding champedak lectin (all at 4 mg/ml).

were detected with porcine thyroglobulin, horseradish peroxidase and human α_1-acid glycoprotein, but not with ovalbumin, human transferrin and human α_1-antitrypsin (Fig. 10c).

3.3.2 Effect of Glycoproteins, Sugars and Other Substances on Lectin M-HRP Interaction

A large number of compounds including glycoproteins, sugars and metal ions have been tested for their capacity to inhibit the interaction between lectin M and HRP (section 2.5.10). In this experiment, it was possible to determine some of the stereochemical requirements of the combining site of lectin M molecule.

3.3.2.1 Effects of Glycoproteins

In order to confirm the interaction of lectin-M with the various glycoproteins, a competitive HRP-binding assay was performed in their presence. Fig. 11 (panel a) demonstrates the results of the assay carried out in the presence of various isotypes of immunoglobulins. The data are in support of our earlier findings that lectin-M interacts strongly with IgE and IgM. In this experiment, IgA2 was also shown to have an inhibitory effect on the HRP-lectin-M interaction although the immunoglobulin did not form a precipitate with the lectin in the double diffusion study. The inhibitory potency of IgA2 was, however, much weaker than that of IgE and IgM. When the competitive HRP-binding assay was carried out in the presence of the other glycoproteins that were tested in the double diffusion experiment, it appeared that all demonstrated inhibitory effects, although these differed in terms of potency (Fig. 11b).

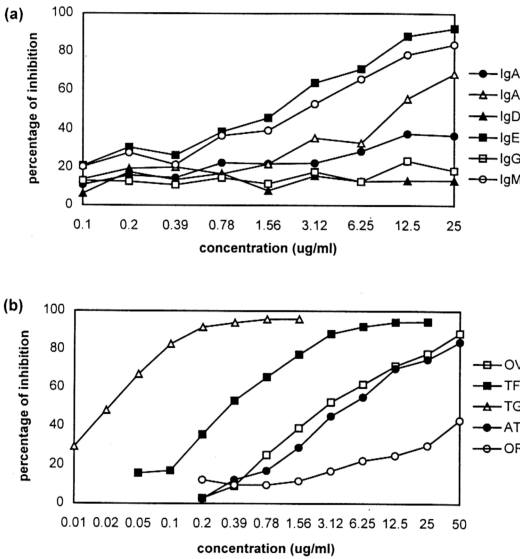


Figure 11. Effects of Glycoproteins on Lectin-M-HRP Binding.

HRP-binding assay was performed in the presence of various isotypes of (a) immunoglobulins and (b) glycoproteins with defined structures of *N*-linked oligosaccharide moieties. OV - ovalbumin; TF - human transferrin; TG - porcine thyroglobulin; AT - human α_1-antitrypsin; OR - human orosomucoid (α_1-acid glycoprotein). Values shown are the average of triplicate readings.

3.3.2.2 Effect of Sugars.

The carbohydrate binding specificity of lectin-M was analysed by determination of the effects of sugars in a similar competitive HRP-binding assay. Twenty two sugars, including mono- and disaccharides, as well as derivatives for mannose, galactose and glucose, were tested. The amount of sugars required to achieve 50 % inhibition of HRP-lectin binding was taken as a measure of the interfering potency of that sugar. Concentrations of carbohydrate required for 50 % inhibition of the lectin-M-HRP interaction are listed in Table 6. Among the sugars tested, eleven displayed inhibitory activity which decreased in the following order: Manα1-3Man, methyl-α-D-Man, Manα1-3Man1-*O*-Me, Manα1-6Man1-*O*-Me, Manα1-6Man, Manα1-4Man1-*O*-Me, D-mannose, Manα1-2Man1-*O*-Me, *N*-Acetyl-Neuraminic Acid, D-Glucose and *N*-Acetyl-D-Glucosamine (Fig. 12 a & b).

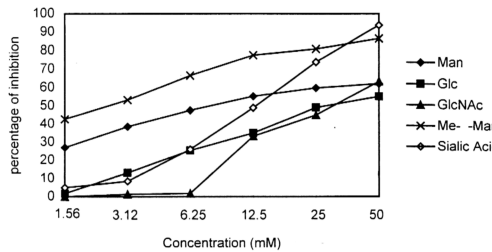
3.3.2.3 Effects of Metal Ions and EDTA.

To assess whether divalent metal ions were required for the expression of lectin activity, the HRP-binding assay was performed in the presence of EDTA. The presence of EDTA apparently induced a inhibitory effect on lectin-M-HRP binding but at a very high concentration. In addition, increased binding was not observed when the assay was carried out in the presence of several metal ions suggesting that the lectin interaction is metal ion independent (Fig. 13).

3.4 LECTIN M-AFFINITY CHROMATOGRAPHY

The ability of lectin to bind selectively and reversibly to certain carbohydrate structure is well known. These properties of lectins permit their use as a tool in the

(a)



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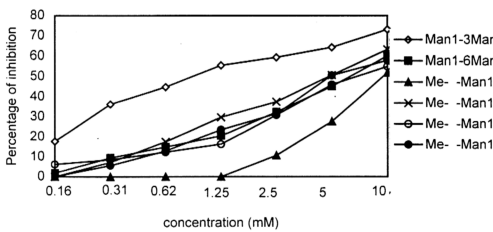


Figure 12. Effect of Sugars on Lectin M-HRP Binding.

HRP-binding assay was performed in the presence of various sugars with concentration ranging from 0.16 mM to 100 mM. Values shown are the average of triplicate readings.

Panel a: Monosaccharides and its derivatives which exhibit inhibitory effect.

Panel b: Mannobioses and its derivatives which exhibit inhibitory effect.

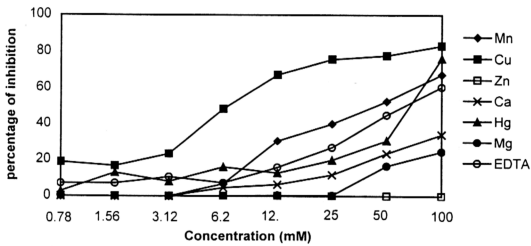


Figure 13. Effects of Metal Ions and EDTA on Lectin M-HRP Interaction.

HRP-binding assay was performed in the presence of various metal ion and EDTA at serially decreasing concentration (100mM - 0.78 mM). Values shown are the average of triplicate readings.

Table 6. Concentration of sugars and various substances that produced 50% inhibition on lectin M-HRP binding.

Sugars etc.	Conc. (mM)	Relative Inhibitory Potency
3-*O*-α-D-Mannopyranosyl-D-Mannopyranose	0.95	8.23
Methyl-α-D-Mannopyranose	2.65	2.95
Methyl-3-*O*-α-D-Mannopyranosyl-D-Mannopyranose	5.00	1.56
Methyl-6-*O*-α-D-Mannopyranosyl-D-Mannopyranose	5.21	1.50
6-*O*-α-D-Mannopyranosyl-D-Mannopyranose	6.59	1.18
Methyl 4-*O*-α-D-Mannopyranosyl-D-Mannopyranose	7.20	1.08
D-Mannose	7.82	1.00
Methyl-2-*O*-α-D-Mannopyranosyl-D-Mannopyranose	9.41	0.83
N-Acetyl-Neuraminic Acid	12.80	0.61
D-Glucose	27.63	0.28
N-Acetyl-D-Glucosamine	31.02	0.25
EDTA	65.72	0.12
$CuSO_4$	6.77	1.16
$MnCl_2$	44.63	0.17
$HgCl_2$	71.03	0.11

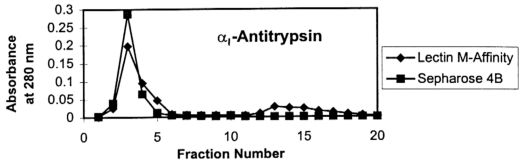
The percentage of inhibition was less than 50 % in the presence of the following sugars and salts tested at a concentration of 100 mM: D-galactose, *N*-Acetyl-D-Galactosamine, *N*-Acetyl-D-Mannosamine, Methyl-α-D-Galactopyranoside, 4-*O*-β-Galactopyranosyl-D-Mannopyranose, Melibiose (6-*O*-α-D-Galactopyranosyl-D-Glucose), Fucose (6-Deoxy-D-Galactopyranose), Rhamnose (6-Deoxy-L-Mannopyranose), D-Mannose-6-Phosphate, D-Mannose-1-Phosphate, D-Mannosamine, 1,6-Anhydro-β-D-Mannopyranose, Methyl-6-*O*-(GlcNAc)α-D-mannopyranoside, $CaCl_2$, $MgCl_2$ and $ZnSO_4$.

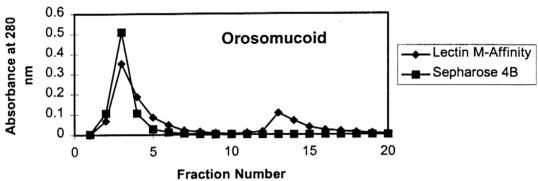
isolation of carbohydrate containing compounds. For example, immobilized-jacalin has been widely used for affinity purification of IgA1, C1 inhibitor and other O-linked oligosaccharide containing proteins. As such, the potential usage of lectin M as a purification technique for proteins containing N-linked oligosaccharide were explored in this study.

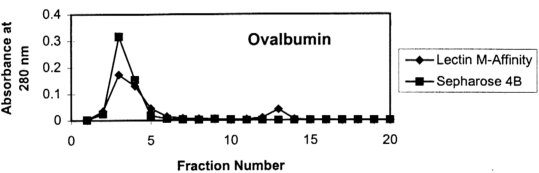
The binding affinity and specificity of lectin M towards glycoproteins with defined N-linked oligosaccharide(s) attached, including α_1-antitrypsin, orosomucoid, ovalbumin, transferrin, thyroglobulin and IgM, were investigated. These glycoproteins were eluted by solutions containing 0.1 M methyl-α-mannopyranose which has been shown to be a potent inhibitor for lectin M interaction. Among all the glycoproteins tested, only transferrin is fully retained by the lectin M-affinity column. For the other five glycoproteins, a large amount appeared to have passed through the column unretained, only approximately 10 - 50 % of the total material as measured by absorbance at 280 nm were bound to the lectin column (Figure 14). The diversity of elution pattern of these glycoproteins was believed to be due to the microheterogeneity of their oligosaccharide chain structure.

3.5 REACTIVITY WITH HUMAN SERUM PROTEINS

In this study, several different approaches were employed in order to determine whether lectin M has any affinity towards other human serum proteins besides the serum glycoproteins (including IgE, IgM, IgA2, transferrin, orosomucoid and α_1-antitrypsin) that were already demonstrated to be lectin-reactive in earlier experiments.







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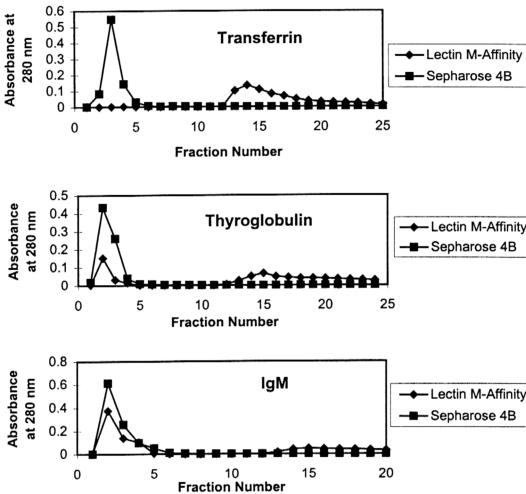


Figure 14. Elution Profiles of N-linked Oligossaccharide-Containing Glycoproteins on Lectin M-Sepharose.

Glycoproteins (1 mg) were applied to the lectin M-Sepharose and Sepharose 4B (control set) columns (0.8 x 4 cm) followed by elution with PBS. The arrow indicates the addition of 0.1 M methyl-α-mannopyranose in the eluent. Fractions of 1 ml were collected. Experiments were performed on lectin M binding proteins including α_1-antitrypsin, orosomucoid, ovalbumin, transferrin, thyroglobulin and IgM.

3.5.1 Immunoelectrophoretic Analysis of Interaction of Lectin M with Human Serum Proteins.

A preliminary study on the interaction of lectin M with serum proteins was carried out by immunoelectrophoresis experiment. Human serum proteins were separated by electrophoresis of NHS on a IEP Hydragel as described in Section 2.5.11. Precipitin arcs corresponding to the respective proteins were formed when lectin or antisera were allowed to react. Figure 15 demonstrates the immunoelectrophoretic profile of interaction of lectin M with NHS. Unlike its galactose-binding lectin which was previously shown to form an IgA1 precipitin arc (Hashim *et al.*, 1991), the mannose-binding *Artocarpus integer* lectin resulted in a clear formation of a distinct precipitin arc with an unknown serum protein. In addition, several faint precipitation lines were also detected indicating interaction with other serum proteins. In this experiment, no IgE and IgM precipitin line was detected, although the double diffusion study has conclusively demonstrated the interaction of lectin M with IgE and IgM. The failure of the lectin M to form precipitin lines with IgE and IgM was most likely due to the low concentrations of the immunoglobulins present in normal human serum.

3.5.2 Identification of Lectin M-Reactive Serum Proteins

In order to further investigate the serum protein components that interacts with lectin M, an affinity chromatography utilising the immobilised lectin was used to separate lectin M interactive and non interactive serum proteins (Section 2.5.6). The bound serum proteins were eluted with 0.1 M methyl-α-mannopyranose in PBS, pooled, and dialysed extensively against PBS. Identification of the lectin M-reactive serum protein was performed using high resolution two dimensional electrophoresis.

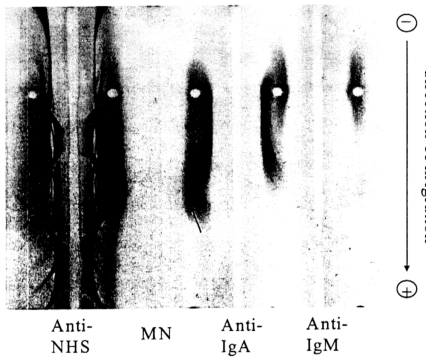


Figure 15. Immunoelectrophoresis Analysis of lectin M Interaction with normal human serum.

Normal human serum was placed in the wells and electrophoresed for about 40 min at a constant voltage of 100V. The troughs were filled with purified lectin M (MN); anti-mu heavy chain (Anti-IgM); anti-alpha heavy chain (Anti-IgA) and anti-whole human serum (Anti-NHS). Arrow indicates the major unknown protein precipitin line.

In an alternative study, serum proteins were initially separated by 2D-electrophoresis, while enzyme-conjugated lectin M was used as a probe to detect the lectin interactive proteins.

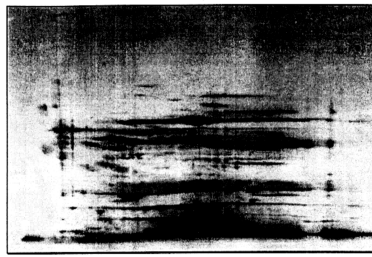
3.5.2.1 Analysis by Two-Dimentional Electrophoresis

The 2D pattern of normal human serum proteins (Fig. 16a) and lectin M-affinity purified serum proteins (Fig. 16b) were analysed after silver staining of the gels. Each protein was identified using the SWISS-2DPAGE as a reference protein map (Sanchez *et al*, 1995). The data clearly indicates that only selective glycoproteins like α_1-antitrypsin, α-heavy chain, haptoglobin-β-chain and a few other unknown glycoproteins had interacted with the immobilised lectin M. Lectin M is thus a useful tool that may be used to separate these glycoproteins from all the other non-interactive serum protein components.

3.5.2.2 Western Blotting

In a separate experiment, instead of using the lectin to isolate serum glycoproteins, enzyme-conjugated lectin M was used to detect the 2D-electrophoresed normal human serum proteins. Fig. 17 demonstrates the result of the experiment. The data indicates that, in addition to α_1-antitrypsin, haptoglobin-β-chain and immunoglobulin α-heavy chain which were shown to be detected in the bound fractions of lectin M-affinity column as described in Section 3.5.2.1, lectin M also demonstrates interaction with μ-heavy chain, γ-heavy chain, transferrin and orosomucoid. However, the interaction of lectin M with IgE which was clearly shown in the double diffusion experiment was not detected in this experiment. Interestingly,

(a)



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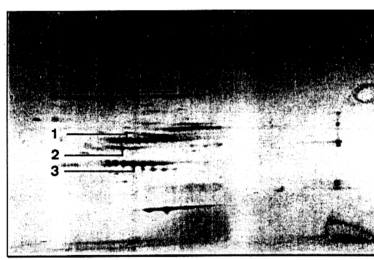

Figure 16. Analysis of Lectin-Reactive Serum Proteins Isolated by Affinity Chromatography

High-resolution 2D electrophoretic was performed to separate the serum proteins from normal human serum (*Panel a*) and lectin M-affinity purified serum glycoproteins (*Panel b*). Proteins were separated in the first dimension by IEF electrophoresis and in the second dimension by SDS-PAGE as described in the Methods and Materials section. Detection of proteins were performed by silver staining and *x* indicates the unknown lectin M-interactive proteins. The identified proteins are: (1) α-heavy chain, (2) α_1-antitrypsin and (3) haptoglobin-β-chain.

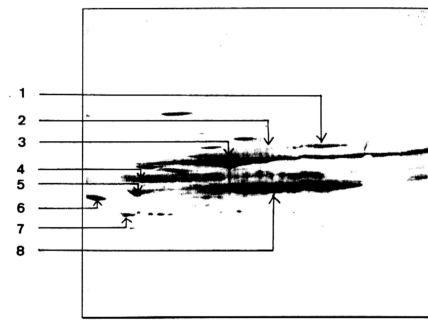

Figure 17. Western Blotting Analysis on Lectin M-Reactive Human Serum Protein.

Human serum proteins were separated by 2D and blotted onto a nitrocellulose membrane. The blot was probe with lectin M-alkaline phosphatase conjugate overnight at 4°C and visualised using BCIP/NBT as described in Section 2.5.14. Shown are the detected lectin M-reactive human serum proteins. (1) Transferrin, (2) μ-heavy chain, (3) albumin, (4) α-heavy chain, (5) α_1-antitrypsin, (6) orosomucoid and (7) haptoglobin-β-chain and (8) γ-heavy chain.

non-specific binding to albumin was also found to have occurred. This binding is believed to be a result of the hydrophobic interaction.

3.6 BIOLOGICAL ACTIVITY OF LECTIN M ON MURINE LYMPHOCYTES

The crude extract of *Artocarpus integer* seeds were previously shown to contain a strong mitogen which selectively stimulated the proliferation of human T cells (Hashim *et.al.*, 1992). In order to identify which lectin in the crude extract that was responsible for the mitogenic activity, a similar study was performed but using purified lectin preparations: lectin C (galactose-binding lectin isolated from crude extract) and lectin M (mannose-binding lectin isolated from crude extract). Due to the difficulty in getting large amount of human T and B cells for the mitogenic study, murine lymphocytes isolated from mouse organ were used as an alternative target in this experiment.

3.6.1 Effect on Proliferation of Murine Thymocytes

Fig. 18 demonstrates the proliferative response of murine thymocytes when stimulated with different purified lectins at different concentrations ranging from 0.3 - 10μg/ml. The data indicates that lectin M exhibits a significant mitogenic effect. While the crude extract exhibits intermediate level of response at the tested concentration range, lectin C fraction induces only a very weak proliferation response. On the other hand, Fig. 19 demonstrate that when thymocytes were exposed to stimulation of lectin C at a higher concentration range (3 - 100 μg/ml), a significant proliferation activity was only observed at concentrations higher than 25 μg/ml. It is

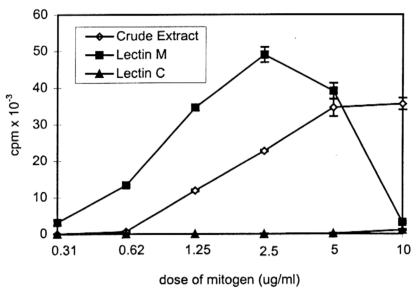

Figure 18. Proliferative Response of Murine Thymocytes Stimulated with Lectins.

Thymocytes were cultured in triplicate at a concentration of 5 x 10^5 cells/well in the presence of different lectin preparations (concentration ranging from 0.3-10 μg/ml). Cultures were incubated for 3 days. Each well was pulsed with 1μCi of [^{3}H]-thymidine 18-24 hr prior to harvest. The incorporation of [^{3}H]-thymidine into DNA is expressed as cpm in stimulated culture minus cpm in unstimulated culture. The standard deviation (SD) of the triplicate is shown by the error bar.

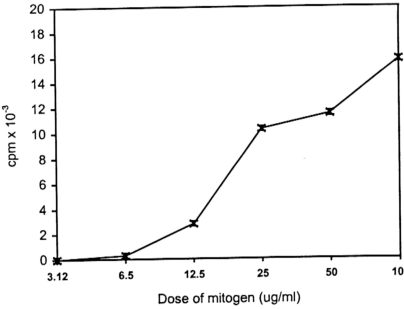

Figure 19. Proliferative Response of Murine Thymocytes Stimulated with Lectin C

Thymocytes (5 x 10^5 cells/well) were cultured for 3 days with different concentrations of lectin C (ranging from 3-100 μg/ml). Cultures were pulsed with 1 μCi of [^{3}H]-thymidine 24 hr before harvesting. The incorporation of [^{3}H]-thymidine is expressed as mean cpm of the triplicate cultures.

very clear at this point that the mitogenic effect of the crude extract as demonstrated previously was due to the lectin M stimulation. The data also demonstrates that a maximum proliferation response was achieved when the thymocyte cultures were induced with 2.5 μg/ml of purified lectin M. However, at higher doses (10 μg/ml), the lectin showed a decrease in mitogenic activity.

Like other phytomitogens, besides having the ability to stimulate the proliferation of lymphocytes, lectin M also appeared to be a lymphoagglutinin. Fig. 20 demonstrates that the degree of cells agglutination caused by lectin M is directly proportional to the degree of cellular proliferation.

3.6.2 Effect of Mannose on Lectin M Stimulation

The effect of mannose on [^{3}H]-thymidine incorporation in the presence of 2.5 μg/ml of lectin M was examined (Fig. 21). The data demonstrates that the proliferation of murine thymocytes induced by lectin M was inhibited in a quantitative manner by mannose. The percentage of inhibition was increased from 26 % to 75 % when the mannose was added at a concentration of 0.62 mg/ml and 2.5 mg/ml respectively. However, the inhibitory effect was not further enhanced by the addition of 5 mg/ml of mannose.

3.6.3 Effect on Proliferation of Murine B Lymphocytes

In order to determine the proliferative effect of lectin M on murine B cells, nude mouse spleen cells (5 x 10^5 cells/well) were cultured in the presence of lectin M. Like artocarpin and Con A, lectin M is not a B cell mitogen as it did not induce any appreciable synthesis of DNA as measured by the incorporation of [^{3}H]-thymidine at

(a) 0 μg/ml

(b) 0.62 μg/ml

(c) 1.25 μg/ml

(d) 2.50 μg/ml

Figure 20. Agglutination of Murine T Cell

Thymocytes were cultured in a 96 wells flat-bottom microtitre plate at a concentration of 5 x 10^5 cells/well in the presence of different concentration of lectin M. Cultures were incubated at 37°C for 3 days in a humidified 5 % CO_2 atmosphere. Cells were observed under an inverted microscope and photographs were taken with an attached camera.

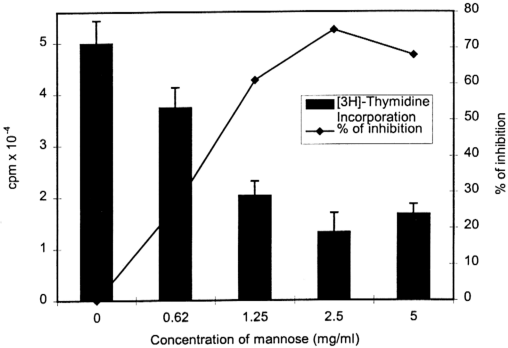

Figure 21. The Effect of Sugars on [^{3}H]-Thymidine Incorporation by Thymocytes Stimulated with Lectin M.

Murine thymocytes (5 x 10^5 cells/well) were cultured for 3 days in the presence of 2.5 μg/ml lectin M and different concentration of mannose. The incorporation of [^{3}H]-thymidine into DNA was determined during the last 24 hr of culturing. The results are expressed as the mean cpm ± SD of triplicate values. Percentage of inhibition caused by mannose at respective concentrations is represented by a line-graph.

the tested concentration (0.3 - 20 μg/ml). The data in Figure 22 indicates that the differences in incorporation between lectin M-stimulated cultures and unstimulated cultures are not significant. The summary of the effect of lectin M on the proliferation of the two murine lymphocyte populations is demonstrated in Figure 23.

3.6.4 Polyclonal Activation of B Cells for Immunoglobulin Secretion

The capability of crude extract and its different fractions to activate B cells for the secretion of immunoglobulins were also studied. Supernatant from lectin M-stimulated mouse spleen cell cultures were collected at different times (1 - 7 days) and assayed for the presence of immunoglobulin isotypes by antibody capture ELISA (Section 2.5.16.2). No significant difference of immunoglobulin secretion was observed between the cultures performed with and without lectin M for a period of 1-7 days.

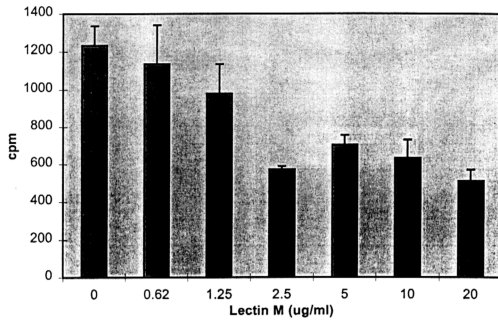

Figure 22. Nude Mouse Spleen Cell Proliferative Response to Lectin M

Purified nude mouse splenocytes were cultured at a concentration of 5 x 10^5 cells/well in the presence and absence of lectin M. The cultures were incubated in a humidified atmosphere with 95 % air and 5 % CO_2 for 3 days and pulsed with 1μCi of [^{3}H]-thymidine 24 hr prior to harvest. The incorporation of [^{3}H]-thymidine are expressed as cpm ± SD of triplicate values.

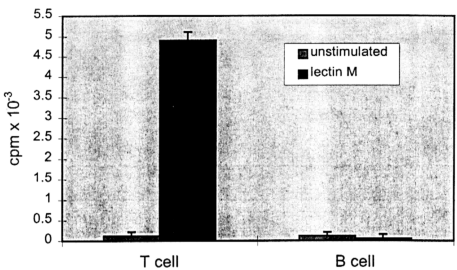

Figure 23. Proliferative Response of Purified Murine T and B cell Stimulated with Lectin M.

[^{3}H]-Thymidine incorporation in purified T and B lymphocytes cultures after 72 hr stimulation by 2.5 μg/ml of lectin M. Results are expressed as mean count per minute.