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SCREENING OF SOY LECTIN: AS NEW ERA CANCER HEALING AGENT

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ABSTRACT: The lectins are glycoproteins or sugar binding proteins of non-immune origin but are barred from sugar binding antibodies and enzymes. Lectins are isolated and purified from seeds of *Glycine max* by soxhlet extraction and dialysis. These collected crude lectins were centrifuged till pH is shifted downward to optimal pH for coprecipitation. Filtration of the same carried out on a Buchner funnel with a pad of Hiflo Supercel on whatman paper. Galactose was added as a ligand to the mixture kept at 250C for 10-20 min. It formed matrix coprecipitation which was centrifuged to remove additional particulates. Supernatant was removed and retained the galactose lectin coprecipitate which finally yields lectins, further purified by dialysis. Encapsulation by spray drying using maltodextrin and lactose along with the Eudragit S100 targeted the drug moiety to colon. Purified Lectins have the binding property of carbohydrate moieties on the surface of erythrocytes which agglutinate the erythrocytes, these lectins were evaluated by the agglutination test using 'A' positive blood group. These lectins showed anticancer activity against the colorectal type of cancer cell lines HCT- 116; proved as new link for developing anticancer drug specific to colorectal adenomas from *Glycine max* seeds. Calculated IC₅₀ value by SRB cytotoxic study which was found 12 compared with capecitabine as standard anticancer drug which was 9.

INTRODUCTION: Lectins and History: Term "lectin" is from the Greek word "legere" was coined by William Boyd in 1954, "legere" means "to select" or "to unite" ¹. The lectins are described as sugar binding proteins or glycoproteins of non-immune origin excluded from sugar binding antibodies and enzymes ². Lectins are widely distributed from bacteria to humans, which have as a minimum one non-catalytic area that shows reversible binding to particular monosaccharides or oligosaccharides. Lectins are easily detected by agglutination of erythrocytes. Lectins are classified on the basis of their overall structure and biochemistry of the subunits viz., merolectins, hololectins, chimerlectins and superlectins.


Soybean and chemistry of soybean lectins:

Soybean (*Glycine max*; Family: Fabaceae) plant is member of legume family native to East Asia. Soybean lectin is a protein, which have ability to bind with the carbohydrate so called as the glycoprotein. They have high binding protein specificity to fatal non-reducing N-acetyl-D-galactosamine but less to D-galactose. They have important role in cell to cell communication ^{5,7}.

Soybean also decreases the chances of breast and prostate cancer. They reduce the protein digestion due to the presence of the protein inhibitor ^{5,7}.

Objectives:

- Generation of crude lectin via extraction
- Characterization of soybean lectin
- Generation of pure lectins
- Anti-cancer activity of soybean lectin via *In-Vitro* studies

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MATERIAL AND METHODS:**Soxhlet Extraction:**

Soybean seeds were finely grounded and was weighed accurately about 100 g, then wrapped in filter paper and placed in soxhlet apparatus for extraction. Different extraction solvents were used separately for isolation of lectins as acetone, 0.015 M phosphate buffer of pH 7.2 in 0.15 M NaCl and methanol (500 ml each). Extraction was carried out for 1-1.30 hour with continuous stirring.

Co-Precipitation Procedure:

Collected lectins processed for centrifugation which was carried out to remove bulk particles. Coprecipitation was formed by shifting pH downward to optimal pH and the solution is kept overnight in the cold room. Remaining particulates removed by a combination of centrifugation; filtration was carried out on a Buchner funnel with a pad of hiflo supercel on whatman no. 1 paper. Obtained clear solution was adjusted to optimal pH. Galactose ligand added to the mixture and kept for 10-20 min. at 25°C to form matrix coprecipitation and was centrifuged to remove additional particulates. Supernatant was removed and retained the galactose lectin coprecipitate, then dissolved in buffer of pH 8 and trapped on anion exchange resin in Cl⁻ form. Received lectins were evaluated and processed for dialysis for purification.

Evaluation of Raw Lectins**TABLE 1: RATIO OF LECTIN AND EXCIPIENTS TAKEN FOR SPRAY DRYING**

Lectins (ml)	Starch (g)	Maltodextrin (g)	Eudragit S100 (g)
10	1	1	2.5
10	3	3	2.5
10	5	5	2.5
10	7	7	2.5

Hemagglutination Assay for Identification of Optimized Batch:

Evaluation test were carried out to evaluate the optimized batch for spray dried lectins. Soybean lectin showed hemagglutination activity with blood group type of A^{+ve}

Screening of Anticancer Activity; In-Vitro Study:**Cell line and Drugs:**

The human colon cancer cell line (HCT 116) purchased from national centre for cell science,

Agglutination Test ²¹: Isolated and collected lectins were processed for agglutination test. Lectins were placed in different tubes and mixed with human blood of A^{+ve} blood group.

Hemagglutination Assay ²¹:

Lectins have the property to agglutinate the erythrocytes. Soybean lectin forms a mesh like structure in between the blood erythrocytes which do not let the blood to be clumped. Assay was performed in two groups as positive and negative control.

Dialysis ¹⁷:

It is common operation in biochemistry and protein biology which assist to separate dissolved molecule by passing through the semi-permeable membrane as per their molecular dimensions.

Spray Dry Method ^{19,20}:

The spray-drying process of lectin done using the various excipients was undertaken in a laboratory scale spray dryer. The feed solution was pneumatically atomized into a vertical, concurrent drying chamber using a two-fluid nozzle at a constant flow rate (5ml. min⁻¹ to 20ml. min⁻¹). The outlet temperature was adjusted from 110°C to 120°C by varying the air inlet temperature. The dried powder was collected in a single cyclone separator.

Pune (N.C.C.S.) and used for *in-vitro* anticancer study.

Chemicals used were; 1, 2-dimethyl hydrazine- Purchased from Sigma Aldrich CAS No.-406-16-2, Capecitabine (Gift sample from Cadila pharmaceuticals Ltd. Ahmadabad)

SRB Assay ²²:**Sample Preparation:**

Stock solution of lectin powder of 1 mM in 0.25% DMSO was prepared and further dilution was done

in 10, 50 and 100µM with phosphate buffer saline (PBS).

Stock solution of Capecitabine of 1 mM in distilled water was prepared and further dilution was done in 10, 50 and 100µM with phosphate buffer saline (PBS).

Experimental Procedure:

- Counted the cells and adjusted the concentration of the cell suspension
- Added 100 µl of a cell suspension to each well in 96 well microplate using serial dilutions. Made a well of only media to measure the background
- Incubated for 24 hours in a CO₂ incubator with 5% CO₂
- If media change is necessary, remove media and add 100 µl of new media to each well including wells for a background measurement
- Added 10 µl of media containing different concentrations of the test substances to each well
- Incubated for set periods of (24 hrs) in a CO₂ incubator
- Added 10 µl of SRB solution to each well in a 96 well microplate
- Placed in a CO₂ incubator 5% CO₂ for 1-4 hours to react
- Measured the absorbance at 450 nm with a microplate reader

Calculation:

Enter the absorbance reading from each well in the equation below to calculate the cell survival rate.

$$\text{Survival rate (\%)} = (A_{\text{sample}} - A_{\text{b}} / A_{\text{c}} - A_{\text{b}}) \times 100\%$$

RESULTS AND DISCUSSION:

Agglutination Test:

Test carried out for agglutination was found positive for lectins in PBS which was used as solvent for soxhlet extraction.

Hemagglutination Assay:

For this assay human blood sample of A⁺ blood group was used. Tests were compared with positive and negative control assay. PBS had shown the positive results for lectins.

Spray Dry Method:

TABLE 2: DRUG-EXCIPIENTS RATIO STUDY FOR SPRAY DRYING

Lectins	Starch	Maltodextrin	Eudragit S100
10*	5*	5*	2.5*

*Selected batch for activity as per agglutination studies result

Screening of Anticancer Activity; Comparative In-Vitro Studies (SRB Assay):

Cytotoxic activity of Methanolic extract of lectin (L. P. A. test) and Capecitabine were investigated against SRB assay and cytotoxicity was reported in terms of lethality concentration (LC₅₀). The LC₅₀ value of batch A, batch B, batch C, batch D and Capecitabine were 80 µg/ml, 75 µg/ml, 15 µg/ml, 50 µg/ml and 9 µg/ml respectively by SRB assay. Show in **Table 3, 4, 5** and **6** and **Fig. 1, 2, 3** and **4** respectively.

TABLE 3: SRB ASSAY FOR BATCH A

Concentration (µM/ml)	% Cell Cytotoxicity (L.A.P)/ Test	% Cell Cytotoxicity Std. Drug
10	19.27±0.7692	50.23±0.5752
50	25.72±0.5391	69.85±0.5275
100	28.75±0.8626	86.53±0.3217
IC ₅₀ Value	80	9

All values expressed in Mean ± S.E.M. (n=3)

TABLE 4: SRB ASSAY FOR BATCH B

Concentration (µM/ml)	% Cell Cytotoxicity (L.A.P)/ Test	% Cell Cytotoxicity Std. Drug
10	25.36±0.8246	50.23±0.5752
50	28.52±0.7421	69.85±0.5275
100	30.82±0.9120	86.53±0.3217
IC ₅₀ Value	70	9

All values expressed in Mean ± S.E.M. (n=3)

TABLE 5: SRB ASSAY FOR BATCH C

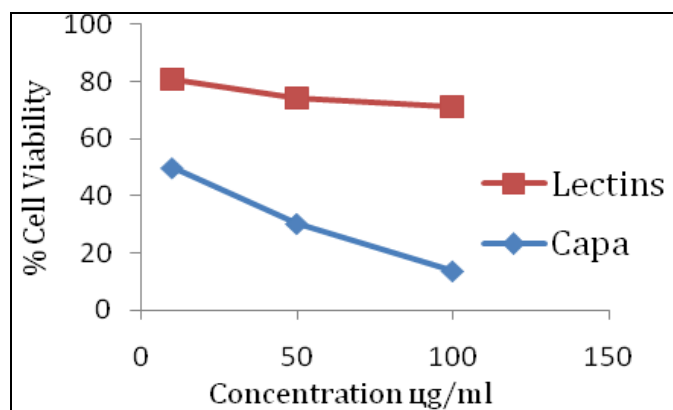
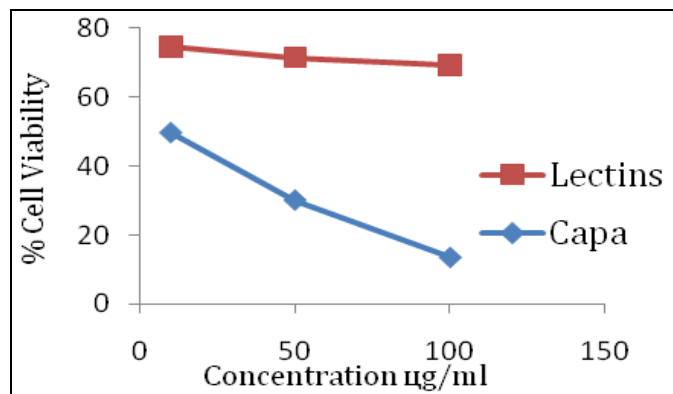
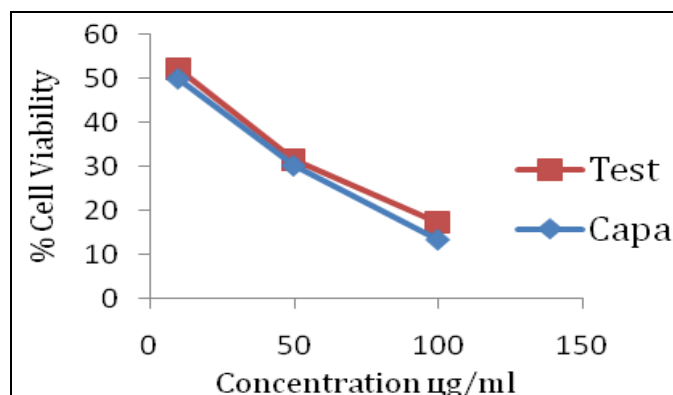
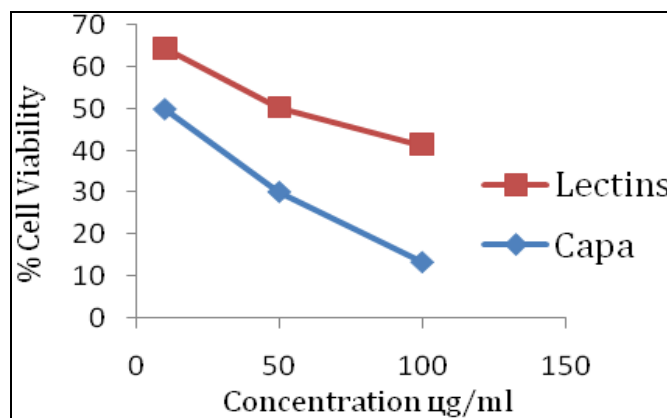
Concentration ($\mu\text{M/ml}$)	% Cell Cytotoxicity (L.A.P)/ Test	% Cell Cytotoxicity Std. Drug
10	47.87 \pm 0.9536	50.23 \pm 0.5752
50	68.56 \pm 0.6894	69.85 \pm 0.5275
100	82.83 \pm 0.3768	86.53 \pm 0.3217
IC ₅₀ Value	15	9

All values expressed in Mean \pm S.E.M. (n=3)

TABLE 6: SRB ASSAY FOR BATCH D

Concentration ($\mu\text{M/ml}$)	% Cell Cytotoxicity (L.A.P)/ Test	% Cell Cytotoxicity Std. Drug
10	35.54 \pm 0.8963	50.23 \pm 0.5752
50	49.76 \pm 0.5839	69.85 \pm 0.5275
100	58.68 \pm 0.3519	86.53 \pm 0.3217
IC ₅₀ Value	50	9

All values expressed in Mean \pm S.E.M. (n=3)

**FIG.1: SRB ASSAY FOR BATCH A****FIG.2: SRB ASSAY FOR BATCH B****FIG.3: SRB ASSAY FOR BATCH C****FIG.4: SRB ASSAY FOR BATCH D**

SUMMARY AND CONCLUSION: The present study was based on finding of anti-cancer activity of lectins obtained from the soya seeds. These days many synthetic agent used for curing many disorders like cancer and other diseases but they are available with many side effects. Consequently it was decided to generate such an agent with least or null side effects and corresponding to natural flora of Gastrointestinal tract.

Finally it was concluded that;

- Crude lectins were obtained from the PBS
- Lectins can be purified by the dialysis method
- Encapsulation with the excipients like starch and maltodextrin along with Eudragit S100 can be used for colon targeting of lectin moiety
- New anti- Cancer activity was screened via *In-Vitro* test successfully

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