Antiproliferative Effect of *Entada rheedii* Crude Lectin Extract on Human Colorectal Cancer Cells

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Abstract

Several studies have proved that leguminous lectins are often explored as a potential anti-cancer agent owing to their substantial anti-cancer properties which include inhibition of cell adhesion, proliferation and colony formation. This group of carbohydrate-binding proteins is seen to cause agglutination and an increase in cancer cell cytotoxicity. The study aims to isolate and purify the lectin present in *Entada rheedii*, a tropical legume and analyse its significance as a potential antiproliferative agent against human colorectal cancer cells. Previously published work confirmed the lactose, galactose and cellobiose specificity of the lectin, and this lectin was isolated, purified and separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Cytotoxic assay was performed to investigate the extent of its strength as an antiproliferative protein. The purified lectin agglutinates processed chicken RBCs with a maximum hemagglutination Unit of 32. The lectin was observed to be stable when stored at 0-4°C. The antiproliferative activity of lectin from *Entada rheedii* was confirmed against human colorectal carcinoma cells HCT 116 with IC_{50} values of 188.72 μg/mL.

Keywords: Antiproliferative, Cancer, *Entada rheedii,* Lectin

1. Introduction

Cancer treatment is a complex field, involving considerations such as specificity, selectivity, toxicity and mechanisms of action in the development of effective anticancer drugs. Some natural compounds or phytocompounds have shown potential in preclinical studies. Some typical examples include taxol analogs, vinca alkaloids such as vincristine, vinblastine, and podophyllotoxin analogs (Choudari *et al.,* 2020).

Lectins are defined as a class of proteins that selectively bind to carbohydrates. These phytocompounds show a high affinity towards glycoproteins, glycolipids and polysaccharides. They are oligomeric in nature, with each lectin presenting at least 2 carbohydrate binding sites. Lectin-carbohydrate interaction resembles antigen-antibody interaction through a non-catalytic, reversible and highly selective domain (Law & Strijdom, 1977; Kasapoğlu & Dere, 2022). All legume lectins share homologies with significant conserved regions of the protein subunits. The 3D structures of all legume lectins exhibit similarities in carbohydrate binding, ligand binding and allosterically interactive sites. Specifically, lectins being metallo-proteins, the 3D structures of leguminous lectins show remarkable similarity in binding to metal ions (Sharon & Lis, 1990).

Lectins are abundant in cotyledons of mature seeds of most legumes. Legume lectins, being ubiquitously available, include the largest and the best characterized family of lectins. Lectins in legumes play a major role in plant immunity against phytopathogens acting as pattern recognition receptors and also have the ability to influence the production of various interleukins mentioned in Tamilarasan *et al*. (2021) and De Mejía & Prisecaru (2005).

A major advantage of dietary lectins is their resistance to gastrointestinal digestion, owing to their compact globular structure (De Mejía & Prisecaru, 2005). This structure protects ingested lectins from protease denaturation, allowing them to retain their structure and activity post ingestion (Kelsall *et al*., 2002). As an added domestic advantage, most plant lectins can retain a significant amount of activity when subjected to gentle cooking methods (Ryder *et al*., 1992).

A unique property of lectins is their ability to agglutinate or clump together certain cells. This agglutination is evident when lectins are tested against red blood cells (RBCs). Specific sugar residues on the outer membrane of erythrocytes have a high affinity for the lectin and cause the surrounding cells to clump together. This property of lectins is used in human blood typing experiments described by Gorakshakar & Ghosh (2016).

Previous research on various legume lectins has demonstrated their ability to induce antiproliferative

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activities in selected cell lines such as HeLa, A549 (Naik & Kumar, 2020) and EAC (Kabir *et al.,* 2021). The induction of apoptosis through type-II programmed cell death in transformed cells is one of the most common abilities of lectins. For example, soybean lectin has been shown to induce autophagy and DNA damage in HeLa cells by generating reactive oxygen species which have a cytotoxic effect on cells (Yau *et al.*, 2015). This tumor specificity of the lectins depends on the type and abundance of carbohydrate moieties expressed on the tumor cells, allowing them to be classified as biomarkers by specifically binding to tumor-associated carbohydrates (Maaroof *et al.*, 2018).

Numerous reports highlight a prevailing trend whereby transformed cells exhibit greater susceptibility to lectin agglutination than their normal cell counterparts. In addition, transformed cells tend to show increased sensitivity to the toxic effects of lectins, a contrast to the relatively reduced impact observed on normal cells (Burger *et al.*, 1969).

In cases involving lectins such as concanavalin A, phytohemagglutinin (PHA), abrin and ricin, their efficacy has been observed in specific tumor systems. Within these systems, the inhibition of tumor growth can be attributed to the greater toxicity of the lectins to tumor cells compared to their effect on normal cells.

It is proposed that the difference in surface membrane structure between normal and transformed cells contributes to the different toxic effects of plant lectins on healthy and cancerous   cells.
It is also proposed that the difference in surface membrane structure between normal and transformed cells contributes to the different toxic effects of plant lectins on healthy and cancerous cells (Esumi-Kurisi *et al.*, 1983).

In their work, Esumi-Kurisi *et al.* suggested that wheat germ agglutinin (WGA) operates *in vivo* by facilitating the proximity of tumor cells and macrophages, achieved by linking the N-acetyl glucosamine groups present on their respective glycoproteins. Notably, this interaction does not extend to normal cells, which lack this particular recognition by macrophages, leading to reduced activity of WGA against them.

Entada rheedii is a tropical liana belonging to the family of Fabaceae, a medicinally relevant subset of African and Indian subcontinental flora (Gurib-Fakim *et al.,* 2013). It is large in size with a hard seed coat containing large cotyledons with ample amounts of proteins. It has been well established to have medicinal, anti-cancer (Li *et al*., 2014), (Panda *et al.*, 2014), (Thies *et al.*, 2008) anti-bacterial (Carvalho *et al.*, 2015; Li *et al.*, 2014) and anti-fungal properties (Sitohy *et al.*, 2007; Phadungsil & Grams, 2021). Traditional medicine, supported by recent scientific studies, shows a wide range of drugs extracted from *Entada rheedii* including treatment for mumps (Shivanna & Rajakumar, 2011) and remedies for diarrhoea (Okba *et al.,* 2013). Entadamine from crude extracts showed pronounced activity against protozoan diseases such as trypanosomiasis and leishmaniasis (Okba *et al.*, 2013).

Prior studies (Naik & Kumar, 2020) on the lectin in *Entada rheedii* revealed the presence of a 19.333 kDa protein that specifically binds to lactose, galactose and cellobiose. This *Entada rheedii* lectin was further examined for its stability in varying temperatures, pH and

in the presence of metal ions. The protein band of interest, as previously analyzed by Naik *et al.*, (2020) confirmed that lectin from *E. rheedii* appeared as a single band with an approximate molecular weight of 20 kD with and without β-mercaptoethanol and was monomeric in nature. The study also included *in vitro* cytotoxicity and morphological analysis of *Entada rheedii* lectin on lung cancer cell line A549, HeLa. Tha results from MTT assay performed on these cell lines showed an IC$_{50}$ value of 28 μg/mL confirming the induced apoptotic activity of *Entada rheedii* lectin (Naik & Kumar, 2020).

This *Entada rheedii* lectin was further investigated for its stability at different temperatures, pH and in the presence of metal ions. The protein band of interest, as previously analysed by Naik & Kumar et al. (2020) confirmed that the lectin from *E. rheedii* appeared as a single band with an approximate molecular weight of 20 kD with and without β-mercaptoethanol and was monomeric in nature.

Further work by Naik & Kumar (2020) demonstrated that when normal cells (African Green Monkey Normal Kidney Cells (Vero) were exposed to different concentrations of Entadin lectin and assessed by the dose-dependent MTT assay, cell viability was largely unaffected. This observation supports the notion that Entadin lectin has a preferential effect on cancer cells without inducing notable toxicity towards normal cells.

The present study focuses on the characterization and evaluation of the anti-cancer properties of the lectin extracted from *Entada rheedii* seeds. Drawing upon insights and findings from previously published research, our primary objective is to investigate the effects of lectins from *Entada rheedii* on the Human colon cancer cell line HCT 116.

2. Materials and Methods

2.1. Extraction of total protein

Seeds of *Entada rheedii* were sustainably harvested from fallen pods from Puttur, Dakshina Kannada District of Karnataka and were identified and confirmed by the taxonomist at The University of Trans-Disciplinary Health Sciences and Technology (TDU, Bangalore). The hard testa was cracked open and the inner contents were crushed to a coarse powder and were stored in an air-tight container at 4 °C. 5 g of seed powder was homogenized thoroughly for 10 minutes with 0.15M phosphate buffer saline of pH 7.2 using a mortar and pestle and stored overnight at 4°C followed by centrifugation at 7200 rpm for 20 minutes. The pellet was washed using 0.15 M PBS and centrifuged again. The supernatants were pooled and filtered using Whatman Filter Paper (Grade 1, 125 mm). Hemagglutination assay was performed to confirm the presence of lectin in the supernatant (Naik & Kumar, 2020).

2.2. Hemagglutination assay

Gorakshakar & Ghosh (2016) describe an efficient method to analyse a sample for the presence of lectins. Following their method, upon addition of the sample and the RBC suspension to a 96-well, V-bottom microtiter plate, the formation of mesh-like structures indicates that specific binding has taken place, and the sample is positive for lectins, whereas the formation of dots indicates the

unbound RBCs have settled at the bottom of the well and the sample is considered negative for lectin content.

Hemagglutination tests were routinely carried out at each step to check the presence and concentration of lectins. Fresh chicken blood was obtained from a local butcher shop and mixed with 0.01M ethylenediaminetetraacetic acid (EDTA). The mixture was centrifuged at 2500 rpm for 10 minutes to remove the EDTA, and the pellet was washed and centrifuged twice with 1 M PBS to prepare a 4% RBC (Adamová *et al.*, 2014).

Hemagglutination assays were performed in a 96-well, U-bottom microtitre plate. 25 µl of 1 M PBS was added to all the wells and a two-fold dilution of the sample was performed with 25 µl of the sample. An equal amount of 4% RBC solution was added to all wells, and the plate was incubated for 30 minutes at room temperature. The Hemagglutination Unit (HU) of the sample was calculated based on the number of meshes formed, and results were analysed in triplicates as described by Grimes *et al., (*2002) in their published protocols.

2.3. Salting out of total protein content

The total protein content in the crude extract was subjected to ammonium sulphate precipitation. Salt saturation was gradually increased from 0% to 30%, 30% to 50% and 50% to 80% with two-hour incubation periods and constant stirring for each saturation increase. After each round of precipitation, the solution was centrifuged at 7200 rpm for 20 minutes. The supernatants were used for the next round of precipitation, and the pellets were resuspended in 2 mL of 0.15 M PBS buffer. The pellets of 30%, 50% and 80% saturation were tested for the presence of lectins by hemagglutination.

The pellets showing hemagglutination activity were pooled together and passed through a 10 mL desalting column (Genei Laboratories Private Limited) which was equilibrated with 2-bed volumes of 0.15 M PBS buffer. The pooled pellets were added to the column and incubated for 1 hour at 4°C. The desalted protein was eluted out with 2 more bed volumes of 0.15 M PBS buffer and collected as 1 mL fractions. Each fraction was tested for the presence of lectin by hemagglutination assay.

2.4. PEG assisted-osmosis driven protein concentration

The fractions collected from the desalting column were tested for their hemagglutination activity using 4% chicken blood. The fractions forming meshes were pooled together and concentrated by osmotically removing excess buffer in the solution. The fractions showing positive hemagglutination results were pooled together and placed in a 17 cm cellulose membrane (HiMedia Dialysis Tubing Size 1 with a molecular weight cut off of 14 kDa) ensuring no leaks (Goldring & Dean Goldring, 2019). This was subjected to dialysis for 3 hours at room temperature using 0.15 M PBS to eliminate any residual salt content. The membrane was then laid on a bed of polyethylene glycol (PEG) 6000 until the solution reduced by four times the original volume. The concentrated protein was checked for lectin content by hemagglutination with 4% RBC.

2.5. Quantification of protein

The amount of protein in crude extract and PEG concentrated protein was estimated by following the standard procedure of Lowry's Assay (Lowry *et al.*, 1951).

2.6. Molecular Mass Separation of Proteins

Proteins present in the concentrated fraction were electrophoretically separated on a discontinuous vertical gel using the Biorad Mini-PROTEAN® Electrophoresis System and stained in Coomassie brilliant blue G-250 overnight. Post destaining, the gel was visualised using the Biorad GelDoc Go System. Himedia Prestained Protein Ladder (MBT092).

2.7. In vitro cytotoxicity test by MTT (3-(4, 5-dimethylthiazol-2)-2, 5-diphenyltetrazolium bromide) analysis

MTT (Liu *et al.*, 1997) analysis of concentrated *Entada rheedii* protein extract was performed. HCT 116 cells were obtained and subcultured from the National Centre for Cell Science Cell Repository, Pune, India. Cultured cells were dislodged by trypsin and centrifuged at 300 g. A solution of 10,000 cells per 200 µl of cell suspension was made. 200 µl of cell suspension was added to all the wells of a 96-well microtitre plate and incubated at 37°C at 5% CO_2 for 24 hours. After aspirating the spent medium, 200 µl of prepared sample concentrations were added (undiluted, 21.5625 µg/mL, 43.125 µg/mL, 86.25 µg/mL, 172.5 µg/mL, 345 µg/mL) to the wells and incubated again at 37°C for another 24 hours. The medium was removed and 200 µl of 10% MTT was added to each well and incubated at similar conditions for 3 hours. The culture media was completely removed and 100 µl of DMSO was added and kept on a gyratory shaker to solubilize the crystals. Absorbance was read using a microplate reader set to 570 nm and percent growth inhibition and IC_{50} values were calculated by plotting protein concentrations represented on the X-axis and the variance in percent cell viability represented on the Y axis. The plot was then fitted to a straight line from which the IC_{50} value was obtained from the equation (1) and (2) ("Science Gateway," n.d.)

$$Y = a*X + b \qquad (1)$$

$$IC_{50} = (0.5 - b)\,a. \qquad (2)$$

In $Y = a*X + b$, a is the slope, representing the rate of change, and b is the y-intercept, indicating the value when X is zero.

The treated cells were imaged using XDFL series biological microscope, Sunny Instruments, China.

3. Results

3.1. Purification of E. rheedii proteins

The *E. rheedii* protein was isolated and purified from a 50% ammonium sulfate saturated crude extract yielding a final concentration of 686.948 µg/mL post PEG assisted-osmosis driven protein concentration which is soluble in 0.15 M PBS. Presence of lectins was confirmed by assessing its hemagglutination activity against 4% RBCs.

3.2. Hemagglutination activity

The purified protein showed hemagglutination activity against 4% chicken erythrocytes. The lectins present in total proteins showed mesh formation when administered as crude extract and after every step of purification (Figure

1). Similar results were observed in earlier studies done on

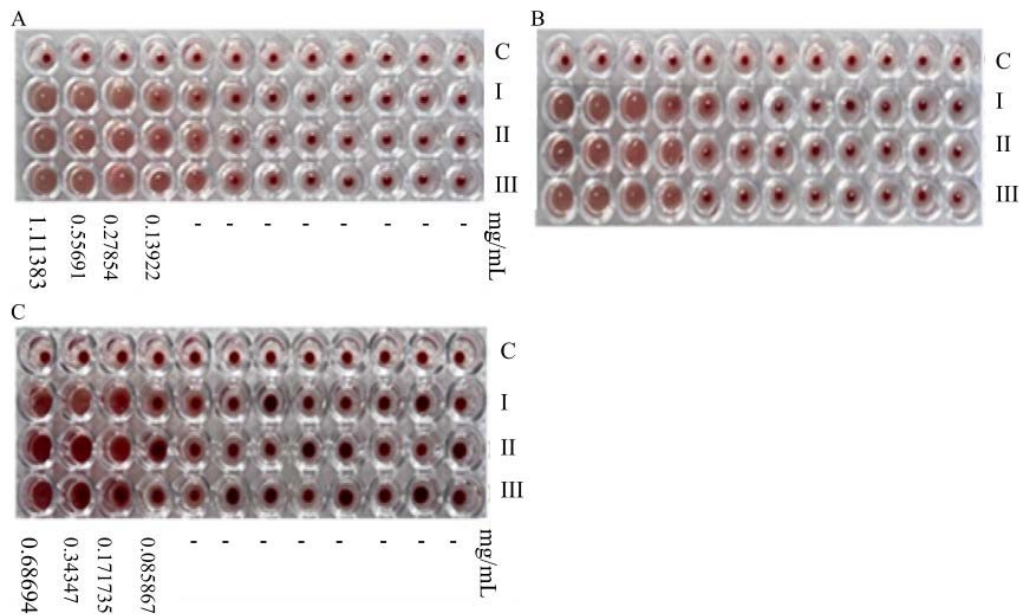lectins present in *E. rheedii* (Naik & Kumar, 2020).

Figure 1. Hemagglutination of Chicken red blood cells by *E. rheedii* lectin shown in triplicates as in I, II, III for each image (A, B, C). (A) Hemagglutination activity of crude extract of *E. rheedii* indicating hemagglutination unit of 32 with a serially diluted concentration of 0.13922 mg/mL. (B) Hemagglutination activity of 50% ammonium sulphate saturated crude extract indicating hemagglutination unit of 16. (C) Hemagglutination activity of concentrated purified protein from *E. rheedii* indicating hemagglutination unit of 8 and a serially diluted concentration of 0.085 mg/mL.

3.3. Molecular mass separation

The high-resolution separation of mixtures of protein was performed on crude extract by SDS-PAGE (Figure:2).
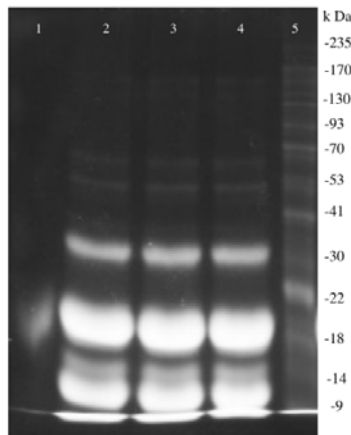

Figure 2. Molecular weight based separation of *Entada rheedii* crude extract shown in triplicates. Lane 1 - Protein Marker Lactoglobulin (18.4 kDa). Lane 2, 3, 4 - *E. rheedii* crude extract (19.33 kDa). Lane 5 - Protein Ladder.

3.4. In vitro cytotoxicity of crude extract of lectins from *E. rheedii* on HCT 116

MTT assay for lectins from *E. rheedii* was performed on HCT 116 cancer cells. The dosage dependent assay administered the lectins present in purified proteins in the concentrations of 345 µg/mL, 172.5 µg/mL, 86.25 µg/mL, 43.125 µg/mL, 21.5625 µg/mL and 686.948 µg/mL.

The assay showed a reduction in cell viability after incubating the cells for 24 hours and the IC$_{50}$ was found to be 188.72 g/mL (Table 1 and Figure 3). There is an observed appearance of apoptotic bodies upon administration of the sample (Figure 4).

Table 1. MTT assay results

HCT 116 cells vs *E.rheedii* Lectin	Blank	Untreated	Test concentration µg/mL				
			21.5625	43.125	86.25	172.5	345
Trial 1	0.002	1.554	1.589	1.046	0.888	1.022	0.022
Trial 2	0.002	1.501	1.518	1.36	1.196	1.021	0.019
Trial 3	0.002	1.518	1.4	1.627	1.176	1.089	0.019
Mean Absorbance	0.002	1.524	1.502	1.344	1.087	1.044	0.02
Mean OD-Mean Blank		1.5223	1.5003	1.3423	1.0847	1.042	0.018
Standard deviation		0.0271	0.0955	0.2908	0.1723	0.0390	0.0017
Standard error		0.0156	0.0551	0.1679	0.0995	0.0225	0.0010
% Standard error		1.0263	3.6207	11.0293	6.5361	1.4781	0.0657
% Viability		100	98.5500	88.1800	71.250	68.450	1.1800
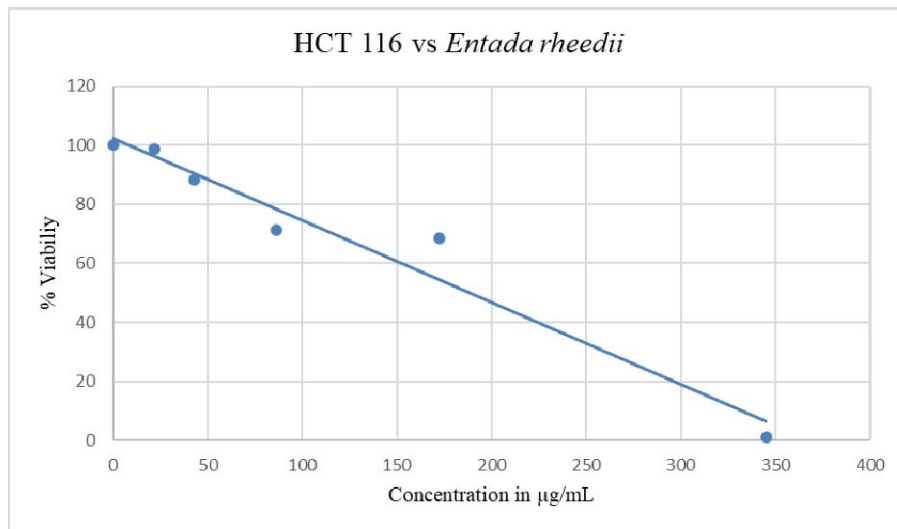
Figure 3. Effect of lectin on cell survival on HCT 116 human colorectal cancer cells. MTT assay. The Y-Axis represents the % cell viability and the X-axis represents the administered dosage in increasing concentrations ranging between 0 to 345µg/mL. The scatter plot also depicts a trend line showing the cytotoxic nature upon administration of *E.rheedii* crude lectin extract.
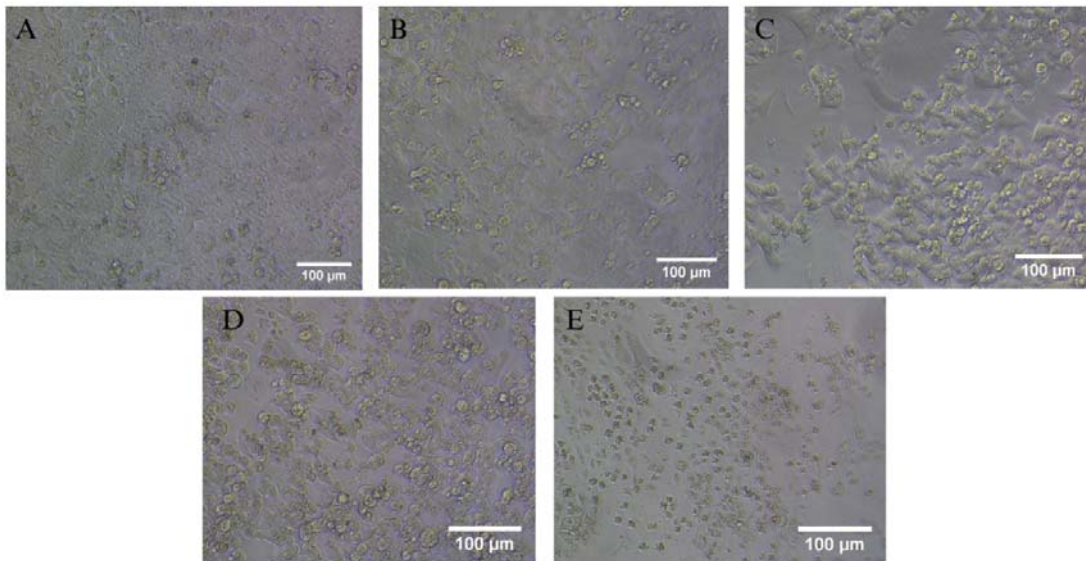
Figure 4. Cytotoxic effect of different concentration of lectin extract of *E. rheedii* administered on HCT 116 - Human colorectal cancer cells: (A). 21.56 µg/mL, (B). 43.12 µg/mL, (C). 86.25 µg/mL, (D). 172.5 µg/mL and (E). 345 µg/mL.

4. Discussion

With increasing occurrences of cancer, there is a dire need for safe alternative therapies; either from nutritional changes or lifestyle changes with the former being a more promising section that allows exploration in the field of medicaments from nature. Extensive review of literature indicated that different seed and plant contents of *E. rheedii* possess medicinally active principles.

In the present study, lectin from seed proteins were purified by ammonium sulphate protein precipitation, desalting and dialysis, followed by PEG assisted-osmosis driven protein concentration yielding a pure and concentrated form.

On account of the above-mentioned "requirement of changes" in nutritional habits as a cancer preventive/treatment measurement, legume lectins included as a part of the diet as a nutritional therapy may possibly prove helpful in detecting, targeting and applying its antiproliferative abilities onto cancers particularly occurring in the course of the digestive system. Since lectins surpass the digestive system without loss of activity or any structural degradation, *E. rheedii,* if included in the diet, upon reaching the colon may provide a fixed and more targeted delivery of the Entadin lectin and may help induce its therapeutic features by acting on the surrounding colorectal cancer cells.

Lectins, including soybean lectins, have been shown to have anti-inflammatory and antioxidant properties, which are associated with chemopreventive effects in colorectal cancer. Lectin-binding patterns in tumors have been strongly linked to their biological behaviour in this type of cancer. Soybean bioactive proteins and peptides, such as lectins, have been studied for their antioxidant and anti-inflammatory properties in relation to colorectal cancer prevention. Research has demonstrated that soybean lectins can boost the immune response, exert chemopreventive effects, and affect protein kinases in cell-based assays by inducing cytotoxic activity, apoptosis, reducing cell proliferation, arresting the cell cycle through the caspase cascade, inhibiting telomerase activity and angiogenesis (Chakrabarti *et al*., 2014, Chen & Hsieh, 2018, Pan *et al*., 2011).

Chemoprevention aims to reduce the risk of cancer by understanding it as a complex, multistep process at the molecular and cellular level. It uses protective compounds to stop or reverse the development of cancerous cells (Greenwald *et al*., 1995). Similarly, from comparative decipherment, it can be hypothesized that bioactive proteins, specifically lectins from *E. rheedii,* being a natural dietary compound, may also exhibit similar chemopreventive properties which can be attributed to its proven anti-inflammatory and antioxidant properties as shown by Okba *et al.,* (2013) and Nzowa *et al.,* (2010).

Performing a cell viability assay, such as an MTT dosage-dependent assay, can help to strengthen the hypothesis that including lectins from *E. rheedii* in the diet can effectively and directly impact colon cancer cells. This would also associate the relevance of utilizing *E. rheedii* lectins as a potential anti-cancer molecule against HCT 116.

A frequently observed glycosylation abnormality in colon cancer and precancerous conditions is the elevated presence of the disaccharide molecule composed of galactose and N-acetylgalactosamine connected by a beta-1,3 glycosidic linkage, within the mucosal layer (Evans *et al*., 2002; Greenwald *et al*., 1995). Further, from earlier studies of Nadia *et al.,* 2011 (Arndt *et al*., 2011) and Naik *et al.,* 2020 (Naik & Kumar, 2020), it is understood that there is overexpression of lactose and galactose moieties on the altered cell surface of HCT 116. In parallel, it is also known that lectin from *E. rheedii* shows carbohydrate specificity towards lactose and galactose. This may possibly be beneficial, as the key aspect of lectins to be able to promote its antiproliferative activity lies in the binding of lectins onto the specific carbohydrates present on the cell surface.

The glyco-biological profile of HCT116 and other colorectal cancer cells reveals the overexpression of Lewis Antigens (LeX and LeA) as glyco-epitopes (Shimizu *et al*., 2023). These LeX and LeA as well as their respective sialylated derivatives play a vital role in cancer cell adhesion, tumour formation and malignant transformation (Durrant *et al*., 2012). Lewis Antigens are well known to bind to selectins (or cell surface C-type Lectins). This leads to another strong hypothesis that the *Entada rheedii* lectin has the potential to selectively target Lewis Antigen expressing colorectal cancer cells (such as HCT116), providing another epitope for targeted induction of necrosis or apoptosis.

MTT analysis conducted on HCT 116 cells exposed to *Entada rheedii* lectin gave an IC_{50} value of 188.72μg/mL. The results showed that a concentration of 345μg/mL of *Entada rheedii* lectin must be used to reduce the viability of colorectal cancer cells to 1.18%. This can be seen in the increase in the number of apoptotic bodies constricted cell shape and darker appearance with increase in concentration (Figure 4). Studies done on *Entada rheedii* by Naik *et al*. (2020) solidifies the theory of *Entada rheedii* lectin being apoptotic in nature towards cervical cancer cells and lung cancer cell lines. HCT 116 cells clearly express Neu5Acα6Gal glycosyl and sometimes mannose complexes.

Other lectins and plant phytocompounds have been studied for their reaction with HCT 116 cells. Lectins from *Eclipta alba* and *Origanum vulgare* all show a similar range of IC_{50} values of 160 μg/mL, 256 μg/mL, 179 ± 0.81 μg/mL and 140.77 ± 2.13μg/mL respectively (Kabir *et al*., 2021), (Nelson *et al*., 2020) and (Jovankić *et al*., 2022). The diethyl ether lectin extract from the plant *Utrica urens* subjected to MTT analysis gave an IC_{50} value of 41.21μg/mL for HCT116 cells (Gaafar *et al*., 2020). Similarly, the *B. papyrifera* lectin showed cytotoxicity towards HCT116 cells with an IC_{50} value of 71.50 ± 9.24 μg/mL (Kumar *et al*., 2014). *Entada rheedii* lectin also falls in the same range with an IC_{50} value of 188.72μg/mL. As a potential drug, it is necessary to consider the toxic effects lectins have on the human body. As previously stated, the toxicity of *Entada rheedii* lectin on normal, non-cancerous African Green Monkey Normal Kidney Cells was analysed by MTT assay in the paper Naik *et al*., 2020 and shown to have no significant effect on the cell viability. The garlic lectin showed cytotoxicity towards U937 and HL60 cells but not towards human leukocytes (Karasaki *et al.,* 2001). Numerous lectins have shown their selective cytotoxicity to cancer cells, but *Entada rheedii* lectins's toxicity towards normal human colon cells needs to be further evaluated.

Through MTT Analysis, cytotoxicity and anti-cancer activity of Entadin has been established against Human colorectal cancer cells, Lung cancer and Cervical cancer cells (Naik *et al.,* 2020). This concludes that *Entada rheedii* can be a potential anti-cancer medicament for more than one cancer type. Being a natural phytocompound, ingestion of Entadin as a prepared colorectal cancer drug should aid in easy drug delivery to the affected colon. More research is needed to understand the anti-tumour pathway induced by Entadin, but this potential should be recognized in future studies.

5. Conclusion

E.rheedii lectins showed significant hemagglutinating activity against chicken erythrocytes. A 0.15 M phosphate buffer saline was used, which is a novel method for the extraction of proteins. The study introduced desalting and PEG-assisted osmosis-driven protein concentration techniques. The carbohydrate binding protein was stable when stored at 0-4 ºC for up to three weeks. This was confirmed by weekly hemagglutination. This study revealed the antiproliferative effect of *E. rheedii* lectins on the HCT 116 cell line, with an IC_{50} value of 188.72 μg/mL, indicating significant therapeutic promise. Moreover, the study successfully isolated, purified, and confirmed the stability of lectin extracts, thereby affirming their potential as effective therapeutic agents targeting transformed colon cells.

Acknowledgment

Conflict of interest

This study was equally contributed to by all authors, and they declare that they have no conflict of interests.

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