



Extraction, Purification and Analysis of Anti cancer activity of Ricin on Colon Cancer Cell Lines and Testing its Efficacy using Insilco Docking

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Abstract:

Ricin, one of the well known plant toxins has played a vital role in the history of medicine. It is usually extracted from *Ricinus Communis*, a common plant. The use of these proteins in medical treatment since ancient times is reviewed. Later the proteins played important roles in the early days of immunological research and some of the fundamental principles of immunology were discovered with toxic proteins of this group. The present study involves extraction of ricin proteins from powdered castor seed using buffer of definite composition, purification of extracted sample of castor seed was done using Dialysis and Ion Exchange Chromatography. The purified samples were tested for their purification by running sample through 8% SDS PAGE. The anti-cancer activity of ricin proteins were observed on human colon cancer cell lines and further studied the protein-protein docking interactions between the ricin protein and km23, the protein mainly involved in colon cancer. Based upon the docking results it can be analyzed whether the ligand receptor pair used is suitable for docking as favored by their energy values.

Keywords: *Ricinus Communis*, Immunological Research, Colon Cancer Cell Lines, Anti Cancer Activity, Docking, Protein Protein Interactions.

Introduction:

Castor belongs to the genus *Ricinus* of the Euphorbiaceae or spurge family (Atsmon, 1985). Plants range in color from bright red stems and leaves rich in anthocyanin to a uniform dark green. Up to 50% of the dry weight of the seed is oil, with approximately 90% of the fatty acids being ricinoleic acid [1]. Detoxified meal of *Ricinus communis* can be fed to ruminants or used as a high nitrogen fertilizer due to its inherent properties. Castor oil is estimated to have a market value six times higher than soybean oil [2]. Ricin is a potent plant toxin composed of a 32 kDa A-chain glycoprotein, and a 32 kDa B-chain glycoprotein, linked by a disulfide bond. The A-chain of ricin is a ribosome inactivating protein, preventing protein synthesis by depurinating an adenine residue found in a 28S ribosomal RNA (Olsnes and Pihl, 1982). A single ricin molecule can inactivate over 1500 ribosomes per minute and kill

the cell [3]. The B-Chain is a lectin which binds specifically to galactose terminals found on the cell (Olsnes and Pihl, 1973). Binding of the B chain to cell surface receptors triggers endocytosis of the protein (Montfort et al., 1987). Due to these cell inactivating characteristics of ricin it can be employed in the treatment of cancer, wherein inactivating or destroying the surplus cells is needed.

Materials and Methods:

Sample Collection:

The seeds from the commonly available castor (*Ricinus Communis*) Plant are obtained and crushed with a specific buffer to maintain its composition. The extracts are collected and stored under incubation conditions (4⁰C) for future consumptions.

Extraction of Ricin Protein in crude form from the sample:

The extract is taken and centrifuged at 6000rpm for 10 minutes and the supernatant is collected. The collected aqueous supernatant is used as a crude extract of the protein that has to be purified further for the analysis. Three types of extracts were collected which includes Methanolic, Ethanolic and Aqueous extracts were collected.

Purification of the protein by Molecular Techniques:

The Crude sample was processed for purification so as to use it for the further analysis. Salt Precipitation was performed using Sodium Acetate salt. The solution is kept for incubation at 4⁰C for 24 hrs. Later the supernatant is collected as the partially pure sample. This is further purified using Dialysis method. The sample is poured into the dialysis membrane and is suspended into water beaker and incubated for 24hrs at room temperature. Later the solution retained in the bag was collected as the pure sample.

SDS PAGE for Identification of Protein:

The samples extracted were processed for performing SDS PAGE, so as to confirm the proteins presence based on its molecular weight using Ladder. Based on the position of the protein band in the gel the

molecular weight is known and the sample is identified.

Estimation of the sample extracted using Colorimetry:

Here the method employed for the protein estimation was Lawry's method. The Blue color developed in the samples due to their reaction with the Folin's reagent is read against the colorimeter at 595nm. The reading obtained is plotted on the graph and the calculations were performed to estimate the concentration of the sample.

S.N	Enzyme	Dt. H ₂ O	Alk CuSO ₄	INCUBATION	Fc .Rgnt	INCUBATION	OD
1	Chloroform Ext	0.4 ml	0.6ml		0.5ml		0.42
2	Methanol Ext	0.4 ml	0.6ml		0.5ml		0.26
3	Ethanol Ext	0.4 ml	0.6ml	0.5ml	0.25		

Ion Exchange Chromatography:

The samples were run through Ion exchange chromatography in order to obtain the protein in the most pure form. The elutes were collected in several intervals. DEAE Cellulose was used as the stationary phase and the mobile phase employed was NaCl of various concentrations.

Estimation of the Protein Concentration in the elutes using Lowry's method:

All the elutes obtained were subjected for the estimation of the protein content to identify the concentration of the NaCl that can extract maximum protein from the column. The concentrations were calculated by colorimetric estimation. The graph was plotted and evaluated.

Testing the efficacy of the sample Ricin on Colon Cancer Cell lines:

The human Colon cancer cell lines MDST8 was maintained at 37°C in 5% CO₂, 95% air and passaged every 3 days in RPMI 1640 culture medium supplemented with 10% heat-inactivated FCS, 1% glutamine, and 1% penicillin/streptomycin. To 100µl of this cell suspension 100µl of each of the 4 elutes are added in 4 different eppendorfs. 1ml of Culture medium was added in all of them. These were incubated for 48 hrs at 37°C in 5% CO₂ and 90% relative humidity in CO₂ incubator. O D was measured at 540nm.

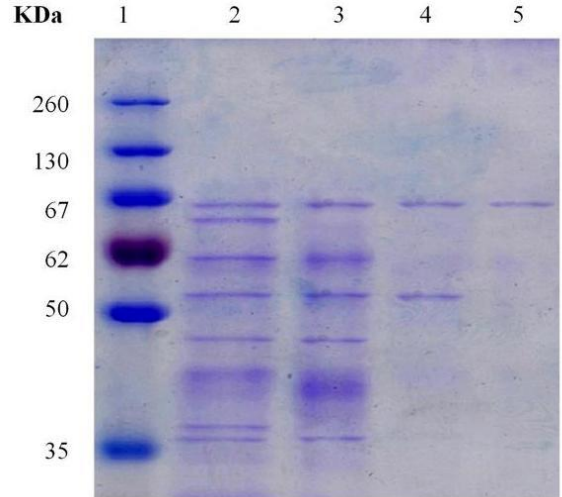
Docking Analysis of the Ricin (Ligand) with the km23, one of the vital proteins in cancer:

In Bioinformatics analysis we do have several softwares that can analyse the docking affinity between the selected Receptor and the Ligand. In the

current work Insilico docking has been employed to detect the docking affinity (binding affinity) between the Ricin (ligand) and KM23 (Receptor). Argus Lab software has been used for the docking study. Lower the docking energy higher would be the binding affinity among the ligand receptor pair.

Results and Discussion:

Fig 1: Identification of the Protein Bands in SDS:



Lane 1: Protein marker

Lane 2: Crude Protein

Lane 3: Salt Precipitation Purified Protein

Lane 4: Dialyzed protein

Lane 5: Chromatography Purified Protein

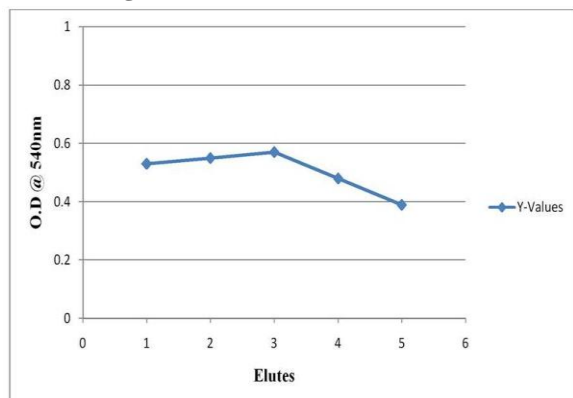
The above Figure 1m shows the protein bands in various purification steps as observed in SDS Page. As the protein becomes pure the band of it becomes sharper, indicating the absence of impurities.

Table 1: Purification of the protein by Ion Exchange Chromatography:

S.No.	Enzyme	D.W.	Alk CuSO ₄	Fc reagent	OD		
Elute-1	0.4ml	0.6ml	3ml	0.5ml	0.53		
Elute-2	0.4ml	0.6ml	3ml	Incubation	0.5ml	Incubation	0.55
Elute-3	0.4ml	0.6ml	3ml	At	0.5ml	At	0.57
Elute-4	0.4ml	0.6ml	3ml	Room	0.5ml	Room	0.48
Elute-5	0.4ml	0.6ml	3ml	temperature	0.5ml	temperature	0.39

The above tables shows the OD Values obtained for different elutes in the experiment.

Fig 2: The graph plotted to calculate the concentration of the protein in different elutes of Ion exchange column:



The above graph shows the values of the OD are obtained for different elutes after chromatography. Using the graph and the standard values the Concentrations of the protein is calculated to be 530µg/ml, 550µg/ml, 570µg/ml, 480µg/ml and 430µg/ml in the elutes 1, 2, 3, 4 and 5 respectively.

Table 2: Showing the OD values of Cell Suspensions Inoculated With Ricin:

S.No	Cell Suspension	Amount of Elute	Culture Medium	OD @ 540nm
No elute	100	-	1	0.38
Elute 1	100	100	1	0.21
Elute 2	100	100	1	0.19
Elute 3	100	100	1	0.11
Elute 4	100	100	1	0.22
Elute 5	100	100	1	0.24

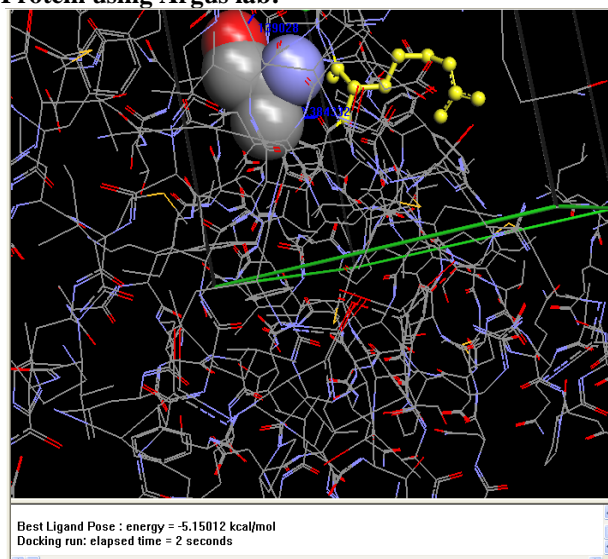
Incubation for 48hrs @ 37°C in 5% CO₂ and 90% relative humidity

From the above OD values it can be inferred that the Ricin addition shows a simultaneous decrease in the OD values due to cell lysis. At the end of the graph an increase in the OD is related to the decrease in the concentration of the ricin in the sample thereby a decrease in the cell lysis and increase in the OD.

Determination of the Anticancer Activity of Ricin using Insilico Docking Analysis:

To confirm the effect of ricin on cancer cells docking analysis is employed. Here ricin is used as the protein ligand to dock against the receptor km23 which is well known protein involved in cancer.

Fig 3: The Docking between km23 and Ricin Protein using Argus lab:



The above figure shows the docking between km 23 and ricin. The energy obtained between the docking pair is -5.15012Kcal/mol.

Discussion:

Based on the above work different observations were made. Regarding the purification of the ricin protein, the maximum concentration of pure protein is obtained in the elute 3. When the elutes were tested against the Colon cancer cell lines, the observations showed that the ricin protein is capable of inhibiting the growth of the cells. This can be read against the colorimeter. To accurately measure the binding affinity of the ricin (ligand) against the cancer vital protein involved in cancer, the km23. The docking studies showed that the docking energy between the pair is -5.15012Kcal/mol. This work lays a foundation to the use of ricin in treating cancers.

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