ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 8, Dec 2022

B-Cell Epitope Prediction From Leishmanolysin Protein Of *Leishmania Donovai* By In Silico Method.

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ABSTRACT:

A neglected tropical disease leishmaniasis is caused by *Leishmania* parasite. There are limited antileishmanial drugs for complete cure of visceral leishmaniasis (kala-azar). In our present study we are focusing on antigenic potential of leishmanolysin (GP63) surface protein for vaccine development. For the vaccine development there is a need of prediction of potential immunogenic B-cell epitope. We constructed Leishmanolysin(GP63) protein structure by homology modeling and predicted potential antigenic epitope against *Leishmania donovani* by using bioinformatics tools.

3D structure of Leishmanolysin(GP63) protein was predicted by homology modeling and its validation was done by ERRAT and VERIFY3D software. Linear B-cell epitope predicted by Support Vector Machine TriP tool, BEPIPRED tools. ElliPro tool was used for conformational B-cell epitope prediction. We detected the total 27 antigenic peptides by Immuno-medicine Group tool. The prep-4 from amino acid 140 to 163 was highly antigenic for immunization and also confirmed by BEPIPRED tools. The prep-4 was also confirmed by Ellipro in which two discontinuous (conformational) B-cell epitopes were predicted.

Keywords: *Leishmania*, Homology modeling, Leishmanolysin, 3D structure, B-cell epitope.

INTRODUCTION:

Leishmaniasis is a vector born parasitic disease, caused by *Leishmania* parasite. It is classified by center of disease control and prevention (CDC) as a neglected tropical disease. *Leishmania* spread by *phlebotomine* sand fly. The digenic life cycle of *Leishmania* consist of motile, flagellated, extracellular promastigote form in the gut of sand fly vector that infects mammalian host and transform into nonmotile, nonflagellated, amastigote form, which survive and multiply within phagolysosomal compartment of macrophages. According to

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World Health Organization (WHO) 2020 report out of 200 countries 98 were found endemic to Leishmaniasis. (Scarpini, S. et al., 2022). The Leishmaniasis classified into three major clinical forms visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and Mucocutaneous leishmaniasis (MCL), these forms differ in immunopathological conditions and level of illness and mortality. From all these clinical forms Visceral leishmaniasis caused by *Lishmania donovani*, which in latter stages may cause post Kala-azar dermal leishmaniasis (PKDL), it is most sever and fatal condition. Another fatal complication of visceral leishmaniasis is hemophagocytic lymphohistiocytosis (HLH) in which mainly children get affected (Varma & Naseem, 2010).

The effective control of leishmaniasis is challenging because less therapeutic options are available and there is no registered vaccine that prevents human leishmaniasis. The treatment depends on chemotherapy, using pentavalent antimonials as first line drug and Amphotericin B and pentamidine as second line drug. Miltefosine is the first recognized oral treatment for leishmaniasis but it has some limitations like host resistance and teratogenicity (Pradhan et al., 2022). Vaccination is potential method of control of leishmaniasis. In human there are the some recombinant vaccines under clinical trial like Leish-F1 but there is no registered antileishmanial vaccine yet (Moafi et al., 2019).

Leishmania donovani surface proteins have antigenic potential for vaccine development (Saha et al., 2022). In our present study we are focusing on antigenic potential of leishmanolysin (GP63) surface protein for vaccine development. Leishmanolysin (GP63) protein has role in mammalian host for infection of macrophages. For the vaccine development there is a need of prediction of potential immunogenic B-cell epitope and this can be done by using bioinformatics tools. But for B-cell epitopes prediction, 3D structure of Leishmanolysin (GP63) protein in PDB file is essential. Homology modeling of GP63 was done (Razzazan et al., 2008 and Waghmare et al., 2015) but protein structure is not available. In this study main aim was to construct Leishmanolysin(GP63) protein structure by homology modeling and its validation for prediction of potential antigenic epitope against *Leishmania donovani*.

Material and Method:

1. 3-D modelling of the protein:

We required 3D model of Leishmanolysin(GP63) protein for the prediction of potential conformational B cell epitopes. The 3D model of Leishmanolysin protein was not available at Protein Data Bank (PDB), so we aimed constructing 3D model of protein. The amino acid sequence of protein was retrieved from protein database (ID: P23223) of NCBI using the URL (https://www.ncbi.nlm.nih.gov/protein/P23223.1?report=fasta). The ProtParam tool (https://web.expasy.org/cgi-bin/protparam/protparam) was used for determination of physiochemical properties like amino acid composition, estimated half-life and instability index, molecular weight, isoelectric point (pI). PSIPRED server (http://bioinf.cs.ucl.ac.uk/psipred/&uuid=700cb3b4-214c-11ed-b4a1-00163e100d53) was

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used for the prediction of secondary structure of protein. Homology modelling approach was used for constructing 3D model of protein; it refers to construction of an atomic resolution model of the target protein by using an amino acid sequence and by using an experimental three-dimensional structure of a related homologous protein. It identifies appropriate template which having maximum identity and its 3D structure should be identified by X-ray crystallography or NMR. For 3D modelling of Leishmanolysin protein suitable template with default parameters was searched in PDB by Swiss server. On the basis of lowest E-value, maximum similarity crystal structure of LEISHMANOLYSIN (PDB ID: 1lml.1) of *Leishmania major* was selected as a template. Using ClustralW2 software target and template sequences were aligned and analysed. 3D modelling was done by using an automated server, Swiss model.

2. Validation of constructed 3D model of Leishmanolysin protein:

The ERRAT and VERIFY3D software with default parameters were used for checking quality and reliability of generated 3D model of protein. PROCHECK was used to check the stereochemical quality of a protein structure by analysing residue-by-residue geometry and overall structure geometry. ProQ server was again used for checking overall quality of protein model (Vedamurthy et al., 2019).

3. Linear B-cell epitope prediction:

Support Vector Machine TriP tool was used for prediction of linear antigenic B- cell epitopes from the sequence of Leishmanolysin protein. Prediction method of this tool is based upon the properties of amino acid like hydrophilicity, as potentially antigenic region which are hydrophilic found on the surface. Antigenic peptides in Leishmanolysin protein sequence were predicted by Immuno-medicine Group tool (http://imed.med.ucm.es/Tools/antigenic.pl), in which antigenic peptides are determined by using the technique of Kolaskar and Tongaonkar (1990). Prediction of epitopes is based on a table that reflects the occurrence of amino acid residues in experimentally known segmental epitopes. Segment size at least of 8 residues is only reported. About 75% accuracy of method is reported. Linear epitope prediction was done by BEPIPRED tools with default settings, which uses combination of a hidden Markov model and a propensity scale method (Larsen et al., 2006).

4. Conformational B-cell epitope prediction:

ElliPro tool was used for prediction of linear and discontinuous B cell epitope by using 3D model of Leishmanolysin protein (http://tools.iedb.org/ellipro/result/predict/). Ellipro provides score for each predicted epitope called as PI value.

Results and Discussion:

1. Physiochemical properties and secondary structure conformation of the protein:

The ProtParam analysis revealed physiochemical properties of Leishmanolysin protein. The molecular weight of the protein was 62950.33 Da, isoelectric point was 6.41. The protein contained total number of negatively charged residues (Asp + Glu) 56 and total number of positively charged residues (Arg + Lys) 51. The instability index was computed, it was 36.14,

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which clarifies the protein is stable as obtained value is less than the cut off of 40. The N-terminal of the sequence considered is M (Met) which indicated that the half-life of protein is 30 hours in mammalian reticulocytes, in vitro, >20 hours in yeast, in vivo, >10 hours in *Escherichia coli*, in vivo. The secondary structure of Leishmanolysin protein contained 29.8 helices (%), 53.6coils (%) 16.6 strands (%) (Fig.1).

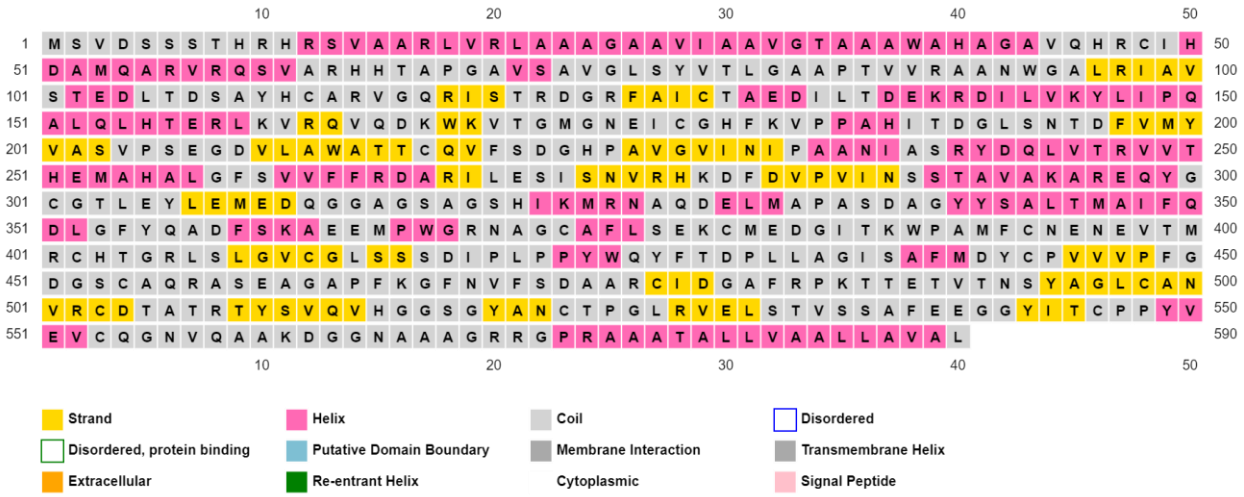

Fig.1 Secondary structure of Leishmanolysin protein predicted by PSIPRED server representing the alpha helix, beta sheet or coil on the basis of colour.

2. 3D modelling of Leishmanolysin protein:

In Swiss server, for 3D modelling of Leishmanolysin protein, crystal structure of LEISHMANOLYSIN (PDB ID: 1lml.1) of *Leishmania major* was selected as a template. The total 50 templates were found in Swiss server. Based on maximum similarity (56%), maximum identity (81.51) and GMQE score (0.82) and maximum query coverage of the alignment between the target and template sequences, we selected 1lml.1 as a template for 3D modelling of a Leishmanolysin protein. The model was constructed by using Swiss model server. The Overall Quality Factor of the predicted Leishmanolysin protein was 91.45 by the ERRAT. Mostly, quality score of 91 or above is considered as a good quality model (Fig. 2a). VERIFY3D result (Fig. 2b) shows 95.99% of the residues of Leishmanolysin protein model have averaged 3D-1D score >= 0.2. It is good at least 80% of the amino acids have scored >= 0.2 in the 3D/1D profile. Ramachandran plot assessment of PROCHECK (Fig. 2c) showed 87.6% residues in most favoured region, 11.4% in additional allowed regions, 0.7% residues in generously allowed regions and only 0.2% residues in disallowed regions. ProQ server predicted LG score 7.284 as LG score>4 extremely good model, which shows that quality of our protein model was good.

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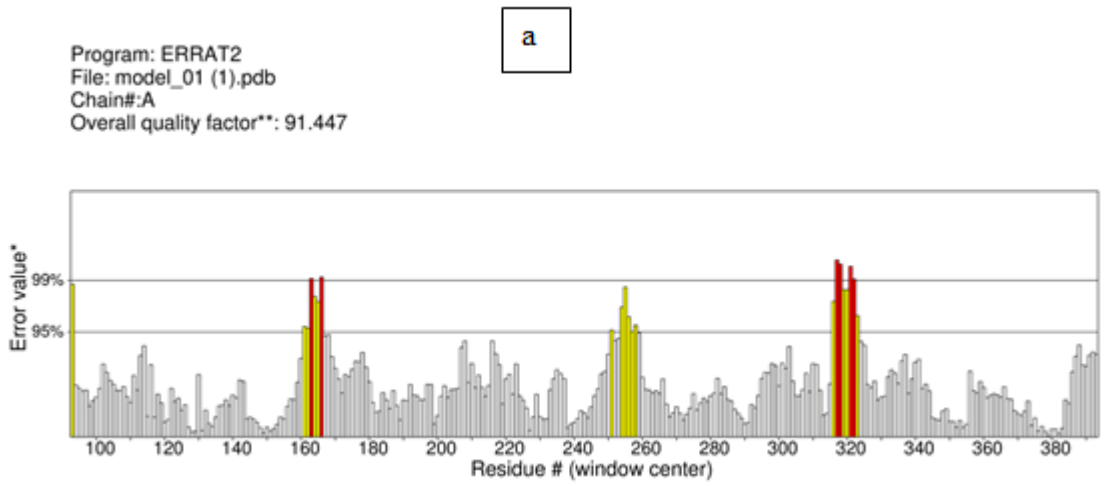
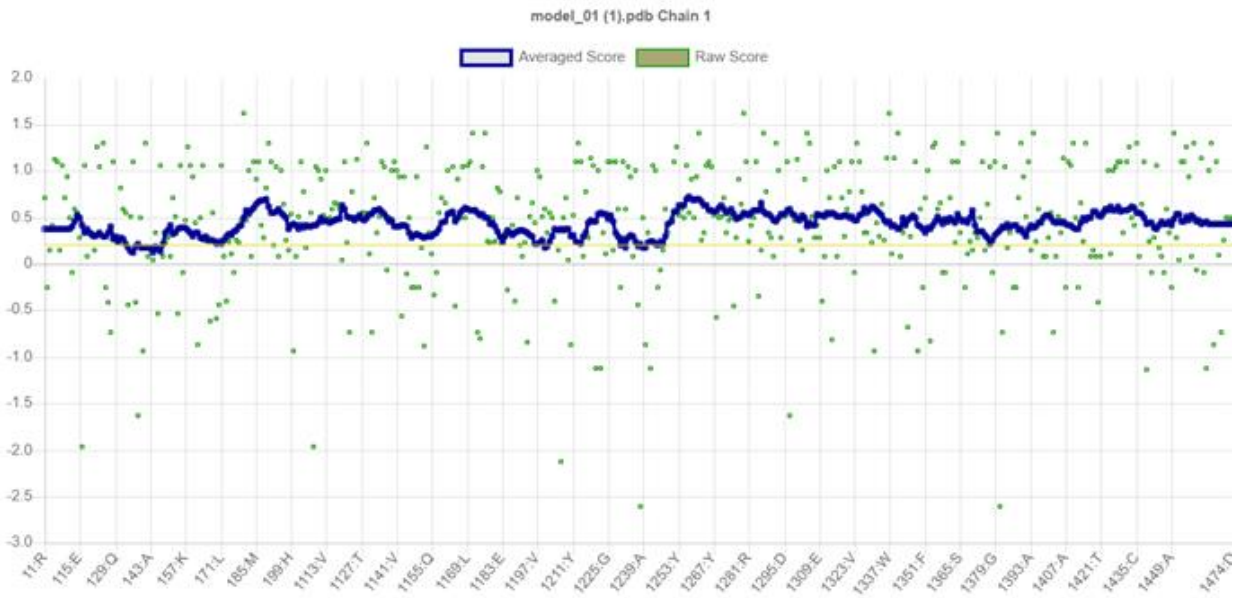
a



b



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c

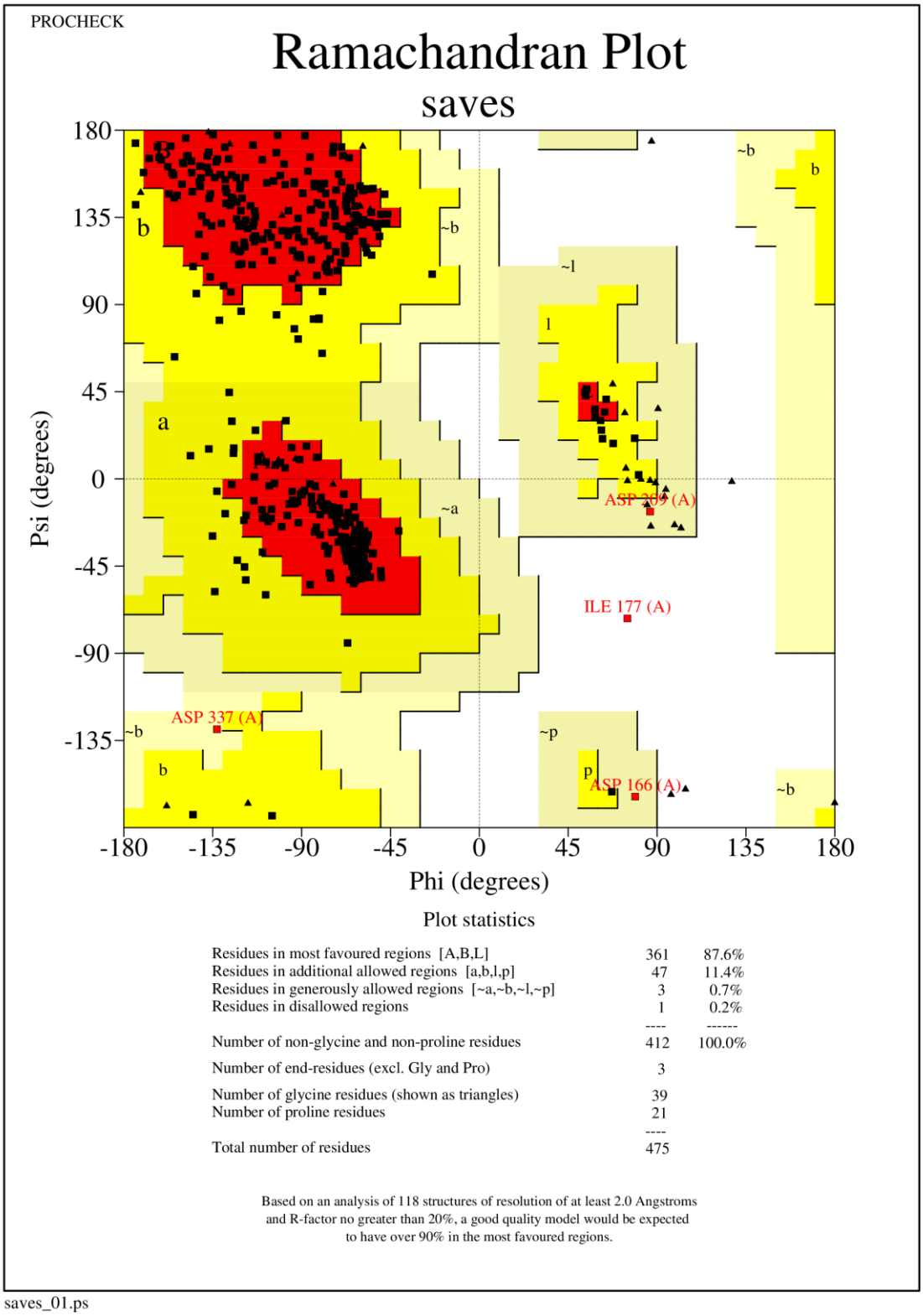

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Fig. 2 a: ERRAT plot, in which x-axis represents the residues number and y-axis represents the error values. The quality of modelled Leishmanolysin protein was very good with overall quality factor 91.45. b: VERIFY3D plot, in which x-axis represents the residues number and y-axis represents the average 3D-1D score. c: Ramachandran plot of Leishmanolysin protein predicted by PROCHECK server in which 87.6% residues in most favoured region.

3. Linear B-cell epitope prediction:

Total 27 antigenic peptides were predicted by Immuno-medicine Group tool (Table-2 and Fig. 3) with average antigenic propensity score of 1.0432. It shows peptides with antigenic propensity score greater than 1.0432 are potentially antigenic. Peptide named prep-1(AFMDYCPVVVPFGDGSCAQ) from the amino acid 438 to 456 shows greatest peak with propensity score 1.25, peptide named prep-2(AAATALLVAALL) from the amino acid 575 to 586 shows second highest peak with propensity score 1.21, peptide named prep-3 (GYITCPPYVEVCQGNVQA) from amino acid 542 to 560 shows propensity score 1.19 and peptide named prep-4 (RDILVKYLIPQALQLHTERLKVRQ) from amino acid 140 to 163 shows propensity score 1.18 which is greater than 1.0432. Therefore these four peptides were considered to be highly antigenic for effective immunization. Also, B-cell epitopes of Leishmaniolysin protein were predicted by SVMTriP tool, which gives 6 peptides out of which 2 qualified to be potentially antigenic, marked by flag (Table-1). The Linear B cell epitopes were also confirmed by BEPIPRED tools in which prep-4 was present in antigenic region (Fig. 4).

Rank	Location	Epitope	Score	Recommend
1	145 - 164	KYLIPQALQLHTERLKVRQV	1.000	
2	238 - 257	ASRYDQLVTRVVTHEMAHAL	0.833	
3	392 - 411	FCNENEVTMRCHTGRLSLGV	0.724	
4	342 - 361	SALTMAIFQDLGFYQADFSK	0.685	
5	474 - 493	AARCIDGAFRPKTTETVTNS	0.671	
6	518 - 537	SGYANCTPGLRVELSTVSSA	0.573	

Table-1 Predicted B-cell epitopes of Leishmaniolysin protein by SVMTriP tool. Epitope predicted inside protein marked by flag.

No	**Start Position**	**Sequence**	**End Position**
1	10	RHRSVAARLVRLAAAGAAVIAAVGT	34
2	36	AAWAHAGAVQHRCIHD	51
3	53	MQARVRQSVARH	64
4	66	TAPGAVSAVGLSYVTLGAAPTVVR	89
5	95	ALRIAVST	102
6	106	TDSAYHCARVGQ	117

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7	125	RFAICTAE	132
8	140	RDILVKYLIPQALQLHTERLKVRQ	163
9	176	EICGHFKVPPAHIT	189
10	196	DFVMYVASVPSEGDVLAWATTCQVFSDGHPAVGVINIPAANI	237
11	239	SRYDQLVTRVVTH	251
12	253	MAHALGFSVVFFRD	266
13	268	RILESISN	275
14	277	RHKDFDVPVINSSTAVAK	294
15	297	EQYGCGTLEY	306
16	335	ASDAGYYSALTM	346
17	348	IFQDLGFYQAD	358
18	371	AGCAFLSE	378
19	403	HTGRLSLGVCGLSSSDIPLPPYWQ	426
20	428	FTDPLLAGI	436
21	438	AFMDYCPVVVPFGDGSCAQ	456
22	466	KGFNVFSDAARCID	479
23	490	VTNSYAGLCANVRC	503
24	508	RTYSVQVHG	516
25	519	GYANCTPGLRVELSTVSS	536
26	542	GYITCPPYVEVCQGNVQA	559
27	575	AAATALLVAALL	586

Table-2: Predicted antigenic peptides of Leishmaniolysin protein by Immuno-medicine Group tool.

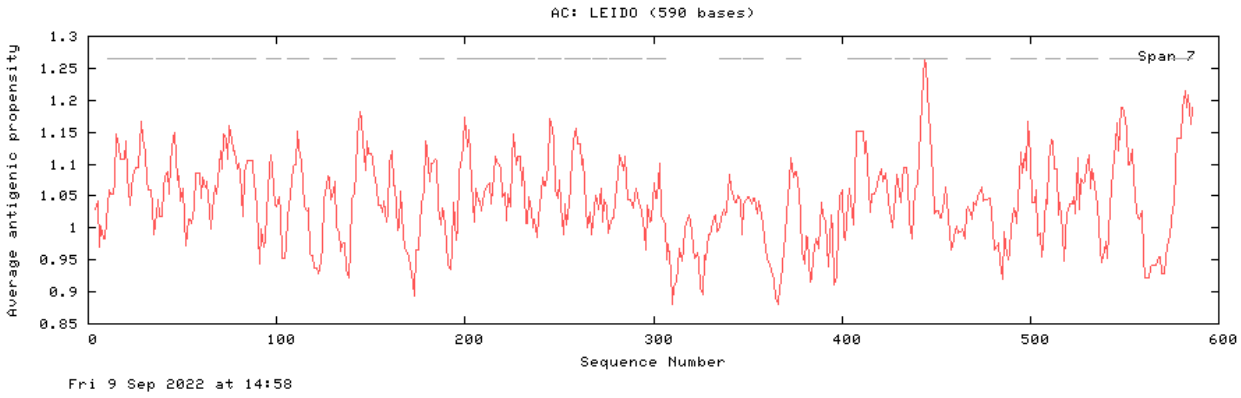

Fig. 3 Predicted antigenic peptides of Leishmaniolysin protein by Immuno-medicine Group tool. In this X-axis represents residues number and Y-axis represents average antigenic propensity.

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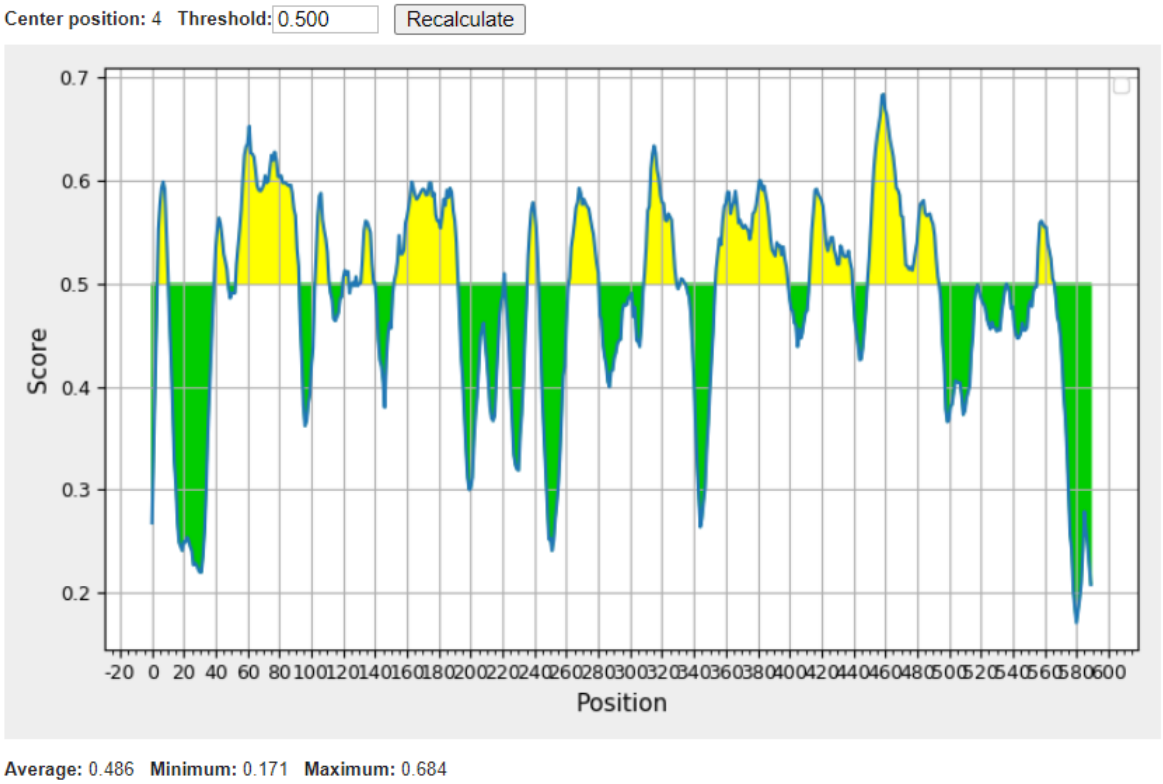

Fig. 4 Lineal B-cell epitopes of Leishmanolysin protein predicted by Bepipred software, in which Y-axis represents score of predicted amino acids in the sequence and X-axis represents the position. The red line is indicating the predicted epitopes threshold value (0.5) in which the linear B-cell epitopes in the sequence represented in yellow region.

4. Conformational B-cell epitope prediction:

For the Leishmaniolysin protein model, conformational B-cell epitope were predicted by using Ellipro(Table-3 and Table-4). Ellipro result shows highest score for epitope No-1(GLSSSDIPLPPYWQYFTDPLLA) from amino acids 413 to 434 and for epitope No-2(KVRQVQDKWKVTGMGNEICGHFKVPPAHITDGLSNTD) from amino acids 160 to 196 is 0.794 and 0.753 respectively. From these two epitopes, epitope No-2 shows some sequence part in antigenic peptide Prep-4 indicated by SVMTriP server and Immuno-medicine Group tool. Therefore antigenic peptide named prep-4 (RDILVKYLIPQALQLHTERLKVRQ) was selected from all other peptides because they were not recommended by Ellipro. 3D strucuture of predicted linear epitope No-1 and No-2 visualized by Jmol viewer and two discontinuous (conformational) B-cell epitopes were predicted by Ellipro (Fig. 5).

Sr. No	Epitopes	Start	End	No. of residues	Score
1	GLSSSDIPLPPYWQYFTDPLLA	413	434	22	0.794
2	KVRQVQDKWKVTGMGNEICGHFKVPPAHIT	160	196	37	0.753

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	DGLSNTD				
3	TEDLTDSAYHCARVGQRISTRDGRFAICTAED ILTDEKRD	102	141	40	0.748
4	RCDTATRTYSVQVHGGSGYANCTPGLRVELS TVSSAFEEGGYITCP	502	547	46	0.748
5	GAFRPKTTETVTNSYA	480	495	16	0.737

Table-3Ellipro server predicted linear B-cell epitopes.

No	Residues	No of residues	Score
1	A:D504, A:T505, A:A506, A:T507, A:R508, A:T509	6	0.717
2	A:R89, A:A90, A:A91, A:N92, A:W93, A:G94, A:A95, A:L96, A:R97, A:T102, A:E103, A:D104, A:L105, A:T106, A:D107, A:S108, A:A109, A:Y110, A:H111, A:C112, A:A113, A:R114, A:V115, A:G116, A:Q117, A:R118, A:I119, A:S120, A:T121, A:R122, A:D123, A:G124, A:R125, A:F126, A:A127, A:I128, A:C129, A:T130, A:A131, A:E132, A:D133, A:I134, A:L135, A:T136, A:D137, A:E138, A:R140, A:D141, A:K145, A:Y146, A:K160, A:V161, A:R162, A:Q163, A:V164, A:Q165, A:D166, A:K167, A:W168, A:K169, A:V170, A:T171, A:G172, A:M173, A:G174, A:N175, A:E176, A:I177, A:C178, A:G179, A:H180, A:F181, A:K182, A:V183, A:P184, A:P185, A:A186, A:H187, A:I188, A:T189, A:D190, A:G191, A:L192, A:S193, A:N194, A:T195, A:D196, A:S203, A:V204, A:P205, A:S206, A:C217, A:Q218, A:V219, A:F220, A:S221, A:D222, A:G223, A:H224, A:P225, A:A226, A:R265, A:D266, A:A267, A:R268, A:I269, A:L270, A:E271, A:S272, A:I273, A:S274, A:N275, A:V276, A:R277, A:H278, A:K279, A:D280, A:F281, A:D282, A:V283, A:N287, A:S288, A:S289, A:T290, A:V292, A:A293, A:R296, A:E297, A:G300, A:C301, A:G302, A:T303, A:L304, A:E305, A:Y306, A:D311, A:L352, A:G353, A:F354, A:Q356, A:A357, A:G368, A:R369, A:N370, A:A371, A:G372, A:C373, A:A374, A:S377, A:E378, A:K379, A:C380, A:M381, A:E382, A:D383, A:G384, A:I385, A:T386, A:K387, A:W388, A:P389, A:F392, A:C393, A:N394, A:E395, A:N396, A:E397, A:V398, A:T399, A:M400, A:G413, A:L414, A:S415, A:S416, A:S417, A:D418, A:I419, A:P420, A:L421, A:P422, A:P423, A:Y424, A:W425,	266	0.691

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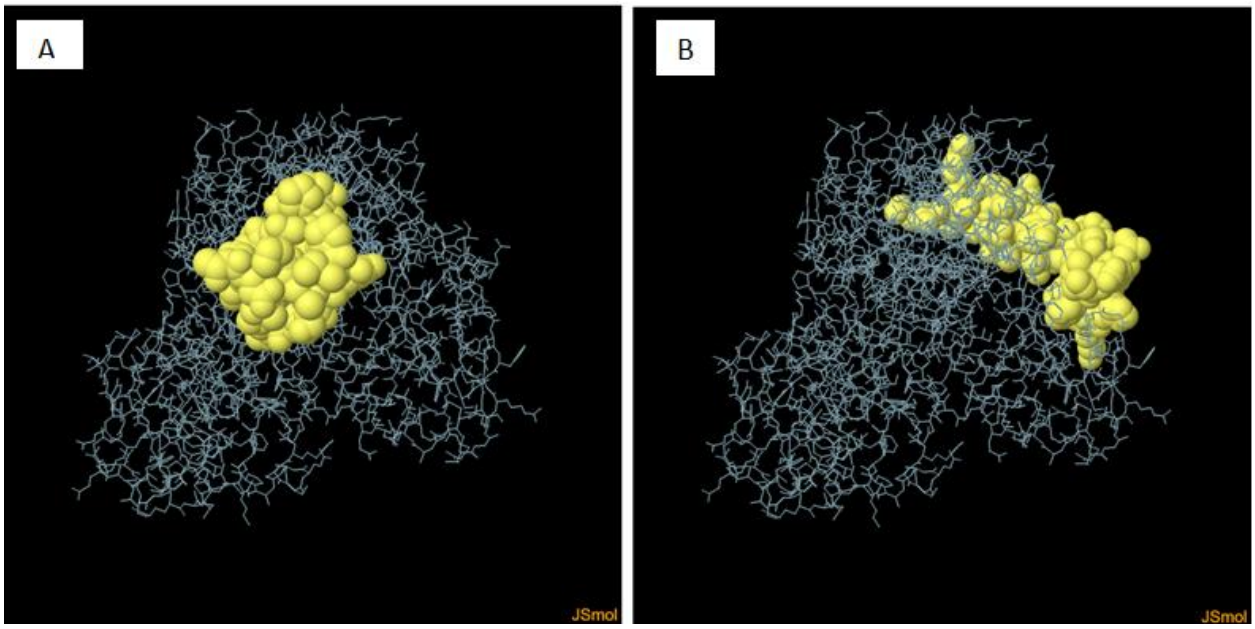
	A:Q426, A:Y427, A:F428, A:T429, A:D430, A:P431, A:L432, A:L433, A:A434, A:D441, A:Y442, A:C443, A:Q456, A:R457, A:A458, A:S459, A:E460, A:A461, A:G462, A:A463, A:P464, A:F465, A:K466, A:G467, A:F468, A:N469, A:F471, A:G480, A:A481, A:F482, A:R483, A:P484, A:K485, A:T486, A:T487, A:E488, A:T489, A:V490, A:T491, A:N492, A:S493, A:Y494, A:A495, A:R502, A:S511, A:V512, A:Q513, A:V514, A:H515, A:G516, A:G517, A:S518, A:G519, A:Y520, A:A521, A:N522, A:C523, A:T524, A:P525, A:G526, A:L527, A:R528, A:V529, A:E530, A:L531, A:S532, A:T533, A:V534, A:S535, A:S536, A:A537, A:F538, A:E539, A:E540, A:G541, A:G542, A:Y543, A:I544, A:T545, A:C546, A:P547, A:P548, A:D562		

Table-4 Ellipro server predicted discontinuous B-cell epitopes.



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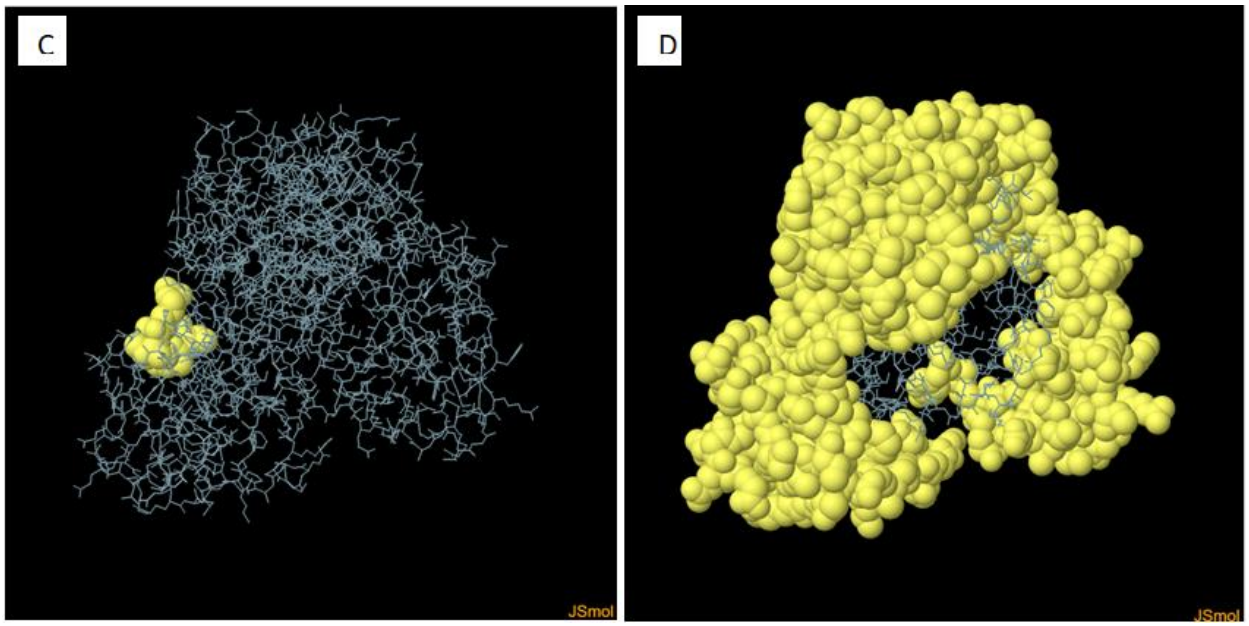

Fig. 5 ElliPro:Image A and B shows 3D Structures for linear epitope No-1(GLSSSDIPLPPYWQYFTDPLLA) and No-2(KVRQVQDKWKVTGMGNEICGHFKVPPAHITDGLSNTD) respectively. Image C and D shows 3D structure of discontinuous B-cell epitope with highest PI score predicted by Ellipro server. In ball and stick model, non-epitope residues of protein showed by white sticks and residues of predicted peptide showed by yellow balls in Jmol viewer.

CONCLUSION:

The main objective of this study is to predict antigenic peptide candidate of leishmanolysin protein for that 3D modeling of leishmanolysin protein from *L. donovani* was done. We have used homology modelling approach for proposing 3D structure of leishmanolysin protein. This predicted structure of protein was used for further B-cell epitope prediction. B-cell epitope predicted by using Immuno-medicine Group tool, SVMTriP tool and Ellipro in which prep-4 of leishmanolysin protein was proved potential antigenic. This peptide may also be helpful for vaccine design for the *L. donovani*.

REFERENCES:

http://bioinf.cs.ucl.ac.uk/psipred/&uuid=700cb3b4-214c-11ed-b4a1-00163e100d53

http://imed.med.ucm.es/Tools/antigenic.pl

http://tools.iedb.org/ellipro/result/predict/

https://web.expasy.org/cgi-bin/protparam/protparam

https://www.ncbi.nlm.nih.gov/protein/P23223.1?report=fasta

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Kolaskar, A. S., & Tongaonkar, P. C. (1990). A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBS letters*, *276*(1-2), 172-174.

Larsen, J. E. P., Lund, O., & Nielsen, M. (2006). Improved method for predicting linear B-cell epitopes. *Immunome research*, *2*(1), 1-7.

Moafi, M., Rezvan, H., Sherkat, R., & Taleban, R. (2019). Leishmania vaccines entered in clinical trials: a review of literature. *International Journal of Preventive Medicine*, *10*.

Pradhan, S., Schwartz, R. A., Patil, A., Grabbe, S., & Goldust, M. (2022). Treatment options for leishmaniasis. *Clinical and experimental dermatology*, *47*(3), 516-521.

Razzazan, A., Saberi, M. R., & Jaafari, M. R. (2008). Insights from the analysis of a predicted model of gp63 in Leishmania donovani. *Bioinformation*, *3*(3), 114.

Saha, S., Vashishtha, S., Kundu, B., & Ghosh, M. (2022). In-silico design of an immunoinformatics based multi-epitope vaccine against Leishmania donovani. *BMC bioinformatics*, *23*(1), 1-28.

Scarpini, S., Dondi, A., Totaro, C., Biagi, C., Melchionda, F., Zama, D., ... & Lanari, M. (2022). Visceral Leishmaniasis: Epidemiology, Diagnosis, and Treatment Regimens in Different Geographical Areas with a Focus on Pediatrics. *Microorganisms*, *10*(10), 1887.

Varma, N., & Naseem, S. (2010). Hematologic changes in visceral leishmaniasis/kala azar. *Indian Journal of Hematology and Blood Transfusion*, *26*(3), 78-82.

Vedamurthy, G. V., Ahmad, H., Onteru, S. K., & Saxena, V. K. (2019). In silico homology modelling and prediction of novel epitopic peptides from P24 protein of Haemonchus contortus. *Gene*, *703*, 102-111.

Waghmare, S., Buxi, A., Nandurkar, Y., Shelke, A., & Chavan, R. (2016). In silico sequence analysis, homology modeling and function annotation of leishmanolysin from Leishmania donovani. *Journal of Parasitic Diseases*, *40*(4), 1266-1269.