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Title:

DESIGNING A MULTI-EPITOPE VACCINE AGAINST *DRACUNCULUS MEDINENSIS* BY EMPLOYING IMMUNO-INFORMATICS AND *IN SILICO* APPROACHES

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Abstract:

Dracunculiasis (also known as Guinea worm disease) is caused by *Dracunculus medinensis* parasite and it spreads by drinking water containing Larvae of Guinea worm. The lack of safe water facilities, preventions and treatments resulted in highly dangerous consequences in its endemic regions. The economy of the affected regions totally falls down due to less production which is the result of agricultural field worker's bad health. In this study, a multi epitope vaccine was designed against *Dracunculus medinensis* by using immune-informatics. The vaccine was designed by using T-Cell and B-Cell epitopes derived from *Dracunculus medinensis* proteins (Lactamase-B domain-containing protein, G-Domain containing protein and Ferrochelatase) in addition to Adjuvants and Linkers. The tertiary structure, physiochemical properties and immunogenic elements of vaccine were achieved. The validation of tertiary structure was accessed, and quality was achieved. In addition, the world coverage of parasite's CTL and HTL epitopes is 95.61%. The stability of the chimeric vaccine was achieved through disulfide engineering. The molecular docking with Toll Like Receptor 4 (TLR-4) of vaccine showed its binding efficiency followed by Molecular Dynamic Simulation. The immune simulation suggested the mediated cell immunity and repeated antigen clearance. At the end, the optimized codon was used in *in silico* cloning to ensure vaccine's higher exposure in bacterium E. coli strain K12. With further assessments, it is believed that the proposed multi epitope vaccine has strong immunogen to control *Dracunculus medinensis* which may result in better social and economic conditions of endemic regions.

Keywords: Immuno-informatics, Multi-epitope, Dracunculiasis

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Introduction:

The disease caused by parasite *Dracunculus medinensis* is very painful. Public health authorities including the CDC have declared the disease an NTD. The disease spreads mainly by contaminated drinking water containing *D. medinesis's* larvae (Hopkins, 1988). The spread varies from region to region. In dry regions, the disease is at peak in rainy season when there is a lot of standing water on the surface while in wet regions the disease is at peak when the dry season comes up and the surface started to dry, and water becomes stagnant. Early, it was considered a Human disease but now it becomes both animal and human disease because both animals and humans eat certain aquatic animals that carry Larvae of *D. medinensis*. The disease occurs depending on sex, age, occupation, village and region (Hopkins, 1988). Possibly because of how and where people of all ages and genders get drinking water. Today, Guinea worm disease in Sudan, Ghana and Nigeria is very high. Together, these three countries compensated 93% of all cases worldwide, with Sudan reporting 73% (Greenaway, 2004).

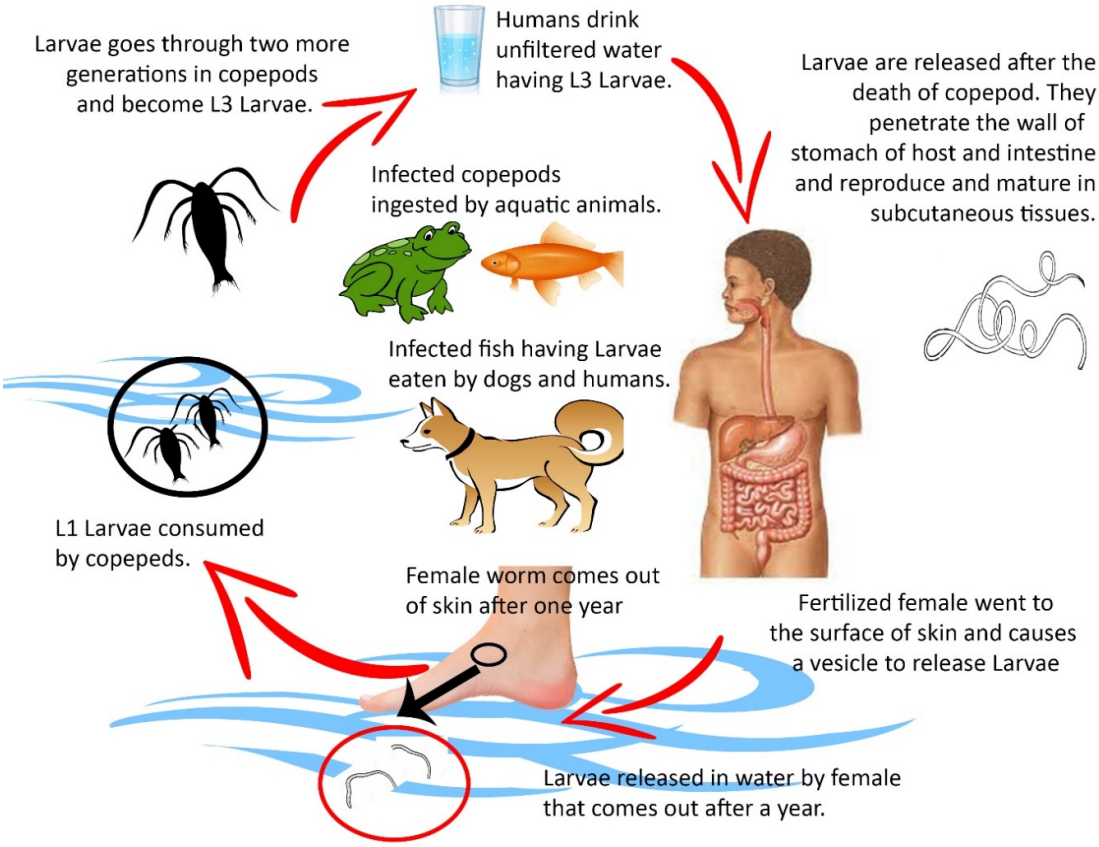

Figure 1: *Dracunculus medinensis's* life cycle.

Humans drink unfiltered water having copepods infected with L3 Larvae of *D. medinensis*. The copepods die after ingestion and with their death, the Larvae are released. Larvae mature and regenerate in subcutaneous tissues after penetrating the wall of the host's stomach and intestine. Then the male worm dies, and females of length 70-120 cm go to the surface of the skin after a year causes a blister and released Larvae L1. The L1 is consumed by copepods and Larvae generates two more generations and becomes L3 (Figure 1). The CDC reports 28 cases in 2018. The cases included one in Angola, seventeen in Chad and ten in South Sudan.

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The World Bank has estimated roughly that after the disorder has been eradicated, the economic return on investment in Guinea worm eradication shall be around 29% per year (Greenaway, 2004). Due to severe illness an infected person cannot go to school and men can't go to work. In Africa, it causes more damage to society, the economy, education and health. A total of 54 cases were reported from four countries in 2019 according to the most recent WHO report: Angola (1 case), Chad (48 cases), South Sudan (4 cases) and Cameroon (1 case, probably imported from Chad).

When the worm grows up the infection is symptomatic. The worms come from the foot, ankle or bottom of the leg and most of the patients get only one worm in the 'Guinean worm phase.' However, worms have been reported to come from other parts of the body, such as the head, neck, abdomen, scrotum, vulva, arm and hip. No powerful or strong immunities develop and grow (Hopkins, 1988). The identification of new immunodominant epitopes for the design of effective multi-epitope peptide vaccines recently gained increased visibility in the light of advances in immune-stating technologies. The present research consists of *D. medinensis* proteome, accompanied by the T- and B-cell epitopes prediction, classify the highest antigenic protein. The population distribution was measured using the T-cell epitopes and the MHC alleles. The build of multi-epitope vaccine was designed to use effective adjuvant epitopes. The building was used for immunogenic and physiochemical profiles and was accompanied by analysis of the tertiary and disulfide structures of the vaccine. Molecular docking of the vaccine with the TLR4 receptor was carried out to measure binding effectiveness. Finally, molecular dynamics and immune simulations have been carried out to estimate the stability of the complex and real-life immunogenic capacity of the vaccine-receptor (Nain et al., 2019). No Disease-related vaccine or therapies are available. Alternative control measures are therefore necessary. The directional method involves the detection of the virulence protein, the collection of peptide parts and an immune response generation (Nain et al., 2019). New methods for vaccination can easily be put forward, such as genome sequencing, comparative proteomics and immune supporting methods. In immune for mapping the possible epitopes of B and T cells, which reduce time and expense, is studying and developing algorithms. Here, we throw some light on available immunology related tools and algorithms.

To predict epitopes, it is usually important to determine the binding score of MHC molecules and antigenic peptides. The experimental methods are hard and time consuming. In consequence, several silicon methods are improved and used to detect epitopes. Matrix approaches, structural binding motifs, a quantitative structure-activity relationship analysis (QSAR), homology models, protein threading, docking techniques, and many machine-learning algorithms and software are used to develop these techniques. The latest study is planned to develop a MEV by using three *Dracunculus medinensis* proteins that are known to protect and can create neutralizing antibodies against *Dracunculus medinensis,* combining B-cell, T-cell and Helper T lymphocytes (HTL). The efficiency of expression and translation of the structure was also determined by the silicon cloning (Kalita et al., 2019).

Materials and Methods:

Immuno-informatics analysis of the antigen

Proteome Retrieval:

The entire proteome in FASTA format of *D. medinensis*, a pathogenic strain was retrieved from UniProt. Then protein duplicated was removed using the CD-HIT Suite (Kar & Srivastava, 2018).

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Screening of Proteins Non-Homologous to Humans:

The BLASTp tool was used for screening. The database chosen for this function was Reference Proteins and the host organism was chosen as Human (Kar & Srivastava, 2018).

Screening Antigenicity of Proteins:
The proteins selected after the previous step were tested for antigenicity and the method used for this step was VaxiJen v2.0. Vaxijen uses an auto-cross-covariance (ACC) prediction strategy to transform protein sequences into uniform vector of major amino acid characters (Kar & Srivastava, 2018). The threshold was set at 0.5 and the selected organism was a parasite.

Prediction and assessment of B Cells:
The three highly antigenic proteins that have already been declared characterized by UniProt selected from the previous stage and B cells have been predicted for them. B-cell epitopes play a key role in the production of adaptive immunity and may therefore be a significant component in the design of vaccines. 2 types of B-cell epitopes exist, linear and conformational. For this purpose, a server that uses an artificial network and predicts linear B-cell epitope regions is an antigenic sequence, as only linear epitopes are considered when designing an ABCpred vaccine server (Saha & Raghava, 2006; Patra et al., 2020). The sequence of each protein was submitted one by one with a threshold set of 0.51 with duration of window 14. Predicted B-cell epitopes were tested using VaxiJen v2.0, AllergenFP v1.0 and Toxinpred servers (Gupta et al., 2013) for their antigenic, allergenic and toxic profiles.

CTL Epitopes Prediction and Evaluation:
Most T-cells express T-cell receptors (TCRs) that recognize a particular antigen (Chaudhri et al., 2009). Therefore, CTL epitopes are essential to the coherent nature of vaccines. A consensus approach and 14mer length in IEDB analysis tool predicted MHC-I binding alloys for every CTL epitope (Calis et al., 2013; Moutaftsi et al., 2006). The percentiles were < 2 when the person was chosen as the MHC source. The suitability of CTL epitopes is known to be a core of the adeptness of the vaccine within their immune capacity (Nain et al., 2019). For their immunogenicity, the forecast epitopes were calculated using the IEDB server MHC-I immunogenicity instrument (Calis et al., 2013). Their antigenicity was evaluated on the Vaxijen 2.0 server to detect the capacity of epitopes to cause an immune response. No allergic reactions may occur to vaccine components. Allertop 2.0 server used to carry out allergic predictions of epitopes. The CTL toxic epitopes have been found on the Toxinpred list.

HTL epitope prediction and assessment:
Support T cells activate B calls to secrete macrophages from antibodies to pathogenic phagocytes and cytotoxic T cells to destroy targeted cells. For the 15-Meter HTL prediction with their binding alles using CONSENSUS technique, the selected protein was subjected to IEDB MHC-II binding tools (Wang et al., 2010) with the percentile rank threshold ≤ 2 (da Silva Pissarra, 2019). Helping T cells produce cytokines like interferon gamma, interlukin-4 (IL-4), and interlukin-10, which cause cytotoxic T cell activation and other immune cells. Thus, cytokine with HTL epitope is decisive for the construction of vaccines. The IFN epitope server was therefore used to evaluate the HTL epitopes produced by IFN-Gamma using Motif and SVM (Dhanda et al., 2013). On the other hand, inducing characteristics for IL-4 and IL-10 have been forecasted on both IL-4 and IL-10, respectively. Both servers have been designed to prevent interleukin peptides by *in-silico*; IL4Pred, IL4Pred (Dhanda et al., 2013) and IL10Pred (Nagpal et al., 2017). Operations with the threshold values 0.2 and -0.3 respectively were performed by SVM method and hybrid method.

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Modeling of peptides and molecular docking studies:

For the construction of the shortlisted HTL and CTL epitopes from scratch, the online PEP-FOLD v.3.0 method was considered. For this reason, the 200 simulated sOPEP sorting scheme were adopted. The 3D structure of peptides is predicted by PEP-FOLD V.3.0 based on the backtrack / taboo probes (Shen et al., 2014). When all 3D epitope structures were ready, they were docked to their binding affinity with a specific human leucocyte antigen (HLA). RCSB PDB has downloaded crystal structures of HLAs (Berman ET AL., 2003). Binding protein and protein-DNA / RNA docking was done by the HDOCK SERVER based on a hybrid modeling and initial docking algorithm. PyMOL visualized the Docked complex. PyMOL is a molecular visualization framework that is funded and distributed by Schrödinger on an open-source basis (Lill & Danielson, 2011).

Population Coverage Estimate:

The distribution and expression of HLA alleles may differ from one region to another (Adhikari et al., 2018). In this analysis, the IEDB population coverage method was used for the distribution of HLA alleles for possible CTL and HTL epitopes (Bui et al., 2006). This server is intended to estimate epitopes from various regions on the basis of the distribution of their MHC-binding alleles. From the involvement *D. medinensis* is poorly known, and for various parts of the world the percentage of population coverage was evaluated. However, we especially highlighted the areas in which it was happened first and triggered outbreaks (Nain et al., 2019).

Multi-epitope vaccine design and assessment

Mapping of the development of vaccines:

The vaccine was developed using adjuvant, T- and B-cell epitopes and suitable connectors as previously defined (Nain et al., 2020). In the multi-epitope series of vaccines, the epitope cells of CTL, HTL and B were included. The selected epitopes were expected to be extremely promiscuous, overlapping, immunogenic and non-allergic for the final vaccine development. In the CTL and HTL epitopes respectively, the AAY and GPGPG linkers were used. The vaccine construct which serves as a supplement to boost vaccine immunogenicity was also supplemented with the Adjuvant cholera enterotoxin subunit B. The EAAAK linker was attached to a multi-epitope chain (Chauhan et al., 2019).

Linear and secondary structural analysis of vaccine:

The vaccine structure's physical and chemical property, which includes molecular weights (MW), isoelectric theoretical points (theoretical PI), and broad average in vitro and in vivo half-life, were evaluated on the ProtParam server.

Modelling of 3D Structure:

The tertiary structure of a protein provides optimum stability with proper binding and twisting in its lowest energy state (Nain et al., 2019). The server I-Tasser was used for the structure prediction (Hajighahramani et al., 2017; Zheng et al., 2019). The vaccine sequence has been compiled and submitted.

Refinement of Tertiary Structure:

The initial structure has been improved by using the Galaxy Network Refine service. The structure has been uploaded to PDB format. The most detailed structure was made by molecular dynamic simulation. The rehashed structure disruption accompanied by complete structural relaxation by dynamic simulation. To confirm our refined structure standard, the Ramachandran plot analysis was performed using the ProCheck api, followed by a structural verification test by the ProSAweb api, which gives an overall quality score. The ERRAT server was used to analyze the statistics of non-bonded interactions in the parasite vaccine model.

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B Cell Epitope Screening:

To find the conformational B-cell epitopes for the final vaccine build, the Ellipro tool provided by IEDB-AR v.2.22 was used to preserve the settings by default (Ponomarenko et al., 2008). It predicts epitopes by estimating residual protrusion index (PI), protein form and neighbor residue clustering. In addition to these linear B-cell epitopes, the Abcpred server predicts 14-mer LBL epitopes.

Vaccine Protein Disulfide Engineering:

Disulphide technology is a modern approach to adding disulphide bonds to protein structure amino acids. The disulphide bond is the covalent bond that gives a protein structure considerable stability by confirming precise geometry. Disulphide was used in disulfide engineering of vaccine protein using Design v2.12 online platform. Refined MEV structure has been uploaded and run for research of the residue-pair, potential residue pairs for mutation have been identified, and cysteine residue has been taken as a final objective. Residues of less than 2.2kcal / mol and an angle of − 87 to + 97 ° were considered for the development of disulphide connections (Craig & Dombkowski, 2013).

Docking of protein vaccine and receptor TLR4:

When an antigen / vaccine bind correctly with the target cells, the host develops an appropriate immune response. The association between vaccines and human immune receptors was thus studied by molecular docking. TLR4 has been comprehensively studied and scientists find that the vaccine docking TLR4 (PDB ID: 4G8A) is basically involved in antiviral immune response. The PyMOL molecular graphic system v.1.3 was used for visualizing the docked complex and drawing figures (DeLano, 2002). In the online PDB database, interactive residues from docked complexes were classified (Laskowski, 2009).

Molecular Dynamic Simulation:

For analyzing the stability of the protein-protein complex in any silicon research the molecular dynamics study is of vital importance. By comparing important protein dynamics with their usual modes, protein stability can be concluded (Van Aalten et al., 1997; Wüthrich et al., 1980). The iMODS server clarified the collective protecting motion through standard mode analysis (NMA) in the internal coordinate (López-Blanco et al., 2014). In deformation, self-value, B factor and covariance, the server evaluated the direction and extent of the inherent motions of the complex. The deformation of the principal chain depends on the deformation of a particular molecule in each of its residues. The normal mode has its own meaning representing its rigidity. This is directly correlated with the energy needed to break the structure and is much simpler if the value is low.

Immune simulation:

C-ImmSim 10.1 Server has been used to authenticate the immunological response of the designed vaccine. C-ImmSim simulates the functional mammal system three main elements (Rapin et al., 2010b). The input parameters for the immune simulations are as follows: volume (10), HLA (A0101, A0101, B0702, B0702, DRB1_0101, DRB1_0101), number of steps (100), random seed (12345), number of injections set to 1. Default was defined for the rest of the parameters.

Optimization of Codon and In-silico cloning:

Codon adjustment is the method to increase the effectiveness of translation in the host for external genes when codon use varies in the two species. The codon optimization and silicon clone were followed after careful assessment of vaccine properties and immune response. The JCAT server has been used to enhance our vaccine codon (Grote et al., 2005) & has been used to create the prokaryotic expression system widely used; E.coli K12 (C. Smith). Additional options were available to prevent: (a) rho-independent termination of transcription, (b) prokaryotic site binding ribosome, and (c) limitation of cleavage sites in

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enzymes. The contents of GC (guanine and cytosine) are mixed with the CAI (Sharp & Li, 1987) were evaluated.

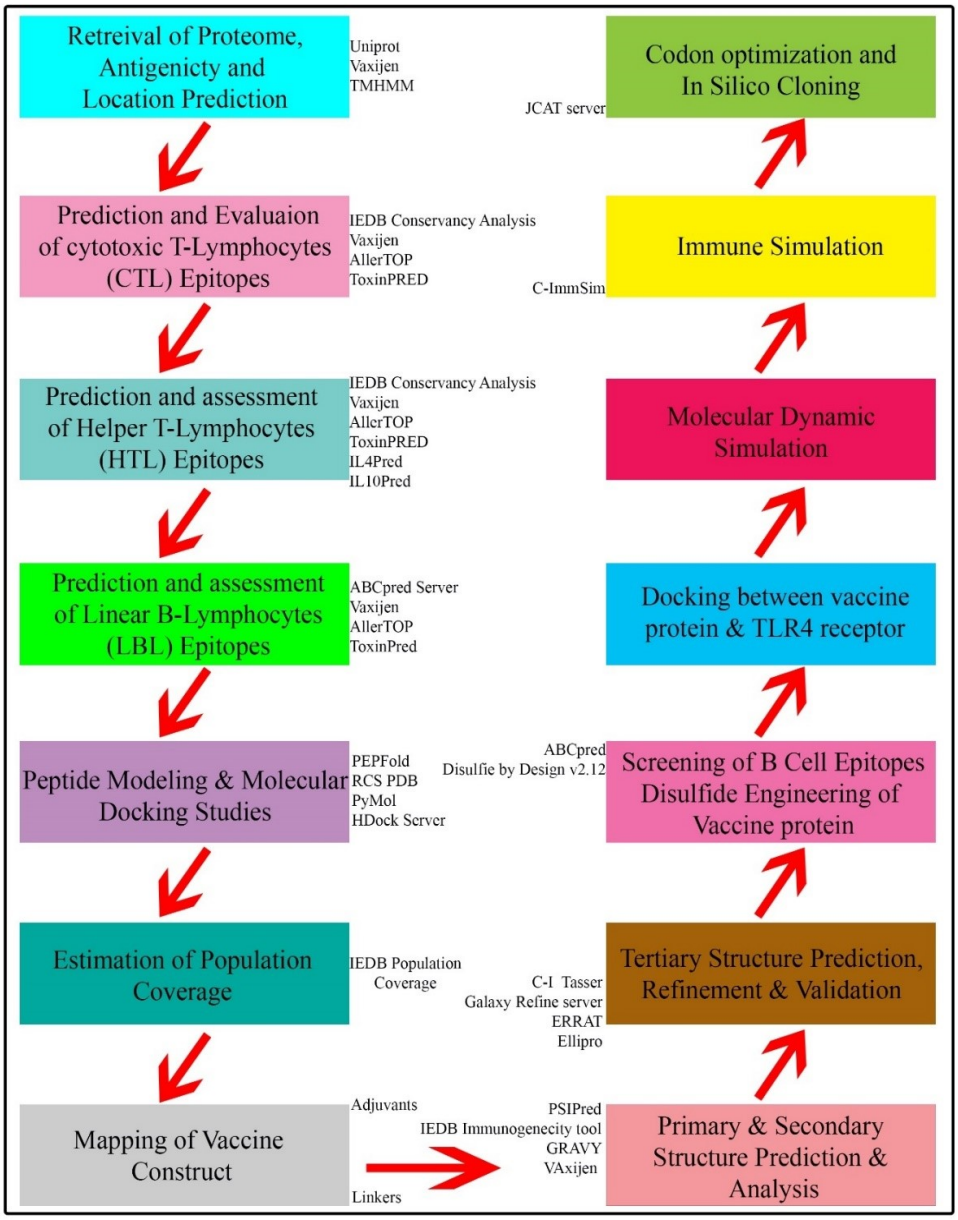

Figure 2: A description of the approach and methods used.

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Results:

Selection of Protein:

The whole proteome was retrieved by UniProt having 10861 proteins and then CD-HIT server was used and 10861 proteins were subjected to CD-HIT server and 10361 proteins left. The essential proteins from whole proteome were 281 and matched with 10361 CD-HIT results. BLASTp was used to remove the homologous proteins between humans and parasite. The criterion for selection of protein is that similarity should not more than 37%. The remaining proteins were 10 after BLASTp and subjected to antigenicity screening and three proteins 1. Lactamase_B domain-containing protein, 2.G-Domain containing protein and 3. Ferrochelatase were selected because they were highly antigenic among others.

Appraisal and Selection of T and B-cell Epitopes:

In order to predict the CTL epitopes and the HTL epitopes, the IEDB-AR v.2.22 consensus network servers were used. The prediction of linear B-cell epitopes was based on the ABCPred server. The criterion for selecting best-possible epitopes is that their preservation should be 100% binding, should not interfere with human proteins; should not lie in the sites of post-transduction alteration or glycosylation in the specific proteins. Their preservation should be 100% antigenic / immunogen. Total: 22 CTL, 21 HTL, 36 linear B-cell epitopes. Epitopes: Total 22 CTL epitopes, 21 HTL epitopes, 36 linear B-cell epitopes. The final selected epitopes for vaccine construct are 7 CTL epitopes, 5 HTL epitopes and 7 B Cell epitopes.

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Table 1: B Cell selected *D. medinesis* antigenic protein epitopes used for the design of the multi-epitope subunit vaccine (MEV) construction.

B cell Epitopes	Protein	Position	Antigenicity	Immunogenicity
KAIFITHAHHDHMM	Lactamase B domain-containing protein	433	1.20	0.40
AEYGENADLLVHEY	Lactamase B domain-containing protein	481	1.33	0.28
DSVFFVSSFTGEGI	G domain-containing protein	362	1.31	0.25
VLQIVTEVRCDKER	G domain-containing protein	440	1.35	0.20
KILWTVILHLFILN	Ferrochelatase	65	1.13	0.65
YDNYLYIGAIKDSI	Ferrochelatase	190	1.56	0.02
ILMVAPGFISDCFE	Ferrochelatase	292	1.53	0.17

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Table 2: Final CTL selected *D. medinesis* antigenic protein epitopes used for the design of the multi-epitope subunit vaccine (MEV) construction.

CTL Epitopes	Protein	Position	Antigenicity	Immunogenicity	Alleles	Binding Score
KQKENYIFILFW	Lactamase_B domain-containing protein	6-17	1.45	0.44	HLA-B*44:03	-201.52
QKENYIFILFWY	Lactamase_B domain-containing protein	7-18	1.72	0.61	HLA-B*44:03	-199.33
KENYIFILFWYF	Lactamase_B domain-containing protein	9-19	2.01	0.65	HLA-B*44:03	-197.59
VRCDKERHARLV	G domain-containing protein	381-392	2.40	0.04	HLA-C*06:02	-188.95
KLVDFTNFLNRK	G domain-containing protein	430-441	0.76	0.30	HLA-A*11:01	-196.06
FPKILWTVILHL	Ferrochelatase	63-74	1.32	0.48	HLA-B*51:01	-183.28
RVIPAFYDNYLY	Ferrochelatase	184-195	1.00	0.18	HLA-A*11:01	-224.00

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Table 3: Final HTL selected *D. medinesis* antigenic protein epitopes used for the design of the multi peptide vaccine (MPV) construction.

HTL Epitopes	Protein	Position	Antigenicity	IFN gamma	IL-4	IL-10	Alleles	Binding Score
KCYFLHNIWNFIDPK	Lactamase_B domain-containing protein	311-325	0.71	Positive	Inducer	Inducer	HLA-DRB1*04:10	-227.02
CYFLHNIWNFIDPKL	Lactamase_B domain-containing protein	312-326	0.83	Positive	Inducer	Inducer	HLA-DRB1*04:10	-219.88
NVSSYLLQLNHHSYV	Lactamase_B domain-containing protein	390-404	0.97	Positive	Inducer	Inducer	HLA-DRB1*04:01	-264.73
VRILCCGQVGGKPIT	G domain-containing protein	213-227	1.05	Positive	Inducer	Inducer	HLA-DRB1*01:02	-180.22
KILWTVILHLFILNS	Ferrochelatase	65-79	1.03	Positive	Inducer	Inducer	HLA-DRB1*11:04	-205.21

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Peptide Modeling and Molecular Docking Studies:

The epitopes chosen must be 100% preserved, overlapping and antigenic for construction of a subunit vaccine. Thus, twelve (Seven Major Histocompatibility Complex Class I and 5 Major Histocompatibility Complex Class II) epitopes were identified, overlaps, retention and highly antigenic and their linkage with their respective HLA alloys by means of the molecular docking approach has been determined (Table 4 & Table 5).

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Table 4: Finally selected antigenic protein epitopes designed with their respective human antigenic leukocytes' alleles for a multipeptide-based subunit framework and their binding detail.

MHC 1 Epitopes	Protein	Position	Antigenicity	immunogenicity	Alleles	Binding Score
KQKENYIFILFW	Lactamase_B domain-containing protein	6-17	1.45	0.44	HLA-B*44:03	-201.52
QKENYIFILFWY	Lactamase_B domain-containing protein	7-18	1.72	0.61	HLA-B*44:03	-199.33
KENYIFILFWYF	Lactamase_B domain-containing protein	9-19	2.01	0.65	HLA-B*44:03	-197.59
VRCDKERHARLV	G domain-containing protein	381-392	2.40	0.04	HLA-C*06:02	-188.95
KLVDFTNFLNRK	G domain-containing protein	430-441	0.76	0.30	HLA-A*11:01	-196.06
FPKILWTVILHL	Ferrochelatase	63-74	1.32	0.48	HLA-B*51:01	-183.28
RVIPAFYDNYLY	Ferrochelatase	184-195	1.00	0.18	HLA-A*11:01	-224.00

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Table 5 Finally selected antigenic protein epitopes designed with their respective human antigenic leukocytes' alleles for a multi-peptide-based subunit framework and their binding detail.

MHC 2 Epitopes	Protein	Position	Antigenicity	IFN gamma	IL-4	IL-10	Alleles	Binding Score
KCYFLHNIWNFIDPK	Lactamase_B domain-containing protein	311-325	0.71	Positive	Inducer	Inducer	HLA-DRB1*04:10	-227.02
CYFLHNIWNFIDPKL	Lactamase_B domain-containing protein	312-326	0.83	Positive	Inducer	Inducer	HLA-DRB1*04:10	-219.88
NVSSYLLQLNHHSYV	Lactamase_B domain-containing protein	390-404	0.97	Positive	Inducer	Inducer	HLA-DRB1*04:01	-264.73
VRILCCGQVGGKPIT	G domain-containing protein	213-227	1.05	Positive	Inducer	Inducer	HLA-DRB1*01:02	-180.22
KILWTVILHLFILNS	Ferrochelatase	65-79	1.03	Positive	Inducer	Inducer	HLA-DRB1*11:04	-205.21

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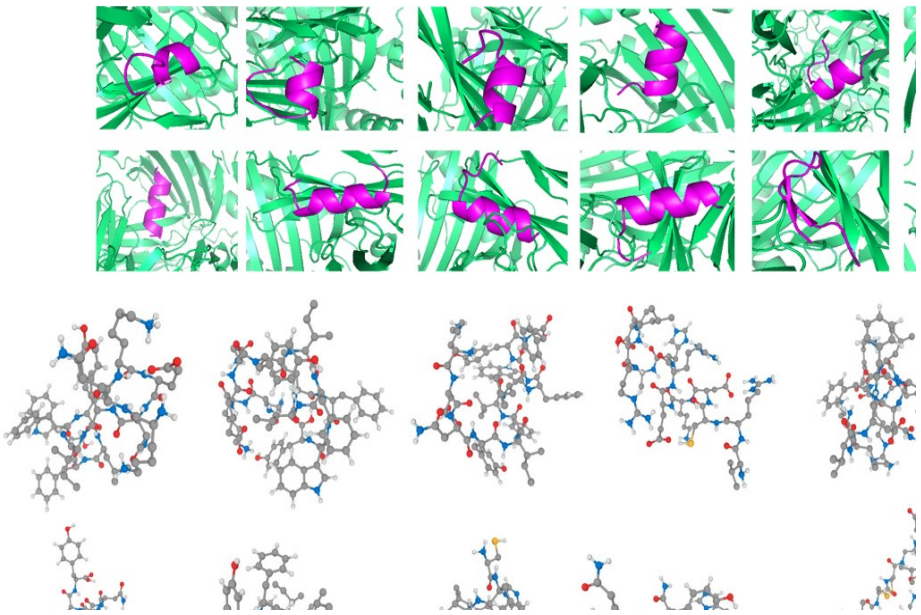

Figure 3: As shown in Table 4 & 5, the 3D binding patterns for the selected 12 epitopes are based on their respective HLA.

Analysis of population coverage:

A population coverage analysis was performed on 7 CTL and 5 HTL finalized epitopes. HLA alleles are varied in terms of ethnic groups and regions of the world. Consequently, they impact the building of the vaccine epitope. The statistical results showed a cumulative distribution of 95.61 % of the world population for our final epitopes since the vaccine will contain both forms of epitopes. The highest coverage in the Europe community, 97.59 percent, has been measured. However, Ethiopia has 35.34% with the lowest population coverage. The coverage is given in Figure 4 below.

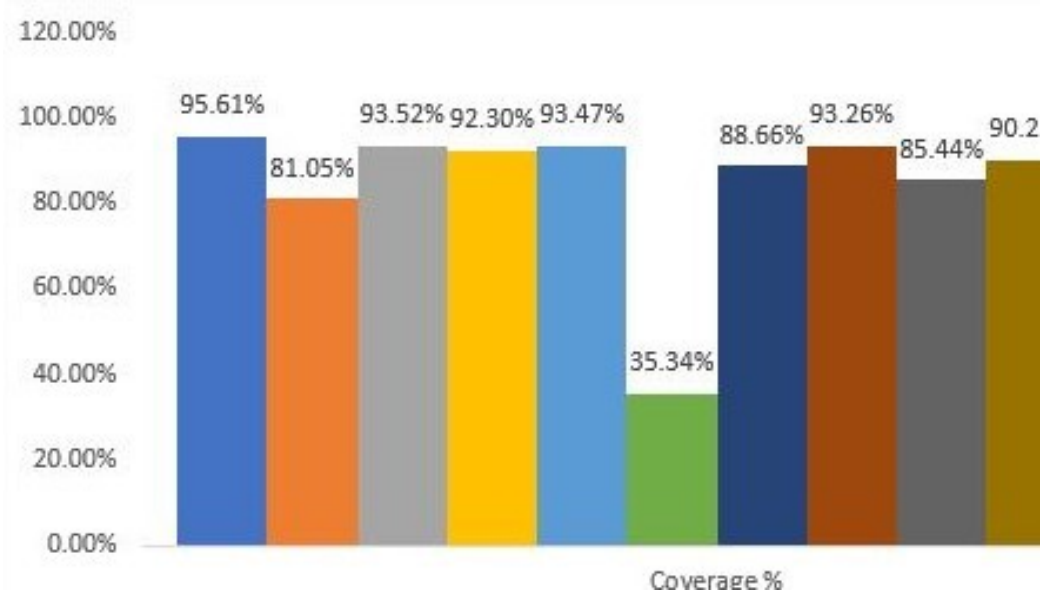

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Figure 4: Multi Epitope vaccine epitopes dependent on their respective HLA alles are collectively coverage in the world population.

Mapped vaccine construct:

AAY linkers linked the selected seven CTL epitopes and the selected five HTL epitopes linked to a GPPG linker, which ultimately connected 7 LBL epitopes to a KK linker. The 124-long residue adjuvant Cholera enterotoxin subunit B (UniProt ID: P01556) has been connected with an EAAK to above CTL. There were 443 residuals of the final vaccine and a map shown in the Figure.

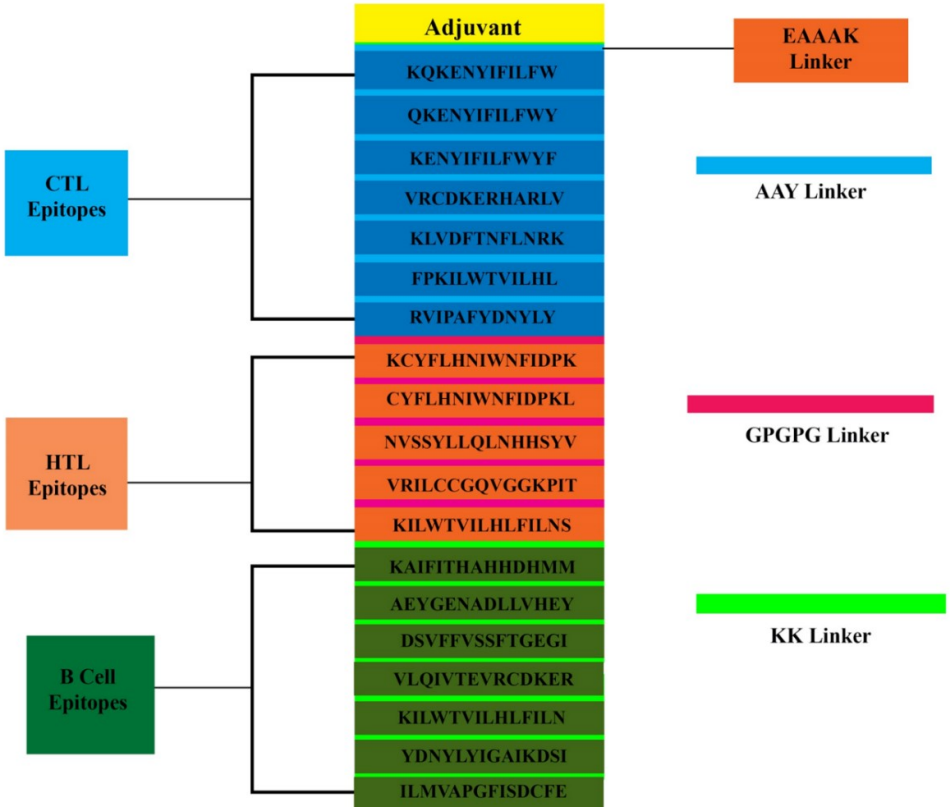

Figure 5: Overview of MEV construct. It contains 443 amino acids which consist of an adjuvant (yellow) connected with EAAAK-cyan at N-terminal MEV. In the HTL epitopes and LBL epitopes with the KK (light green) connector AAY (blue) linker was used to join the GPGPG (pink) linker.

Profiles of physiochemistry and immunogenicity:

First of all, the BLASTp study was alone against Homo-sapiens proteins, and the results showed that our parasite proteome had no resemblance to any human protein. After this, a study of the allergenicity, antigenicity and toxicity of the vaccine build was performed. Results have shown that our vaccine model is strongly antigenic (0.7186 at 0.5 per cent threshold), non-allergenic and non-toxic. First, ProtParam looked at the physicochemical properties of the vaccine construct. The theoretical PI was 9.31 KDa and the molecular weight was 50898.58 KDa. The mean half-life of our vaccine construct was evaluated as 30 hours in vitro, >20hours (yeast, in vivo) and >10hours (E.coli in vivo) the grand average hydropathicity (GRAVY) was estimated -0.003, negative sign in this value is showing the hydrophilic nature of vaccine construct. All these outcomes showed that vaccine builds as a potential contender for vaccine.

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Interpretation of the secondary structure:

PSIPRED was utilized to examine the secondary structure of vaccine. According to the examinations, alpha-helix was developed by 236 residues consisting of 53.27% of the overall sequence, for beta-strand formation 61 residues participated which are 13.76% and for coils, 146 amino acids which constitute 32.95% of the whole vaccine construction participated.

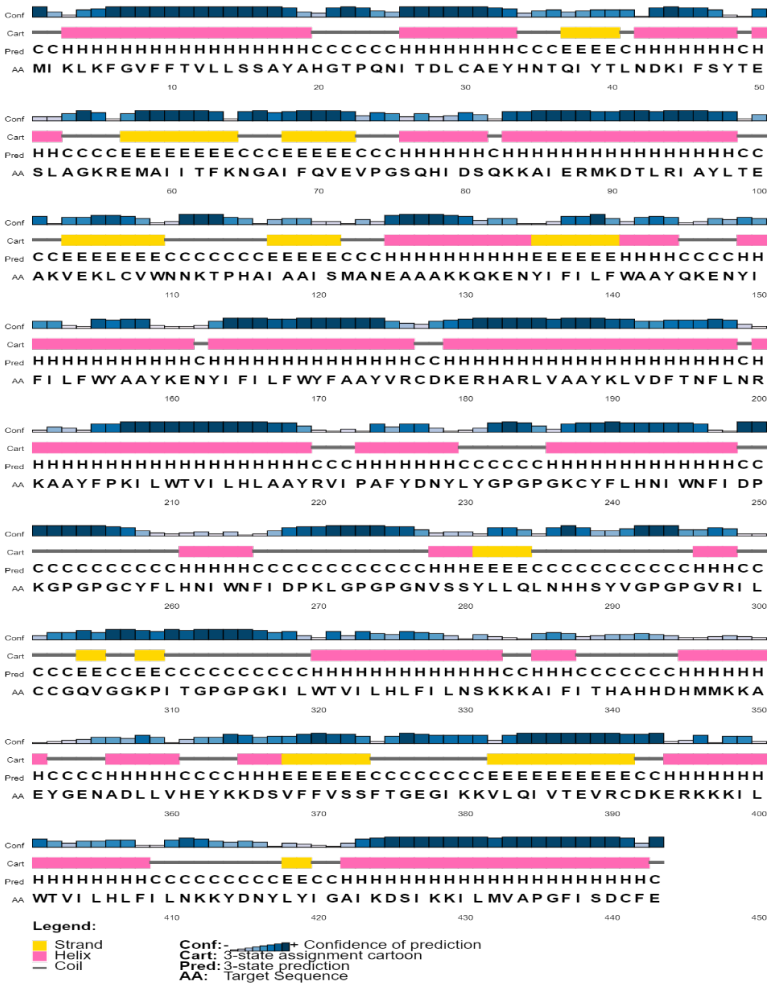

Figure 6: A graphic multi-peptide illustration of secondary structure prediction. Here are marked yellow, pink and blue colors for the β-strands, α-helix and r coils respectively.

Tertiary Structure Prediction, Refinement and Validation:

I Tasser was used to predict the tertiary vaccine structure. Galaxy Refine Z server refined the predicted structure. The enhanced Ramachandran model analysis revealed that 79.7% of the amino acids are in favored regions, 15.7% residues in approved areas and 2.8% in unauthorized regions. A PROSA web-based Z-score is -5.54. In ERRAT quality control study, the refined model score was 68,8095. The high quality of the refined model has shown these consequences.

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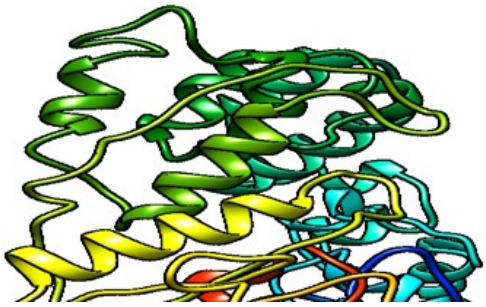
A)

MIKLKFGVFFTVLLSSAYAHGTPQNITDLCAEYHNTQIYTLNDKIFSYTESLAGKREMAI
GSQHIDSQKKAIERMKDTLRIAYLTEAKVEKLCVWNNKTPHAIAAISMANEAAAKKQI
KENYIFILFWYAAYKENYIFILFWYFAAYVRCDKERHARLVAAYKLVDFTNFLNRKAAYFI
RVIPAFYDNYLYGPGPGKCYFLHNIWNFIDPKGPGPGCYFLHNIWNFIDPKLGPGPGN
VGPGPGVRILCCGQVGGKPITGPGPGKILWTVILHLFILNSKKAIFITHAHHDHMMK
EYKKDSVFFVSSFTGEGIKKVLQIVTEVRCDKERKKILWTVILHLFILNKKYDNYLYIGAI
ISDCFE

B)



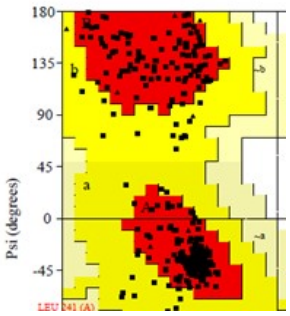


Figure 7: Prediction and validation of the MEV structure in three-dimensional (3D): (a) MEV sequence. The series of epitopes is black. The adjuvant sequence is highlighted by red color, the linker sequence is emphasized in the green color of the EAAAK, the AAY linkers are highlighted in the blue color, and the GPGPG linkers is highlighted as the purple color; (b) the MEV builds a refined 3D structure; (c) the plot analysis for the predicted structure of Ramachandran, where 79.7 per cent of the residues in the favored region are present.

Screening of B Cell epitopes:

Three conforming / discontinuous (Figures 8) and 26 linear / continuous (Table 6) B-cell sequence epitopes were predicted without modifying the prediction parameters in ElliPro and the ABCPred 2.0, respectively (Lund, 2008).

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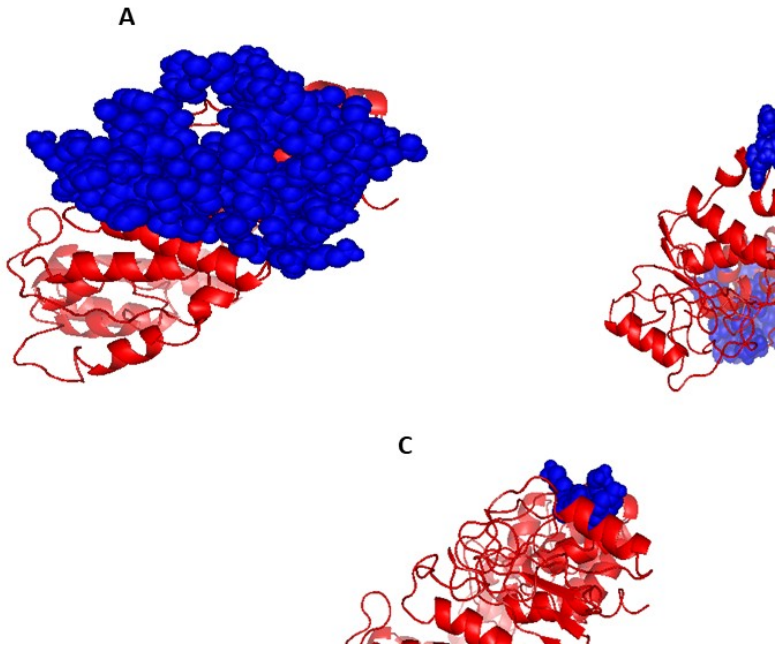

Figure 8: Conformal B-cell epitopes (blue) found in the final MEV vaccine (red): (A) 68 residues (B) 162 residues (C) 4 residues.

Table 6: LBL epitopes in the final form of the MEV vaccine.

LBL Epitopes	Position	Antigenicity
LRIAYLTEAKVEKL	93	0.5543
GPGPGCYFLHNIWN	252	0.7466
GPGVRILCCGQVGG	294	0.8397
YFLHNIWNFIDPKL	258	0.7706
KENYIFILFWYAAY	146	1.5086
FILNSKKKAIFITH	327	0.9749
TVILHLFILNSKKK	321	0.6887
PKILWTVILHLAAY	206	0.8201
EVPGSQHIDSQKKA	72	0.6600
NGAIFQVEVPGSQH	65	1.2055
WYAAYKENYIFILF	155	0.8933
CDKERHARLVAAYK	177	1.2389
LLSSAYAHGTPQNI	13	0.9360
HHSYVGPGPGVRIL	287	0.5867
LCCGQVGGKPITGP	300	0.8119
AFYDNYLYGPGPGK	224	0.8750
HDHMMKKAEYGENA	343	1.0682
KPITGPGPGKILWT	308	0.6826
IFILFWYFAAYVRC	164	2.3443
KAEYGENADLLVHE	349	1.2130
WYFAAYVRCDKERH	169	1.5518
FILFWAAYQKENYI	137	0.7548
KKKAIFITHAHHDH	332	1.0212
AKKQKENYIFILFW	128	1.2749

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SVFFVSSFTGEGIK	367	1.3026
HEYKKDSVFFVSSF	361	1.0870
IKLKFGVFFTVLLS	2	0.9557

Disulfide engineering of vaccine:
Disulfide by design v2.12 for the stabilization of the modeled structure of the final vaccine building was used to conduct the disulphide engineering. In disulfide engineering a total of 54 residue pairs may be used. However, only the remaining two pairs of residues, as their value fell below the acceptable range, namely less than 2.2 kcal / mol and angles of β3 should be between 87 and +97 ° ALP at angles + 96.66 and 1.65 kcal / mol, ASP with an angle of angles +88.95 and energy 1.87 kcal / mol and GLY having angular, were concluded after the assessment of more parameters, such as energy score and X3 angle.

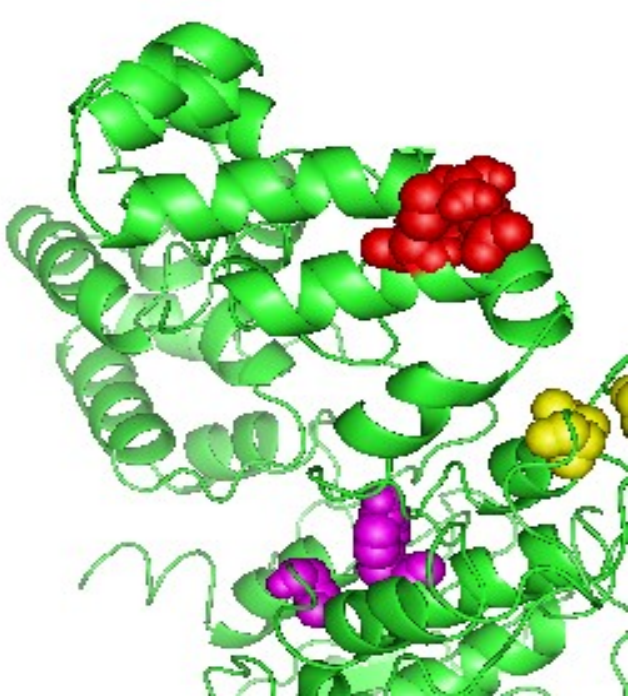

Figure 9: Displaying the 3 remaining pairs in the set. MEV is green with red, purple and yellow sets. The MEV is in green color and pairs are in red, purple and yellow.

Docking between vaccine protein and TLR4 receptor:
In order to trigger an immune response, proper interaction between the immune receptor molecules and the antigen molecule is required. The vaccine is then docked with TLR4 human immune receptor using the HADDOCK v. 2.2 server. After bacterial recognition, TLR4 can effectively produce an immune response. The docking study showed significant vaccine-TLR4 interactions. A score of −-86.9 + /- 5,7 kcal / mol binding on TLR4 was provided. The docking statistics are shown in the following table.

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Table 7: Top of the TLR4-MEV cluster docking statistics.

Parameters	Values
HADDOCK score	-86.9 +/- 5.7
Cluster size	21
RMSD from the overall lowest-energy structure	1.8 +/- 0.1
Van der Waals energy	-112.5 +/- 6.0
Electrostatic energy	-278.8 +/- 4.1
Desolvation energy	-76.3 +/- 14.3
Restraints violation energy	1576.3 +/- 95.47
Buried Surface Area	3491.4 +/- 49.3
Z-Score	-1.4

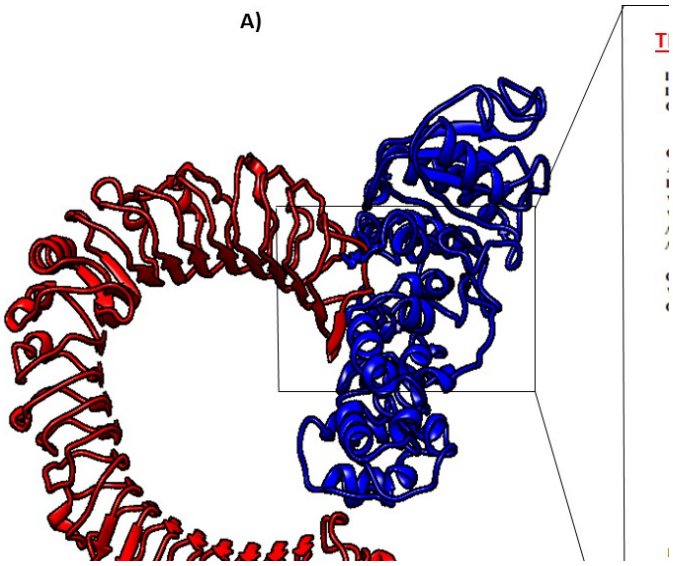

Figure 10: MEV construct docking with human TLR4

Molecular Dynamics simulation:

The (NMA) for stabilization and large protein mobility was conducted. Based on the internal coordinates of the docking complex, this assessment was carried out by the server iMODS. Deformability depends on the distortion, described by chain hinges, of each residue. (Figure 12). The eigenvalue found for the complex was 3.415964e-06 (Figure 12 (a)). The variance correlated with each normal mode was inverted to the eigenvalue (Kovacs, Chacón, & Abagyan, 2004). B-factor values were proportionate to RMS produced by normal mode analysis NMS (Figure 12 (c)). The pairing of residues pairs was shown in the covariance matrix with separate pairs of connected, anti-correlation or uncorrelated motion represented by the colors red, blue and white (Figure 12(d)). Also, the result was an elastic network model characterizing the atom pairs connected by fountains. The diagram shows each dot between the corresponding atom pairs, which is colorful in the level of rigidity. The darker the greys, the springs were stiffer.

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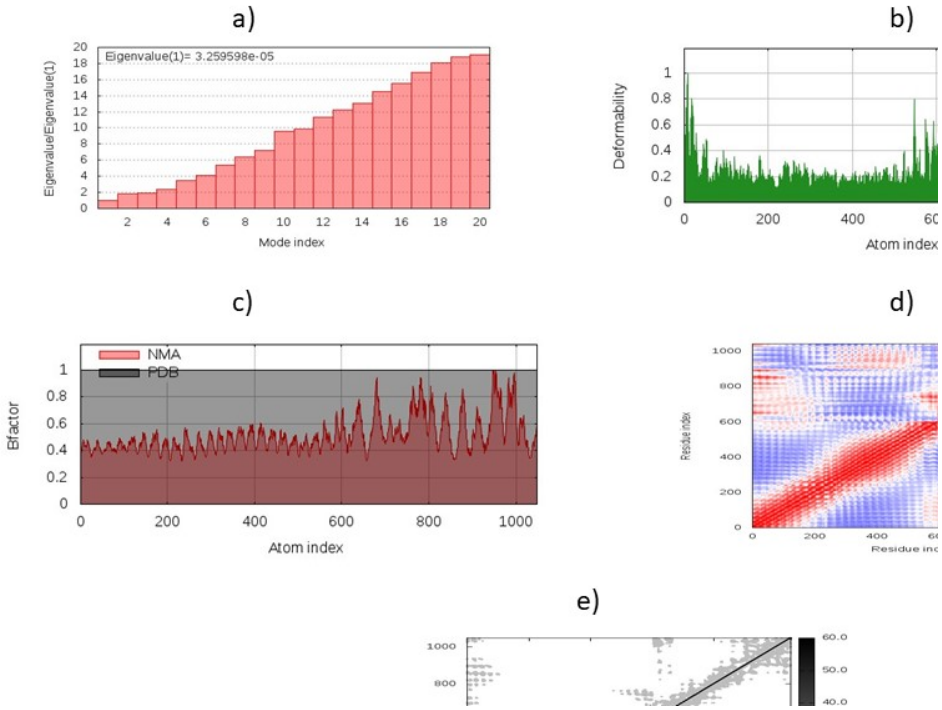

Figure 11: Simulation of vaccine-TLR4 composite molecular dynamics with showing (a) eigenvalue value; (b) deformability; (c) its B-factor; (d) its matrix covariance; (e)elastic network analysis.

Immune simulation studies:

Both secondary and primary immune reactions appear to substantially contribute to the pathogen and likely to the main immune reaction. The antigen reaction of the in-silico host is demonstrated. The primary response was described as high IgG + IgG and IgM concentrations followed by Immunoglobulins M, Immunoglobulins1 + ImmunoglobulinsG2 and ImunoglobulinsG1 at both secondary and primary stages with concomitant antigen reduction. Furthermore, there have also been significant interleukin and cytokine responses. All this indicates the efficient immune reaction and clearance after good vaccine interactions

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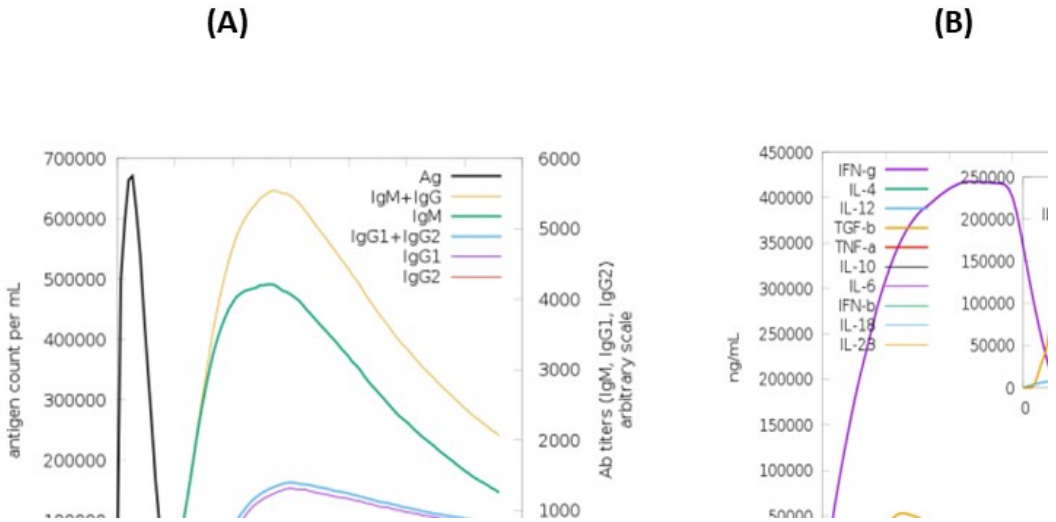

Figure 12: Using multi peptide vaccine showing immune response as an antigen: (a) generation of immunoglobulins and B-cell isotypes following exposure to antigen (B) production of cytokines and interleukins in different states with Simpson index.

In silico cloning between vaccine & E. coli host:

Cloning in silicon was done to ensure the expression of *D. medinensis* vaccine derived from the commonly used vaccine E. coli host. Second, vaccine-constructed codons have been modified as per codon utilization of E. Expression system of coli. The JCAT server was used to optimize the E-based vaccine codons. Coli (K12 train). The optimized vaccine design contained an ideal range of GC content of 50.79 per cent (30 per cent −70 per cent) and CAI value of 0.9 (0.8−1.0) and demonstrated a high potential for positive protein expression and reliability.

Conclusion:

It is really important to eliminate the disease. Economic, socializing or education-related illnesses are very risky. It is not just people but the whole of an ecosystem that have an impact. Food supply is disrupted by diseases, education is disrupted, and the nation's health and sources are compromised. One of the most important and effective methods of pathogens prevention worldwide is vaccination. In the research, subunit vaccines were used in comparison to other pathogen-derived vaccines. The immune reaction can be better and greater since sub-unit vaccines consist of multiple immunogenic parts of pathogens. In the research, subunit vaccines were used in comparison to other pathogen-derived vaccines. Since subunit vaccines consist of different immunogenic sections of pathogens, the immune response can be safer and stronger. Multi-epitope vaccines eliminate unnecessary components compared with conventional vaccines, which can either lead to harmful or adverse effects immune responses (Zhang, 2018). The more complex way of improving the functioning of the immune system and avoiding infectious disorders today is the vaccine. However, the expensive production and delivery of live or attenuated vaccines can take years to produce. In addition to the fact that a large antigenic strain is put in the attenuated vaccine, the disease tends to interfere with allergic reactions (Li et al., 2014). Multi-epitope vaccines

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are used to delete worthless elements that can have abnormal or adverse effects compared to conventional vaccines (Zhang, 2018). Improved protection, cost efficient efficacy, possibility to evolve a similar epitope for greater strength and scope, and ability to concentrate immune responses on retained epitope are the positive benefits of multi-epitope vaccines (Shey et al., 2019). Researchers have worked for many years to minimize costs, times and side effects of development of the vaccine. Many approaches are currently available on a voluntary basis for designing and developing safe, trained epitope based vaccines for new generations (María et al., 2017; Seib et al, 2012). Researchers have used immunology techniques to include advanced Ebola virus, Hepatitis C virus, Oropouche virus, Dengue virus, multi-epitope-driven vaccine models (Adhikari et al., 2018; Ali et al., 2017; Dash et al., 2017; Fernald & Clyde, 1970; Ikram et al., 2018; Jensen, Senterfit, Chanock, Smith, & Purcell, 1965; Smith et al., 1967; Zhang, 2018). We concentrated on integrating B cell epitopes because of its role in antibody development and in efficiency mediation (MacLennan et al., 2008). But antigens can easily resolve the mood of memory B cell, over time, while lifelong immunity precedes cell-mediated immunity (T-cell immunity) (Bacchetta et al., 2005). CTL limits pathogen transmission through the recognition and destruction of infected cells and the secretion of special antibacterial cytokines (Garcia et al., 1999). Consequently, the build of vaccines predicts B and T cell epitopes. Based on their antigenicity, allergenicity, immunogenicity and toxicity, vaccine candidates were selected from the HTL, CTL, and B- cell epitopes. T-cells like interferon gamma (IFN-β), interleukin 4 (IL-4) and interleukin 10 (IL-10), which can help to resolve pro-inflammatory response and thereby reduce tissue damage, may be supported. T cells help to support B-lymphocytes, cytotoxic T cells and others by the innate immune system. T cells help to protect B-lymphocytes, cytotoxic T cells and others through the innate immune system. Cytokine (i.e., IFN-μ, IL-4 and IL-10) were also assessed for the selection of candidates. After the CTL, HTL or B-cell epitopes were joined, with AAY, GPGPG and KK linkers, the vaccine was completed. Linkers are used to increase expression, folding and stabilization as an important factor in the production of vaccine proteins (Shamriz et al., 2016). When used alone, Multiepitope-based vaccines are weakly immunogenic and require coupling with adjuvants (Meza et al., 2017). Adjuvants are additives added to vaccine formulations that have an effect on specific immune reactions to antigens, their design, stability and durability and protect against infection (Lee & Nguyen, 2015). They also get great attention because the immune response to humoral and cell-mediated immune reactions can be controlled exclusively (Bonam et al., 2017). Although the vaccine size tends to be long as a peptide vaccine, there have been some studies where the size of the vaccine is much longer (Chatterjee et al., 2018; Kalita et al., 2019; Rahmani et al., 2019). Therefore, we conclude that it would not be a concern in terms of continuity and understanding. When we tested the vaccine construct, we found that the non-adjuvant structure showed less antigenicity with the help of a Vaxijen server than the adjuvant structure which clearly shows that the vaccine adjuvant is essential for the chimera. Our vaccine applicant's molecular weight is ~50898.58 kDa, a typical molecular vaccine weight. Solubility of the already-expressed recombinant protein is one of the main aspects of the host in many biochemical and functional studies (Khatoon et al., 2017). The vaccine protein produced has been found to be soluble which ensures easy access to the host. The vaccine's fundamental essence is shown by its numerical value of PI. Furthermore, the predicted instability index suggests that the protein stays stable after expression and thus the capacity to be used is further increased. Hydrophilicity and thermostat show the GRAVY score and the aliphatic index. 3D structure modelling needs a complete understanding of how the protein elements, the complexities, ligand interactions and other proteins are arranged 3D. After extraction, the vaccine generates the positive properties. The Ramachandran plot indicates that most residues in the preferred and permitted regions (79.7% and 15%) with very little residue are found in the

disadvantaged region and this indicates that the overall quality of the model is satisfactory. The good quality of our constructed vaccine construction is also demonstrated by the values of the GDT-HA, RMSD, MolProbity, Clash Score and Poor Rotamers. Different structure authentication methods were used to recognize errors in the model design of the vaccine. The Z-score (-5,54) and ERRAT (68,8095 percent) consistency factor showed that the overall refined vaccine structure was satisfactory. To achieve mass coverage, the T-cell epitope can bind with multiple HLA alleles. Therefore, we have taken CTL and HTL epitopes with their appropriate HLA methods to quantify the worldwide distribution of alleles. The results show that in many regions of the world, the selected epitopes and their alleles are the most advanced. The population of Europe was 97.59 percent, and when combined, these epitopes and their proper HLA sites cover 95.61 percent of the world's population. Data-driven protein-receptor docking analysis and molecular dynamics simulation were performed to determine a possible immune association and stability between TLR4 and the vaccine protein, taking into account the use of a TLR4 agonist as an adjuvant in the chimera being built. Energy minimization was performed to reduce the potential energy of the entire system for the full stabilization of the docked vaccine protein-TLR4 complex in conformity. By replacing individual protein atoms, the energy minimizes unsuitable structural geometry, thus making the structure more stable with sufficient stereochemistry. Test of immunoreactivity through serological evaluation is one of the first steps in justifying a candidate vaccine (Gori et al., 2013). Recombinant protein expression is required within a stable host E. Coli. Expression systems for the recombinant protein production are developed (Chen, 2012; Rosano & Ceccarelli, 2014). To ensure that our recombinant vaccine protein in E has a high level of expression. Coli, optimization of codon has been carried out. Both the index (0.9) and GC (50.79) of adaptability were favorable for high-level protein expression in bacteria. Improving protein stability is essential in various biomedical and mechanical applications. In this research, the multi-epitope vaccine has been bridged with disulfide to improve protein thermostability, modify its functional characteristics and support the analysis of the genetic elements. Fact proves traditional immune responses were shown by immune simulation. After repeated antigen exposure, the general increase in the immune answer occurred. Memory was evident with respect to the production for months of B-cells and T-cells. All of the consequences were adequate and stable structural and functional chemistry.

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