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### Original Article

## A Novel Chimeric Antigen as a Vaccine Candidate against *Leishmania major*: In silico Analysis

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### **Abstract**

**Background:** *Leishmania* is a mandatory intracellular pathogen and causing neglected disease. Hence, protection against leishmaniasis by a development vaccine is an important subject. This study aimed to design a poly-epitope vaccine for cutaneous leishmaniasis.

**Methods:** The present study was conducted in the Parasitology Department of Tarbiat Modares University, Tehran, Iran during 2017-2019. Several bioinformatics methods at online servers were used for prediction of different aspects of poly-epitope, including, physico-chemical attributes, allergenicity, antigenicity, secondary and tertiary structures, B-cell, T-cell and MHC (I, II) potential epitopes of LACK, LEIF, GP63 and SMT antigens of *L. major*.

**Results:** After designing the construct (GLSL), the outputs of PTM sites demonstrated that the poly-epitope had 57 potential sites for phosphorylation. Furthermore, the secondary of GLSL structure includes 59.42%, 20.94% and 19.63% for random coil, extended strand and alpha-helix, respectively. The GLSL is an immunogenic protein with an acceptable antigenicity (0.8410) and non-allergen. Afterward, 20 potential epitopes of LACK, LEIF, GP63 and SMT antigens were linked by a flexible linker (SAPGTP), then was synthesized, and sub-cloned in pLEXY-neo2. The results were confirmed the expression of 38.7 kDa poly-epitope in secretory and cytosolic sites, separately.

**Conclusion:** A good expression in the *L. tarentulae* and confirmation of the GLSL poly-epitope could be a basis for developing a vaccine candidate against leishmaniasis that should be confirmed via experimental tests in BALB/c mice.

### Introduction



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**L***eishmania* is as an obligate intracellular parasite within mammalian hosts that replicates in macrophages and spread by sand flies (1). Approximately 350 million people in 98 countries are exposed to the risk of this infection. Despite several drugs such as antimonials compounds, toxic drug side-effects and drug resistance has led to suggest designing a vaccine to prevent the disease (2).

Accordingly, different vaccination methods have been evaluated, such as live virulent form of vaccine (leishmanization), killed parasites, live attenuated of the *Leishmania* parasites, dendritic cells based vaccines (3), recombinant proteins to polyproteins, DNA based vaccines (4) and salivary antigen-based vaccines (5). It is urgent need to find out the appropriate polypeptide vaccine candidates against leishmaniasis using several gene groups.

The current study was conducted to use some immunoinformatics approaches for analyzing the structure, phosphorylation, physicochemical parameters, cellular site, immunogenicity, position of transmembrane, and transmembrane domains of some genes to introduce a novel chimeric antigen as a vaccine candidate against *L. major*.

## Materials and Methods

The present study was conducted in the Parasitology Department of Tarbiat Modares University, Tehran, Iran during 2017-2019.

### Sequence retrieval

The complete amino acid sequence of Glycoprotein 63 (ACL01096.2), *Leishmania* activated protein kinase c receptor (AAB88300.1), *Leishmania* eukaryotic initiation factor (XP\_001681281.1) and sterol 24-c-methyltransferase (XP\_001686815.1) was obtained from a publicly available sequence database, (NCBI) at FASTA format for future analysis.

### Epitopes prediction

The epitopes from the GP63, LACK, LeIF and SMT of *L. major* genes were identified by the Immune Epitope Data Base (IEDB) and analysis resource, which has a high affinity for epitope mapping of B-cell and T-cell.

### B-cell

The continuous (linear) and discontinuous (conformational) of B-cell epitopes was predicted by BCPREDS server (6). Features of all predicted B cell epitope have length of 14 amino acids. Moreover using physicochemical, the linear B-cell epitopes were predicted based algorithm Bcepred an online server ([http://crdd.osdd.net/raghava/bcepred/bcepred\\_submission.html](http://crdd.osdd.net/raghava/bcepred/bcepred_submission.html)).

This server can predict epitopes with 58.7% precision, using polarity, hydrophilicity (7), surface accessibility (8) and flexibility (9), combined at a threshold of 2.38 (6, 10). Mean-time, ABCpred server (6, 11) was used. This server can predict epitopes by usage of recurrent neural network with 65.93% accuracy. Moreover, we used the IEDB, at <http://tools.iedb.org/bcell/> to predict the Bepired linear epitope (12), beta-turn (13) and antigenicity (14), hydrophilicity (7), surface accessibility (8) and flexibility (9).

### MHC-I and MHC-II binding epitopes

The half maximum inhibitory concentration (IC50) of attachable peptides to the major histocompatibility complex (MHC) class II and class I were analyzed by IEDB online service. All alleles were selected for MHC source species.

### Prediction of cytotoxic T lymphocyte (CTL) epitopes

The CTLpred online server (8, 15) was employed for prediction the CTL epitopes.

### Final construct of GLSL poly-epitope

GP63, LACK, SMT, Leif genes of *L. major* have abundance epitopes. For each gene, one epitope for CTL and MHCI, two epitopes of MHCII and one for B cell were chosen. The epitopes were joined by SAPGTP linker and finally a construct (GLSL) with 38.7 kDa molecular weight was used for further analysis.

### ***Secondary and tertiary structure prediction***

The protein secondary structure forecasting was done by using Garnier-Osguthorpe-Robson (GOR) secondary structure prediction online service (16). The disulfide bonds were prediction by DiNNNA online service (15, 17). In addition, SWISSMODEL was used for designing the three-dimensional models of the GLSL construct, by using online server (18, 19), Solvent accessibility of different residues was anticipated via SCRATCH online server (15).

### ***Tertiary structure refinement***

The best model (made by SWISS-MODEL) was selected with ModRefiner (20). It can describe the initial models closer to their native state in periods of the diverse properties such as side-chain positioning, backbone topology, hydrogen bonds, etc. with considerable improvement in the physical quality of local structures. (21).

### ***Tertiary structure validation***

The confirmation of tertiary structure was assessed by the production of Ramachandran plot using RAMPAGE online (22).

### ***Prediction of physico-chemical parameters of GLSL***

Several physico-chemical parameters of poly-epitope including, molecular weight (MW), number of amino acids, total number of positive and negative charged residues, theoretical isoelectric point (pI), estimated in vitro and in vivo half-life, extinction coefficients, instability index, grand average of hydropathicity (GRAVY) and aliphatic index

were predicted using the Expasy ProtParam online server (23).

### ***Post-translational modifications (PTMs) sites of poly-epitope***

Phosphorylation of poly-epitope was computed via NetPhos 3.1 Server. CSS-Palm Online Service was used for prediction of GLSL acylation (20).

### ***Evaluation of allergenicity and antigenicity and solubility***

SOLpro server (24) was predicted solubility of construct. The antigenicity of final poly-epitope was evaluated by ANTIGENpro (25) and VaxiJen v.2.0 (26). It described that antigenicity of protein exclusively based on the physicochemical properties of amino acid properties. The accuracy of this server varies from 70% to 89% that varies according to different types of organisms (26). Auto cross-covariance (ACC) transformation of protein sequence into similar vectors of main amino acid properties is the bases of allergen prediction. SOLpro server can predict solubility of the poly-epitope (24).

Furthermore, AlgPred server (27) was used for allergenicity prediction. We applied different approaches for investigation of poly-epitope, such as, the crossbred approach (SVMc+IgE epitope +ARPs BLAST+MAST), which had 85% accuracy at a threshold -0.4 (27).

### ***Prediction of mRNA secondary structure***

The mfold tool predicted the RNA secondary structure and computed the free energy associated with the 5' end in the mRNA of the construct gene (28).

### ***Expression of poly-epitope in *Leishmania tarentolae****

#### ***Cloning of GLSL poly-epitope***

After designing the GLSL poly-epitope, the target sequence was synthesized and cloned in PEGFP- N1 (Cloning Sites: Sall- AgeI) via the Gene synthesis. The GLSL poly-epitope was

sub-cloned in pLEXY-neo2, in order to express in eukaryotic expression system. The target expressing system has two sites for cytosolic (NotI and BglII enzymes) and secretory (NotI and SalI enzymes) expression position. For this purpose, the recombinant plasmid GLSL was digested at secretory and cytosolic sites, NotI, SalI and NotI, BglII enzymes, respectively. After enzymatic digestion, digested fragment were purified by gel purification Genet bio kit (cat. No. K-8000). Eventually, the DNA extracted from the recombinant plasmid GLSL were ligated into pLEXY-neo2 by T4-ligase enzyme.

#### **Transfection of GLSL constructs into *L. tarentolae***

Promastigotes of *L. tarentolae* (Tar II (ATCC 30143) strain) in logarithmic phase ( $3.5 \times 10^7$ ) were suspended in 350  $\mu$ l of electroporation buffer (21 mM HEPES, 137 mM NaCl, 5 mM KCl, 0.7 mM Na<sub>2</sub>HPO<sub>4</sub>, 6 mM glucose; pH 7.5) and mixed with 10  $\mu$ g purified linear (gel extraction kit, cat. No. K-8000) pLEXY-GLSL via SmaI restriction enzyme (Fermentas, USA). Digested fragment electrophoresed by Bio-Rad Gene Pulser Ecell, (Germany) at 450 V and 500  $\mu$ F in electroporation cuvette. The transfected promastigotes were recovered in 5ml of RPMI-20% FCS antibiotic free medium and incubated at 26 °C for 24 hours. Then the recombinant promastigotes were screened with adding 5  $\mu$ g/ml of Geneticine (G418) (Sigma, USA).

#### **RNA extraction and Reverse - Transcription PCR**

Total RNA's promastigote of *L. tarentolae* was extracted with the Sina Clon RNX Plus kit (cat. No. RN7713C) based on manufacturer's instructions. Before synthesis of the cDNA, the concentration of extracted RNA was discerned via UV absorbance (NanoDrop™ 2000, Thermo Scientific). The

extracted total RNA, was used for cDNA synthesis via ROJE kit (cat. No. EB983028-S). The cDNA was amplified by universal forward (CCGACTGCAACAAGGTGTAG) and reverse (CATCTATAGA-GAAGTACACGTAAAAG) primers. RT-PCR products were evaluated by electrophoresis on a 1.2% agarose gel.

#### **Western blot analysis**

One ml of cultured of wild type and transfected *L. tarentolae*- GLSL (secretory and cytosolic samples) were centrifuged at 3000 rpm for 12 minutes. The pellets were washed in PBS and were mixed by 5X SDS-PAGE sample buffer. The samples were incubated at 90 °C for 5 min. Finally, specimens were loaded on SDS-PAGE (5% stacking and 12.5% separating gels). Eventually transferred to 0.2  $\mu$ m immune-Blot™ polyvinylidene difluoride membranes (Cat No: 162-017777; Bio-Rad Laboratories, CA, USA) and western blotting was done based on the standard procedure (29).

#### **Ethical approval**

Approval of ethics application with appropriate experimental protocols was taken from Medical Ethic Committee of Tarbiat Modares University, Tehran, Iran.

## **Results**

#### **Gene specifications and physico-chemical properties of poly-epitope construct**

Selected epitopes based on the CTLpred<sup>a</sup> online server (Epitope CTL), IEDB online service (MHC I and MHC II alleles)<sup>b,c</sup> and Bcepred<sup>d</sup> (Bcell Epitopes) sites then functional epitope were chosen (Table 1). The epitopes were linked together via SAPGTP linker and eventually the final construct was obtained.

**Table 1:** Selected epitopes from antigens of *L. major* after analysis

Variable	Epitope	Peptide Rank	Start Position	Length	Score
CTL	HSNYVSTVT <sup>1</sup>	3	193	9	1
	VHDAMQARV <sup>2</sup>	2	49	9	1
	TVTLKAENN <sup>3</sup>	3	325	9	1
	PNDEYHRTI <sup>4</sup>	1	216	9	1
MHCI	IAFSPNRFWMCVAT <sup>1</sup>	0.75	241	14	-
	LSTVSNAFEGGGYI <sup>2</sup>	0.13	543	14	-
	HDVLMATMLNGAAI <sup>3</sup>	0.22	142	14	-
	GDYSSLQGLRSTPI <sup>4</sup>	0.44	280	14	-
MHCII	MCVATERSLSVYDLE <sup>1</sup>	2.56	250	15	-
	LESKAVIAELTPDGA <sup>1</sup>	5.32	263	15	-
	RRRCVAARLVRLAAA <sup>2</sup>	1.80	10	15	-
	RCVHDAMQARVRQSV <sup>2</sup>	5.85	47	15	-
	EHLKAVEIMKLRHLV <sup>3</sup>	6.8	176	15	-
	LNGAAIMDAALLLIA <sup>3</sup>	21.6	150	15	-
	IFTPSFYIRARKPSK <sup>4</sup>	24	337	15	-
	PKGTYKATEVLEEEAA <sup>4</sup>	18.6	312	15	-
B cell	EHPIVVSGSWDNTI <sup>1</sup>	-	163	14	0.851
	AHTAAAADPRPGSA <sup>2</sup>	-	84	14	0.999
	KSKDGGKSTAKRVQK <sup>3</sup>	-	392	14	1
	VGCGVGGPARNIVR <sup>4</sup>	-	109	14	1

<sup>1</sup>LACK, <sup>2</sup>GP63, <sup>3</sup>LEIF, and <sup>4</sup>SMT antigens

The poly-epitope contained 382 amino acids with the MW of 38.7 kDa, theoretical pI=9.44. The total number of positively charged residues (Arg+Lys) were 33 and the number of negatively (Asp + Glu) were determined 24. The bio half-life was computed 3.5 h (mammalian reticulocytes, in vitro), >10 h (*Escherichia coli*, in vivo) and 10 min (yeast, in vivo). The extinction coefficients of this poly-epitope in water at 280 nm with units of M<sup>-1</sup> cm<sup>-1</sup> were

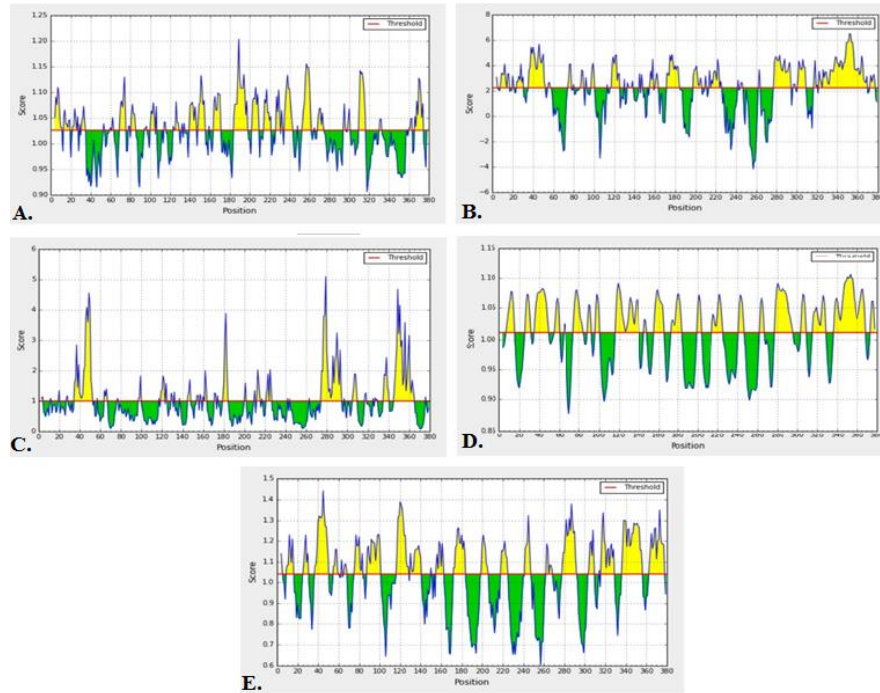
21680. The aliphatic index and instability index (II) were estimated to be 68.61 and 32.92, respectively. Moreover, Grand average of hydropathicity (GRAVY) was computed -0.201. Bcepred server online predicted the continuous B-cell epitopes such as, flexibility, hydrophilicity, exposed surface, antigenic propensity, accessibility and polarity are arranged in Table 2.

**Table 2:** Prediction of Epitopes in poly-epitope protein by 6 parameters based on Bcepred server

Prediction parameter	Epitope sequence
Hydrophilicity	KAENNSAPGTPPNDEYHRT; APGTPGDYSS; ESAPGTP; TPDGASAPG; SAPGTPE; ARKPSKAPGTPPKGT; SAPGTPE; ADPRPGSA; PGTPKSKDGGK-STAKR; QKSAPGT
Flexibility	VTLKAEN; CVATERS; ASAPGTPRR; FYIRARKPSKSAPGTPP; AAADPRPG-SASAPGTPKSKDGGKSTAKRVQ
Accessibility	TLKAENNSAPGTPPNDEYHRTIS; GTPLESKA; APGTPRRRCV; MQARVRQS; GTPEHLKA; FYIRARKPSKSAPGTPPKGTYKATEV; PGTPEHP; ADPRPG-SASAPGTPKSKDGGKSTAKRVQKSAPGTP
Antigenic Propensity	SNYVSTVTS; SLSVYDLE; GTPRCVHD; MKLRHLVS; PEHPIVVSGSW; TPVGCGVGG
Exposed Surface	PNDEYHR; RARKPSKSAP; PPKGTYK; GTPKSKDGGKSTAKRVQKSA
Polarity	PNDEYHRTIS; GTPEHLKAVEIMKLRHLVS; FYIRARKPSKSA; PKSKDGGK-STAKRVQKSA

The accuracy and threshold of this site were 58.7% and 2.38, respectively. By <http://tools.iedb.org/bcell/> online service, the thresholds (mean score) of GLSL were

evaluated, in order, surface accessibility (2.00), turns (1.04), hydrophilicity (2.234), flexibility (1.01), and antigenicity (1.026) (Fig. 1).

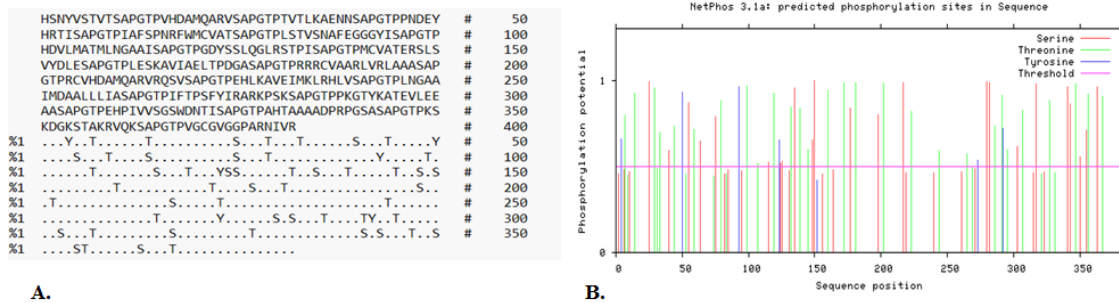


**Fig. 1:** Trend scale plots of GLSL protein. A. Antigenicity; B. Hydrophilicity; C. Surface accessibility; D. Flexibility; E. Beta-turn. Horizontal red line showed the average score or the threshold. Green color (under the threshold) show the inadequate zones related to the attributes of interest. Yellow colors (above the threshold) demonstrate desirable regions related to the properties of interest

### Bioinformatics analysis of the phosphorylation and acylation

NetPhos 3.1 Server analyzed the phosphorylation and displayed phosphorylation sites

(Ser: 23, Thr: 28, Tyr: 6). CSS-Palm Online Service was employed for prediction of GLSL acylation but no acylation point was found (Fig. 2).

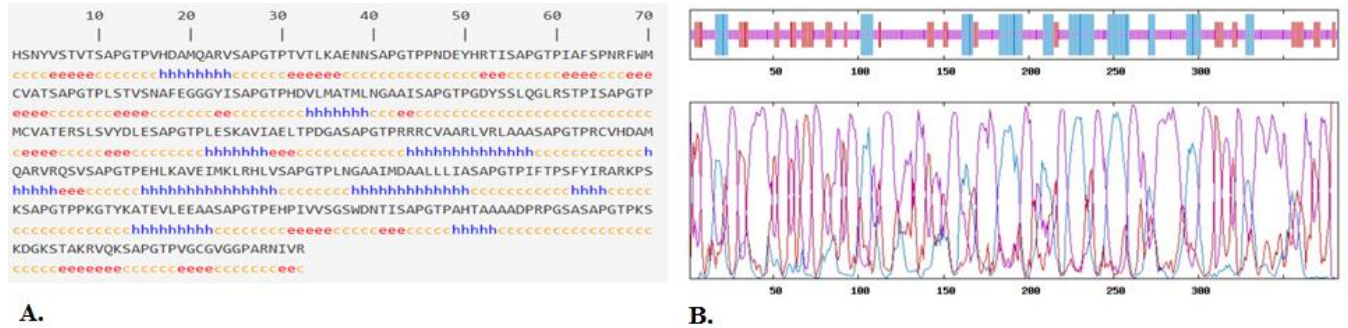


**Fig. 2:** Bioinformatics analysis of the phosphorylation sites of poly-epitope. A. Prediction of the residues with a score above the threshold are demonstrated by 'T', 'S' or 'Y'; Non-phosphorylated of the residue has a score below the threshold or the residue is not, Thr, Ser and Tyr that is signed via a dot ('.'). B. Predicted phosphorylation sites in Poly-epitope sequence

**Secondary and tertiary structure prediction**

The GOR IV online service was determined 382 amino acids in sequence length and predicted secondary structure of poly-epitope in-

cluding random coil (59.42%), extended strand (20.94%) and alpha-helix (19.63%) (Fig. 3).



**Fig. 3:** A. Predicted secondary structure via GOR IV online service. e=extended strand, h=helix, c=coil; B. Prediction of graphical results for secondary structure of GLSL by GOR IV

SCRATCH server predicted distribution of solvent availability regions via polarity and hydrophobic aspects of residual patterns. The outcomes revealed solvent availability features of poly-epitope was acceptable. In prediction of 3D structure of poly-epitope, three 3D models were constructed, that one of them

with the greatest sequence identity (45.16%) was selected. The results of SWISS-MODEL including, local quality estimate, universal quality estimate, model pattern alignment, sequence identity and the 3D model constructed for poly- epitope are presented in Fig. 4.



**Fig. 4:** Output of SWISS-MODEL. A. Model-template alignment; B. Sequence identity and coverage; C. Global quality estimate; D. Comparison with non-redundant set of PDB structures. E; Local quality estimate; F; The 3D model constructed for GLSL

The results of disulfide bonds prediction with the DiNNNA indicated 5 cysteines in our sequence at 71 - 205 and 142-186 posi-

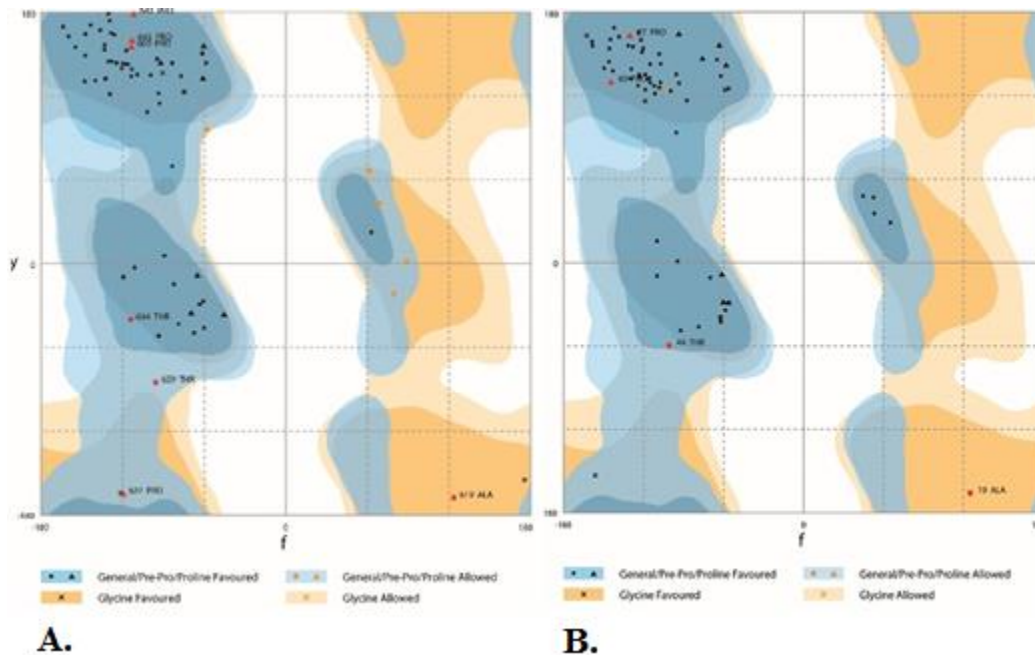
tions to form the disulfide bond. The more details data indicated in the Table 3.

**Table 3:** Predicted disulfide bonds

<i>Cysteine sequence position</i>	<i>Distance</i>	<i>Bond</i>	<i>Score</i>
71 – 142	71	NRFWMCVATSA-PGTPMCVATER	0.01417
71 – 186	115	NRFWMCVATSA-TPRRRCVAARL	0.01101
71 – 205	134	NRFWMCVATSA-PGTPRCVHDAM	0.01288
71 – 371	300	NRFWMCVATSA-GTPVGCYVGGP	0.01095
142 – 186	44	PGTPMCVATER-TPRRRCVAARL	0.34855
142 – 205	63	PGTPMCVATER-PGTPRCVHDAM	0.01914
142 – 371	229	PGTPMCVATER-GTPVGCYVGGP	0.01078
186 – 205	19	TPRRRCVAARL-PGTPRCVHDAM	0.01408
186 – 371	185	TPRRRCVAARL-GTPVGCYVGGP	0.01135
205 – 371	166	PGTPRCVHDAM-GTPVGCYVGGP	0.01053
Weighted matching			
71 – 205		NRFWMCVATSA-PGTPRCVHDAM	
142 – 186		PGTPMCVATER-TPRRRCVAARL	

**Tertiary structure validation (Refinement)**

The Ramachandran plot before and after the tertiary structure of poly-epitope was validated and the results of them are shown in Fig. 5.



**Fig. 5:** Validation of the three-dimensional structure of GLSL by usage of Ramachandran plot. A. initial model (before refinement) of Ramachandran plot statistics showed 83.3% amino acid residues in the favored regions, whilst in allowed and outlier regions of plot are 6.9% and 9.7%, respectively. B. The RAMPAGE results of after refinement were changed: 1.4% in allowed regions, 5.6% in outlier regions and 93.1% of residues in favored regions

**Assessment of allergenicity and antigenicity and solubility**

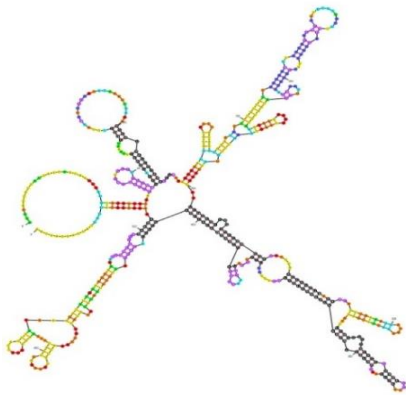
Antigenicity of poly-epitope protein that were analyzed by ANTIGENpro was

0.930975 and 0.8410 by VaxiJen v. 2.0 (Threshold for this model: 0.5). SOLpro server was computed 0.938054 of the protein solubility.



### Prediction of mRNA secondary structure

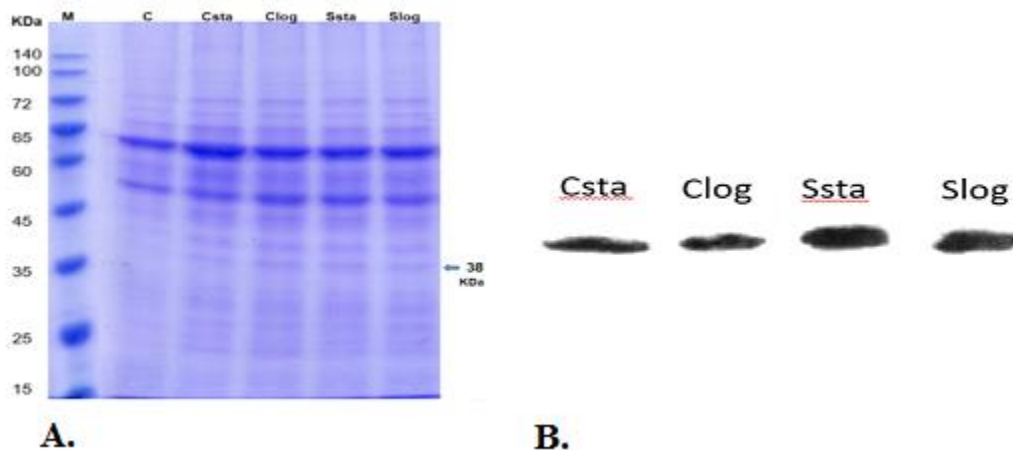
By usage of the mFold tool, the minimum free energy (MFE) for secondary structures composed via RNA molecules was assessed.  $\Delta G$  was evaluated and showed that the almost good-predicted structure had -166 kcal/mol and was observed that, there was no stable hairpin or pseudoknot in the first nucleotides at the 5' end (Fig. 6).



**Fig. 6:** The predicted of RNA secondary structure for GLSL poly-epitope (by usage of mFold tool) has not any hairpin and pseudoknot at 5' site of mRNA

### Expression of GLSL-pLEXY-neo2 in *L. tarentolae*

After transfection of GLSL-pLEXY-neo2 into promastigotes of *L. tarentolae*, expression of GLSL protein was assayed and confirmed via RT-PCR and western blot methods. The supplementary information of them is determined in Fig. 7. The 1146 bp band and 38.7 KDa were confirmed the expression of GLSL-pLEXY-neo2 in promastigotes of *L. tarentolae* as secretory and cytosolic protein.



**Fig. 7:** A. SDS-PAGE analysis outcome of the expression of synthesized GLSL sequence that sub-cloned in pLEXY-neo2 and expressed in *L. tarentolae*. Lane1: Protein molecular weight marker (10-140 kDa); Lane 2: Control (*L. tarentolae* secretory specimen), Lanes 3, 4, 5 and 6: Stationary phase of cytosolic specimen, Logarithmic phase of cytosolic specimen, Stationary phase of secretory specimen and Logarithmic phase of secretory specimen, respectively. B. Western blot analysis

### Discussion

Bioinformatics procedures for the analysis of protein have become an essential instrument for vaccine researches. The mentioned

methods are economical and effectual and can decreased experimental expenditure, considerably (6, 30). The poly-epitope vaccines provide an anticipant choice for the control of various pathogens (31). In this study, the first chosen

gene was LEIF. It is a part of the DEAD proteins and is highly conserved in the *Leishmania* genus which is a natural adjuvant for inducing Th1 and producing protective cytokines such as IL-12 and IFN- $\gamma$  in mononuclear cells of patients with leishmaniasis (32). LEIF antigen stimulates the cytokine effects of IL-12 and reduces IL-4 secretion in cell culture of BALB/c mice lymph nodes infected with *Leishmania major* (33). This antigen as a part of the fusion recombinant protein, has been reported significantly reduces the number of parasites and lesions size in mice and primates (34). Another gene used in this study was the enzyme sterol 24-c-methyltransferase (SMT). It is needed for the biosynthesis of ergosterol. Ergosterol is the main membrane sterol in *Leishmania* parasites. SMT and ergosterol are not exist in mammals so this enzyme can be a target for *Leishmania* vaccine and drugs. SMT is available in another *Leishmania* species and the amino acid sequence's in many species is extremely conserved (35). The combination of SMT with MPL-SE (monophosphoryl lipid A) led to Th1 immune responses to IFN- $\gamma$  and IL-2 production and decreased the parasitic load in the liver and spleen of vaccinated mice (35). Next gene, glycoprotein 63 (GP63) or leishmanoyisin is another target gene that is an ectoenzyme. GP63 induces IFN- $\gamma$  signaling in cutaneous leishmaniasis (36).

Using CpG-ODN as adjuvant in the gp63-based vaccine against susceptible BALB/c mice with VL and CL has been effective. In addition, Glycoprotein 63 has an innate potency to stimulate protective immunity (37). The fourth gene selected was LACK. It has different functions, such as signal transduction, DNA replication, RNA synthesis and cell cycle control in the eukaryotes that expressed in promastigotes and amastigotes. Vaccination by LACK (DNA or protein vaccine) be able to produce the protective response against CL due to shifting from Th2 cytokine responses to Th1. The main reason for this efficient response is the induction of IFN- $\gamma$  production by interleukin 12 (38). These antigens were

evaluated for CTL, T-cell (MHCI and MHCII), and B cell epitopes and the best rank or score selected for final construct as GLSL poly-epitope with 20 different epitopes which linked by SAPGTP. Finally, the various bioinformatics approaches were applied for analyzing the diverse aspects of GLSL poly-epitope. GLSL poly-epitope has 382 amino acids and molecular weight 38.7 KDa.

The GRAVY and aliphatic index of GLSL-poly-epitope sequence were estimated -0.201 and 68.61, respectively. The negative value of GRAVY demonstrates hydrophilicity of protein for better interaction with environs water molecules. The high aliphatic index indicated the stability of the protein in a wide range of temperatures. The PTMs have an important duty in cellular control mechanisms (33). Our results indicated that there are 57 potential PTM sites for phosphorylation on the sequence. These points offers that which are likely to regulate some of the functions and activity of the protein, such as the ability of proteins to remain inbound within a cell because the negative phosphorylated location disallows their permeability through the cellular membrane (34). The secondary structure of the poly-epitope was investigated via GOR IV method. The results of the constructional aspects of the poly-epitope included random coil (59.42%), extended strand (20.94%) and alpha-helix (19.63%). Alpha-helix and beta-turn which have a high hydrogen-bond energy are placed in the interior section of the protein and conserve the structure of a protein and thus create a potent interplay with antibodies (39). Analysis of secondary structure has a fundamental task in refinement of tertiary structure via merging specific restraints beta turn and alpha helix (40). Prediction of tertiary structures is the definitive purpose of forecasting a protein's frame. Consequently, it is very significant to comprehend the structures of proteins and the junction among functions and structures (19, 35).

Antigenicity is one of an important attributes of protein that enables detection it via immune system (28). The obtain results from allergenicity and antigenicity appraisalment GLSL poly-epitope showed that the protein was a potent immunogenic and non-allergen. Protein expression is the most main aspects of poly-epitope design in the hosts, so the proteins with a low expression have not enough efficiency (36). In this study, negative MFEs of -166 kcal/mol showed expression of the synthesized GLSL gene in the *L. tarentulae*.

## Conclusion

Our results were confirmed the expression of 38.7 kDa poly-epitope in secretory and cytosolic sites, separately. A good expression in the *L. tarentulae* and confirmation of the GLSL poly-epitope could be a basis for developing a vaccine candidate against leishmaniasis that should be confirmed via experimental tests in BALB/c mice.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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