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IN SILICO CHARACTERISATION AND COMPUTATIONAL MODELLING OF NEURO-TOXINS OF INDIAN COBRA

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ABSTRACT: Snake venom is cocktail of various enzymes, toxins. Snake venom components have major role as therapeutics application in current drug industry. Snake venom toxins plays vital role as one of the major component in case of bite and most of the toxins are lethal which are of various types such as cardio-toxins, myotoxins and neurotoxins etc. The Indian Cobra neurotoxins sequences were retrieved form Swissprot Database. The ten neurotoxins were characterised and three were modelled using *in silico* approach whose structure were not available on Protein Data Bank and protein model portal. The neurotoxins were modelled using homology modelling approach and energy minimisation was carried out for all the three neurotoxins. The stearic hindrance was removed using chiron server. The Ramchandran plot was used to validate the modelled structure which provides idea of the modelled structure conformation and configuration and it was satisfactory. Further the modelled structures will be used to understand docking with the suitable receptor.

INTRODUCTION: Snake venom is complex mixtures of proteins and low molecular weight compounds like peptide, nucleoside & metal ions. The exact number of compounds venom contains is still not known [1].

The Indian Cobra (Naja Naja) mainly contains a powerful post-synaptic neurotoxin and cardiotoxin. Cobras have both a short and long neurotoxin as well as a cardiotoxin. These snake neurotoxins act on the neuromuscular junction and block neuromuscular transmission.

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Cobra toxin interferes with this process by binding to Acetylcholinesterase (AChE) and to the Acetylcholine receptors on the muscle cells on synaptic gaps of the nerves there by paralyzing muscles and in case of severe bites leading to respiratory failure or cardiac arrest [2]. It is also known since long time that toxicity of cobra venom can withstand boiling which reflects the heat stability of various components present in the venom.

Thus, venom contains substance designed to affect the vital process such as the function of nerves and muscles, the action of heart, circulation of blood and the permeability of the membrane [3]. Cobra venom contains a large number of pharma-cologically active substances with a specific mode of action [4]. Such compounds can be of great value in the investigation and may be useful as potent or a novel approach of therapeutics.

Toxins are studied to better understand how inhibitors work. Specifically, they have helped us gain a greater understanding of muscle and nerve function.

MATERIALS AND METHODS:

1) **Neurotoxin Protein Sequences:** Neurotoxin protein sequences from Indian Cobra were retrieved from the manually curated public protein database Swiss-Prot. The search result yielded 10 Neurotoxin protein sequences which were Q9PTT0, P25668, P25669, P25671, P25672, P25673 P29179, P29180, P29181 and P29182.

   Structure was available for P25669, P25671, P29179, P29180, P29181, P29182 and Q9PTT0 rest three neurotoxins viz. P25668, P25672, P25673 were computationally modelled.

2) **Computational Tool and Servers:** The physico-chemical parameters, the amino acid compositions, percentages of hydrophobic and hydrophilic residues were calculated from the primary structure analysis results and are tabulated in table 1. Theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, half-life, instability index, aliphatic index and grand average hydrophathy (GRAVY) was computed using the Expasy's ProtParam (http://us.expasy.org/tools/protparam.html) prediction server. The SOPMA server was used for the secondary structure prediction shown in table 2 [5]. The TMHMM server [6] was used for the identification of transmembrane regions. The presence of disulphide bridges (SS bonds) in Neurotoxin protein sequence P25671, P29180, Q9PTT0, P29179, P29181, P29182, P25668, P25669, P25672 and P25673 is predicted by the tool CYS_REC [7]. CYS_REC identifies the position of cysteines, total number of cysteines present and it also predicts the most probable SS bond pattern of pairs in the protein sequence shown in table 3. The 3D structure of 3 Neurotoxin proteins P25668, P25672 and P25673 were generated by homology modelling using Swiss workbench server. The energy minimisation of modelled neurotoxins was done using Chiron server [8]. The modelled 3D structures were evaluated using the online servers Rampage server [9] and are shown in **figure 1**. The software UCSF Chimera was used to visualize the modelled 3D structures of three neurotoxins, P25668, P25672 and P25673 Neurotoxins were shown in **figures 2, 3 and 4** respectively. The hydrophobicity and atomic configuration of P25668, P25672 and P25673 were shown in **figure 5, 6 and 7** respectively.

RESULT AND DISCUSSION: The physiochemical parameters are shown in **Table 1**. The average molecular weight of neurotoxins was found to be 7824.4. The computed pI value of all the neurotoxins P25671, P29180, Q9PTT0, P29179, P29181, P29182, P25668, P25669, P25672 and P25673 was found pI >7 which indicates that these neurotoxin proteins are basic in nature. The computed isoelectric point (pI) is extremely useful for developing buffer systems for purification by isoelectric focusing method.

TABLE 1: PHYSICOCHEMICAL CHARACTERISATION OF NEUROTOXINS

Swissprot ID	Length	M.W	AI	II	+R	-R	GRAVY	pI	Extinction coefficient
P25671	71	7833.0	53.52	22.25	10	8	-0.287	8.11	7615
P29180	65	7567.9	68.92	21.43	14	6	-0.534	9.13	5095
Q9PTT0	83	9262.4	65.66	48.72	9	8	-0.510	7.51	10470
P29179	62	6943.2	73.87	35.02	9	4	-0.111	8.75	3605
P29181	65	7636.9	55.54	34.64	15	7	-0.692	9.17	3605
P29182	65	7580.8	55.54	31.12	14	7	-0.668	9.03	3605
P25668	71	7847.0	52.11	22.66	10	8	-0.327	8.11	7615
P25669	71	7820.9	46.62	28.08	10	8	-0.401	8.11	7615
P25672	71	7889.0	52.11	17.79	10	9	-0.365	7.64	7615
P25673	71	7862.9	46.62	28.57	10	9	-0.439	7.64	7615

TABLE 2: SECONDARY CHARACTERIZATION OF THE NEUROTOXINS

Accession number	P25673		P25672		P25669		P25668		+P29181		P29179		Q9PTT0		P29180		P25671		P29182	
Characteristics	No. of a.a.	%distribution	No. of a.a.	%distribution	No. of a.a.	%distribution	No. of a.a.	%distribution	No of a.a.	%distribution	No. of a.a.	%distribution	No. of a.a.	%distribution	No. of a.a.	%distribution	No. of a.a.	%distribution	No. of a.a.	%distribution
Alpha helix (Hh)	2	2.82%	1	1.41%	6	8.45%	0	0.00%	0	0.00%	0	0.00%	10	12.05%	0	0.00%	0	0.00%	0	0%
3_{10} helix (Gg)	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0%
Pi helix (Ii)	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0%
Beta bridge (Bb) :	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0%
Extended strand (Ee)	21	29.58 %	17	23.94%	18	25.35%	20	28.17%	17	26.15 %	21	33.87%	27	32.53%	18	27.69 %	19	26.76 %	18	27.69 %
Beta tum (Tt)	6	8.45%	2	2.82%	4	5.63%	2	2.82%	4	6.15%	2	3.23%	4	4.82%	3	4.62 %	2	2.82%	3	4.62%
Bend region (Ss)	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00 %	0	0.00%	0	0%
Random coil (Cc)	42	59.15 %	51	71.83%	43	60.56%	49	69.01%	44	67.69 %	39	62.90%	42	50.60%	44	67.69 %	50	70.42 %	44	67.69 %
Ambiguous states (?)	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00 %	0	0.00%	0	0%

Although Expasy's ProtParam computes the extinction coefficient for a range of (276, 278, 279, 280 and 282 nm) wavelength, 280 nm is favoured because aromatic amino acids like tryptophan and tyrosine present in protein show maximum absorption at 280nm and other amino acids do not show such maximum absorption at 280nm. Extinction coefficient of neurotoxin protein at 280 nm was ranging from 3605 to 10470 M^{-1} cm^{-1} with respect to the concentration of Cys, Trp and Tyr.

The high extinction coefficient of Q9PTT0 indicates presence of high concentration of Cys, Trp and Tyr. The computed protein concentration and extinction coefficients help in the quantitative study of protein–protein and protein–ligand interactions in solution. The aliphatic index (AI) which is defined as the relative volume of a protein occupied by aliphatic side chains (A, V, I and L) is regarded as a positive factor for the increase of thermal stability of globular proteins. The high aliphatic index of P29179, P29180 and Q9PTT0 neurotoxin infers that this neurotoxin may be stable for a wide range of temperature.

The lower aliphatic index of P25669 and P25673 is indicative of a more flexible structure when compared to other neurotoxins. Grand Average hydropathy (GRAVY) Index of Neurotoxin protein are ranging from –0·1 to –0·6. The very low GRAVY index of Neurotoxin protein P29181 and P29182 infers that these neurotoxins could result in a better interaction with water. On the basis of instability index Expasy's ProtParam classifies the Q9PTT0 neurotoxin as unstable (Instability index > 40) and other neurotoxin as stable (Instability index < 40).

The results in Table 2 tell about secondary structure of the neurotoxins. Secondary characterization was done by using SOPMA. The neurotoxin P25673 composed of 2.82% of alpha helix, 29.58% of extended sheet, 8.45% of βturn and 59.15% of random coil. The neurotoxin P25672 composed of 1.41% of alpha helix, 23.94% of extended sheet, 2.82% of βturn and 71.83% of random coil. The neurotoxin P25669 composed of 8.45 % of alpha helix, 25.35 % of extended sheet, 5.63 % of β turn and 60.56 % of random coil. The neurotoxin P25668 composed of 28.17 % of extended sheet, 2.82 % of β turn and 69.01% of random coil. The neurotoxin P29181 composed of 26.15 % of extended sheet, 6.15 % of βturn and 67.69 % of random coil.

The neurotoxin P29179 composed of 33.87 % of extended sheet, 3.23 % of βturn and 62.90% of random coil. The neurotoxin Q9PTT0 composed of 12.05 % of alpha helix, 32.53 % of extended sheet, 4.82 % of β turn and 50.60 % of random coil. The neurotoxin P29180 composed of 27.69 % of extended sheet, 4.62% of β turn and 67.69 % of random coil. The neurotoxin P25671 composed of 26.76 % of extended sheet, 2.82 % of βturn and 70.42 % of random coil. The neurotoxin P29182 composed of 27.69% extended sheet, 4.62 % of β turn and 67.69% of random coil.

From **table 3**, it can be concluded that the cysteine residue of neurotoxin was found using cysteine recognition server. Ten cysteine residues were found in P25671, P25668, P25669, P25672 and P25673 and the disulphide bond is between 14-41, 26-30, 45-56, and 57-62. Ten cysteine residues were found in P29180 and the disulphide bond is between 3-42, 6-17, 11-24, 46-57 and 46-57.

TABLE 3: RESULTS OF CYSTEINE RECOGNITION ONLINE TOOL FOR NEUROTOXINS

Query Protein ID	No. of Cys residues	Position	Probable pattern pairs
P25671	10	3, 14, 20 26, 30, 41, 45, 56, 57, 62	14-41, 26-30, 45-56, 57-62,
P29180	10	3, 6, 11, 17, 24, 42, 46, 57, 58, 63	3-42, 6-17, 11-24, 46-57, 58-63
Q9PTT0	9	15, 24, 38, 45, 62, 64, 75, 76, 81	24-45, 38-62, 64-75, 76-81
P29179	10	3, 6, 11, 17, 24, 40 44, 54, 55, 60	3-40, 6-11, 17-24, 44-54, 55-60
P29181	10	3, 6, 11, 17, 24, 42, 46, 57, 58, 63	3-24, 6-17, 11-42, 46-57, 58-63
P25668	10	3, 14, 20 26, 30, 41, 45, 56, 57, 62	14-41, 26-30, 45-56, 57-62
P25669	10	3, 14, 20, 26, 30, 41, 45, 56, 57, 62	14-41, 26-30, 45-56, 57-62
P29182	10	3, 6, 11, 17, 24, 42, 46, 57, 58, 63	3-11, 6-24, 17-42, 46-57, 58-63
P25672	10	3, 14, 20, 26, 30, 41, 45, 56, 57, 62	14-41, 26-30, 45-56, 57-62
P25673	10	3, 14, 20 26, 30, 41, 45, 56, 57, 62	14-41, 26-30, 45-56, 57-62

P29181 also showed presence of 10 cysteine residues and the disulphide bond is between 3-24, 6-17, 11-42, 46-57 and 58-63.Ten cysteine residues were found in P29179 and the disulphide bond is found between 3-40, 6-11, 17-24, 44-54 and 55-60.Ten cysteine residues were found in P29182 and the disulphide bond is found 3-11, 6-24, 17-42, 46-57, 58-63. Nine cysteine residues were in Q9PTT0 and the disulphide bonds were found between 24-45, 38-62, 64-75 and 76-81.Transmemebrane regions were absent in all neurotoxins hence not shown.

The neurotoxins were visualised using UCSF chimera software. The energy minimisations of neurotoxins were carried out using online Chiron server which also removes clashes in the atoms and also helps in structure refinement.

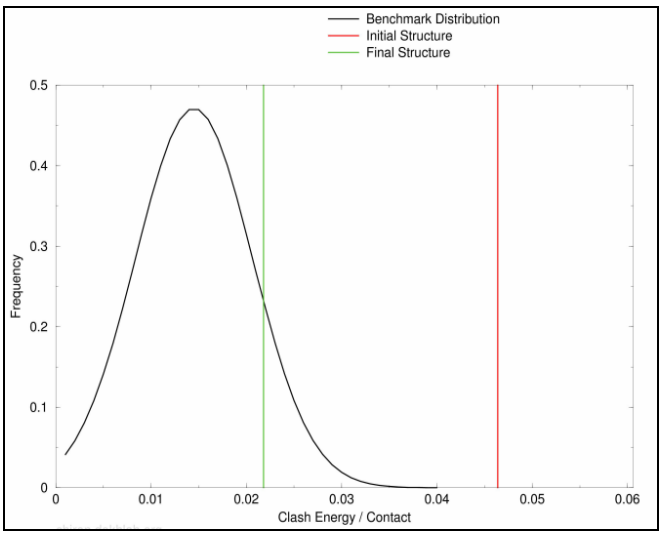


FIGURE 1: (A) P25668

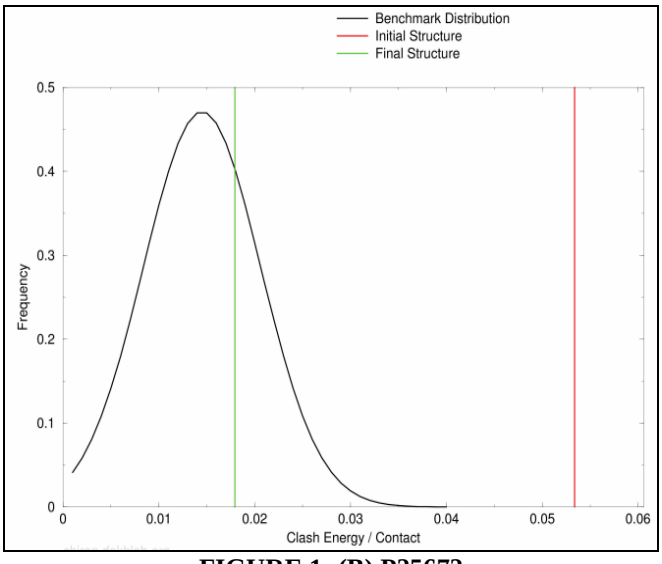


FIGURE 1: (B) P25672

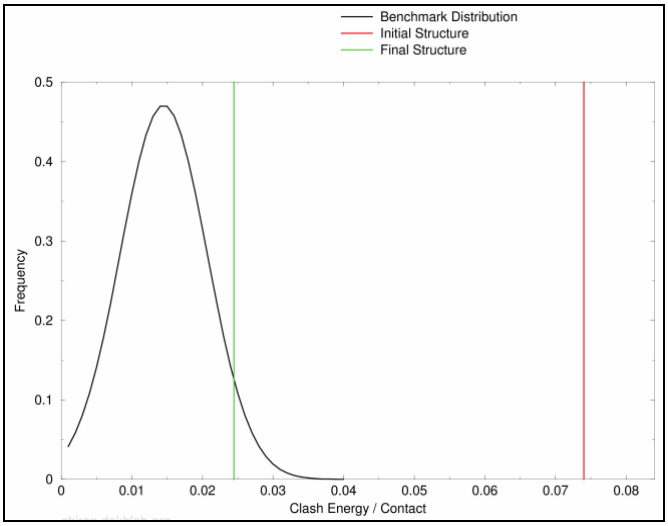


FIGURE 1: (C) P25673

FIGURES 1: (A), (B), (C) SHOWING INITIAL STATE OF THE STRUCTURE AND REFINED STATE AS FINAL STATE OF STRUCTURE IN TERMS OF ENERGY FOR NEUROTOXIN USING CHIRON

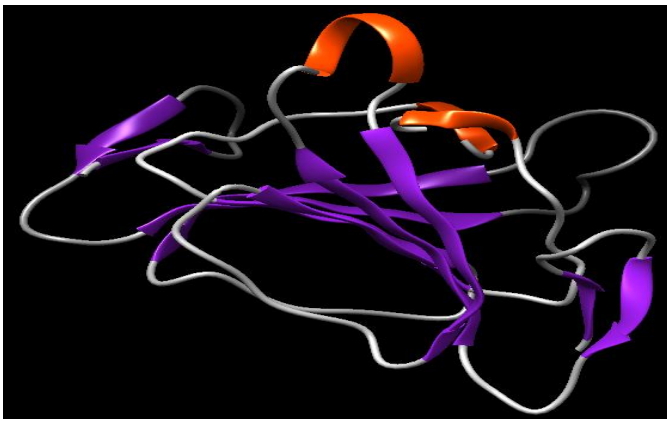


FIGURE 2: MODELLED STRUCTURE OF P25668 SHOWN IN UCSF CHIMERA SOFTWARE-SECONDARY STRUCTURE AS COIL IN GREY AND STRAND IN PURPLE COLOR

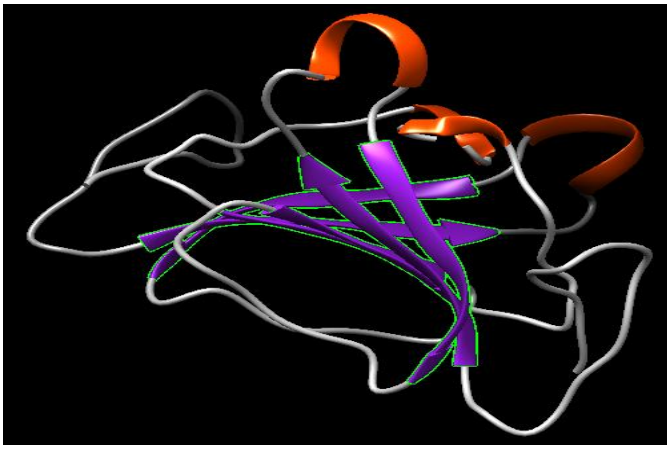


FIGURE 3: MODELLED STRUCTURE OF P25672 SHOWN IN UCSF CHIMERA SOFTWARE-SECONDARY STRUCTURE AS COIL IN GREY AND STRAND IN PURPLE COLOR

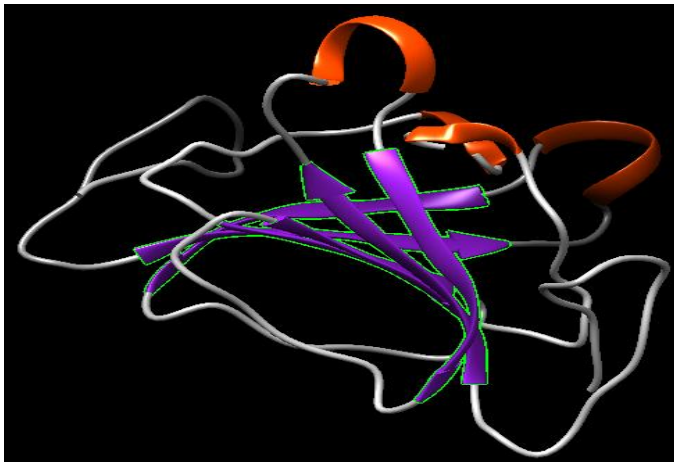
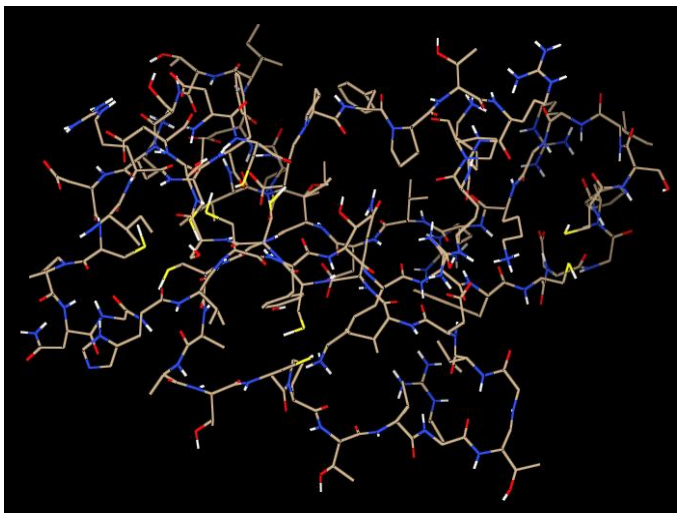
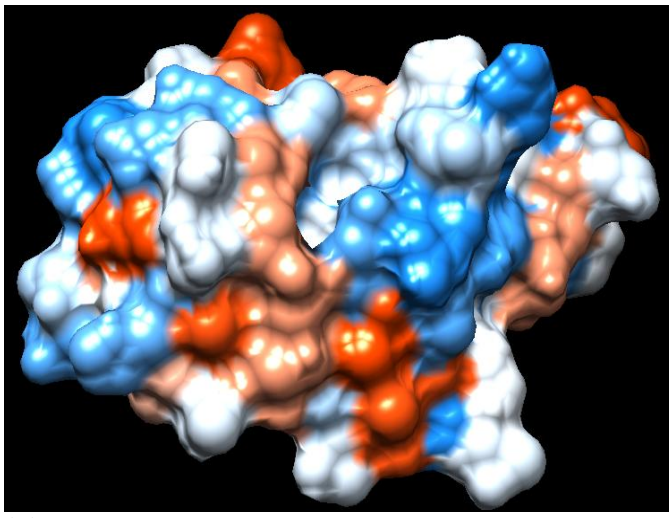


FIGURE 4: MODELLED STRUCTURE OF P25673 SHOWN IN UCSF CHIMERA SOFTWARE- SECONDARY STRUCTURE AS COIL IN GREY AND STRAND IN PURPLE COLOR

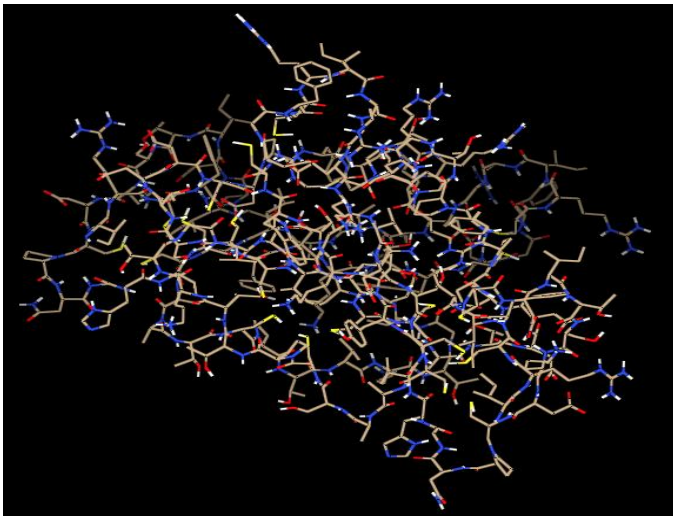


(A) STRUCTURE SHOWN IN ATOMIC CONFIGURATION

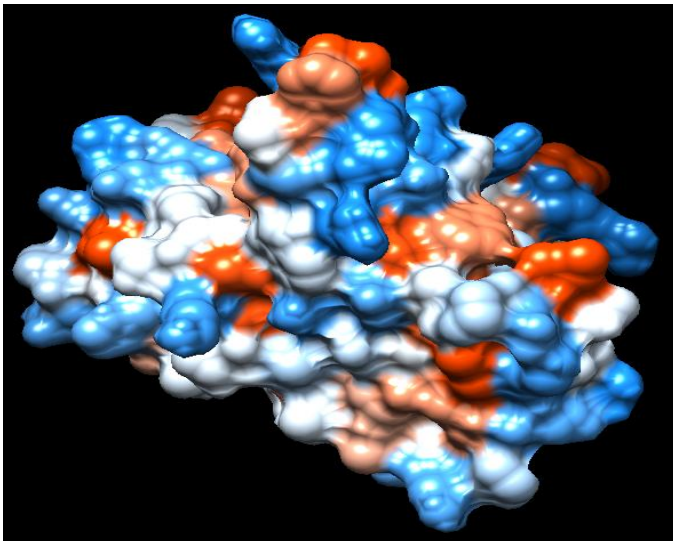


(B) HYDROPHOBIC SURFACE

FIGURE 5: P25668, STRUCTURE IN ATOMIC CONFIGURATION AND HYDROPHOBIC SURFACE

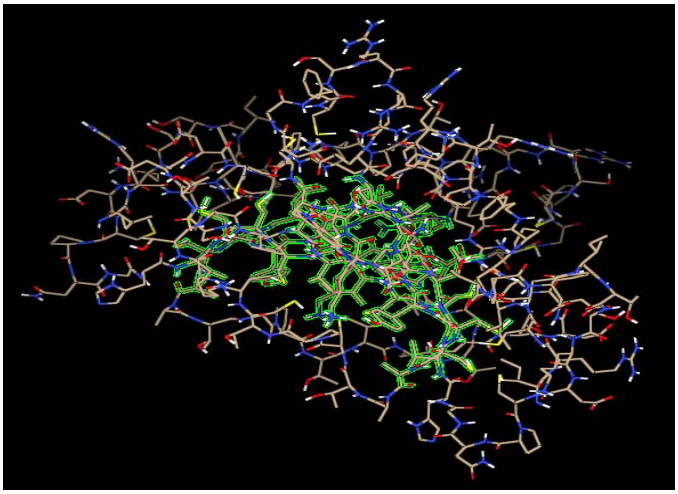


A) STRUCTURE SHOWN IN ATOMIC CONFIGURATION

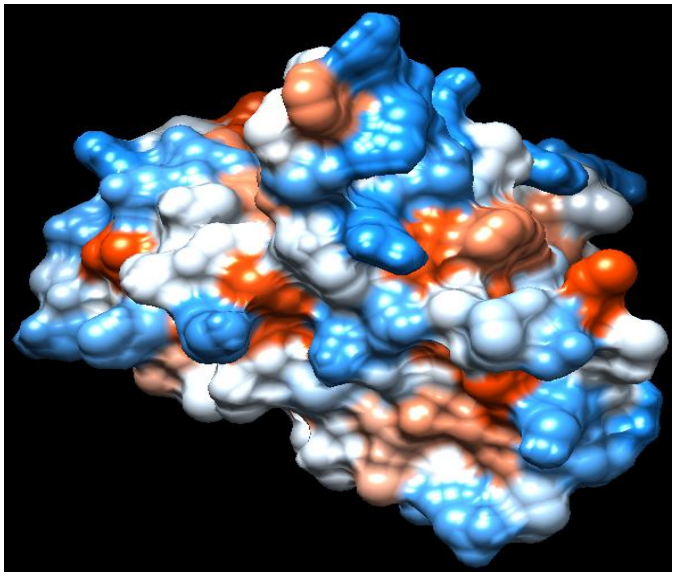


(B) HYDROPHOBIC SURFACE

FIGURE 6: P25672, STRUCTURE IN ATOMIC CONFIGURATION AND HYDROPHOBIC SURFACE



(A) STRUCTURE SHOWN IN ATOMIC CONFIGURATION



(B) HYDROPHOBIC SURFACE

FIGURE 7: P25673, STRUCTURE IN ATOMIC CONFIGURATION AND HYDROPHOBIC SURFACE

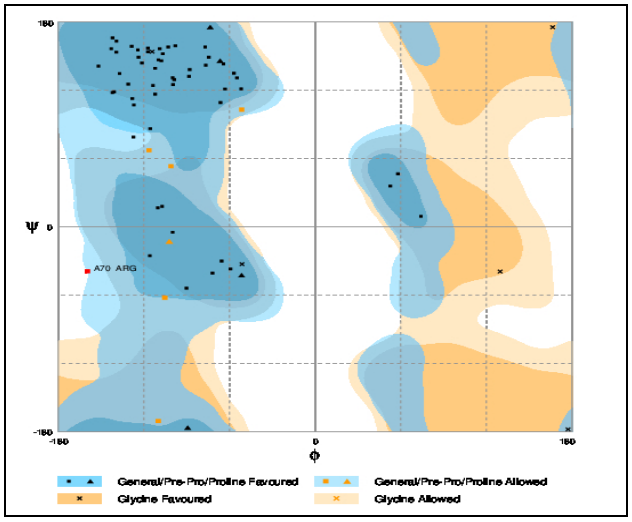


FIGURE 8: RAMCHANDRAN PLOT

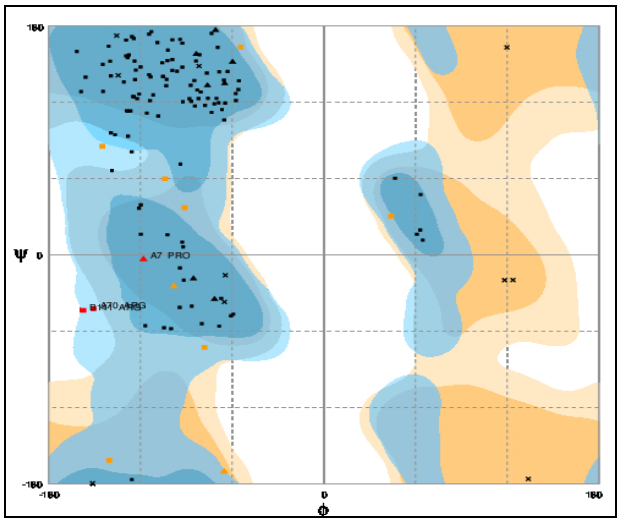


FIGURE 9: RAMCHANDRAN PLOT ANALYSIS OF P25672

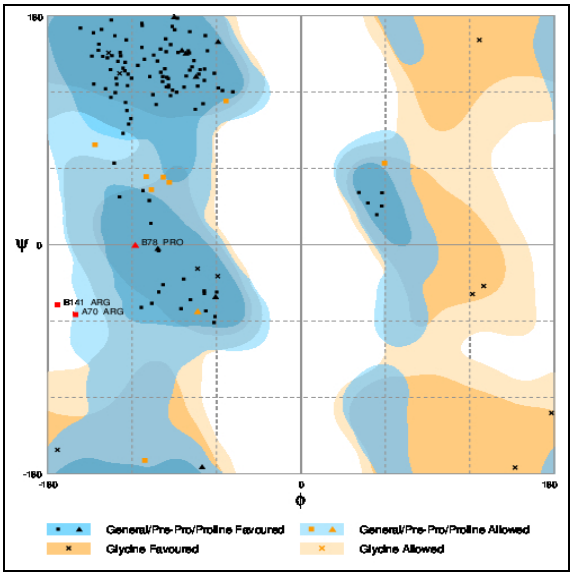


FIGURE 10: RAMCHANDRAN PLOT ANALYSIS OF P25673

TABLE 4: RESULT GIVEN BY RAMCHANDRAN PLOT IN PERCENTAGE WISE MANNER BY RAMPAGE SERVER FOR P25668

Evaluation of residues		
Number of residues in favoured region	:	62 (89.9%)
Number of residues in allowed region	:	6 (8.7%)
Number of residues in outlier region	:	1 (1.4%)

TABLE 5: RESULT GIVEN BY RAMCHANDRAN PLOT IN PERCENTAGE WISE MANNER BY RAMPAGE SERVER FOR P25672

Evaluation of residues		
Number of residues in favored region	:	126 (91.3%)
Number of residues in allowed region	:	9 (6.5%)
Number of residues in outlier region	:	3 (2.2%)

TABLE 6: RESULT GIVEN BY RAMCHANDRAN PLOT IN PERCENTAGE WISE MANNER BY RAMPAGE SERVER FOR P25673

Evaluation of residues		
Number of residues in favored region	:	127 (92.0%)
Number of residues in allowed region	:	5 (3.6%)
Number of residues in outlier region	:	6 (4.3%)

CONCLUSION: The neurotoxins of Indian Cobra venom was characterized using bioinformatics tools. This information obtained can be further used for understanding their effective role in neuro and cardial function and also for designing new therapeutic agents against varoius diseases like Alzhimer's, Parkinson's, Heart disease, Cancer and many more thus, becoming a boon in the development of medicinal science.

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