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Research Article

BioChemistry for New drug discovery

Predicted Mechanism of Action of Venom Toxins On Testicular Toxicity: An In-Silico Approach

Karthik N Awathade[1], Kumar J. R[1*], Govindaraju Shruthi[2], Kavitha Raj Varadaraju[1] and Kiran K. S[1]

[1]*Division of Biochemistry, School of Life Sciences, JSS AHER, Mysuru-570015*

[2]*Glimetomics Bioresolve Pvt. Ltd., Hootagalli Industrial Area, Mysuru-570018*

Abstract: Snake envenomation is considered as a neglected disease from world health organization. Recent survey states India has the highest rate of around 1.2 million deaths. As a pathophysiological effect, snake bite is contributing for major toxicity on human organ systems. As a secondary outcome of snake bite, testicular toxicity on the later stages of envenomation gaining more interest in recent years. But till date none of the studies have explored the field with the major toxins of the viper venom. Based on the review, our aim of the study was to predict the possible mechanism of action of the viper toxins against testicular toxicity. Hence to achieve the aim of the study, the objectives framed to perform the in-silico docking of major viper venom toxins namely VRV-PL-V and VRV-PL-VIIIa against human testicular tight junction and extra cellular matrix proteins. Docking results of our data demonstrated the prominent interaction of VRV-Pl-VIIIa with claudin, occludin, TGF-β and Tubulin α/β with the strong hydrogen bonds where as VRV-PL-V exhibited very poor hydrophobic interactions. VRV-PL-V structure was available and hence the structure was predicted and its stability was confirmed before docking. Occludin, TGF-β and Tubulin-α proteins shared multiple hydrogen bonds with the toxin VRV-PL-VIIIa whereas claudin and Tubulin-α had numerous hydrophobic interactions with the VRV-PL-VIIIa toxin. On discussion, our docking studies state the impressive binding intensity of VRV-PL-VIIIa over VRV-PL-V where it showed binding ability only with Tubulin α and β that too with weak interactions. In conclusion, our overall studies justify the previous reports of VRV-PL-VIIIa on testicular toxicity.

Keywords: Docking, Snake Bite, Viper, Claudin, Occludin, TGF-B and Tubulin A & B

***Corresponding Author**

Kumar J. R , Division of Biochemistry, School of Life Sciences, JSS AHER, Mysuru-570015

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1. INTRODUCTION

Snakebite - a global burden, is classified under neglected public health issues by WHO. India is the highest contributor towards morbidity and mortality in Asian countries tolling to around 58000 deaths a year and most go unrecorded.[1] And India has 1.2 million, the highest snakebite envenomation in the world.[2] WHO estimates around 81000 - 138000 people die each year from snake bite worldwide.[3] The burden of snake envenomation estimates at 6 million disability-adjusted life years globally[4]. Envenomation owing to the high variability of venoms of different snake species, clinical manifestations of bite vary from local to systemic toxicities caused by the biological concoction, a composite of active substances like Phospholipase A_2, Snake venom metalloprotease, hree-finger toxins, Snake venom serine protease, Hyaluronidase, vasoactive peptides & dendrotoxins.[5] Snake venom PLA_2's display numerous pharmacological activities including myotoxic, neurotoxic, anticoagulant. Hypotensive, hemolytic, anti-platelet aggregating activity, bactericidal, and pro-inflammatory activities.[6] This has inspired towards extensive research to understand the underlying mechanisms. Venom PLA_2 can be either specific or non-specific in their action with a high potency of toxicity or non-toxicity. In spite of high degree of sequence and 3D structural homologies all these variations occur. Since PLA_2's is phospholipid hydrolyzing enzymes they potentially act by disrupting the membranes of tissue & organs. VRV-PL-VIIIa is a basic PLA_2 and a major fraction of Vipera russelli pulchela snake venom from the south Indian region which makes up to about 30% of the total venom protein having a lethal potency of approximately 5.4 mg/kg body weight.[7] It attacks vital organs such as the liver, lungs, kidney & muscle contributing to the whole venom toxicity provoking biological effects such as edema, platelet aggregation, hemolysis, and pulmonary hemorrhage[8]. Snake venom toxin administration in a study resulted in severe structural damage to testes.[9] The abnormalities observed were, reduction in the intracellular concentrations of calcium, potassium & phosphorus in testis cells[10]. Significant degenerative alterations caused severe damage to seminiferous tubules resulting in severe disarray of the spermatogonia and the malformation of spermatozoa disrupting the spermatogenesis through Sertoli cells.[11] Furthermore, studies have shown that Pit Viper's highly toxic venom has the ability to reverse puberty.[12] It showed that 29% of people who survived bites later suffered from hypopituitarism, which resulted in men losing their sex drive fertility.[13] Loss of libido and erectile dysfunctions was also reported following viper bites. Case studies of snakebite, report the reproductive toxicity as a secondary effect.[14] Even by the neutralizing effect of the anti-venin, these secondary effects were observed in the survived victims[15]. Studies shows that many other toxins like bee venom toxins and hazardous chemicals which shows testicular toxicity are having a direct effect on tight junction proteins and on blood testes barrier.[16] But none of the studies till date have explored the snake venom toxins against tight junction proteins. Hence in the present study by using virtual docking methods, we have predicted the possible mechanism of venom testicular toxicity via binding of tight junction proteins.

2. MATERIALS AND METHODS

2.1 Protein Structure Retrieval

2.1.1 Venom Toxins

The structure of phospholipase A_2 VRV-PL-V protein was predicted using the protein sequence of *Daboia russelli pulchella* phospholipase A_2 VRV-PL-V obtained from Genbank Database. The structure was predicted using Modeller protein structure prediction server. The structure of phospholipase A_2 VRV-PL-VIIIa (*Daboia ruselli pulchella)* with PDBID: 1TH6.

2.1.2 Tight Junction Proteins

Structure of human occludin with PDBID: 1WPA, structure of PDZ domain of claudin with PDBID: 3VQF, structure of Tubulin α and Tubulin β with PDBID: 7LXB were procured from protein data bank.

2.2 Protein Structure Validation

The predicted structure of phospholipase A_2 VRV-PL-V protein was validated through Ramachandran plot using Rampage webtool, and Z-score was predicted using ProSA server [17]

2.2.1 Binding Site Prediction

The binding site of the phospholipase A_2 VRV-PL-VIIIa Claudin, Occludin, Tubulin α and Tubulin β protein structures were analyzed through ligand explorer of RCSB PDB server. Whereas, the binding site of phospholipase A_2 VRV-PL-V, protein structure was determined using GalaxySite tool from GalaxyWeb server [18].

2.2.2 Molecular Interaction Studies

The procured crystal structures of the Phospholipase A_2 VRV-PL-VIIIa (PDB ID: 1TH6) and VRV-PL-V, Claudin, Occludin, Tubulin α and Tubulin β protein structures were further refined by removing water residues and other cofactors. The interaction of Phospholipase A2 VRV-PL-VIIIa (PDB ID: 1TH6) and VRV-PL-V with Claudin, Occludin, TGF β, Tubulin α and Tubulin β were analysed using Z-dock server [19]. Where, the binding site residues were selected based on the binding site data procured from ligand explorer of RCSB PDB.

2.3 Visualization of Interactions

The hydrogen bond interaction and hydrophobic interactions between Phospholipase A_2 VRV-PL-VIIIa (PDB ID: 1TH6) and VRV-PL-V with Claudin, Occludin, TGF-β, Tubulin α and Tubulin β were analyzed through UCSF Chimera tool [20].

3. RESULTS

3.1 Interaction of VRV-PL-Viiia with Tight Junction and Extra Cellular Matrix Proteins

Out of the selected protein from of tight junction and extracellular matrix protein Claudin, occludin, TGF-β and tubulin showed the interactions.

3.1.1 Interaction with Claudin

VRV PL VIIIa exhibited high interaction with tight junction protein claudin, indicative of possible modification of normal functioning of the tight junction of sertoli cells, through constitutive activation of claudin.

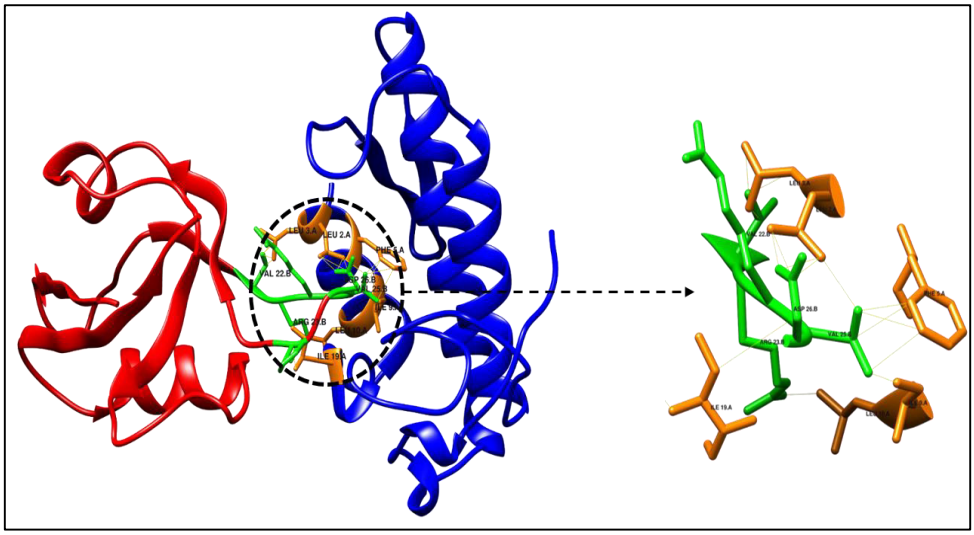
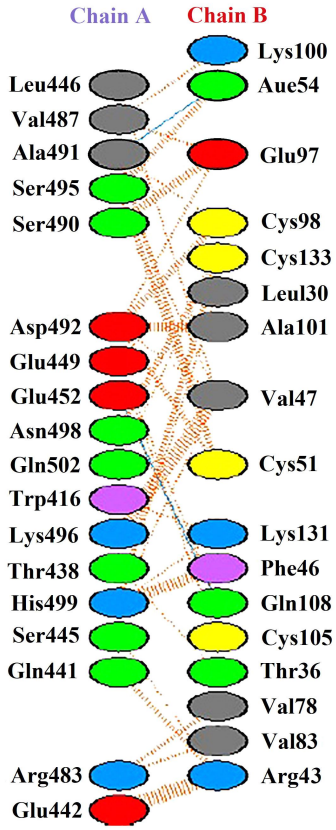


Fig-1: Exhibiting molecular docking of VRV PL VIIIa with Claudin, where the contact sites have been illustrated in green and orange hue.



The amino acid residues LEU2, LEU3, PHE5, ILE9, LEU10 and ILE19 of VRV PL VIIIa interact with VAL22, ARG23, VAL25 AND ASP26 of Claudin through hydrophobic interaction more closely when compared to other hydrophobic interactions between VRV PL VIIIa and claudin, depicted in figure 2.

Fig-2: Representative image of various interactions seen at binding site of VRV PL VIIIa with (Chain B) Claudin (Chain A).

3.1.2 Interaction with Occludin

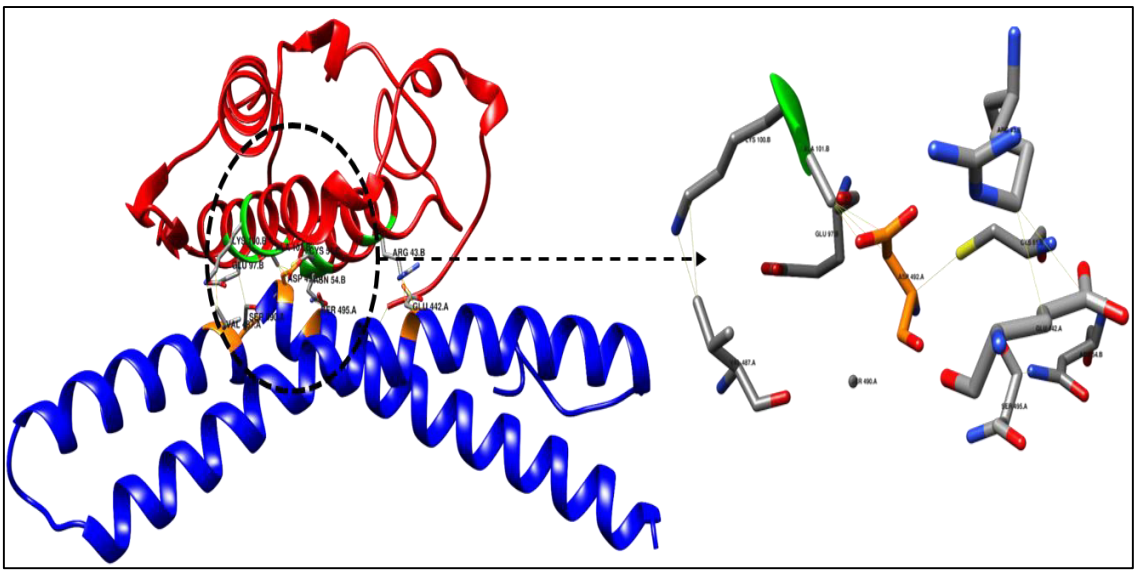
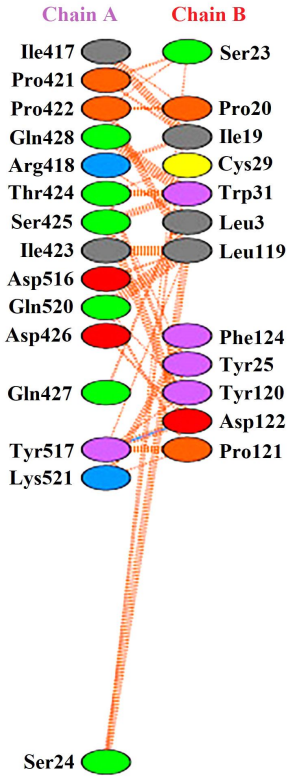


Fig-3: Exhibiting molecular docking of VRV-PL-VIIIa with occludin, where the contact sites have been illustrated in green and orange hue.



The amino acid residues ARG43, CYS51, ASN54, GLU97, LYS100 and ALA101 of VRV PL VIIIa interact with GLU442, VAL487, SER490, and SER495 of occludin through hydrophobic interaction more closely when compared to other hydrophobic interactions between VRV PL VIIIa and claudin, depicted in figure 4.

Fig-4: Representative image of various interactions seen at binding site of VRV-PL-VIIIa (Chain B) with Occludin. (Chain A)

3.1.3 Interaction with Tgfβ

VRV PL VIIIa was found to interact with the heterodimers of TGF-β simultaneously forming a triad kind of complex upon binding to chain A and chain B of TGF-β.

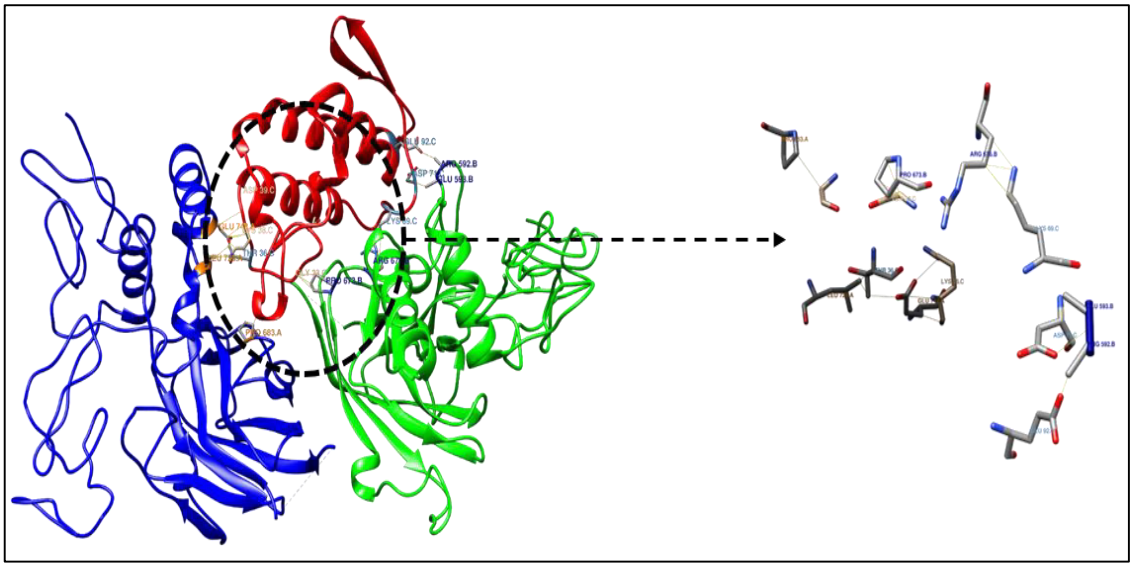
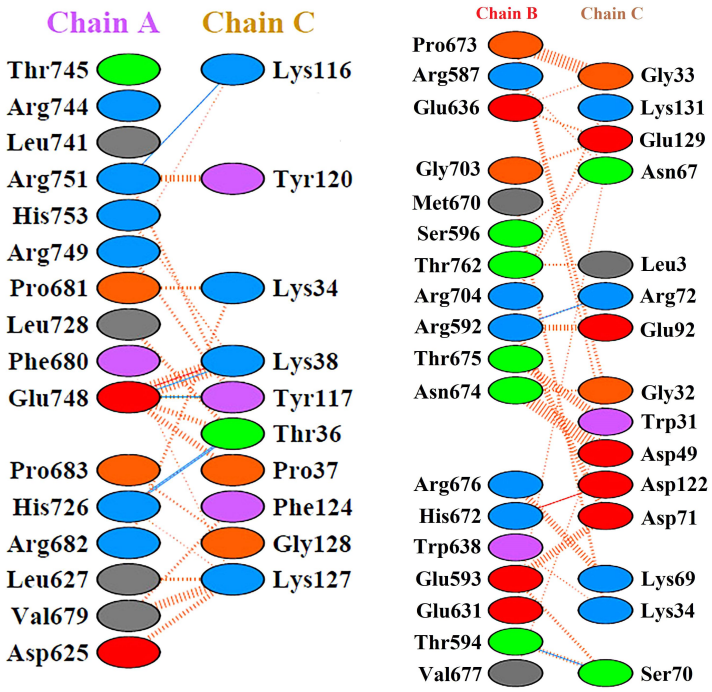


Fig-5: Exhibiting molecular docking of VRV-PL-VIIIa with TGF-β, where the contact sites have been illustrated in orange, grey and blue hue.



VRV PL VIII A binds to TGF β at the interface of chain A and chain B, where GLU92, ASP71, LYS69 and THR36 amino acid residues of VRV PL VIII A interact with GLU748, PRO683, LEU728 of chain A and PRO673, ARG592, GLU593 and ARG587 of chain B respectively.

Fig-6: Representative image of various interactions seen at binding site of VRV-PL-VIIIa (Chain C) with TGF-β (Chain A and B)

3.1.4 Interaction with Tubulin A

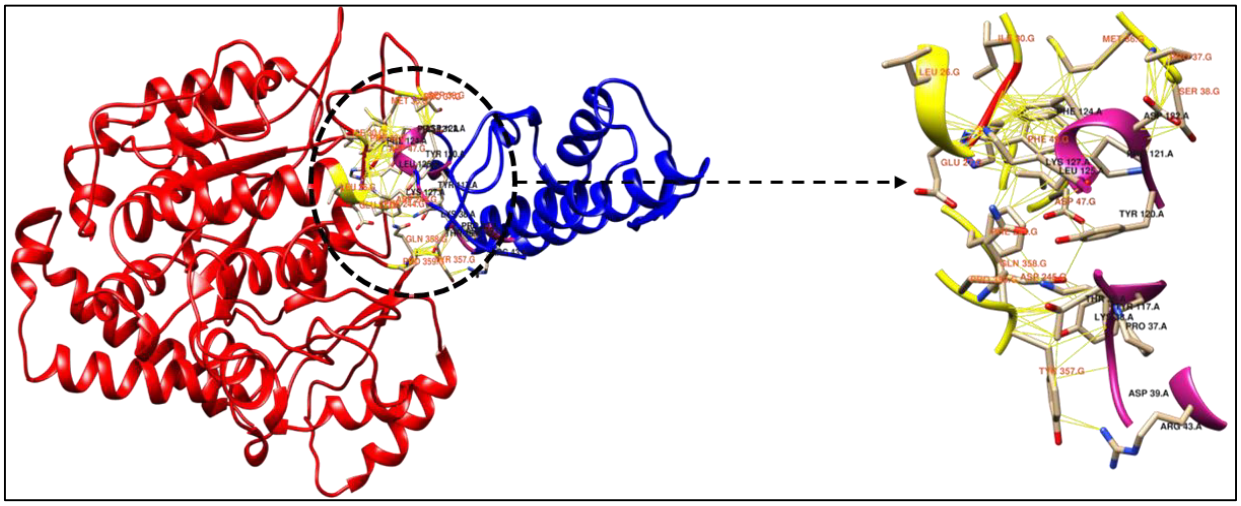
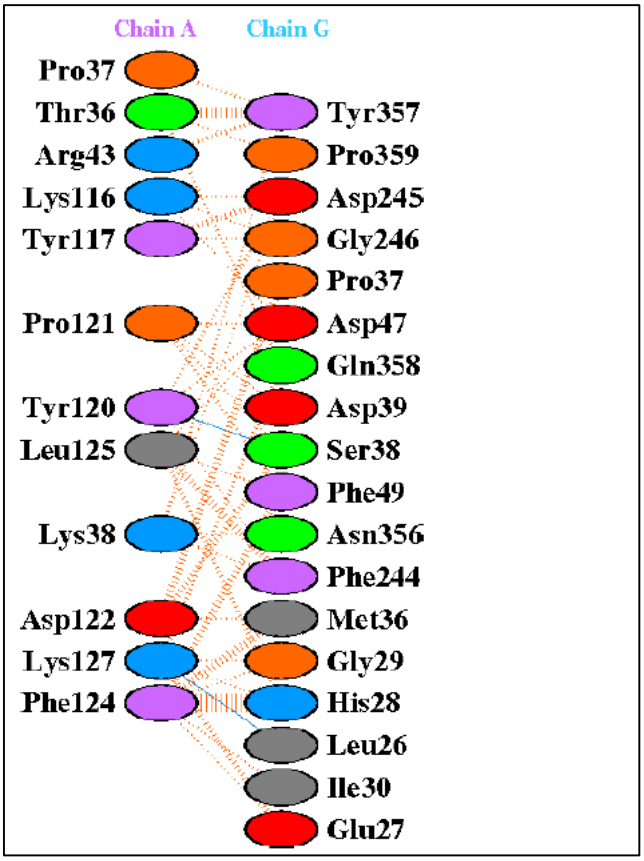


Fig-7: Exhibiting molecular docking of VRV-PL-VIIIa with Tubulin α, where the contact sites have been illustrated in yellow and Pink hue



VRV PL VIII A binds to Tubulin α, where LYS127, LEU125, PHE124, ARG121, TYR120, THR36, TYR117, LYS38, PRO37, ASP39, ARG43 amino acid residues of VRV PL VIII A interact with TYR357, ASP245, PRO359, GLN358, PHE70, ASP47, GLU27, PHE41, LEU26, ILE30, MET36, PRO37, AND SER38 of Tubulin α.

Fig-8: Representative image of various interactions seen at binding site of VRV-PL-VIIIa with Tubulin α, where the contact sites have been illustrated in yellow and pink hue

3.1.5 Interaction with Tubulin B

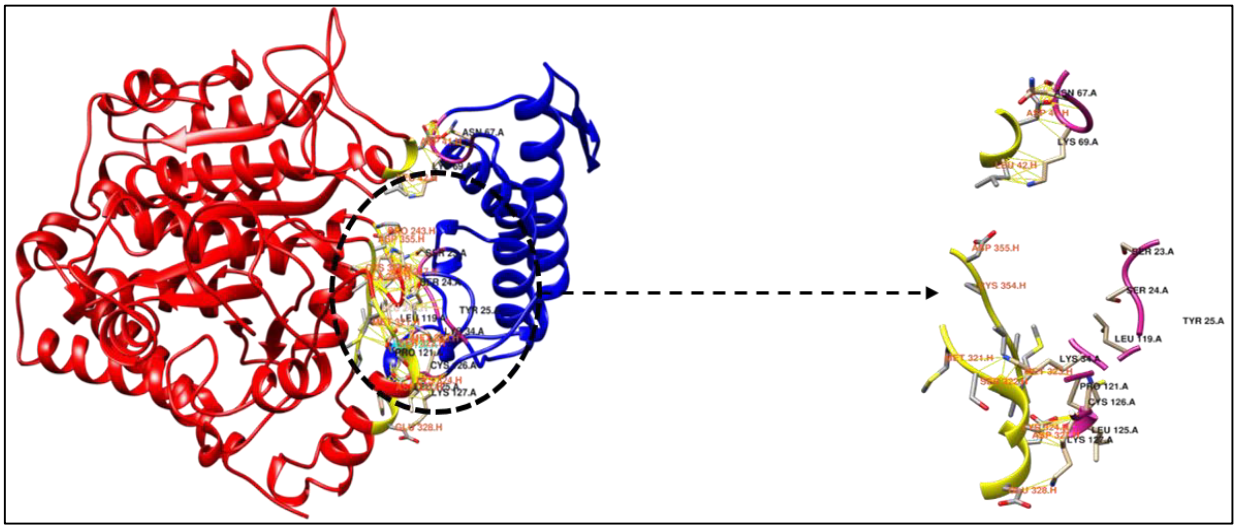
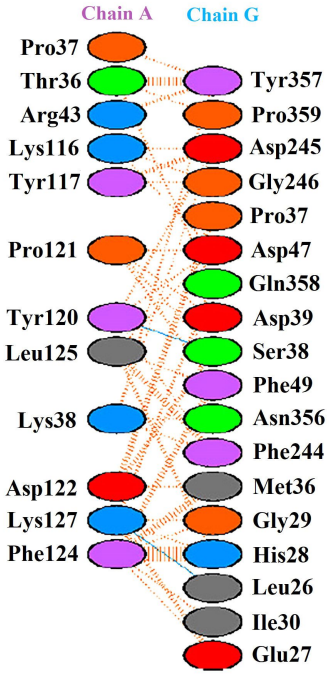


Fig 9: Exhibiting molecular docking of VRV-PL-VIIIa with Tubulin β, where the contact sites have been illustrated in yellow and pink hue.



VRV PL VIII A binds to Tubulin B, where ASN67, LYS69, SER23, SER24, TYR25, LEU119, LYS34, PRO121, CYS126, LEU125, and LYS127 amino acid residues of VRV PL VIII A interact with ASP41, LEU42, ASP355, LYS354, MET321, SER322, MET323, LYS324, ASP325, AND LEU328 of Tubulin B.

Fig 10: Representative image of various interactions seen at binding site of VRV-PL-VIIIa (Chain A) with Tubulin β (Chain B), where the contact sites have been illustrated in yellow and pink hue

3.2 Structure of VRV PL V

The other toxin VRV-PL-V of viper venom was not available on PDB. Hence by taking the sequence of VRV-PL-V structure was predicted using modeller software.

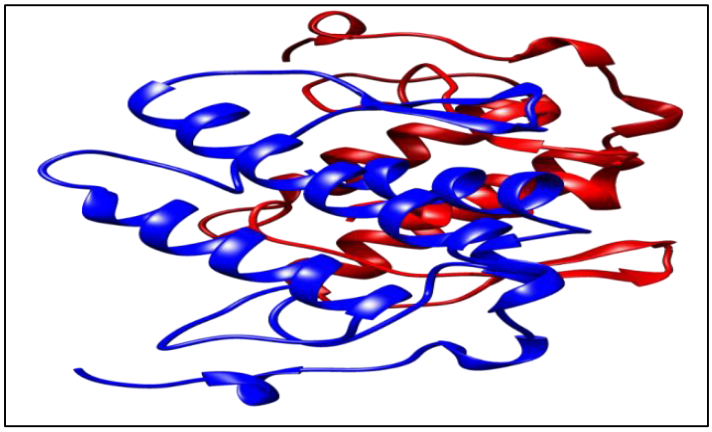


Fig 11: Predicted structure of VRV-PL-V

3.2.1. Validation of Protein Structure

The predicted protein structure was validated for structural integrity through Ramachandran plot prediction. It was found that all the amino acids were in stable region authenticating the stability of the predicted structure.

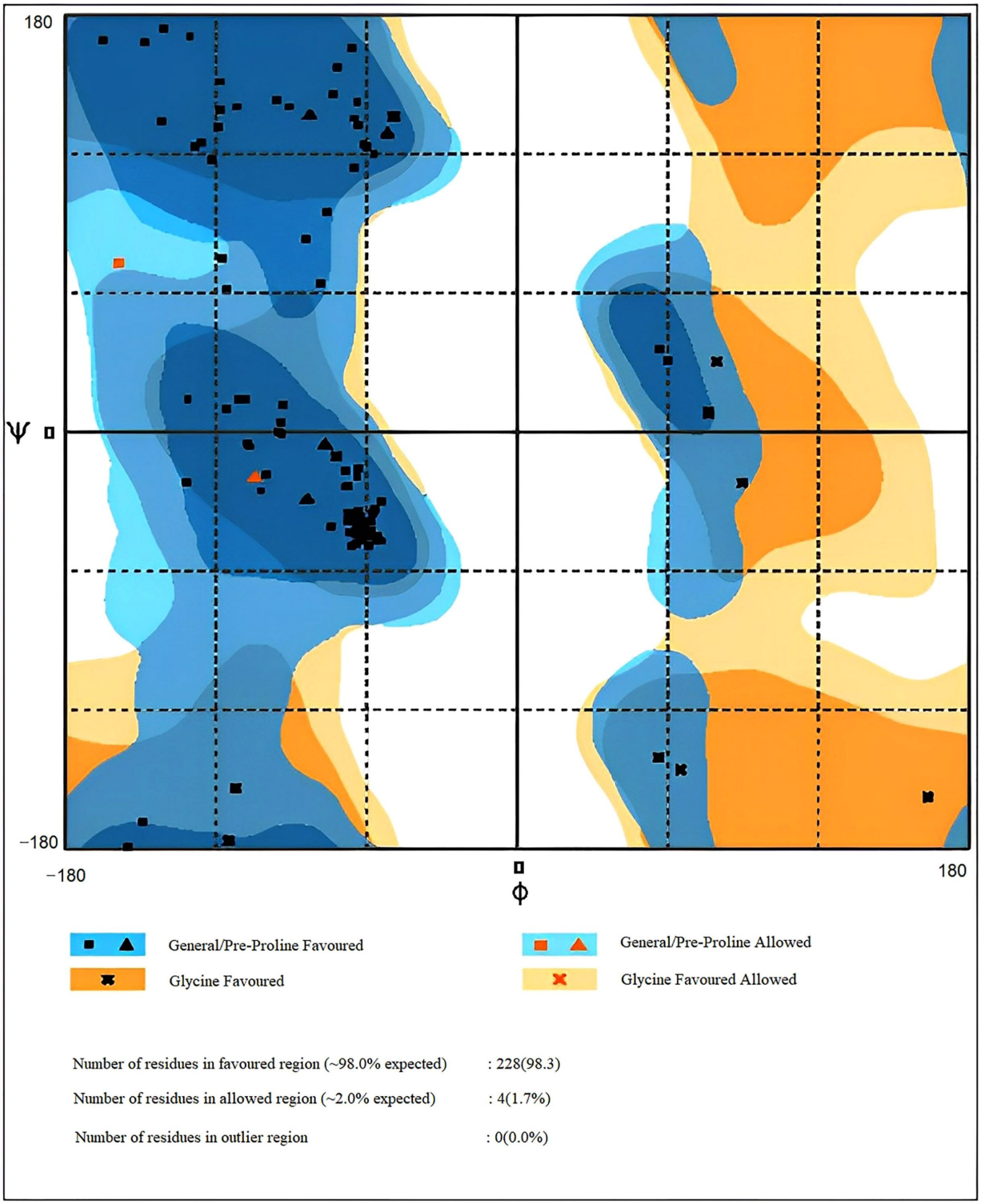


Fig 12: Ramachandran plot for VRV-PL-V

3.3 Interaction of VRV-PL-V with Tubulin A and B

VRV-Pl-V toxin was not binding with any of the proteins other than Tubulin α and β. Hence only that data is been projected in the below results section.

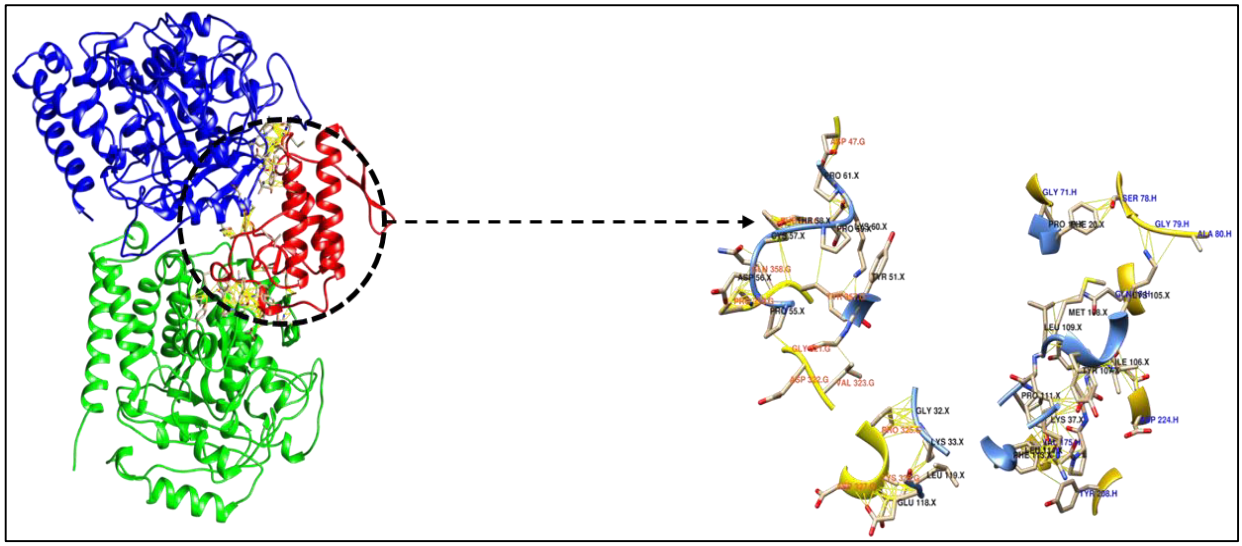


Fig 13: Exhibiting molecular docking of VRV-PL-V with Tubulin α and β, where the contact sites have been illustrated in yellow hue.

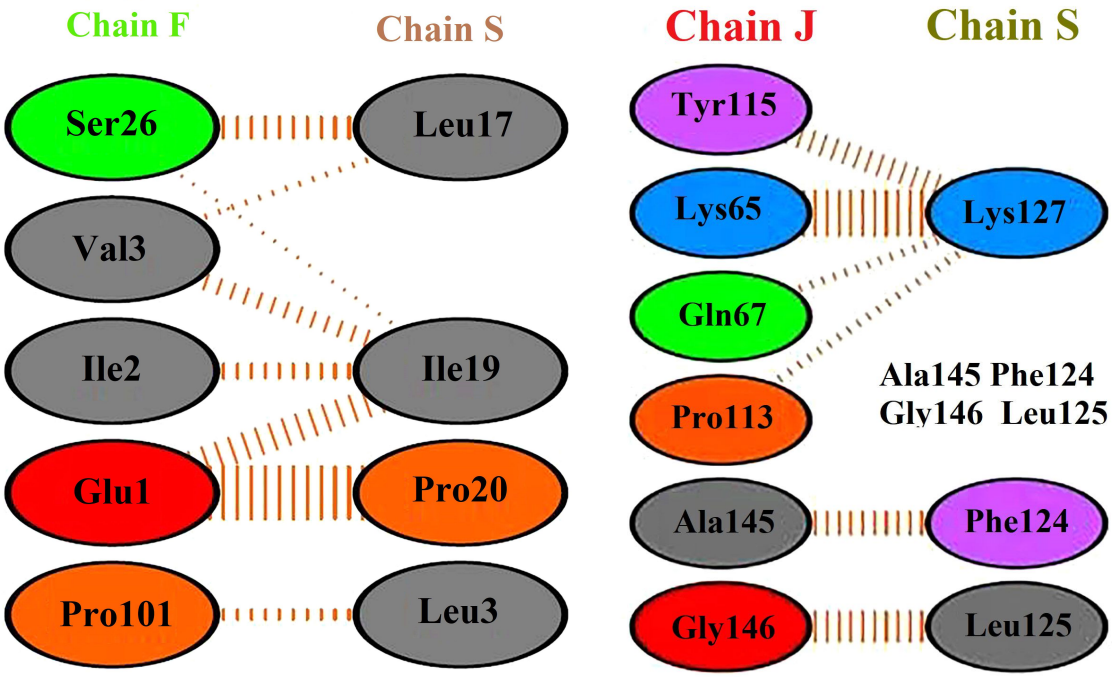
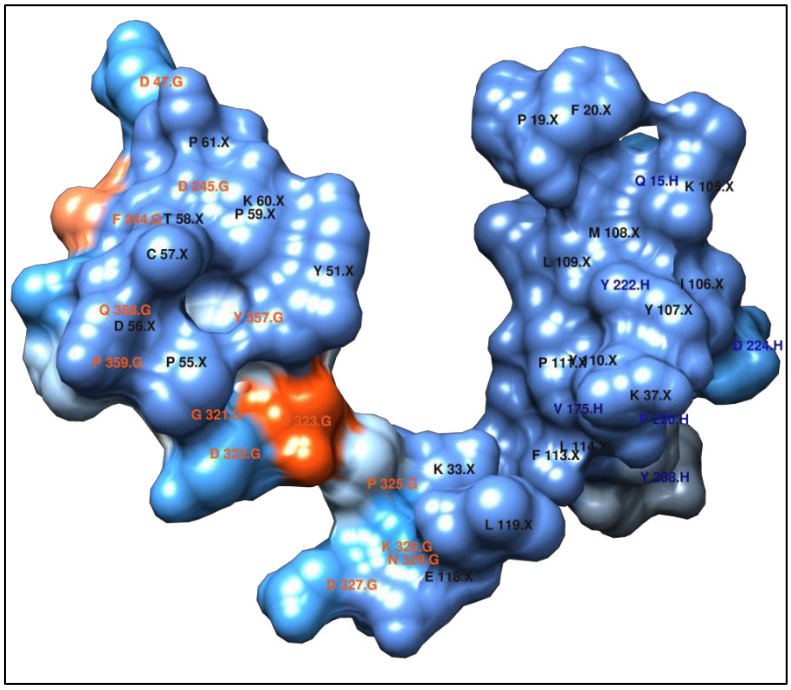


Fig 14: Representative image of various interactions seen at binding site of VRV-PL-V (Chain S) with Tubulin α and β (Chain F & J), where the contact sites have been illustrated in yellow and blue hue



VRV-PL-V was found to interact with GLY321, ASP322, VAL323, PRO325, ASP327, LYS328 of G chain of Tubulin α and ß. GLY71, SER78, GLY79, ALA80, GLN171, ASP224 VAL175 and TYR208 of chain H of Tubulin α and ß through hydrophobic interactions. It can be noted that the VRVPL-V interacts with both chain G and chain H simultaneously at the site of intersection of chain G and Chain H.

Fig 15: Hydrophobic interactions seen at binding site of VRV PL V with Tubulin α and β, where the contact sites have been illustrated.

Table - 1 Exhibits the binding site interaction between VRV PL VIII A, VRV PL V with various cell junction proteins, with binding energy, number of non-bonded interactions, hydrogen bond interactions between each toxin and respective cell junction proteins.

Sl.no	Protein Name	Toxin Binding Site	Protein Binding Site	Binding Energy	No of non-bonded interactions	Hydrogen bond interaction	
						Toxin	Protein
		VRV PL VIIIa					
1	Claudin	LYS100, ASN54, GLU97, CYS98, CYS133, LEU130, ALA101, VAL47, CYS51, LYS131, PHE46, GLU108, CYS105, THR36, VAL78, VAL83, ARG43	LEU446, VAL487, ALA491, SER495, SER490, ASP492, GLU452, ASN498, GLN502, TRP416, LYS496, THR438, HIS499, SER445, GLN441, ARG483, GLU442	-12.4 kcal/mol	117	-	-
2	Occludin	SER23, PRO20, ILE19, CYS29, TRP31, LEU119, PHE124, TYR25, TYR120, ASP122, PRO121	ILE417, PRO421, PRO422, GLN428, ARG418, THR424, SER425, ILE423, ASP516, GLN520, ASP426, GLN427, TYR517, LYS521, SER24	-9.5 kcal/mol	112	GLN108, GLU449, ALA491	GLU452, CYS105, ASN54
3	TGF-β	LYS116, TYR120, LYS34, LYS38, TYR117, THR36, PRO37, PHE124, GLY128, LYS127, GLY33, LYS131, GLU129, ASN67, LEU3, ARG72, GLU92, GLY32, TRP31, ASP49, ASP122, ASP71, LYS69, LYS34, SER70	CHAIN A: THR745, ARG744, LEU741, ARG751, HIS753, ARG749, PRO681, LEU728, PHE680, GLU748, PRO683, HIS726, ARG682, LEU627, VAL679, ASP625	-15.1 kcal/mol	115	TYR117, LYS38, ARG72, SER70, LYS116	GLU748, ARG592, THR 594, ARG751
			CHAIN B: PRO673, ARG587, GLU636,		96		

			GLY703, MET670, SER596, THR762, ARG704, ARG592, THR675, ASN674, ARG676, HIS672, TRP638, GLU593, GLU631, THR594, VAL677				
4	Tubulin α	PRO37, THR36, ARG43, LYS116, TYR117, PRO121, TYR120, LEU125, LYS38, ASP122, LYS127, PHE124	CHAIN G: TYR357, PRO359, ASP245, GLY246, PRO37, ASP47, GLN358, ASP39, SER38, PHE49, ASN356, PHE244, MET36, GLY29, HIS28, LEU26, ILE30, GLU27	-21.3 kcal/mol	238	TYR120	SER38
5	Tubulin β	TYR22, ILE9, ILE19, LEU2, PHE5, GLY30, SER23, ALA18, LEU3, ARG72, LEU17, LEU10	PRO28, GLY29, GLU27, VAL25, ARG24, ASP26, PHE31, SER65, ARG23, GLU49, VAL22, PHE33, GLU67	-10.4 kcal/mol	159		
		VRV-PL-V					
1.	Tubulin α and β	LYS127, PHE124, LEU125, LEU17, ILE19, PRO20, LEU3	CHAIN F: SER26, VAL3, ILE2, GLU1, PRO101 CHAIN J: TYR115, LYS65, GLN67, PRO113, ALA145, GLU146	-9.7 kcal/mol	238		

4. DISCUSSION

Tight junction (TJ) proteins of testes are found in blood testes barrier (BTB).[21] The term blood-testis barrier, also known as the Sertoli cell seminiferous epithelium barrier.[22, 23] BTB is unique from the other tissue barriers as it not only comprised of TJ's but also they co-exist and co-function with desmosomes[24], gap junctions and ectoplasmic specializations [25] to create a specific environment for meiosis to occur among the spermatids to develop into spermatozoa.[26] It's the main route for any toxin or any external materials to interfere with the spermatogenesis.[21,27] Many previous studies have reported the interaction of the toxicants with the tight junction proteins impacting on reproductive toxicity in males.[28, 31] The interruption in the BTB of sertoli cells cause damage to the normal physiological functions of testes namely, spermatogenesis and cause loss of spermatids.[32] Previous research on reproductive toxicity in male have shown the direct interaction or the damage TJ's proteins leading to testicular toxicity[16]. Many case studies on snake bite has confirmed the secondary effect of toxins on reproduction in males showing disruption in spermatogenesis.[33] Even with the notable case reports, none of studies was conducted on the mechanism of action of snake venom toxins on TJ's. Hence in the present study, we predicted the possible mechanism of action of major viper venom toxins against TJ proteins and extra cellular matrix proteins. Literatures on snake bite evidenced mainly the viper venom bite on testicular toxicity. And the research studies on toxins from viper venom reported VRV-PI-V and VRV-PL-VIIIa as the lethal toxins.[34] Hence these two toxins were considered for the whole study. However, for the study, the major proteins of the sertoli cells involved in normal testicular spermatogenesis were identified through a thorough literature review. Based on their location and function proteins present in the tight junction (TJ) of sertoli cells, were considered along with the Extracellular matrix proteins (EMP) of the seminiferous tubule.[21] Hence proteins such as Claudin, Occludin, TNFα, TGFβ, Tubulin α and β were considered for molecular interaction studies with venom toxins. In-silico docking studies of VRV-PL-VIIIa shows a very interactive binding to the major sites of Claudin (Figure -1 & 2), Occludin (Figure -3 & 4). Toxin was found to interact with the heterodimers of TGFβ simultaneously forming a triad kind of complex upon binding to chain A and chain B of TGFβ (Figure -5 & 6). Tubulin α & β interaction with the VRV-PL-VIIIa also exhibited a significant interaction (Figure -7 & 8). The major hydrogen bonding was found in between the toxin and protein of interest. These interactions may contribute for the inhibition of the protein functioning as its proved that changes in Claudin results in the lack of functional redundancy in BTB[35]. Likewise, VRV-PL-V toxin was considered for the studies. Due to the unavailability of the VRV-PL-V structure, by using homology modelling, VRV-PL-V structure was predicted (Figure-11). Again the predicted protein structure was validated by rechecking the predicted protein for its stability using Ramachandran plot (Figure-12). VRV-PL-V toxin didn't show any interaction with the other TJ or EMP than Tubulin α and β. But the data represents only the hydrophobic interactions between VRV-PL-V and Tubulin α and β of sertoli cells (Figure-13, 14 & 15). The binding interaction between the toxin and the sertoli cells TJ or EMP proteins were summarized in table-1. The overall docking of major toxins of viper venom with the TJ or EMP demonstrates the prominent

interactions between them. Among both the toxins, VRV-PL-VIIIa represents maximum strong hydrogen bonding interpreting the possible inhibitory mechanism of the TJ or EMP proteins. Even this prediction is justified by the previous results were among the two toxins, VRV-PL-VIIIa has been experimentally proved for reproductive toxicity in male mice.[34] Hence our overall studies signifies the possible mechanism of toxins on testicular toxicity. Further experimental studies should be conducted to justify the above present in-silico study.

5. CONCLUSIONS

The predicted mechanism of toxins in effecting the testicular toxicity by docking studies explained the possible strong interactions between the venom toxin and the tight junction proteins through hydrogen bonds and hydrophobic interactions. Whereas VRV-PL-VIIIa presented the more interfaces than VRV-PL-V. These data helps us to conclude that the testicular toxicity by viper snake venom is mainly by aberration of the tight junction proteins by venom toxin proteins. These findings have paved a way for further exploration of the toxins on male reproductive system in-turn in future it would be helpful in the treatment strategy against snake bite.

6. ACKNOWLEDGEMENTS

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7. AUTHORS CONTRIBUTION STATEMENT

Mr. Karthik N Awathade conceptualized and designed the study and Dr. Kavitha Raj V and Dr. Govindaraju Shruthi curated the data and prepared the original draft. Dr. Kumar J R and Dr. Kiran K S discussed the methodology and analyzed the data and provided valuable inputs towards designing of manuscript. All authors read and approved the final version of the manuscript.

8. CONFLICT OF INTEREST

Conflict of Interest declared none.

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