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Identification of Potential Lead Compounds Against Snake Neurotoxin in *Rauvolfia Serpentina* Through Molecular Docking

Y. N. Joshi[1], Iravati Sodal[2], Tejaswini Kale[3], Shraddha Patange[4], Vinay Gote[5]

[1,3&4]Department of Bioinformatics, Walchand Centre for Biotechnology, WCAS, Solapur, Maharashtra, India

[2&5]Department of Biotechnology, Walchand Centre for Biotechnology, WCAS, Solapur, Maharashtra, India

ABSTRACT

In all over the world including India, snake bite is a serious health problem which possess singnificant amount of mortality as well as abnormality. In world *Bangarus caeruleus* (Indian krait), *Naja naja* (Indian cobra), *Dendroaspis polylepis polylepis* (Black mamba),*Oxyuranus microlepidotus* (Inland taipan) are most venomous snake species belonging from neurotoxin family. Primarily neurotoxins affect the nervous system by strongly binding with receptor such as nicotinic acetylcholine (nAchRs). In modern medicine, immunotherapy is only treatment for snake bite but it has many limitations which includes adverse side effects to the patient. Herbal medicine is another way for snake bite victims which causes less side effects than antivenom therapy. For the identification of lead compounds and to demonstrate the drug activity, *In-silico* technique is the best option. In current studies, with the help of *In-silico* tools, we identified various potential lead compounds from *Rauvolfia serpentina* against snake neurotoxins. In present investigation chemical molecules present in *Rauvolfia serpentina* were docked with each of four neurotoxin venom protein using HEX software. Most potential lead compounds found after docking are 2,6-dimethoxy benzoquinone and Thebaine.

Keywords : Neurotoxins, Venom Protein, *Rauvolfia Serpentina*, Lead, Docking.

I. INTRODUCTION

Snakes have been residents of this planet well before ancient humans dwelled the earth having a figure of fear. Venomous snakes causes notable mortality and abnormalities to the victim throughout the world [1]. Venom composition consists of different kinds of digestive enzymes, nuclease, bioactive proteins and polypeptides which is used for defense or to immobilize it's prey [2]. Among the toxin families neurotoxins attack the central nervous system of the victim's body resulting in heart failure, tissue damage, respiratory blockage and paralyses the body, kidney failure, coma and death [3].

For snake envenomation treatment, immediate administration of specific polyvalent antivenom is most effective and accepted therapy. But this treatment has risk of adverse side effects. To achieve the national requirements, authorized venom dealers have a challenge for a reasonable amount antivenom production to provide sufficient amount of quality venom. It requires ideal storage conditions and the development is time consuming process as well as costly. With this reference, herbal treatment is only available option for snakebite treatment as these herbs are common, easily available and cheaper [4]. From ancient times we know that plants are rich source of a variety of chemicals with nutritive and therapeutic properties. Dependence on plants is basically due to their lush availability all the times,

cheaper, safety, effectiveness, and cultural preference [5]. On the basis of venom species belonging from neurotoxin family *Bangarus caeruleus* (Indian krait), *Naja naja* (Indian cobra), *Dendroaspis polylepis polylepis* (Black mamba), *Oxyuranus microlepidotus* (Inland taipan) are selected against the plant *Rauvolfia serpentina*. *Rauvolfia serpentina* is an evergreen, woody, medicinal shrub. The plant bears white or pinkish colored flowers. The root bark of this plant contains more 90% of total alkaloids in roots. Reserpine is major alkaloid present in this plant [6]. In modern medicine, immunotherapy is the only treatment for snake bite but it has many limitations which includes adverse side effects to the patient [7]. For the identification of lead compounds and to demonstrate the drug activity, *In-silico* technique is the best option because it doesn't need any kind of live raw material, bulk amount of investment [8]. *In-silico* methods gives clear molecular level theoretical intuition about the drug activity and identify the exact lead molecule for further identification. Docking is widely applied screening method in *In-silico* methods.

Bioinformatics study involves access to venom data of multiple databases, inspection for errors, analysis and classification of venom toxin sequences and their structures and design, use of predictive models for simulation of laboratory experiments. A clean and comprehensive collection of venom data enriched with structural and functional information provides means for more detailed analysis [9]. In present investigation, chemical molecules present in Rauvolfia were docked with each of four neurotoxin venom protein such as Basic phospholipase A2 beta-bungarotoxin A2 chain, Cytotoxin 1, Kunitz-type serine protease inhibitor dendrotoxin E, Basic phospholipase A2 paradoxin-like alpha chain using HEX software.

II. METHODS AND MATERIAL

1. Retrieval of sequences :- The protein sequences of *Bungarus caeruleus* (Indian krait), *Naja naja* (Indian cobra), *Dendroaspis polylepis polylepis* (Black mamba), *Oxyuranus microlepidotus* (Inland taipen) were retrieved from UniProt sequence database.UniProt is a freely accessible database of protein sequence and functional information, many entries being derived from genome sequencing projects. It contains a large amount of information about the biological function of proteins derived from the research literature. [10].
2. Physicochemical analysis:- Primary structures of four snake neurotoxin sequences were analyzed by ProtParam tool. ProtParam is one among the protein analysis tool avaible on the ExPasy server. It computes various physico-chemical properties of provided protein. The parameters computed by ProtParam include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY). [11]
3. Secondary structure prediction:- The secondary structures of snake protein sequences were predicted by GOR4 secondary structure prediction tool. The GOR method analyzes sequences to predict alpha helix, beta sheet, turn, or random coil secondary structure at each position based on 17-amino-acid sequence windows. [12]
4. Three dimensional structure prediction and model validation:- The 3D structures of four snake protein sequences were predicted using SWISS-MODEL server. SWISS-MODEL is a structural bioinformatics web-server dedicated to homology modeling of 3D protein structures. Homology modeling is currently the most accurate method to generate reliable three-

dimensional protein structure models and is routinely used in many practical applications. Predicted models were validated using PROCHECK. It is a suite of programs to check the stereochemical quality of protein structures. [13].

5. Retrieval of phytochemicals:- The structures of molecules in *Rauvolfia serpentina* were retrieved from PubChem database. PubChem is a database of chemical molecules and their activities against biological assays. The structures of molecules were saved in SDF form and were converted in PDB format by Open Babel. Open Babel is computer software, a chemical expert system mainly used to interconvert chemical file formats.[14].
6. Molecular docking:- Each of four snake venom proteins were docked with phytochemicals Hex 6.12 software. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. [15].

III. RESULTS AND DISCUSSION

1. Retrieval of Sequences :

The sequences of four snake species were retrieved from UniProtKB database and sequences were saved in FASTA format. The each species with UniProtKB ID, protein names and sequence length were shown in Table 1.

Table. 1 Retrieval of Sequences

Name of organism	Name of protein	Uniprot ID	Sequence length
Bungarus caeruleus (Indian krait)	Basic phospholipase A2 beta-bungarotoxin A2 chain	Q8QFW3	
Naja naja (Indian cobra)	Cytotoxin 1	P01447	60
Dendroaspis polylepis polylepis (Black mamba)	Kunitz-type serine protease inhibitor dendrotoxin E	P00984	59
Oxyuranus microlepidotus (Inland taipan) (Diemenia *microlepidota)*	Basic phospholipase A2 paradoxin-like alpha chain	Q45Z42	146 146

2. Physicochemical Analysis

The physicochemical properties were analysed using ProtParam tool; the results were shown in Table 2. As per table all protein sequences are basic in nature. Protein of *Bungarus caeruleus* is more stable while others are comparatively less stable.

Table 2. Physicochemical Analysis

Parameters	*Bungarus caeruleus* (Indian krait)	*Naja naja* (Indian cobra)	*Dendroaspis polylepis polylepis* (Black mamba)	*Oxyuranus microlepidotus* (Inland taipan) (Diemenia microlepidota)
Molecular Weight	16119.56	*6791.21*	*6619.70*	*16361.91*
Theoretical PI	7.88	9.24	9.39	8.49
Instability index	32.50	51.27	50.00	48.56
Aliphatic Index	73.06	79.50	49.83	61.51
GRAVY	-0.092	-0.192	-0.403	-0.286

3. Secondary structure prediction

The secondary structural elements were predicted using GOR4 tool, the percentage of alpha helix, extended strand, and random coil were calculated in Table 3. From the table percentage of random coil is more than extended strand followed by alpha helix.

Table 3. Secondary Structure Prediction

Name of toxin	Alpha helix (%)	Extended strand (%)	Random coil (%)
Basic phospholipase A2 beta-bungarotoxin A2 chain	4.00	32.65	63.27
Cytotoxin 1	0.00	45.00	55.00
Kunitz-type serine protease inhibitor dendrotoxin E	0.00	27.12	72.88
Basic phospholipase A2 paradoxin-like alpha chain	3.42	36.99	59.59

4. Three dimensional structure prediction and model validation

Homology modeling was done using SWISS-MODEL workspace and predicted models were validated using PROCHECK tool which shown in Table 4 and graphical representation of 3D models were visualized in RasMol which shown in Fig. 1-4. The structures predicted by SWISS-MODEL are of best quality.

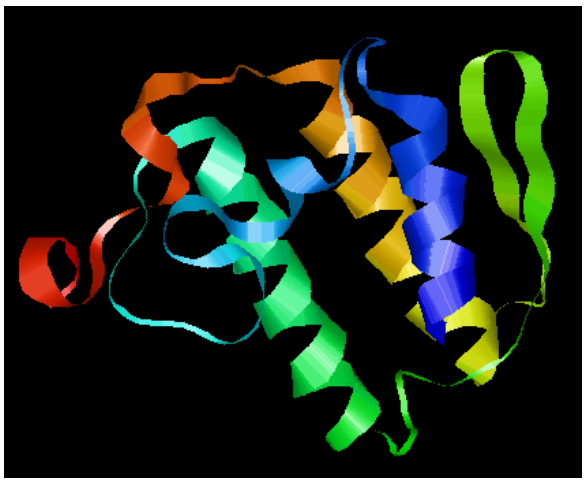

Fig. 1 Bungarus caeruleus

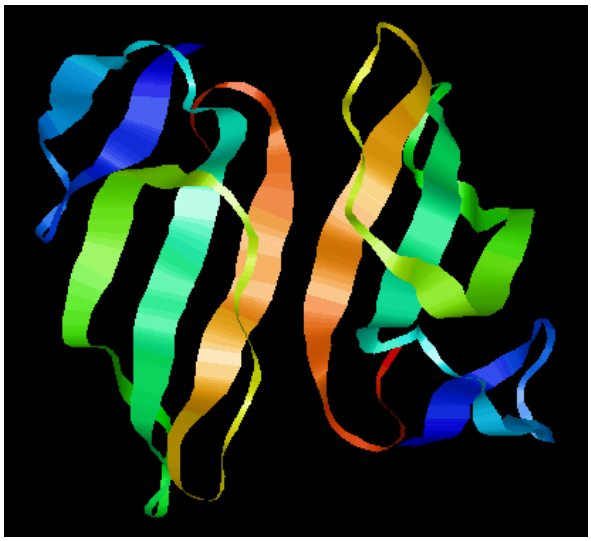

Fig. 2 Naja naja

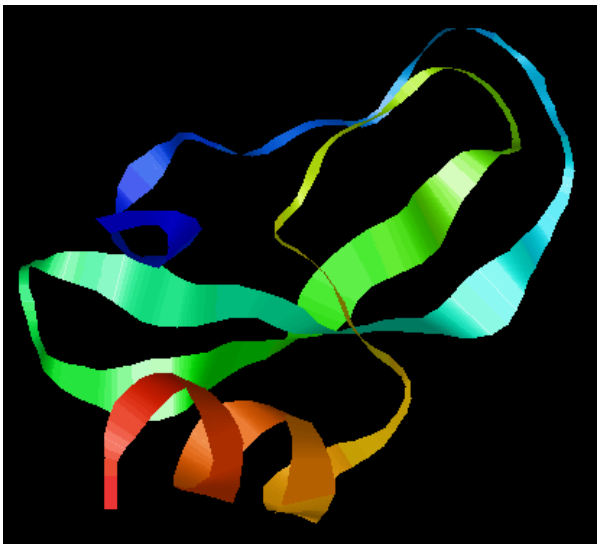

Fig. 3 Dendroaspis Polylepis polylepis

Fig. 4 Oxyuranus microlepidotus

Table 4. Validation Score

Name of Organism	*Bungarus caeruleus*	*Naja naja*	*Dendroaspis polylepis polylepis*	*Oxyuranus microlepidotus*
Validation Score (%)	84.4	85.6	86.4	90.4

5. Retrieval of phytochemicals:

The total 36 structures of molecules in Rauvolfia serpentina were retrieved from PubChem database and stored in SDF format. The structures of phytochemicals were shown in Fig.5-6

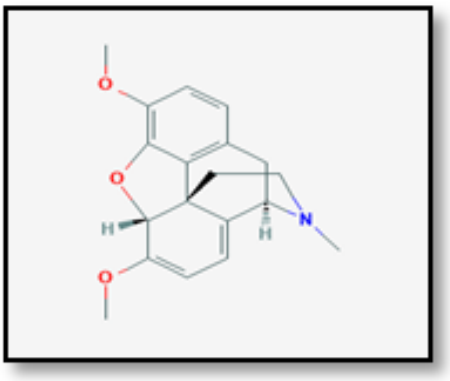

Fig.5 2,6 – dimethoxy benzoquinone

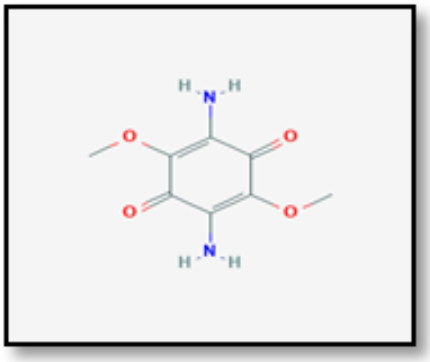

Fig.6 Thebaine

Molecular docking:

All selected 36 phytochemicals were docked into the binding site of each of the four snake venom proteins using Hex6.12 software package. The top ranked hit molecules with each venom protein were selected and results were shown in Table 5. and fig 7.As per table 2,6-dimethoxy benzoquinone is potential lead compound against *Naja naja*, *Dendroaspis polylepis* and *Oxyuranus microlepidotus* and Thebain is potential against *Bungarus caeruleus*.

Table 5. Docking score

Name of phytochemicals	Black mamba	Naja naja	Indian krait	Inland taipan
2,6-dimethoxy benzoquinone	-127.8	-131.1	-16.8	-135.8
3,4,5-trimethoxy benzoic acid	-144.2	-150.0	0.0	-138.1
Ajmalicine	-187.1	-207.9	-27.5	-194.3
Ajmalidine	-162.3	-174.1	-23.9	-168.6
Ajmalimine	-215.6	-227.3	-36.3	-221.7
Ajmaline	-182.5	-198.4	-26.3	-191.0
Alloyohimbine	-181.7	-202.1	-32.5	-200.5
Aricine	-192.5	-209.1	-32.3	-209.1
Coryanthine	-181.7	-202.1	-32.5	-200.5
Desperidine	-262.1	-267.4	-43.2	-263.0
Eudesmic acid	-140.1	-148.2	0.0	-149.6
Isoajmaline	-163.1	-174.1	-22.6	-168.5
Isorauhimbine	-181.7	-202.1	-32.5	-200.5
Isoreserpiline	-246.4	-258.8	-30.1	-260.6
Methyl reserpate	-222.4	-233.5	-38.5	-244.4
Ophioxylin	-192.8	-203.6	-20.8	-200.9
Papaverine	-289.2	-309.0	-59.5	-314.8
Raubasine	-342.9	-349.1	-99.5	-376.4
Rauwolfinine	-162.5	-172.7	-22.9	-166.4
Rauwolscine	-222.9	-172.7	-22.9	-166.4
Rescidine	-264.1	-282.8	-44.0	-273.5
Rescinnamidine	-295.6	-292.1	-70.9	-274.0
Rescinnamine	-384.7	-470.7	-153.9	-432.4
Reserpiline	-268.4	-280.3	-32.5	-282.9
Reserpine	-265.3	-282.7	-45.9	-273.0
Reserpinine	-292.2	-296.6	-70.3	-288.6
Reserpoxidine	-213.4	-238.7	-22.4	-212.5
Sandwicine	-230.7	-243.2	-24.3	-240.3
Sarpagine	-230.7	-198.8	-24.7	-185.7
Secologanin	-203.9	-198.8	-24.7	-185.7
Serpentina	-199.6	-223.3	-23.4	-204.3
Serposterol	-219.9	-239.6	-20.3	-209.7
Tetraphyllicine	-219.9	-239.6	-20.3	-209.7
Thebaine	-170.8	-196.1	-15.1	-172.4
Vomalidine	-179.9	-197.1	-30.9	-176.9

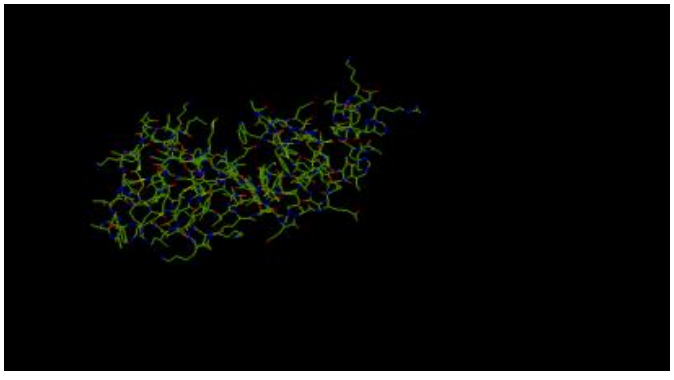

Fig. 7. Molecular Docking

IV. CONCLUSION

Snake bite is one of the most common and many a times potentially fatal phenomenon. Anti-snake venom being the only therapeutic option available, but having many drawbacks, herbal plants provide a solid platform for the natural treatment of this serious issue. Data mentioned above clearly envisage that the herbal medications have excellent potential to treat snake bite. Herbal medicinal plants are an important element of indigenous medical systems globally, due to their safe, non-toxic, cost-effective and ubiquitous nature. The neurotoxin sequences of four snake species were selected for in-silico analysis. In the present study molecular phylogenetics and structural insight of snake neurotoxin species give the detailed knowledge of venom toxin composition and their biological properties. The primary and secondary structures of snake proteins were predicted from ExPaSy server. The 3D structures of these neurotoxins were predicted in SWISS-MODEL server and validated using PROCHECK server. The molecular docking of these neurotoxins with phytochemicals from Rauvolfia serpentina depicts high binding affinity. Further study will carry by identification of binding sites and lead likeness properties through Computer Aided Drug Design (CADD).It is helpful among the researches and venom toxinologists working in this area to search for other substitutive treatment modes to resolve the issues related to snake venom poisoning.

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