RESEARCH ARTICLE

STRUCTURAL UNDERSTANDING OF CYTOTOXIN 1 OF *NAJA SPUTATRIX*: A POTENTIAL ANTICANCER AGENT

Panda Subhamay*[1, 2], Kumari Leena[3], Panda Santamay[2, 4]

[1]Department of Pharmacy, Gupta College of Technological Sciences, Ashram More, G.T. Road, Asansol-713301

[2]Indian Institute of Human and Social Sciences, Sitarampur, Asansol-713359

[3]Department of Pharmaceutical Technology, Jadavpur University, Kolkata, 700032, India

[4]Department of Physics, NSHM Faculty of Engineering & Technology, NSHM Knowledge Campus, Durgapur-713212

* *Corresponding author: E-mail: subhamay_panda@rediffmail.com*

ABSTRACT:

Snake venom cytotoxin from different *Naja* species possesses significant cytotoxic activity on tumor cells. The cytoxin from snake venom can exert a plethora of biological activities including depolarization and muscular cell contraction, lysis of variety of cells such as red blood cells, epithelial cells and fetal lung cells, and also apoptotic activity on certain types of tumor cells. In the present article, we have effectively utilized comparative modeling approach to propose the first molecular model structure of cytotoxin 1 of *Naja sputatrix*. The charge distribution on the structure and distribution of secondary structural elements were also investigated with the aid of *In silico* based approach. A homology structural model of the protein was generated and analyzed to deduce molecular enrichment strategy. The model data and other relevant post model analysis data provides a clear understanding of molecular structure of cytotoxin 1 of *Naja sputatrix* and its relevant cytotoxic potential for the development of a beneficial anticancer natural lead compound.

Keywords: Cytotoxin 1, *Naja Sputatrix,* Anticancer Agent, Snake Venom, Structural model, Malayan cobra.

INTRODUCTION

Over the past several years, the anticancer drugs exhibited their effects on tumor cells by combating cancer cells proliferation. Since the last decade, however, it has been evident that anticancer drugs are capable of inducing apoptosis in proliferating tumor cells and this mechanism is attributable to the exertion of their cytotoxic effects. Consequently, anticancer therapies focus on accelerating apoptosis in cancer cells and possess significant therapeutic activity [1, 2]. Based on this strategy, a plethora of research work is going on globally for synthesizing novel effective apoptogenic compounds or isolating these therapeutic agents from natural sources [3]. However, one of the major drawbacks of these chemical and synthetic anticancer drugs involves numerous toxic side effects and in the present scenario, researchers are focusing on the discovery of potential novel anticancer agents from natural sources such as animal venoms and toxins without possessing any potential necrotic effects [4, 5].

Snake venom comprises of biologically active polypeptides and non-polypeptide constituents [6, 7]. Among the various venomic constituents snake venom cytotoxins and short neurotoxins are generally nonenzymatic polypeptide moieties having a molecular weight of 5-10 kDA [6]. Fifty percent of the dry weight of cobra venom attributes to its cytotoxin constituents [8].

So far, numerous cytotoxins were isolated from different species of snake venom and investigated for their potential cytotoxic activity and other related mechanisms. From the earlier investigations, it was evident that basic residues in cytotoxins structure (namely Lys and Arg) interact with phospholipids of cell membrane and form pores on cell membranes that may result into severe membrane damage and necrotic cell death [9, 10, 11, 12].

WŰster investigated that the spitting cobras of Southeast Asia, formerly known collectively as *Naja sputatrix* (earlier known as Malayan cobra) generally comprises of three distinct species: *Naja sumatrana* (Equatorial spitting cobra) in Peninsular Malaysia and Sumatra, *Naja siamensis* (Indochinese spitting cobra) in Thailand, and *Naja sputatrix* (Javan spitting cobra) in Java and southern Indonesia [13].

Earlier research experimnets on six *Naja sputatrix (N. sputatrix)* cytotoxin isoforms manifested that they revealed varying degrees of cytolytic potency [14]. By comparing the primary sequences of these cytotoxins, it was analyzed that the majority of the conserved amino acid residues plays a vital role in structural organization, forming the three- finger loop conformations, thereby conserving the structural scaffold, whereas the variable residues around the tips of the loops are involved in exhibiting their significant biological activities [15]. Loop II is known to be the most crucial cytolytic domain for these cytotoxins [14].

With this present scenario the objective of this study was to determine a homology based structure of Cytotoxin 1 of *N. sputatrix*. The other contributory objective was to investigate the charge distribution on the structure and distribution of secondary structural elements with the aid of *In silico* based approach. The generated and validated structure may be utilized in the future as a template for the development of vaccine and other beneficial therapeutic development.

MATERIALS AND METHODS

The starting material i.e., amino acid sequence of cytotoxin 1 of *N. sputatrix* was collected from National Centre for Biotechnology Information (http://ncbi.nlm.nih.gov) [16]. Comparative molecular model of cytotoxin 1 of *N. sputatrix* was created with the help of iterative implementation of the threading assembly refinement algorithm [17]. Energy minimization procedure for structural betterments of molecular model of cytotoxin 1 of *N. sputatrix* was performed by Swiss-PDB Viewer [18]. Validation of structural model obtained by comparative modeling strategy was analyzed by PROCHECK algorithm, ProSA and QMEANclust tool [19, 20, 21]. Position of secondary structural components and distribution of positive and negative charge over the structure was performed with the aid of UCSF Chimera package [22, 23].

RESULTS AND DISCUSSION

The three-dimensional structures of the three-fingered toxins (cytotoxins) signify that the backbone of members of this family comprises of a globular core possessing four disulphide bridges and three-fingers emerging from the core. By comparing the structural and evolutionary behavior, it was demonstrated that the globular core retains the overall structure of these toxins, while major structural plasticity appears at the tips of the loops [24, 25, 26, 27]. Recently, several researches

have suggested that the slight alterations in these loop regions might lead to the functional differences among the toxins [28]. By investigating several biological activities of cytotoxins, their cytolytic and/or haemolytic functions are the most widely explored. The lytic activity of cytotoxins is attributable to their binding to cell membranes and resultant damage to the membranes [24].

Snake venom cytotoxins are extremely hydrophobic and basic polypeptides. It is interesting to note that cytotoxins can exhibit a wide range of biological activities, such as depolarization and muscular cell contraction, lysis of several types of cells including red blood cells, epithelial cells and fetal lung cells, and also selective destruction of certain types of tumor cells [29, 30, 31].

PROCHECK analysis or Ramachandran plot evaluation is a good standard for validation purpose of protein structure models. Ramachandran plot for cytotoxin 1 of *N. sputatrix* has been illustrated in Figure 1. In summary 100% of the residues of the cytotoxin 1 structure were observed in allowed and favored regions, which successively substantiate the predication of generated protein structural model. PROCHECK tool also displayed 74.5% of residues in the most favored regions, with 23.5% residues in additionally allowed regions, respectively (Figure 1). This validated that the protein structural backbone dihedral angles, phi and psi, in the cytotoxin 1 of *N. sputatrix*, were reasonably accurate. This asserts that the three dimensional modeled structure of cytotoxin 1 of *N. sputatrix* is commensurate and satisfactory (Figure 2).

As shown in Figure 3 the Z-score (ProSA tool) of cytotoxin 1 of *N. sputatrix* was – 4.39. The result was properly inside the range of scores normally regarded for proteins of equal size, demonstrating highly reliable structures. The QMEANclust algorithm also hints an appropriate quality of the structural co-ordinates of cytotoxin 1 of *N. sputatrix* with a QMEANscore of 0.628 and Z-Score of -0.533.

The visual inspection of cytotoxin 1 of *N. sputatrix* postulates that the total protein is composed by 60 numbers of amino acids. The presence of total number of positively charged amino acids is 11 (Figure 4). In contrast to that the total number of negatively charged amino acids is only 2 (Figure 5). The molecular weight and theoretical pI of cytotoxin 1 of *N. sputatrix* is 6729.2 and 9.38 respectively.
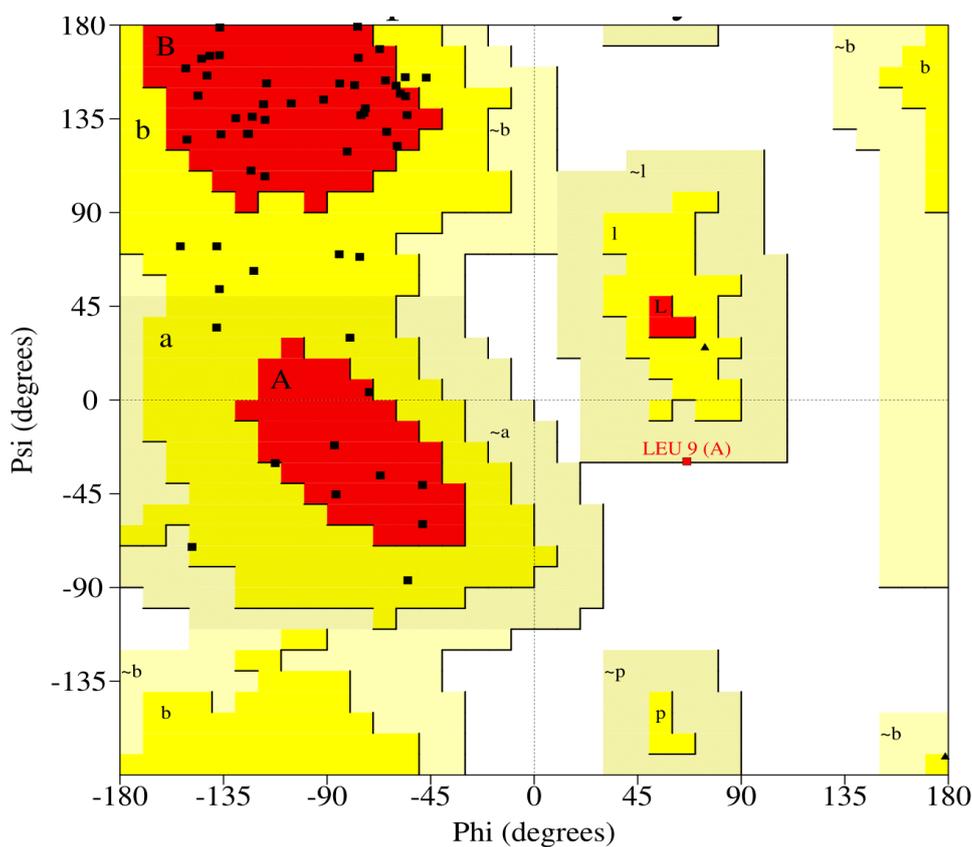
Plot statistics

Residues in most favoured regions [A,B,L]	38	74.5%
Residues in additional allowed regions [a,b,l,p]	12	23.5%
Residues in generously allowed regions [~a,~b,~l,~p]	1	2.0%
Residues in disallowed regions	0	0.0%
	----	------
Number of non-glycine and non-proline residues	51	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	2	
Number of proline residues	5	

Total number of residues	60	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms
and R-factor no greater than 20%, a good quality model would be expected
to have over 90% in the most favoured regions.

Figure 1: Ramachandran plot analysis of molecular model of Cytotoxin 1 of *Naja sputatrix*.

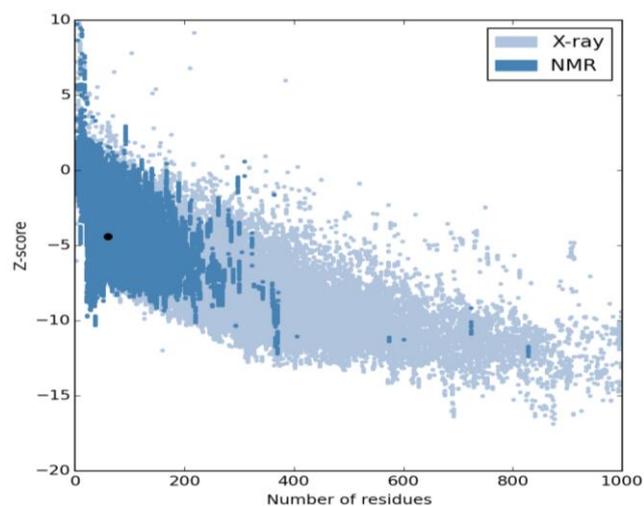**Figure 2: Three-dimensional modeled structure of Cytotoxin 1**

Figure 3: Stereo-chemical analysis of modeled structure of Cytotoxin 1 of *Naja sputatrix*.
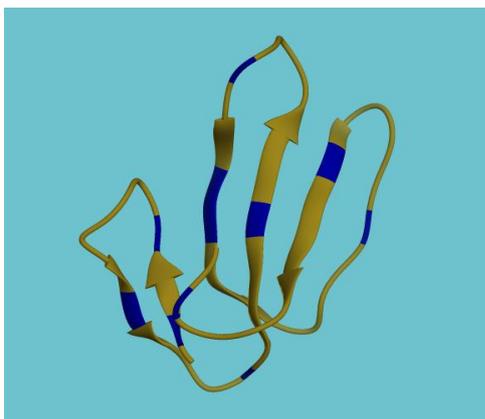
Figure 4: Positively charged amino acid distribution on the modeled structure of Cytotoxin 1 of *Naja sputatrix*.
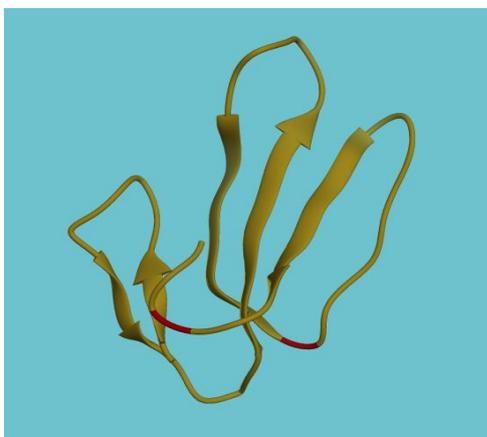

Figure 5: Negatively charged amino acid distribution on the modeled structure of Cytotoxin 1 of *Naja sputatrix*.

CONCLUSION

In the present study, we have effectively employed comparative modeling approach to propose the first molecular model structure of cytotoxin 1 of *Naja sputatrix*. The cytotoxin 1 of *Naja sputatrix* plays a significant role in *Naja sputatrix* venom to assault different preys and in has prospective to turn into a benefical anti-cancer natural compound. Therefore, it would be an interesting approach to deduce its molecular function and propose mechanism of action. Consequently, a homology structural model of the protein was developed and further analysis was carried out to infer molecular enrichment strategy. The model data in addition to other relevant post model analysis data put forward molecular insight to cytotoxic property of cytotoxin 1 of *Naja sputatrix* function towards the clear understanding of anti-cancer natural lead molecule.

ACKNOWLEDGEMENT

DECLARATION OF INTEREST

The authors do not have any conflict of interest.

REFERENCES

1. Reed JC. Dysregulation of apoptosis in cancer. Journal of clinical oncology. 1999; 17(9):2941-53.
2. Ebrahim K, Shirazi FH, Mirakabadi AZ, Vatanpour H. Cobra venom cytotoxins; apoptotic or necrotic agents? Toxicon. 2015; 108:134-40.
3. Extermann M, Chen H, Cantor AB, Corcoran MB, Meyer J, Grendys E, et al. Predictors of tolerance to chemotherapy in older cancer patients: a prospective pilot study. European journal of cancer. 2002; 38(11):1466-73.
4. Mann J. Natural products in cancer chemotherapy: past, present and future. Nature reviews Cancer. 2002; 2(2):143-8.
5. Xiao Z, Morris-Natschke SL, Lee KH. Strategies for the Optimization of Natural Leads to Anticancer Drugs or Drug Candidates. Medicinal research reviews. 2016; 36(1):32-91.
6. Phui Yee JS, Nanling G, Afifiyan F, Donghui M, Siew Lay P, Armugam A, et al. Snake postsynaptic neurotoxins: gene structure, phylogeny and applications in research and therapy. Biochimie. 2004; 86(2):137-49.
7. Panda S, Chandra G. Sequence analysis and phylogenetic study of some toxin proteins of snakes and related non-toxin proteins of chordates. Bioinformation. 2013; 9(5):259-66.
8. Dubinnyi MA, Dubovskii PV, Utkin Iu N, Simonova TN, Barsukov LI, Arsen'ev AS. [ESR study of the interaction of cytotoxin II with model membranes]. Bioorganicheskaia khimiia. 2001; 27(2):102-13.
9. Konshina AG, Dubovskii PV, Efremov RG. Structure and dynamics of cardiotoxins. Current protein & peptide science. 2012; 13(6):570-84.
10. Konshina AG, Boldyrev IA, Utkin YN, Omel'kov AV, Efremov RG. Snake cytotoxins bind to membranes via interactions with phosphatidylserine head groups of lipids. PloS one. 2011; 6(4):e19064.
11. Dubovskii PV, Konshina AG, Efremov RG. Cobra cardiotoxins: membrane interactions and pharmacological potential. Current medicinal chemistry. 2014; 21(3):270-87.
12. Panda S, Chandra G. Physicochemical characterization and functional analysis of some snake venom toxin proteins and related non-toxin proteins of other chordates. Bioinformation. 2012; 8(18):891-6.
13. Wuster W. Taxonomic changes and toxinology: systematic revisions of the Asiatic cobras (*Naja naja* species complex). Toxicon : official journal of the International Society on Toxinology. 1996; 34(4):399-406.
14. Jeyaseelan K, Armugam A, Lachumanan R, Tan CH, Tan NH. Six isoforms of cardiotoxin in malayan spitting cobra (*Naja naja sputatrix*) venom: cloning and characterization of cDNAs. Biochimica et biophysica acta. 1998;1380(2):209-22.
15. Dufton MJ, Hider RC. Conformational properties of the neurotoxins and cytotoxins isolated from Elapid snake

venoms. CRC critical reviews in biochemistry. 1983; 14(2):113-71.

16. Sayers EW, Barrett T, Benson DA, Bolton E, Bryant SH, Canese K, et al. Database resources of the National Center for Biotechnology Information. Nucleic acids research. 2012; 40(Database issue):D13-25.

17. Zhang Y. I-TASSER server for protein 3D structure prediction. BMC bioinformatics. 2008; 9:40.

18. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis. 1997; 18(15):2714-23.

19. Laskowski RA, Rullmannn JA, MacArthur MW, Kaptein R, Thornton JM. AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. Journal of biomolecular NMR. 1996; 8(4):477-86.

20. Sippl MJ. Recognition of errors in three-dimensional structures of proteins. Proteins. 1993; 17(4):355-62.

21. Benkert P, Schwede T, Tosatto SC. QMEANclust: estimation of protein model quality by combining a composite scoring function with structural density information. BMC structural biology. 2009; 9:35.

22. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera--a visualization system for exploratory research and analysis. Journal of computational chemistry. 2004; 25(13):1605-12.

23. Goddard TD, Huang CC, Ferrin TE. Visualizing density maps with UCSF Chimera. Journal of structural biology. 2007; 157(1):281-7.

24. Ma D, Armugam A, Jeyaseelan K. Cytotoxic potency of cardiotoxin from *Naja sputatrix*: development of a new cytolytic assay. The Biochemical journal. 2002; 366(Pt 1):35-43.

25. Brown LR, Wuthrich K. Nuclear magnetic resonance solution structure of the alpha-neurotoxin from the black mamba (*Dendroaspis polylepis polylepis*). Journal of molecular biology. 1992; 227(4):1118-35.

26. Hatanaka H, Oka M, Kohda D, Tate S, Suda A, Tamiya N, et al. Tertiary structure of erabutoxin b in aqueous solution as elucidated by two-dimensional nuclear magnetic resonance. Journal of molecular biology. 1994; 240(2):155-66.

27. Albrand JP, Blackledge MJ, Pascaud F, Hollecker M, Marion D. NMR and restrained molecular dynamics study of the three-dimensional solution structure of toxin FS2, a specific blocker of the L-type calcium channel, isolated from black mamba venom. Biochemistry. 1995; 34(17):5923-37.

28. Ricciardi A, le Du MH, Khayati M, Dajas F, Boulain JC, Menez A, et al. Do structural deviations between toxins adopting the same fold reflect functional differences? The Journal of biological chemistry. 2000; 275(24):18302-10.

29. Teng CM, Jy W, Ouyang C. Cardiotoxin from *Naja naja atra* snake venom: a potentiator of platelet aggregation. Toxicon : official journal of the International Society on Toxinology. 1984; 22(3):463-70.

30. Zaheer A, Braganca BM. Comparative study of three basic polypeptides from snake venoms in relation to their effects on the cell membrane of normal and tumor cells. Cancer biochemistry biophysics. 1980; 5(1):41-6.

31. Dubovskii PV, Utkin YN. Antiproliferative activity of cobra venom cytotoxins. Current topics in medicinal chemistry. 2015; 15(7):638-48.