Review Article

A Review on Biological Toxins: Their Pharmacological Significance and Structural Importance

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ABSTRACT

Toxins are poisonous substances produced by living organisms from emergent microscopic species to well developed species mainly for protection and predation. Biological toxins produce wide range of health effects to mankind, for example, neurotoxins play an essential role by modulating the physiology of nervous system of target species. Toxins are not only harmful but also possess much valuable pharmacological significance and are used for several beneficial applications. The compounds identified against their function will be of great help in preventing lethality due to envenomation. Moreover, neurotoxins are wonderful tools for investigating the ion channel structure and function. Besides toxin bioassays aiming at the molecular mechanism of toxins as well as target biological receptors, the 3D structure prediction using computational molecular modeling approach is considered to be an ultimate methodology and provide an effective solution for the identification of structural and functional role of a massive number of neurotoxins. Moreover it is speculated that the homology modeling of neurotoxin proteins will be an initiative for structure based drug designing.

Keywords: Biological toxins, neurotoxin, homology modeling, 3-D structure, pharmacology.

INTRODUCTION

Toxins from biological origins produce wide range of health effects to mankind. In the recent years toxin bites are increasing in large numbers due to several causes and the cases are reported as mild to severe health effects including partial disability to death[1]. Comparatively animal toxins produce massive number of mortality than plant toxins and microbial toxins.

Biological toxins

Toxins are poisonous substances produced by living organisms from emergent microscopic species to well developed species (Figure 1). Toxin can be of any chemical complexity from simple compound such as formic acid produced by ants to multimillion Dalton protein toxins produced by several bacteria and other living organisms which are mainly used to kill or sedate the other species or cause diseases. Toxins play an important role for the survival of venomous species through successful defense and predation[2].

The term toxin was first used by organic chemist Ludwig Brieger between 1849-1919[3]. Biological toxins are unique biological molecules that are mainly used for protection and predation which affect cells or organs or an entire system of a target species. Most commonly all biological toxins are proteins and especially those are capable of modulating biological process by a number of ways, interacting with the biological receptors and blocking their activities. Some of the toxins are non specific, but most of them have the target specificity.

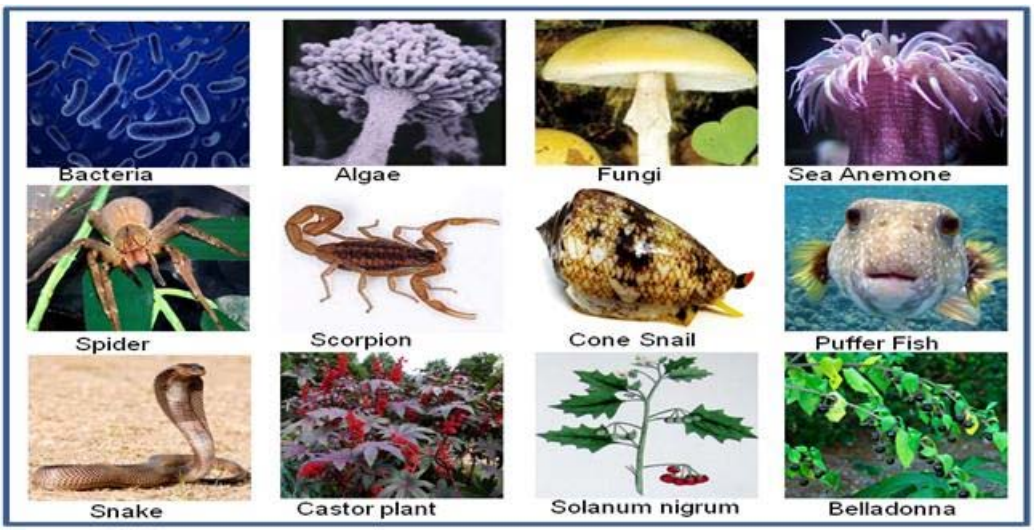

Figure 1: Some sources of biological toxins

Source of biological toxins

Several bacterial species produce large number of toxins. When compared to plant toxins, microbial and animal toxins are reported massive in number. As early as in 1897, van Ermengem identified the botulinum toxin from bacterial origin [4]. Apart from bacterial neurotoxins, many species of fungi also produce toxins called as mycotoxins which affect other living species in different ways. Among the several kinds of fungal toxins, two well known toxins are T-2 and deoxynivalenol (or vomitoxin)[5,6].

Algae are another important source of toxins and algal toxins are considered to be highly poisonous substance which may produce severe damage to the affected species. Saxitoxin is one of the well known phycotoxins that contribute to paralytic shellfish poisoning by inhibiting influx of sodium ions into cells with minimum lethal doses, 25 µg/kg[7].

Scorpions are one of the most ancient animal species with very long evolutionary background and it has more than 1500 species with their conserved morphological characters almost unchanged. Among the 1500 scorpion species, 50 are identified as dangerous to human [8]. In 1971, a huge number of scorpion toxins have been identified and characterized and in later years structural and functional information were derived[9].

Gertsch[10] observed only four species including *L. laeta, L. recluse, L. rufescens* and *L. gaucho* have been shown to be the cause for envenomation in human. Escoubas *et al.*[11] reported that spider venoms contain greater than ten million bioactive peptides based on the strange taxonomic diversity of spiders and the number of existing species predicted to be more than 1,00,000 and the expression that some venoms contain more than 1,000 distinctive peptides. This is a larger pharmacologically important species than that of all other venomous animals.

Types of biological toxins

Toxins are having diverse nature, it includes small molecules, peptides, lipopeptides, cyclic peptides, alkaloids, carbamate alkaloids, organophosphates, proteins, etc. There are two kinds of toxins called as endogenous and exogenous toxins. The endogenous toxins are produced by the body as a byproduct of biochemical processes, and may tend to accumulate in the joins or various muscle groups. The exogenous toxins are ingested or absorbed by an organism into the body from environmental sources, including food, water, etc.[12]

Toxins are classified into four major categories based on their target and mode of action namely hemotoxin, necrotoxin, cytotoxin, and neurotoxin. These toxins act on a specific target and collapse the molecular mechanism of target species.

(i) **Hemotoxins** act on red blood cell (RBC) by lysis activity on erythrocytes. Moreover this type of toxin disrupts blood clotting and destroys the function of blood leading to severe damage to the internal organs and other body tissues.

(ii) **Necrotoxin** is a toxin produced by several venomous species like spider and a few venomous snakes. They cause tissue necrosis especially on epidermal tissues leading to several serious complications to the affected species[13,14].

(iii) **Cytotoxin** is a toxin which has toxic effects on cells which mainly targets a specific type of cell or organ. The cells have been affected and destroyed by a cytotoxin in several different ways including loss of cell integrity in the cell membrane and apoptosis. Cytotoxins are produced by several species including bacteria (Eg. *Helicobacter pylori*), number of plant species and their extracts (Eg. Ribosome inactivating protein or ricin).

(iv) **Neurotoxins** are poisonous toxins play an essential role by modulating the physiology of nervous system of target species. In general, neurotoxins block the transmission of the nerve impulse. Some of the neurotoxins act presynaptically or postsynaptically by binding and blocking the functions of the acetylcholine receptor or ion channels. Among the several types of toxins, neurotoxin causes large number of mortality to the affected species. Neurotoxins are produced by different organisms including several species of venomous snakes, scorpion, etc.

Classification based on ion channels

Based on their target of action, neurotoxins are further classified in to calcium channel toxins, potassium channel toxins, sodium channel toxins, calcium channel blockers, potassium channel blockers, sodium channel blockers, calcium activated potassium channel blockers, chloride channel toxins and so on[15-22].

Functional aspects of ion channels

The history of ion channel research begins in late 1800s. In 1880s Sidney Ringer used a solution of water and ran it via the vessels of an isolated heart from a frog and discovered role of minerals like sodium, calcium, potassium, etc.[23] In 1907, John N. Langley proposed the concept of receptors on the surfaces of nerve and muscle tissues in an attempt to explain the direct effects of certain chemicals such as tetanus toxin on them. The exact mechanism of Na^+ and K^+ ions flow through cell membrane is studied by Cole[24] who developed an instrumentation to demonstrate the function of ion channels. In 1972, Bezanilla[25] proposed the structure of Na^+ ion channels from squid neuron and opening and closing of ionic pores by a "ball and chain" model. Neher and Sakmann[26] proposed the first ion channels and measured their unitary conductance using patch clamp method.

Catterall[27] observed several kinds of neurotoxins targeting Na^+ ion channels. In 1998, the first crystal structure of an ion channel, the K^+ ion channel *KscA* from bacteria is published by Doyle. Several approaches are

developed to explain the structural and functional information of K^+ channels[28]. The three dimensional structure of VGPC shaker from *Drosophila* consists of a tetramer of identical subunits, each contains six transmembrane segments[29].

VGSCs are integral part of plasma membrane proteins composed of a pore forming α-subunit associated with four auxiliary β subunits[16]. Several research studies proved the action of neurotoxins its blocking action on Na^+ ion channels[30].

Ca^{2+} channels are plasma membrane proteins composed of several subunits, including α (1), α (2) δ, β and γ. Although the principal α(1) subunit (Ca(v)α(1)) contains the channel pore, gating machinery and most drug binding sites, the cytosolic auxiliary β subunit plays an essential role in regulating the surface expression and gating properties of HVA Ca^{2+} channels[31]. VGCCs play a key role in regulating diverse cellular functions including neuronal communication[32,33]. Functional hotspots in membrane proteins including several ion channel structures have been studied by Gromiha *et al.*[34]

Mechanism of toxins action

Scorpion envenomation cause severe effects and it induce the release of catecholamines and increased angiotensin-II and also arrest the insulin secretion[35]. In several observed cases scorpion bites also induce the cardiac complications in children and lead to death[36-38]. Malhotra *et al.*[39] described the acute renal failure caused of scorpion venom. Amitai *et al.*[40] revealed the risks of scorpion envenomation to the children and it produces several health effects including pulmonary odema, respiratory distress, myocardial problem, etc.

Gordon *et al.*[41] reported that a lot of highly venomous species targeting ion channels and cause malfunctioning leads to adverse side effects and death. Venom from scorpion consists of various major classes of peptide and short chain neurotoxins (SCNs) are most popular and they targets either K^+ or Cl^- ion channels. But the long chain neurotoxins (LCNs) mostly act on Na^+ ion channels and several studies were carried out by many research groups[22,42,43]. Most of the scorpion neurotoxins target K^+ and Na^+ ion channels to produce acute neurological complications[22,44,45]. Apart from the ion channel binding and modulating action, toxins are used to study the structural and physiological role of ion channels[11].

Pharmacological significance of toxins

Human beings are affected with venomous species bites by accidental way however the life threatening lethality is purely based on envenomation and lethal concentration of the toxin[46]. Toxins are not only harmful for organisms but also possess much valuable pharmacological significance and used for several beneficial applications. It includes treatment of many human diseases like neurological complications, treatment of certain kinds of cancer, pesticides, etc. Huang et al.[47] identified the feasibility of using botulinum toxins produced by a bacterial species called *Clostridium botulinum* as therapeutic agents which is widely used for alleviating several neurological disorders[48]. The active composition of botulinum toxin formulation is named as botulinum neurotoxin type A (BONT-A). Botox is also used for cosmetic procedure carried out in United States since 2008[49].

Another important pharmacological toxin is from marine source, conotoxin from Australian predatory cone snails. Conotoxins have high affinity with neuronal receptors and ion channel proteins in the brain cells as well as nervous system and are used for the pharmacological applications[50,51]. The toxic venom of cone snails consists of large variants of ion channel blockers. The conotoxin discovered and used as a drug for medicinal purposes is ziconotide, an synthetic ω-conotoxin MVIIA act on N-type calcium channels involved in pain pathways and are used as a drug for chronic pain and spinal cord injuries[52,53]. Alpha conotoxins is used as an antagonist of niconitic acetylcholine receptor (nicotinic AChR) which is involved in Alzheimer's disease and Parkinson's disease. κ-conotoxins efficiently block the K^+ channel which is directly linked with hypertension and epilepsy. Apart from this a large number of venomous neurotoxins from marine origin are used to treat autoimmune diseases, analgesics, cancer treatment and so on[54].

Chronic pain is treated with several highly venomous species like spiders, snakes, centipedes, scorpions etc. Snake venom neurotoxins are wonderful pharmacological agents having medicinal values to treat many diseases including thrombosis, arthritis, cancer and many other diseases neuronal aberration[55-57]. For example crotoxin from *Crotalus durissus terrificus* is used as an analgesic, anti-inflammatory, anti-microbial, immune modulatory and anti-tumor agent[58]. Mambalgin from black mamba is used as active blocker of acid-sensing ion channels (ASICs) and used as a potent analgesic agent[59]. Alpha-cobratoxin from the snake venom of *Naja naja* is used as a potential drug candidate for the treatment of pleural mesothelioma[60,61]. It is proposed that neurotoxin from cobra venom can be used for cancer therapy by targeting α7 subtype of nAChRs which are present in most of the cancer cells and they also found the feasibility of using cobra toxins against lung cancer[61]. Australian elapid snakes are used for drug development and the active venom component is named as textilinin, an anti-fibrinolytic agent used to reduce the blood loss associated complexities during surgeries[62].

Highly venomous spiders such as *Phoneutria nigriventer* are also considered to be a rich source of medicinal properties. The cock-tail of toxins acts on more than one ion channels and used as anti-inflammatory agents[63]. Scorpion toxins are also suggested to be a good drug source for a lot of human ailments like human gliomas and tumors therapies[64-66].

Toxins isolated from plant species are also used for several useful applications. Ricin toxin, otherwise called ribosome-inactivating proteins (RIPs) from *Ricinus communis* is found to have potential antiviral, antifungal and insecticidal activity. RIPs have been linked or fused with proper antibodies or other carriers to form immunotoxins[67, 68].

Apart from the pharmaceutical applications of toxins, many neurotoxins are used as potential pest-control agents. Especially toxins, veratridine and brevetoxin, from scorpion which contain alpha-toxins binding at homologous receptor sites on insect sodium channels are the new targets for development of highly selective insecticides[69]. Anti-insect selective peptidomimetic peptide are being developed using venomous toxins[70]. Insect-specific neurotoxin from the scorpion *Androctonus australis* is used to add with fungal insecticide from *Metarhizium anisopliae* has showing excellent activity against some of the harmful insects like *Manduca sexta, Melachacka jeseri, Aedes aegypti* [71,72].

Plants to treat venomous bites

The plant kingdom provides life to many living species in the earth. Medicinal plants are the major sources for the therapeutic remedies of various ailments. Their active phytocostituents are mainly responsible for these potential medicinal effects [73]. Plants are good source of bioactive inhibitors which deactivate toxin actions. Plant derived drugs are used in folk medicine for the treatment of venomous bites caused by highly venomous animals especially, scorpions and snakes[74]. *Aristolochia elegans* is a pharmacologically active plant species, its raw extract shown to have good activity against scorpion sting is used to treat scorpion envenomation[75]. *Ocimum santum*, *Andrographis paniculata* and *Achyranthes aspera* are some of the important plant species that possess several pharmacological properties used in the treatment of scorpion and snake bites[76-78]. Apart from these pharmacologically important plant species *Atropa belladona, Argemone ochroleuca* and *Martynia annua* are also have neutralizing action on venoms of snake and scorpion species[79,80].

Computational studies on toxins

Since many toxicological studies solely depend on structural information which is vital for predicting the functional role and biological mechanisms of neurotoxins the three dimensional structures of toxins from various scorpions and snakes are determined [81-85]. Though experimental structure determination provides 3D structures with high resolution yet time consuming and expensive, large scale structure predictions are always a concern. So combination of experimental and homology model building techniques tend be an significant approach[86].

Computational structure prediction is one of the dedicated ways of predicting large quantity of structures in very short and cost effective manner[87,88]. The computational large-scale structure prediction is one of the distinguished approach for revealing structure and functions which are applied for several research studies and they are vital for drug discovery[54,89,90]. Unfortunately massive number of neurotoxin protein's structures and functions are not determined and it is speculated that the homology modeling of neurotoxin proteins will be an initiative for structure based drug designing[91,92]. The recent developments in homology modeling method delivered high percentage of accuracy and consistent prediction of protein three dimensional structures[93,94].

Another important technique used for molecular function determination is computational molecular docking. Molecular interaction on NTxs and their interacting partners are studied in the past using various levels of computational analysis including molecular dynamics simulation, pharmacokinetics studies, evolutionary relationship analysis etc. and designing of drug candidates[43,95,96]. The evolutionary relationship and divergence of toxins are predicted through using phylogeny using bioinformatics tools[97].

Short chain neurotoxins

The envenomation and intensity of each neurotoxin is unique and varies from species to species[98]. Short chain neurotoxins (SCNs) are the toxic proteins are having around 60-62 amino acids in length. SCNs are specific which are mainly blocks the ligand-binding pocket of nAChR subunits and affects either presynaptic or postsynaptic activity[99]. Neuronal acetylcholine receptors (nAChRs) are very important biological receptors which performs a vital role through transmitting the signals between two adjacent neurons in nervous system[100]. SCNs mainly target the calcium activated potassium (Ca^{2+} activated K^{+}) ion channels[101]. Apart from the toxic activities, SCNs are also having potential pharmacological properties, which can be used to alleviate from usual pain to harmful diseases like multiple sclerosis, cancer, neurological diseases, some of the autoimmune diseases, etc.[61] Most of the SCNs were found in several species of scorpions and snakes along with long chain neurotoxins[22,102].

Scorpion toxins

Scorpion stings are a major public health issue in many underdeveloped and developing tropical countries, especially in Africa, south India, middle east countries, Mexico, south America, etc.[103] Earlier Miranda et al.[104] isolated and characterized eleven neurotoxins from the venoms of the scorpions *Androctonus australis hector*, *Buthus occitanus tunetanus* and *Leiurus quinquestriatus quinquestriatus.* Some of the scorpion toxins whose structures are obtained through computational modelling are as follows:

i) Slotoxin from *Centruroides noxius*

Slotoxin from Maxican Hoffmann scopion *Centruroides noxius*, is a 37 amino acid peptide belongs to the

charybdotoxin sub family which actively blocks Maxi K channels[105]. It reversibly blocks the high conductance Ca^{2+} activated K^+ channels comprised of only alpha subunit and irreversibly blocks the high conductance Ca^{2+} activated K^+ channels composed of alpha and beta-I subunit and irreversibly and weakly blocks high conductance Ca^{2+} activated K^+ channels composed of alpha and beta-IV subunit and has no activity on other K^+ channels or voltage independent Ca^{2+} activated K^+ channels[106]. The positively charged C-terminal surface of slotoxin has a specific short range interaction with negatively charged pore region of K^+ channel leads channel blockade, with hydrophobic residue-residue interaction between slotoxin and MaxiK channels[105]. Calcium ion plays a very vital role in potassium permeability of human blood cells and this channel play several physiological function like heart muscle functioning and smooth muscle activations[38,107,].

The 3D structure of slotoxin is obtained as a result of modelling is shown in Figure 2 which contains a helix and two strands. The six cysteine amino acid residues present in the structure makes conformation of the three dimensional structure more stable. Further analysis shows the helical region of the toxin molecule is mainly involved in receptor binding. The shape complementarity docking of $Ca2^+$ activated K^+ ion channel-slotoxin complex showed some of interesting results that the hot spot residues are actively involved in binding. Among the seven active residues, cysteine and threonine generally participate in receptor binding and conformational change[109].

Figure 2: 3D structure of slotoxin (Uniprot Sequence Id: P0C182)

ii) Tamulotoxin from *Buthus tamulus*

Indian red scorpion (*Buthus tamulus*) is a dangerously venomous species (especially to children)[110]. The active fractions of *Buthus tamulus* or *Mesobuthus tamulus* venom have been characterized to act on various ion channels. Tamulotoxin (TmTx) is a toxin isolated from *B. tamulus* venom and it belongs to the short scorpion toxin super family having 36 amino acids in length and blocks Ca^{2+} activated K^+ channels[111,112]. Tamulus toxins also act on protease inhibitors and histamine releasers apart from channel blocking activity[113].

Figure 3: 3D structure of Tamulotoxin (Uniprot Sequence ID: P0C173).

The resultant structure obtained using computational modelling (shown in Figure 3) shows that a single helix and three extended strands and a coil is found in tertiary level of tamulotoxin protein. The predicted protein structure of tamulotoxin is stabilized by several hydrogen bonds. The predicted tamulotoxin structure is analysed using various tools and from the overall analysis it is identified that the key residues responsible for inhibitions as Thr1, Cys7, Thr8, Lys11, Cys28, Lys31 and Tyr36[114].

iii) Charybdotoxin-C from *Leiurus quinquestriatus hebraeus*

The *Leiurus quinquestriatus hebraeus*, yellow scorpion, envenomation is potentially severe, even it cause death to children by producing toxin charybdotoxin-C (ChTx-C). It is a low molecular weight protein (4318 Dalton) with 37 amino acids and it comes under the category of short chain neurotoxins(SCNs) which greatly affects the Ca^{2+} activated K^+ channels essential for the regulation of several key physiological processes including smooth muscle tone and neuronal excitability[115]. It mainly cause the hyperexcitability of the nervous system especially heart beats of eukaryotes by ionic imbalance. Charybdotoxin-C is highly stabilized with six cysteine amino acids and they are conserved in all animal neurotoxins which are responsible for structural stability and function toxins[116,117].

Figure 4: 3D structure of Charybdotoxin-c (Uniprot sequence ID: P59944)

The homology derived three dimensional structure of Charybdotoxin-c is given in Figure 4. Further analysis

shows that 89.1% of the residues are conserved in Charybdotoxin-C and folding patterns are almost similar with other Charybdotoxin proteins.

Toxin databases

Due to the technological advancements massive amount of data is being accumulating and a result many number of biological databases are evolving and each one concern about a purpose and expertise. Similar the case for toxins and several toxin databases are also available on the web. For example animal toxin database (ATDB) and Tox-Prot, provides the information on toxins which are functionally annotated[118,119]. Toxin target database (T3DB) is a database which provides the basic information on toxin from both natural (from venomous species) and synthetic origin (pesticides, pollutants, drugs and food toxins)[120]. Toxin-antitoxin database (TADB) is an integrated database containing type 2 toxin and antitoxin gene loci and its related genomic information of bacteria and archaea[121]. In addition to these databases, some of the databases are developed specific to the species. It includes Arachnoserver, a manually curated database for spider toxins. This database contains sequential and structural information on arachinidae family toxins (spider toxins)[122]. Conoserver is a database which contains information on most of the marine cone snails and their conopeptides, both sequence and structure[123]. SCORPION database have the information of sequences, structures, and functions of each toxin belong to scorpion species[124,125]. DBETH is another kind of toxin database providing information on bacterial exotoxins and their sequence, structure, interaction network and analytical information[126]. MvirDB database contains information on microbial infection and virulence nature, genes responsible for antibiotic resistance, etc. in a detailed way with their primary data resources[127]. ZINC is a free database of commercially available compounds for virtual screening[128]. Though several toxins databases are available, all are species specific and no particular database is available for neurotoxins. For this purpose, a separate comprehensive resource is being developed which aims to possess information primarily the toxin structural information through computational structure prediction methodologies for all types of animal neurotoxins so as to understand the function of neurotoxins.

CONCLUSION

Biological toxins are unique biological molecules however most of the toxins and their functional roles are still unclear because of the lack of structural information. As one of important fields of science several bioinformatics tools and techniques including computational molecular modelling of toxin protein structures would throw more light on the toxins starting from structure predictions to functional analyses at molecular level.

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