

# Use of Animal Venom Peptides/Toxins in Cancer Therapeutics



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## Abstract

Animal venoms possess variety of toxins/proteins/peptides which act as ionic channel inhibitors and target vital physiological processes. Toxin peptides isolated from honey bee, wasp and scorpion show membrane binding, growth inhibition and strong cytolytic properties. These also inhibit angiogenesis and induce caspase-dependent apoptosis in melanoma cells through the intrinsic mitochondrial pathway protecting the experimental animals against tumor development. These toxin peptides exert cytotoxic effects on human oral cancer cells by inducing apoptosis via a p53-dependent intrinsic apoptotic pathway. These can be used as cancer therapeutic agents by loading them in liposomes. This lead to increase in their target specificity against cancer cells, shorten cell survival, and produce higher reactive oxygen species (ROS), and does enhancement in the number of apoptotic cells. In addition, toxins enhance G0/G1 enrichment upon treatment of cancer cells. Further, encapsulated honey bee, wasp and scorpion venoms exert much potent anti-cancer effect on many cancer cells lines.

Upon slight chemical modifications animal toxins could gear up higher selectivity, show much improved target specificity and lesser toxic effects because of charge optimization. After their improvement they show superiority over the conventional chemical drugs as an increase in net charge of the peptide, its hydrophobicity and anionicity and fluidity of the target cell membranes. It primarily imparts effect through biophysical interaction with the target cell membrane. Toxin peptides are best candidate as they selectively target malignant gliomas and play significant role in GBM immunotherapy. Venom toxins/peptides are good natural sources of compounds/molecules which could act as prototype or template which can be used for development of new therapeutic agents and best tools for diagnosis of not only for cancer but also for other diseases.

**Keywords:** Animal toxins peptides; Channel blockers; Anti-cancer effects; Drug delivery; Therapeutic applications

## Introduction

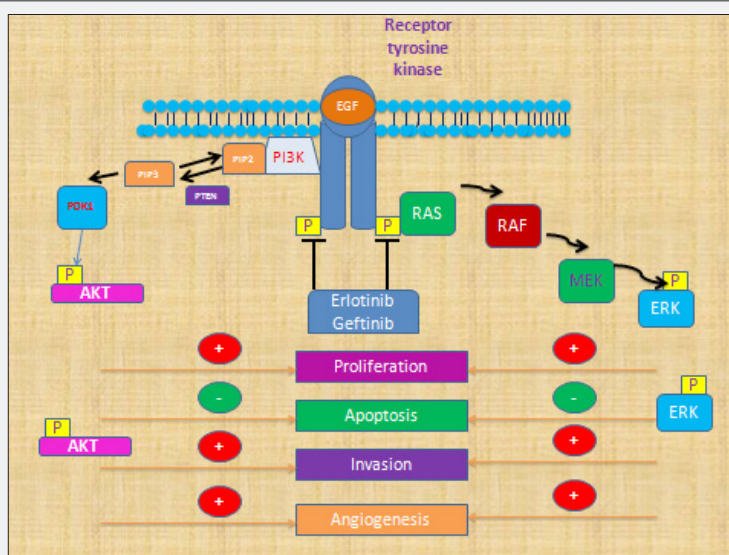


Figure 1: Showing various steps of cancer progression and its strong inhibition sites.

Cancer is a complex disease that shows abnormal cell growth with invasion of surrounding cells and tissues. Cancer is caused by a progressive accumulation of multiple genetic mutations which

are evoked due to environmental stress, microbial infection, food adulteration, smoke, tobacco, ionizing radiation, heavy metal exposure, and multiple genetic reasons. Approximately 5–10%

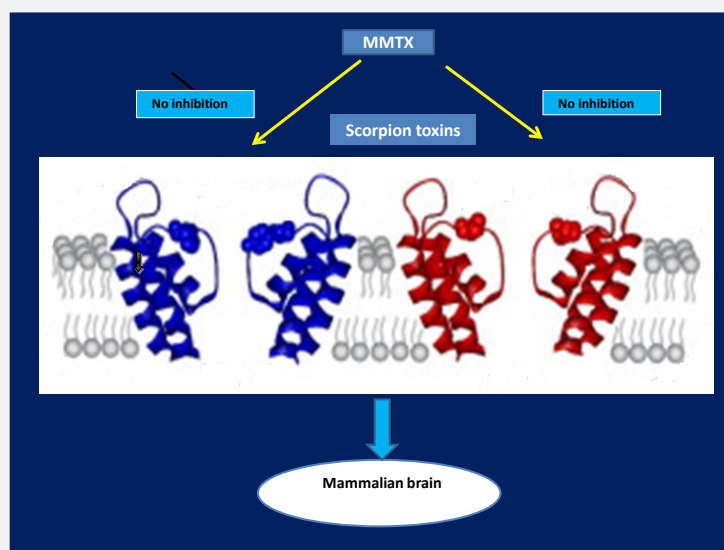
of cancers are due to inherited genetic defects inherited from parents. Cancer is preventable if proper care and therapeutic dietary measures are maintained (Figure 1). Most cancer cells are derived from a single neoplastic cell possessing a regulatory defect, which is inherited by the clonal progeny. Unregulated growth in vivo: Cancer cells do not respond to normal regulatory mechanisms, they can proliferate indefinitely and show unregulated growth. Cancerous activity alters tissue-specific affinity that enables some cancer cells to grow beyond the boundaries of their tissue of origin. These cells can metastasize and grow in diverse tissue sites. Other cancer cells appear to acquire affinity for specific but “wrong” tissues; this property may account for the tendency of certain cancers to metastasize preferentially to particular tissue sites.

There are specific categories of oncogenes that inhibit cellular proliferation and regulate programmed cell death. Some tumors showed altered MHC expression and the wrong biological consequence regarding cell growth and cell proliferation. There are three important attacking points to control cancer invasion of tissues i.e. by activating tumor suppressor oncogenes, stop adhesion of cancer cells, growth and proliferation. Treatment of normal cultured cells with chemical carcinogens, irradiation, and certain viruses can alter the morphology and growth properties of the cells. In some case this process, referred to as transformation, makes the cells able to induce tumors when they are injected into animals; such cells are said to have undergone malignant transformation, and they often exhibit in vitro culture properties similar to those of cancer. They have decreased requirements for growth factors and serum, are no longer anchorage dependent, grow in a density-independent fashion—and they are immortal.

The process of malignant transformation of cells could be used by using animal toxin peptides because they show cytotoxicity to cancer cells and work in a concert at several target sites and show wider therapeutic applications (Figure 1).

Animal venom is unique natural products synthesized by venomous animals. It is complex mixture of various toxins/proteins, peptides, certain elements and acids. Animals inflict venom in self defense or to immobilize the prey for predation and digestion. Venom toxins cause multiple symptoms in stung animals and impart inhibitory effects on electrophysiology of ion channels. They act very fast and also cause respiratory paralysis or cardiac arrest in prey. There are wide varieties of natural toxins, i.e. neurotoxins, myotoxins, cardiotoxins, hematoxins and catalytic enzymes which act by diverse mechanisms on different cell types and cause multiple dysfunctions on the target cell cells, eventually leading to death of the prey [1].

The variety of proteins and peptides have been isolated from honey bee venom and wasp venom which include melittin, adiapin, apamine, bradykinin, cardiopep, mast cell degranulating peptide, mastoparan, phospholipase A2 and secapin. There are several venom proteins and peptides with possible therapeutic applications from bees and wasps and scorpions. Some of these act very fast and target ion channels use as diagnostics and are therapeutically important [2]. Certain naturally occurring toxins from scorpion venom are used as molecular probes for investigating the potassium (K<sup>+</sup>) and calcium binding channels. Many of these toxins are selective for particular K<sup>+</sup> channel subtypes and gear a quick physiological effect on mammalian brain by attacking on cell membranes (Figure 2).



**Figure 2:** Showing action of scorpion neurotoxins on neuron membrane and its effect on mammalian brain.

Wasp venom is also a rich source of therapeutically important toxins which includes short cationic peptides, kinins and polyamines (Table 1). These are used in the treatment of pain, inflammatory disease, and neurodegenerative diseases such as epilepsy and aversion. Mastoparan is  $\alpha$ -helical and amphipathic

cationic tetradecapeptide which is isolated from the venom of the wasp *Vespula lewisii*. It exhibits a wide variety of biological effect mainly tumor cell cytotoxicity and induce histamine release from mast cells. Mastoparan causes melanoma cell death by induction of caspase-dependent mitochondrial apoptosis pathway in murine

model of B16F10-Nex2 cells. It shows cytolytic activity in tumor cells. Upon conjugation to poly (L-glutamic acid) PGA polymer through specific cleavage sequences it binds to over-expressed tumor proteases, such as the metalloproteinase-2 (MMP-2) and cathepsin B and become active.

**Table 1:** Showing Toxin Peptides Isolated from Different Animals, with their Physiological Effects and Therapeutic use.

Animal	Name of Toxin Peptide	Physiological Effect	Toxin Category	Therapeutic use
wasp venom <i>Vespa</i> , <i>Parapolybia</i> , <i>Protonectarina</i> , <i>Polistes</i> and <i>Protopolybia</i> .	Kinins and polyamines	Inflammation	Cytotoxic	Anti-rheumatic dilates blood vessels via the release of
Wasp venom	Mastoparan	Membrane-active amphipathic peptide, it causes the secretion of histamine from mast cells	Cytotoxic, hemolytic insert into the membrane bilayer	Anticancer effects and induce caspase-dependent apoptosis in melanoma cells,
Wasp and honey bee venom	Melittin	Severely acts upon motor nerve terminals and muscle cells	Neuro and cytotoxic, damages skeletal muscles and inhibits cell regeneration	Melittin displays anti-proliferative activity to cancer cells <i>in vivo</i>
Wasp venom	Bradykinin	Impose inflammatory reactions, bradykinin elevate vascular permeability and cause vasodilatation in some arteries and vein	Show biological effects via G protein – coupled receptors.	Antimicrobial and anticancer agents
Honey bee venom and wasp venom	Adiadin, apamine	Polycationic peptides	Antimicrobial and anti-cancer agents	Activities against other gram-negative bacteria and Gram-positive
Wasp and honey bee venom	Cardiopep	Polycationic peptides	Antimicrobial and anti-cancer agents	Hyperalgesic and edematogenic
Wasp and honey bee venom	Mast cell degranulating peptide	Polycationic peptides	Activation of G protein	Antimicrobial and anticancer agents
Wasp and honey bee venom	Phospholipase A2	Polycationic peptides	Cytotoxic	Antimicrobial and anticancer agents
Wasp and honey bee venom	secapin	Africanized honeybee ( <i>Apis mellifera</i> ) venom	Induces inflammation and pain	Potent dose-related hyperalgesic and edematogenic responses
Wasp <i>Agelais pallipes</i>	Protonectin	Promotes mast cell degranulation activity, antibiosis against Gram-positive and -negative bacteria,	peptide isolated from the venom of the, and chemotaxis in polymorphonucleated leukocytes	Antitumor effects, inhibit the growth of both bacteria and fungi
Wasp and honey bee venom	PLA2	Acts synergistically, breaking up membranes of susceptible cells and enhancing their cytotoxic effect	Cytotoxic	PLA2 as a novel anticancer conjugate, hydrolyse the membrane of various cells.
Wasp and honey bee venom	Anoplin (GLLKRIK-TLL-NH <sub>2</sub> )	Candidate because of its small size as well as its antimicrobial and nonhemolytic properties	Cytotoxicity in Friend virus-induced leukemia cells murine erythroleukemia	Novel and selective anticancer agent.
Wasp and honey bee venom	Acyl Polyamines	Non-competitive inhibition at glutamate receptors, leading to paralysis	Neurotoxic	Exhaustive release of neurotransmitters at nerve endings cause
<i>Polibia polista</i>	Phospholipase, hyaluronidases, mastoparans	Genotoxic, mutagenic and damages in the genetic material	Cytotoxic, hemolytic activity, exhibit potent anticancer activity	Broad spectrum antibacterial, and human glioblastoma multiforme cells
<i>Polibia polista</i>	Cationic peptides ( <i>Polibia</i> MP-I)	Antifungal activity	Cytotoxic	Exhibit potent anticancer activity toward human glioblastoma multiforme cells
<i>Vespa tropica</i>	Chemotactic peptides	Antimicrobial activity activate cell mediated antiviral immune response	Cytotoxic	Anticancer agent

<i>Vespa flavitarsus</i>	Hornetin protein	Hemolytic activity in RBCs and presynaptic neurotoxicity	Neurotoxicity, antimicrobial, anticancer agents	Treatment of pain, inflammatory disease, and neurodegenerative diseases such as epilepsy and aversion.
<i>Polibia scutellaris</i>	Hypoallergic variants	Antigen 5 allergen, hypoallergic variants	Cytotoxic	Allergic
<i>Nasonia vitripennis</i>	Polistine peptides,	Reduce thiol group of cystine residue do not form disulphide bridge,	Cytotoxic	Promote programmed cell death, elicited detoxification responses and induced immune responses
<i>Agelaia pallipes</i>	Cationic peptide	Inhibits the growth of gram positive and gram-negative bacteria	Cytotoxic	Antimicrobial activities, anti-tumors drugs
<i>Vespa orientalis</i>	Crude venom	Inhibits the growth of gram (+) and gram (-) bacterial cells	Cytotoxic	Anticancer agent
<i>Vespa affinis</i>	Allergic proteins	Local reaction, IgE mediated anaphylaxis, systemic reaction	Cytotoxic	Anticancer agent
<i>Polibia paulista</i>	Common peptides	Defensive tools to protect the colonies of insects against attacks of the predators.	Neurotoxic	Anticancer agent
<i>Vespa crabo</i> (L)	Crude venom peptides	Local reaction	Allergic	Anticancer agent
<i>Vespa vetulina</i>	Hemostasis-impairing toxins	Allergic and toxic effects	Cytotoxic	Anticancer agent
<i>Nasonia vitripennis</i>	Steroids, cytokine IL-1 $\beta$	Alters the expression of some drug targets, NF- $\kappa$ $\beta$ pathway	Cytotoxic	Anticancer agent
<i>Anopolis samariensis</i>	Alpha-PMTX-13 amino acid peptides	Both excitatory or inhibitory synaptic transmission	Neurotoxic	Anticancer agent
<i>Vespa vulgaris</i>	Phospholipase A, hyaluronidase A, and antigen 5	Severe local swelling accompanied by fever	Cytotoxic	Anticancer agent
<i>Vespa germanica</i>	Phospholipase A, hyaluronidase A, and antigen 5	Severe local swelling accompanied by fever	Cytotoxic	Anticancer agent
<i>Chelonus inanitis</i>	Potentially lineage-specific protein (hyaluronidases and allergen 5)	Parasitoids from encapsulation by hosts nutritional physiology and induce a developmental arrest in pre-pupal stage.	Cytotoxic	Anticancer agent
<i>Agelaia pallipes</i>	Cationic peptides, antimicrobial peptides-crude venom	Antimicrobial inhibits the growth of gram positive and gram-negative bacterial cells.	Cytotoxic	Antitumor activity
wasp and honey bee venom	Polybia-MPI	selectively inhibit the proliferation of prostate and bladder cancer cell	shows good antitumor activity	
Sea snail <i>Conus geographus</i>	$\mu$ - Conotoxin ( $\mu$ - CTX)	Specifically target voltage-gated sodium channels.	Receptor site /Na <sup>+</sup> channel blocker	Propagate electrical signals in muscles and nerves
sea anemone toxins	ASVI, AX I, AX II and AS II.	AS II is one of the most abundant sea anemone toxins	works on mammalian Na <sup>+</sup> channels	Anticancer
<i>Haplopelma hainanum</i>	Hainantoxin IV	Inhibit tetrodotoxin-sensitive voltage-gated sodium channels, thereby causing blockage of neuromuscular transmission and paralysis	Neurotoxins from the venom of the Chinese bird spider binds to sodium channels, block and inactivate them	Anticancer
Spider	Jingzhaotoxin-I	Neurotoxin	Preferentially inhibits cardiac function in rat.	Exhibit better anti-cancer efficacy on the colorectal cancer cell line
North African scorpion, <i>Androctonus australis Hector</i>	Lqh2 and Lqh $\alpha$ II AaH <sup>TH</sup> 4 has	$\alpha$ -insect, antimammalian, and bind to voltage-gated sodium channels and slow down channel inactivation	$\alpha$ neurotoxins	Anticancer

<i>Androctonus bicolor</i> (AB),	AahI-Aahl'-AahII: Toxins of Aah Venom	Induces cell cycle arrest and apoptosis	Exhibits cytotoxicity	Anticancer property
<i>Leiurus quinquestriatus</i> (LQ)	Lqh	Cytotoxic and apoptotic effects	Stimulation of cardiac effects	Toxin acts at the membrane level anticancer
scorpion venom	Chlorotoxin (CTX) peptide	Selectively blocks glioma Cl(-) channel activity	Target malignant gliomas.	Cytotoxic

As it is established fact that toxins/peptides from animal venom typically target ion channels and receptors of both the central and peripheral nervous system, interfering with action potential conduction and/or synaptic transmission. These peptides showed very high degree of sequence homology/conservation of their molecular targets, which makes these toxins active at human receptors. The high selectivity and potency displayed by some of these toxins could be used as pharmacological tools as well as diagnostic probes for detection of morbidity. In present review article important biological effects of toxins, their hydrophobic and ionic interactions, voltage gated channel binding and receptor interactions in cell membranes have been explained. Though animal venom toxins from wasp, bees and scorpion act as potential therapeutic agents and display profound anticancer effects but partial modification in their structure could increase their anticancer potential. These modifications are possible in hydrophobicity, surface charges, and amino acid substitutions in active site regions, topological folding, hydrophobic pockets and

binding affinity. For this purpose, modifications like site-specific mutations and gene rearrangements could provide more active recombinant toxin peptides that will show much cytolytic activity and any drug.

Few antigenic shifts in toxins cold remove their toxicity against normal animal cells and might behave as cell-penetrating peptide that will affect cancer cell proliferation, migration, invasion, apoptotic activity and neo-vascularization. It also highlighted miRNA-based therapeutics, with possible directions for improvement in target specificity of toxins when to be used as a complete therapeutic tool against tumor and cancer. Because of their high selectivity, these toxins can be employed as pharmacological tools in disease diagnosis and therapeutic interventions for gliomas (Figure 3). This article also highlighted encapsulation and conjugation of toxins with onconase, drugs, and lipids to develop new highly efficacious therapeutic delivery system.

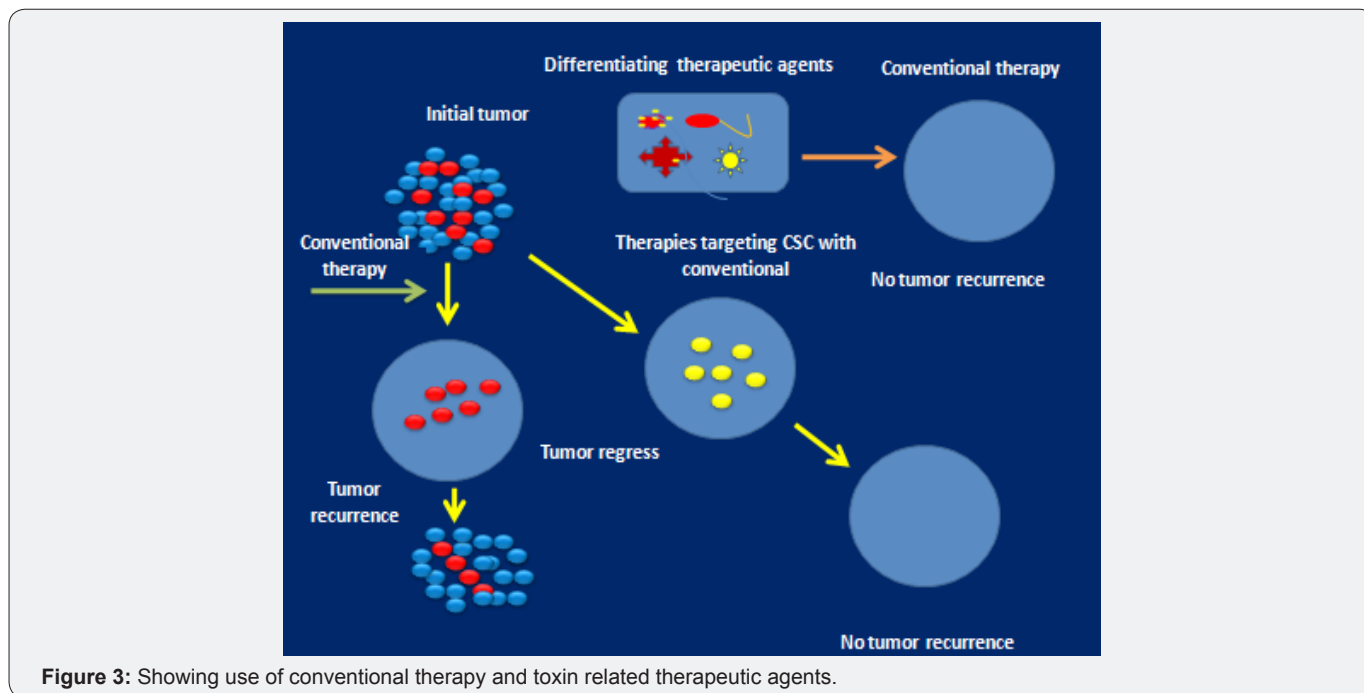


Figure 3: Showing use of conventional therapy and toxin related therapeutic agents.

**Mode of Action**

The mode of action of animal toxins and their physiological consequences vary greatly according to the structural variability in the active site region of toxins. Physiologically animal toxins block ion various channels and breach the normal barrier to free the movement of molecules across cell membrane. Besides this, some toxins are enzymatic in nature and hydrolyze membrane

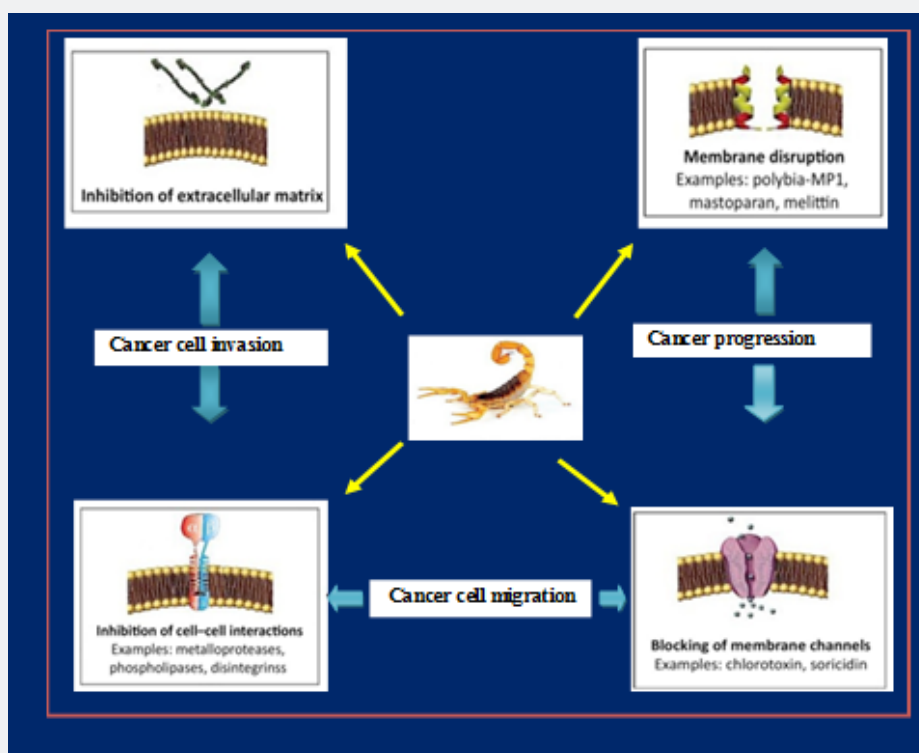
phospholipids and form channels through which small molecules may pass. Melletin of bee venom is an example of such type of toxin. Few toxins cause enormous hemolysis of RBCs and damage nerve cells. Venom toxins specifically act upon neurons, nicotinic acetylcholine receptors and neuromuscular junctions.

A phospholipase toxin severely acts upon motor nerve terminals and muscle cells. It damages skeletal muscles and

inhibits cell regeneration. Different animal groups have different channel inhibitors. Some toxins are inhibitors of metabolic enzymes and have hydrophobic pockets in their secondary structure by which they bind at specific substrate binding sites. Protease inhibitors found in animal venom bind to protease enzymes and prevent their activity. These also inhibit fibrin activity in arthritic joints and induce chronic arthritis in patients. Besides this, venom toxins induce important pathological effects such as immune-modulatory, cardio-respiratory, analgesic and hemopoietic in experimental animals. Moreover, venom factors also cause acute and chronic inflammatory responses in laboratory animals. Histamine inhibits vaso-dilation in mast cells of lungs, liver and gastric mucosa with allergic hyper-sensitivity and inflammation. More specifically citrate present in arthropod venom inhibits phospholipase A2 activity [3].

After few seconds of envenomation toxins cause sudden inflammation in body cells with a severe pain and do massive inhibition of axonal transmission in neurons. Toxins also affect

activity of ATP driven Na<sup>+</sup>-K<sup>+</sup> ATPase pump, which plays a key role in maintaining cell volume and intra cellular ionic composition specially Na<sup>+</sup> and K<sup>+</sup> gradients. This pump actively transports ions across the cell membrane and also helps in excitation of nerves and does phosphorylation and de-phosphorylation in muscle cells. In this mechanism some trans-membrane proteins/enzymes utilize the energy stored in molecules of ATP to move K<sup>+</sup> into the neuron. Na<sup>+</sup>-K<sup>+</sup> pump helps the neurons to maintain resting potential for which pump allows interior negative charge and exterior positive charge on neurons by pumping Na<sup>+</sup> outside the cell and K<sup>+</sup> inside the cell (Figure 4). Both Na<sup>+</sup> and K<sup>+</sup> channels are competitively blocked by these toxins and induce the release of transmitters and cause repetitive firing of the axons (Table 2). Toxins also change the orientation and affinity of ion binding sites change ion permeability mediated by the nicotinic Ach receptors. Snake, neurotoxins such as  $\alpha$ -bungarotoxin and cobra toxin block neuromuscular transmission, affect sodium and calcium exchanges and block ion channels forming a tight ring [4].



**Figure 4:** Shows interaction of scorpion venom toxins to membrane its various phases of disruption.

The toxicity of venom toxins depend upon the sequence of the amino acid residues present in the active site regions, topological folding, hydrophobic pockets and binding affinity. Basically modifications like site-specific mutations and rearrangements occurred in the active site region in response to gradual environmental changes in due course of time have resulted in structural and functional diversifications of toxins. Therefore, different biologically active toxins have been evolved even within a single animal species. However, most of the toxins are identified as neurotoxic, which block various types of ion channels. Different animal groups produce different toxins but each of them shows

significant evolutionary relationship in structure especially in their active site regions.

### Na<sup>+</sup> Channel Inhibitors

Voltage gated Na<sup>+</sup> channels are the key elements of signal transduction in membranes of excitable cells. These voltage-gated channels are transmembrane proteins responsible for rising phase of action potential in most excitable cells (Figure 1). These channels get rapidly activate and inactivate upon depolarization of cell membrane, which result in transient and selective increase in Na<sup>+</sup> conductance. Besides this, Na<sup>+</sup> channels are the targets of

the several kinds of neurotoxins [5] including scorpion toxins. Buthidae family of scorpion contains two major classes -  $\alpha$  - and  $\beta$  toxins among which, Lqh2 and Lqh  $\alpha$  II are the  $\alpha$  neurotoxins that are toxic to mammals and insects respectively [6]. These toxins are the modifiers of the voltage sensitive  $\text{Na}^+$  channels. Lqh  $\alpha$  II is an efficient bacterial expression system of a scorpion  $\alpha$ - neurotoxin with exceptionally high insecticidal activity [7]. Lqh  $\alpha$  II slows the  $\text{Na}^+$  current inactivation in excitable membrane in a manner

characteristic of other  $\alpha$ - toxins [8]. A new anti insect AaHTH4 has been isolated from the venom of North African scorpion, *Androctonus australis* Hector (Table 2A). A similar toxin BMKM 1 is isolated from *E. coli*, which causes paralysis of rear legs, loss of balance, respiratory abnormality, incontinence and eventually death in insects (Table 2A). Different toxin/s groups showed distinct electrophysiological properties, but most of them bind to a common site on the  $\text{Na}^+$  channels of insect.

**Table 2:** Important  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  channel inhibitors in animals.

**Table 2A:**  $\text{Na}^+$  Channel Inhibitor.

Name of Toxin	Name of Species	Effect
$\mu$ -Conotoxin	<i>Conus geographus</i>	Blocks cardiac and skeletal muscle
Toxin I & II (AT X I & II)	<i>Anthopleura xanthogramica</i>	Do
veratridine (APB)	<i>Anemone sulcata</i>	Do
Tetrodotoxin	<i>Tetraodon cutcutia</i>	Acts on human cardiac muscle
Anti-insect Toxin (AaH1 T4)	<i>Androctonus australis</i> (Hector)	Acts on $\alpha$ and $\beta$ sets of mammalian $\text{Na}^+$ Channel
Chrotamint	<i>Crotalus durissus</i>	Local myonecrosis
TSII	<i>Tityus serrulatus</i> (Scorpion)	Induce convulsion in rat
BMKMI	<i>E. coli</i>	Paralysis of rear leg, Loss of balance, respiratory death
AaHTH4	<i>Androctonus australis</i>	Acts on insects $\text{Na}^+$ channel as well as on $\alpha$ and $\beta$ type of the mammalian Sodium channels
Bg II / Bg III	<i>Bunodosoma granulifera</i>	Prolong action potential
Caliotoxin	<i>Calliactis parasitica</i>	Neurotoxic, $\text{Na}^+$ Channel blocker

$\mu$  - Conotoxin ( $\mu$  - CTX) is a receptor site / $\text{Na}^+$  channel blocker isolated from sea snail *Conus geographus* that specifically inhibit  $\text{Na}^+$  flux in skeletal muscles and eel  $\text{Na}^+$  channels with high affinity by physically occluding the channel pores.  $\mu$  - CTX also blocks cardiac and brain  $\text{Na}^+$  channel.  $\mu$  - CTX is a peptide consisting of 22 amino acid residues with six cysteins, which form three disulphide bridges. This toxin blocks cardiac and nerve cell activity. Voltage gated  $\text{Na}^+$  channels are composed of a pore forming  $\alpha$  subunit (26 KDa) and 1-3 auxiliary  $\beta$  subunits (Table 2). The  $\beta 2$  subunit (21 KDa) is disulphide linked to  $\alpha$  subunit while  $\beta$  I (23KDa) subunits are non-covalently attached.  $\mu$ -conotoxins do potential inhibition of tetrodotoxin-sensitive voltage gated sodium channels [9]. Delta conotoxin isolated from *Conus ermineus* selectively act upon vertebrate neuronal  $\text{Na}^+$  channels [10].  $\mu$  -conotoxin ( $\mu$  CTX) isolated from snail *Conus geographus* specially inhibit  $\text{Na}^+$  flux in skeletal muscles and bind to  $\text{Na}^+$  channel with high affinity binding to block the channel pore.  $\mu$  - cono toxin specifically occludes the pore of voltage dependent  $\text{Na}^+$  channel [11].  $\mu$  -CTX is peptides consisting of 22 amino acids residues with six cysteines that form three internal disulphide bonds, impart extreme rigidity to the toxin molecule [12].

Tetrodotoxin is a highly potent poison that is isolated from puffer fish. It (TTX) blocks the conduction of nerve impulses along axons and in excitable membrane of nerve fibres, which lead to respiratory paralysis. Tetrodotoxins are insensitive (TTX-I) to voltage dependent  $\text{Na}^+$  channels and are critical for initial rapid

upstroke of the cardiac action potential and are responsible for most of the  $\text{Na}^+$  current that occurs in mammalian heart. Similarly protein kinase C acts as inhibitor of tetrodotoxin-resistant  $\text{Na}^+$  channels in small dorsal root ganglion and sensory neurons in rats [13]. Besides this, sea anemone toxins interact with large variety of excitable membranes including myelinated and non-myelinated axons. There exist four sea anemone toxins namely ASVI, AX I, AX II and AS II. Among which AS II is one of the most abundant sea anemone toxin that works on mammalian  $\text{Na}^+$  channels. Sea anemones produce cardio and neurotoxins, which mainly cause inactivation of  $\text{Na}^+$  channels, slow down sodium permeability and do prolongation of action potential. In nerve, cardiac and skeletal muscle cell in culture animal toxins effectively inhibit transmission and do make failure of contraction and excitation. Besides these, Bg II and Bg III toxins have been purified and sequenced from the Sea anemone *Bunodosoma granulifera*. BgII specially affect the insect  $\text{Na}^+$  channel (Table 1). Both Bg II and Bg III cause the inactivation of voltage gated  $\text{Na}^+$  channels [14]. ATXII from *Anemonia sulcata* and Ap B from *Anthopleura xanthogrammica* inhibit the inactivation process of  $\text{Na}^+$  channel but shows cardiotoxic and neurotoxic effects.

Similarly Jingzhaotoxin-I a novel spider neurotoxin preferentially inhibits cardiac function in rat [15], target voltage gated sodium channels and inactivate them [16]. Besides this, few paralyzing peptides have been isolated from sea anemone *Bunodosoma caissium* [17] and *Phoneutria nigreventer*. These are identified as neuronal sodium, channel inhibitor and interact

with micro-conotoxin binding sites [18]. Similarly a neurotoxic lipopeptide kalkitoxin interacts with voltage sensitive sodium channels in cerebral granule neurons [19]. Similarly an alpha like scorpion neurotoxin BmK I play an important role in voltage gated Na<sup>+</sup> channels in excitation-contraction coupling of heart rate [20]. Besides this, hainantoxin IV binds to sodium channels, block and inactivate those [21] (Table 2).

Similarly delta-atracotoxin target the voltage gated sodium channels [22]. This polypeptide has a toxic effect on insects and mammals and is capable of competing with anti-insect scorpion toxins for binding to the Na<sup>+</sup> channels of insects. It also modulates the binding of alpha and beta type anti-mammal scorpion toxin to the sodium channels [23]. This toxin shows biological activity only in insects and cause contraction paralysis in them. Electrophysiological and binding studies showed that pharmacological target of anti mammal toxin is the voltage dependent Na<sup>+</sup> channels of excitable cell [24]. Similarly, effects are also noted after inflection of saxitoxin (STX), produced by a marine dinoflagellate. Physiologically TTX (tetrodotoxin), STX (Saxitoxin), and  $\mu$ -conotoxin block Na<sup>+</sup> channels from different tissues with vastly different affinities (Table 2). In contrast brain and skeletal muscle channels show a different magnitude of binding and are more sensitive to TTX and STX than are heart channels found in heart muscles [25].

### K<sup>+</sup> Channels Inhibitors

Potassium channels occur in almost all living organisms and form potassium selective pores which span cell membranes [26]. Due to their high ionic interaction they control a wide variety of cell functions. There is a family of K<sup>+</sup> channel inhibitors that includes charybdotoxin, noxiustoxins, kaliotoxins and iberiotoxins. Among these some are voltage-gated channels and some are pure ionic channels. All these channel inhibitors are short peptides of 28-35 amino acids in length and show different toxicity in different animals. Few scorpion toxins are K<sup>+</sup> channel inhibitors, which bind to a receptor site at the cell membrane or extra cellular vestibule and

prevent conduction of cellular fluid and ion across the membrane. These toxins also variably affect the activity of ventricular myocytes in rat. Iberiotoxin is a small globular polypeptide isolated from *Buthus tamulus*, which shows high potential when added to extracellular fluid [27,28]. The same activity has also shown by TSTX -K isolated from *Tityus serrulatus* [29]. Besides these, there are some low conductance Ca<sup>+</sup> activated K<sup>+</sup> channels, which show mild toxicity. In this category PO5, a polypeptide and apamin, a bee toxin shows similar physiological properties and block K<sup>+</sup> channels. Bmpo2 is a 28 amino acid residues peptide purified from the venom of the Chinese scorpion *Buthus mortensi* Karsh, which shows very low inhibition of apamine sensitive calcium activated potassium channel [30]. Bgk toxin isolated from sea anemone blocks membrane potential and suppresses K<sup>+</sup> current in rat neurons [31] (Table 2B). Another toxin Shk from sea anemone inhibits K<sup>+</sup> channels in T-lymphocytes in very low concentration [32] (Table 2).

Charybdotoxin (ChTX) and naxiustoxin bind to an extra cellular receptor site and prevent ion conduction by occluding the pore. Both toxin bind by a pore binding mechanism established high conductance of Ca<sup>+</sup> activated K<sup>+</sup> channels. It is formed due to presence of similar amino acid sequences especially in active site region [33]. Single amino acid modifications in toxin binding region can either increase or decrease the affinity of a given toxin for a specific channel. ChTX, agiotoxin (AgTX) and kaliotoxin (KTX) are the potent inhibitors of the shaker K<sup>+</sup> channels (Kd <1nM) (Table 2B). A K<sup>+</sup> channel inhibitor containing 37 amino acid residues from the sea anemone *Bunodosoma granuifera* (BgK toxin) shows an apparent Ki of 0.7 nM and suppresses K<sup>+</sup> current in rat dorsal ganglion, while ShK toxin isolated from the sea anemone *Stichodactyla helianthus*, shows an apparent Ki value of 0.7nM for inhibition. Thus ShK toxin also blocks Kv 1.3 type K<sup>+</sup> channels in Jukrat T- lymphocytes at very low concentration. Similarly a new conotoxin superfamily has been identified which act upon K<sup>+</sup> channels [34] and induce confirmation changes in potassium channels [35] (Table 2).

**Table 2B:** K<sup>+</sup> Channel Inhibitors.

Name of Toxin	Name of Species	Effect
BTK - 2	<i>Buthus tamulus</i>	Inhibitor of K <sup>+</sup> channel
Arthropod venom	<i>Apis mellifera</i>	Phospholipase A2
BmBKTx1	<i>Buthus martensi</i>	K <sup>+</sup> blocker
Sq KVIA	<i>Tityus serruiatus</i>	K <sup>+</sup> channel blocker
ChTX	<i>Buthus mortensi</i>	Bind to a receptor site in the extra Cellular vestibule and prevention ion Conduction by occluding the pore
BmTX I	<i>Buthus mortensi</i>	Block high conductance Ca <sup>+</sup> activated K <sup>+</sup> channel
NTX	<i>Buthus mortensi</i>	do
Kaliotoxin	<i>Buthus mortensi</i>	Potent inhibitor for K <sup>+</sup> channels
Iberitoxin	<i>Buthus tamulus</i>	Block squid K <sup>+</sup> Channels
BgK	<i>Bunodosoma granulifera</i>	Suppresses K <sup>+</sup> current in rat dorsal ganglion



ShK	<i>Stichodactyla heliantus</i>	Blocks Kv 1.3 type K <sup>+</sup> channels in Jurkat T-lymphocytes
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© Cl channel inhibitors

Chlorotoxin (ClTx)	<i>L. quinquestratus</i>	Rat colonial epithelial cells
Chlorotoxin (ClTx)	<i>Crotalus scutulatus</i>	Block chloride channels
Chlorotoxin (ClTx)	<i>Ampulex compressa</i>	Blocks synaptic transmission

### Cl<sup>-</sup> Channel Blockers

Chlorotoxin (ClTx) is a small neurotoxic peptide isolated from the venom of *L. quinquestratus*. It contains 36 amino acids with eight cysteine residues [36]. It acts as a specific reversible inhibitor of chloride channels of rat colonial epithelial cells [37]. Similarly toxin isolated from Mojave rattlesnake *Crotalus scutulatus* inhibits calcium channels [38] while toxin from wasp *Ampulex compressa* venom inhibit synaptic transmission and block chloride channels in cockroach [39] (Table 2C). Besides this, hemolysins secreted by pathogenic bacteria are also seem to function like chloride channel blockers which form pores in the plasma membrane of erythrocytes and through these pores pathogens enter inside the erythrocytes and affect ionic permeability and hemoglobin concentration. Another category of extra cellular toxins is leukocidins, which interrupt the phagocytosis of bacteria by leukocytes. Other proteins are streptomycin O and streptomycin S, which attack the cell membrane of many cells (Table 2).

### Action of Animal Toxins on Cancer Cells

Animal venom toxins display profound anticancer effects and are potential therapeutic agents. Toxins purified from snake; bee and scorpion venoms effect cancer cell proliferation, migration, invasion, apoptotic activity and neo-vascularization. Their action mechanism on cancer cells is similar to that of chemotherapeutic agents. Lebein is a snake venom disintegrin which generates anti-angiogenic effects by inhibiting vascular endothelial growth factors (VEGF). Scorpion (*Androctonus bicolor*) venom exhibits cytotoxicity and induces cell cycle arrest and apoptosis in breast and colorectal cancer cell lines. BmKn-2 scorpion venom peptide demonstrates specific membrane binding, growth inhibition and apoptogenic activity against human oral cancer cells.

BmKn-2 toxin peptide isolated from scorpion species is a good candidate which has been therapeutically tested for treatment on oral cancer. It exerts selective cytotoxic effects on human oral cancer cells by inducing apoptosis via a p53-dependent intrinsic apoptotic pathway [40]. It induces potent cytotoxic effects towards both HSC4 and KB cells with the associated induction of apoptosis and showed minimal effects on healthy tissue. The venom of *A. bicolor* scorpion does selective induction of apoptosis in MDA-MB-231 and HCT-8 cells and arrest cell cycle in them. Encapsulated scorpion venoms from *Androctonus bicolor* (AB), *Androctonus crassicauda* (AC), and *Leiurus quinquestratus* (LQ) [41] exhibit better anti-cancer efficacy on the colorectal cancer cell line.

Malignant gliomas are rarely curable malignant tumors arise in the central nervous system. Chlorotoxin (CTX) peptide isolated from scorpion venom, selectively target malignant gliomas. It blocks glioma Cl<sup>-</sup> channel activity and shows anti-angiogenic properties (Table 1). Similarly, an analgesic-antitumor peptide (AGAP), derived from scorpion toxin polypeptides showed antitumor activity. Polybia-MPI shows good antitumor activity [42]. Hemilipin a heterodimeric phospholipase A2 (sPLA2) from *Hemiscorpius lepturus* scorpion venom displays anti-angiogenesis both *in vitro* and *in vivo* [43]. This property remains intact even chemical treatment with p-bromophenacyl bromide that abolishes its enzymatic activity.

### Honeybee Toxins

#### Melittin: an Anticancer Peptide

Bee venom is a mixture of proteins, polypeptides and low molecular weight aromatic and aliphatic constituents in variable amounts. It also contains some important enzymes i.e. phospholipase A, hyaluronidase, acid phosphatase and D-glucosidase which are highly antigenic. Honey bee wasp venom contain many toxic substances such as melittin, adiapin, apamine, bradykinin, cardiopep, mast cell degranulating peptide, mastoparan, phospholipase A2 and secapin. These toxin components have wider therapeutic applications [2]. Melittin triggers lysis of intracellular membrane found in mitochondria while PLA2 and melittin act synergistically, breaking up membranes of susceptible cells and enhancing their cytotoxic effect [44]. This cell damage, in turn, triggers the release of other harmful compounds, such as lysosomal enzymes from leukocytes, serotonin from thrombocytes, and histamine from mast cells which leads to sever pain. Mellitin also interacts with RBC membranes and induce biochemical changes/disorders in lipid protein matrix both in hydrophobic core of lipid bilayer and at polar/non-polar interface of RBC membranes. Moreover, mellitin does rigidization of lipid bilayer and alter reorganization of lipid assemblies and membrane protein rearrangements. It consequently changes the lipid protein interactions. In bees, concentration of mellitin production varies with the weather changes. Honeybee toxins cause allergy by immune-stimulation of the body [45]. These also show antimicrobial [5,46], anti-cancer [47] and antitumor activity both *in vitro* and *in vivo* [48]. Animal toxins act as antibiotics against microbial infections [49,50] (Table 1).

Honey bee venom inhibits significantly nonenzymatic lipid peroxidation and displays a considerable hydroxyl radical

scavenging activity [51]. Melittin causes apoptosis, necrosis and lysis of tumor cells and finally inhibits the tumor growth [48]. It also affects functions of several cell types such renal, lung, liver, prostate, and bladder, breast, and leukemia cancer cells. When melittin is treated with natural detergent it form tetramer aggregates on membranes, it leads to disorders in the structure of phospholipid bilayers. It causes changes in membrane potential, and does aggregation of membrane proteins, and does induction of hormone secretion [52]. It causes membrane disruption and interact with enzymatic systems, such as G-protein [53], protein kinase C [54], adenylate cyclase [55], and phospholipase A [56]. Melittin inhibits calmodulin, a calcium-binding protein that plays a crucial role in cell proliferation [57].

Tumoral cells contain anionic phospholipids, mainly phosphatidylserine on the external leaflet of the plasma membrane [58]. To these leaflets melittin binds preferentially as it possesses cationic charges more than normal cells. It displays anti-proliferative [59,60] and anti-angiogenic activity against cancer cells *in vivo* [61-63]. Conjugation of melittin to an antibody enhances its target specificity and minimizes cellular effects on normal cells. Further, for minimizing the cellular effects pro-cytolytic melittin systems are developed by associating melittin to transporting carriers. However, to enhance action of melittin on cancer cells melittin-based recombinant immunotoxins are prepared by fusion of genes that encode an antibody fragment derived from the murine monoclonal antibody K121 with an oligonucleotide encoding melittin [64]. Recombinant immunotoxins of melittin are also prepared and an anti-asialoglycoprotein receptor (ASGPR) single-chain variable fragment antibody (Ca) is attached to them. It increases target specificity and ASGPR-specific cytotoxicity to hepatocellular carcinoma cells [65]. MMP2 cleavable melittin/avidin conjugates are also (pro-cytolytic) prepared by conjugation with tumor matrix metalloproteinase 2 which usually found over-expressed on cancer cell membranes [66]. Though, when melittin coupled to avidin becomes inactive but upon release from the conjugate it induces immediate cell lysis [67]. Further, mixture of melittin with the anionic detergent sodium dodecyl sulfate formulated into poly (D, L-lactide-co-glycolide acid) (melittin-loaded nanoparticles) shows better inhibitory action against breast cancer MCF-7 cells [68] (Table 1).

Pegylated immune-liposomes are melittin carriers made by coupling to a humanized anti-hepatocarcinoma single-chain antibody variable region fragment loaded with a bee venom peptide fraction [69]. A similar immunoliposome is also prepared by using antibody trastuzumab as targeting agent and melittin as main cargo. Both components combat HER2-overexpressing human breast cancer cell lines [70]. These nanoparticles in spite of goodness are not suitable for systemic administration because melittin is released in blood vessels during transport. It is disrupted by the lytic peptide [71]. Further, ultra-small diameter melittin-nanoparticles ( $\alpha$ -melittin-NP <40 nm) are also prepared which work *in vivo* with few side effects [72,73]. This nanoparticle comprises 1,2 dimyristoyl-sn-glycero-3-phosphatidylcholine

(DMPC) decorated with the hybrid peptide formed by peptide D-4F and melittin via a GSG linker. This peptide D-4F mimics high-density lipoprotein (HDL) [74]. Serine-substituted melittin (Mel-S) showed more cytotoxic effects than asparagine-substituted melittin (Mel-N) against *E. coli* [75]. Interaction between melittin and the red blood cells is electrostatic that also does collapse of the membrane structure and liberation of the cell contents [76]. Though, peptide remains in a low alpha-helical conformation which forces its stabilization.

### Wasp Venom

Wasp venom possesses various short peptides and enzymes which are similar to honey bee. It also contains higher percentage of mastoparan and bradykinins. Mastoparan is a membrane-active amphipathic peptide containing Ile-Asn-Leu-Lys-Ala-Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH<sub>2</sub> in its main amino acid chain [77]. It is rich in hydrophobic and basic residues that form its amphipathic helical structures. Mastoparan possess capacity to make pores in membranes that induces a potent mitochondrial permeability transition that affects tumor cell viability [78]. Its mode of action depends on cell type, but it also involves exocytosis. Upon interaction mastoparan induces secretion of histamine from mast cells, platelets and chromaffin cells secrete serotonin and catecholamines. Its action in the anterior pituitary leads to prolactin release [79]. In histamine secretion, mastoparan interference with G protein activity, stimulate the GTPase activity in subunits. It also promotes dissociation of bound GDP from the protein, enhance GTP binding. Mastoparan shortens the lifespan of active G protein and induces G protein-mediated signaling cascade. It leads to intracellular IP<sub>3</sub> release and maintain influx of Ca<sup>2+</sup> [80]. It inserts into the membrane bilayer causing membrane destabilization with consequent lysis [81]. It also perturbs transmembrane signaling [82-84]. Upon direct interaction it stimulates phospholipases, does mobilization of Ca<sup>2+</sup> from mitochondria and sarcoplasmic reticulum, and causes cell death by necrosis and/or apoptosis [85,86]. Mastoparan induced caspase-dependent apoptosis in melanoma cells through the intrinsic mitochondrial pathway protecting the mice against tumor development [87]. Mastoparan also interacts with the phospholipids found in mitochondrial membrane. It induces permeabilization in cyclosporine A-sensitive and insensitive manners but does not interact with any specific receptors or enzymes [88]. Both PS (phosphatidylserine) and PE (phosphatidylethanolamine) lipids synergistically combine and enhance membrane poration by MP1. Presence of these lipids in the outer leaflet of cancer cells is highly significant for MP1's anticancer action [42] (Table 1).

### Partial Modifications

#### Change in Hydrophobicity

Amino acid substitutions in hydrophobic region of toxins lead to increase in ionic interaction with membranes. Further, hydrophobic interactions also increase target specificity of toxins. Hydrophobic effect is responsible for the separation of a mixture of oil and water into its two components. It also leads to formation of protein complexes with small molecules and also assists in vesicles

formation, protein folding, and insertion of membrane proteins into the nonpolar lipids [89-92]. In proteins hydrophobic amino acids such as alanine, valine, leucine, isoleucine, phenylalanine, tryptophan and methionine are found in cluster. Normally water soluble proteins possess a hydrophobic core in aqueous medium, and its side chains buried in water. It stabilizes and keep protein molecule in folded state. More exceptionally charged polar side chains are situated on the solvent-exposed surface where they interact with surrounding water molecules. More often, protein folding depends on minimum number of hydrophobic side chains exposed to water [93-95]. Further, substitutions of hydrophobic amino acids with non hydrophobic amino acids increase protein folding and formation of hydrogen bonds within the protein which stabilizes protein structure [96,97]. But L-Pro substitutions in polybia-MPI induce significant reduction of antitumor activity because of alpha-helix conformation. Amino acid substitutions in positions 2, 3, 5, 6, 8, 9, and 10 in anoplin analogs increase their surface charge/hydrophobicity that increases hemolytic activity [98].

### Interaction of Toxins with Protein Receptors or Protein-Protein

For maintaining biological and pharmacological activities membrane-peptide interactions are highly important. More often, membrane potential plays a key role in ionic transport across membrane. Most cell membranes are electrically polarized; they possess inside negative charge nearly 260 millivolts (mV). Their excitability depends on ionic transport across membrane and energy conversion mainly chemical transport through impulse conduction. During membrane transport some molecules pass through cell membranes because they dissolve in the lipid bilayer. In animal cells a high concentration of  $K^+$  and a low concentration of  $Na^+$  relative to the external medium are found. This ionic gradient is generated by  $Na^+-K^+$  ATPase pump which operates specific transport system. Upon hydrolysis of ATP this pump provides the energy required for the active transport of  $Na^+$  out of the cell and  $K^+$  into the cell that also generates gradients. ATPase, requires  $Mg^{2+}$  for hydrolysis of ATP.

Modification of surface charges contributes to modulate biological activities of toxins. It also leads to increase the receptor interactions and formation of membrane-peptide complexes [99]. For formation of membrane-peptide complexes, mode of interaction is highly important. It assists in analyzing dynamic properties and the contact residues of the membrane-bound peptide [99]. Mastoparan is an amphiphilic tetradecapeptide extracted from social wasps *Polistes flavus* that is cytotoxic in nature. It interacts with the lipid bilayer using its hydrophobic side chain. This replacement of hydrophobic side chains with basic amino acids found in MP fragments enhanced the inhibitory effects on ACh-evoked catecholamine release [100]. Mastoparan activate GTP-binding regulatory proteins (G-proteins) that couple phospholipase C [79]. Mastoparan-X C-terminal 12 residues take  $\alpha$ -helical conformation upon binding to the phospholipid bilayer. Similar conformation-activity relationships are also observed in mastoparan analogs as activators of G-proteins [101].

Mastoparan, induces various biological functions including histamine release from rat peritoneal mast cells. In this process acidic residues play an important role in modulating the peptides' lytic and biological activities [102]. More specifically, for the activation of mast cells by mastoparan, at least two positively charged side chains are required on the hydrophilic side of the amphiphilic structure of the peptide [103]. Mastoparan enhances the GTPase activity of recombinant G(o) alpha 5-fold in phospholipid vesicles.

### Removing Toxicity of Peptides

Animal toxins despite their toxic effects to mammalian cells they could be used as antibiotics if their toxicity is removed. They can be used template to design new potential drugs. For example inclusion of an arginine and an isoleucine residue at positions 5 and 8 reduce toxicity of mastoparan, turning it into a potential drug for infectious diseases [104]. They have been proved better candidates due to their lesser toxic effects and higher selectivity upon chemical modification and charge optimization. For lowering the toxic effect, the toxin part of the toxin molecule could be modified either into simple non toxic group, or fused to an antibody or carrier ligand. Such molecules can be used to target malignant brain tumor in patients. Contrary to this, amino acid substitution in toxin peptides increases its biological action. When melittin an insect-derived antimicrobial peptide (AMPs) isolated from *Apis mellifera* and *Apis cerana* faces substitution of serine (Ser) to asparagine (Asp). Serine-substituted melittin (Mel-S) showed more cytotoxic effect than asparagine-substituted melittin (Mel-N) against *E. coli*. Similarly, Mel-N and Mel-S showed different inhibitory effects on the production of IL-6 and TNF- $\alpha$  inflammatory factor in BV-2 cells [75].

The specific biological activity of animal toxins can be practically substitution of amino acids. Though all proteins are inactivated by heat treatment but they become pharmacologically unfit. For example low toxicity of the soybean Agglutinin when given orally, as compared to its rather high toxicity when injected, is noteworthy, because the observations with other bean agglutinins are quite different. For reduction of toxic effects of animal toxins non-toxic or random peptides are obtained from SwissProt and TrEMBL and amino acid substitutions are made in natural toxins accordingly. With this minimum mutations in peptides are being made for increasing or decreasing their toxicity, and toxic regions in proteins are cleaved or change to non toxic region.

It is highly advantageous in small peptides because it enhance their specificity, high penetration, and therapeutic efficacy. Further, prediction models are prepared by using various databases and shortening of certain residues like lys, Cys, His, Asn, and Pro are abundant as well as preferred at various positions in toxic peptides. From natural toxin proteins/peptides various motifs are extracted this information is used for development of dipeptide-based model or a hybrid model that shows low toxicity in vivo experiments. ToxinPred is a unique *in silico* method of its kind, which will be useful in predicting toxicity of peptides/proteins. In addition, it will be useful in designing least toxic peptides and discovering toxic regions in proteins (Figure 5).

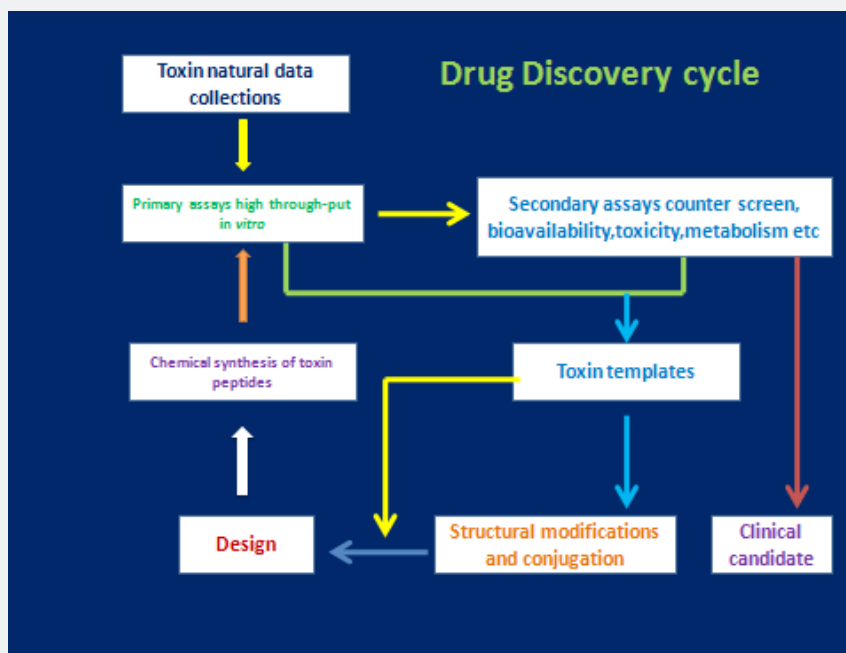


Figure 5: Use of toxin templates for discovery of anticancer drugs.

### Toxin and Lipid Interactions

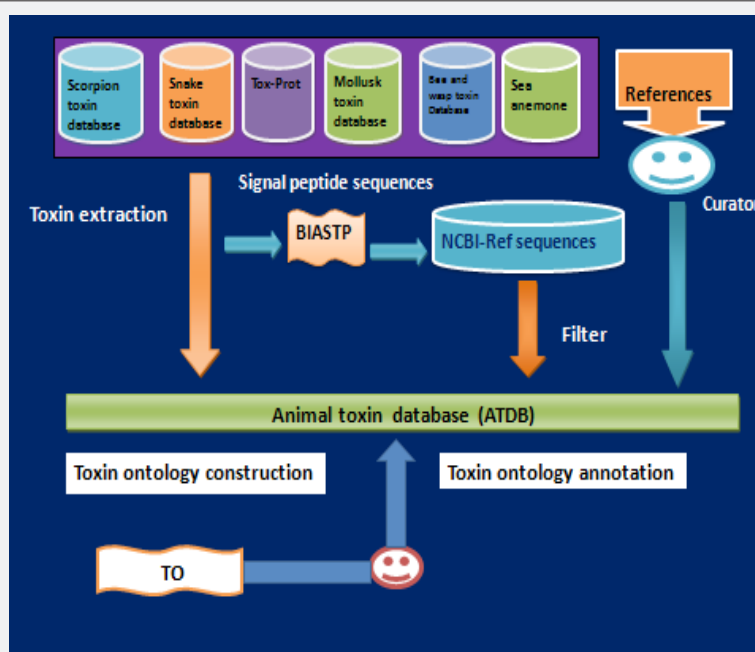


Figure 6: Showing use of structure and activity related information about toxins from animal toxin database for preparing ontology construction.

The maintenance of cell membrane integrity is a dynamic process regulated by the living cell in order to provide discrete extracellular and intracellular compartments and within the cell, separation into subcellular organelles. A major component of this integrity is the preservation of the ionic gradients which are necessary for cell function. Toxin molecule insertion into the lipid bilayer generates pores; disturb its ionic equilibrium mainly fine balance of the ion gradients across membrane. It also

makes changes in the intracellular water content that results in cell death. A large number of toxin peptides interact directly with lipid components (lipid domains) of the cell membrane and form pores [105,106]. These pore-forming toxins are typically amphipathic polypeptides containing both hydrophilic/polar domain (s) and hydrophobic/non polar domain(s) structures. Though, these vary in size from small peptides to oligomers and up to large macromolecules. These pore forming toxins display

increased permeabilization and do disruption of the cell plasma membrane though they show different biological origin, structure or size (Figure 6). The pore formation by toxins leads to a colloid-osmotic process of the cell followed by swelling. This is a primary event in toxin-cell plasma membrane interaction, starts with pore formation. An example is pardaxin is a small pore forming peptide toxin secreted by *Pardachirus marmoratus* fish, which targets the plasma membrane via hydrophobic/lipophilic interactions with plasma membrane phospholipids and penetrates into the cell membrane [107]. Furthermore, pore formation triggers few secondary events such as  $\text{Ca}^{2+}$  overload, PLA2 activation, eicosanoid production, secretion, endonuclease activation, and cytokine release and protein phosphorylation. All these events constitute secondary cascades of toxin action which eventually lead to cell death [108].

Similarly, protonectin, a peptide isolated from the venom of the wasp *Agelaia pallipes pallipes*, promotes mast cell degranulation and chemotaxis in polymorphonucleated leukocytes. It possesses amphipathic features which lead to increase in its biological activity [109]. It shows preferential interaction with the micelle and presents a higher helical structure in aqueous medium. Melittin is a amphiphilic helical molecules that binds with calmodulin. Similarly, mastoporan also binds to the two  $\text{Ca}^{2+}$  binding proteins troponin C and calmodulin [110]. Enzyme phospholipase A2, specifically a soluble form (sPLA2) is secreted in venom of insects, reptiles and mammals. PLA2 belongs to family of housekeeping enzymes that specifically cleave the sn-2 fatty acid of phospholipids attached to the glycerol backbone. This enzyme causes severe damage to the lipid membrane. PLA2 operate in phospholipid cellular catabolism, and does hydrolysis of phosphatidylcholine, generate lyso-phosphatidylcholine and AA [111]. Besides this, membrane-associated iPLA2 also affect number of activities such as lipid metabolism and membrane remodeling, maintenance of mitochondrial integrity, signal transduction, cell differentiation, proliferation and viability [112]. Some of the toxic effects of PLA2 render its binding to specific receptors to which other venom components and the mammalian sPLA2 binds [113]. These sPLA2 enzymes are  $\text{Ca}^{2+}$ -dependent and contain a His-Asp motif in the catalytic domain. Individual sPLA2 exhibit unique tissue and cellular distribution and provide precursors for pro and anti-inflammatory lipid mediator synthesis. It regulates membrane lipid composition. Snake and bee venoms contain different forms of sPLA2 toxins. One of the helices of group III sPLA2 enzymes is hydrophobic that enables its binding to the lipid bilayer membrane [114].

PLA2 found in bee venom consists of a single polypeptide chain of 130 amino acid residues folded by four disulfide bridges and a glycosyl residue at Asp-13 with a molecular weight of 15.8 kDa. The PLA2 from bee venom causes a variety of inflammatory-associated pathologies, including rheumatoid arthritis, septic shock, psoriasis, and asthma [115]. PLA2 stimulates PLA2R1 receptors which mediate the neurotoxic effect. A mild dose of PLA2 (6.2 mg/kg) causes edema, myonecrosis, prolonged the partial thromboplastin time and reducing the plasma fibrinogen

concentration [116]. Similarly, melittin isolated from honeybee *Apis mellifera* is a small linear peptide composed of 26 amino acid residues. Its amino-terminal region predominantly is hydrophobic while its carboxy-terminal region is hydrophilic due to the presence of a stretch of positively charged amino acids. This amphiphilic property is responsible for its cationic surfactant properties and cytolytic activity [117]. Contrary to this, monomeric melittin does not contain sPLA2 activity by itself. Actually, it facilitates the activation of the endogenous cell housekeeping PLA2 activity of the target via rapid binding to the membrane by hydrophobic interaction with zwitterionic phospholipids. This causes disruption of the phosphate group orientation and changes in physical and steric properties of the phospholipid, which result in dislodging of membrane barrier function [117].

PLA2 forms melittin-phospholipid domains after dissolving into the hydrocarbon region of the bilayer. The highly charged C region and the amino phosphate dipole have a strong electrostatic interaction that results in membrane perturbation, rendering the lipid target susceptible to PLA2 cleavage [118]. It also leads to alteration of the membrane surface results in antibacterial and hemolytic activity. Melittin stimulates PLA2 in various cells and tissues, mainly human erythrocytes, anterior pituitary tissue, pancreas, rat adenohypophysis, vascular endothelial cells, human leucocytes, platelets [119] and neurons [120]. Melittin dansylated analogue (DNC-melittin) binds to natural membranes and show cytolytic activity [76]. Due to these properties melittin is used as a neurochemical tool to recognize the role of sPLA2 in modulating synaptic transmission [120]. Bee venom group III sPLA2 shows protective immune responses against a wide range of diseases including asthma, Parkinson's disease, and drug-induced organ inflammation [121]. This is widely used as a component of anti-venom and for desensitization therapy against bee stings [122]. Due to membrane penetration activity and cytotoxic effects melittin is used in cancer therapy. It is used either as an active ingredient or as an absorption enhancer [123].

### Interaction with the Plasma Membrane

Voltage-gated ion channels open or close depending on the setting of voltage gradient across the plasma membrane. Normally for vital functions ions are conducted through the channels down their electrochemical gradient. It depends on ion concentration and plasma membrane potential. These ion channels at large are selectively targeted by certain toxins scorpion [124] and snake venoms [125]. Voltage-Gated Potassium Ion Channels (Kv) The voltage-gated  $\text{K}^+$  channels are trans-membrane channels specific for  $\text{K}^+$  ions and are sensitive to voltage changes in the membrane potential [126]. These channels are known mainly for their role in repolarizing (voltage-dependent Kv) the cell membrane following action potentials, which regulate cellular processes such as  $\text{Ca}^{2+}$  signaling, cell volume, secretion, proliferation and migration [127]. Kv channels usually have a homo-tetrameric structure. The transmembrane domain, the  $\alpha$ -subunit, consists of six helices (S1-S6) forming two structurally and functionally different parts

of the tetrameric channel: a potassium ion-conducting domain (pore domain), helices (S5–S6) located in the channel center and a domain sensible to changes in the membrane potential (voltage-sensing domain, VSD), helices (S1–S4) located on the channel periphery. A large number of polypeptide ligands of Kv channels have been found in the venoms. Apamin and Tertiapin from bee venom target mostly KCa and Kir channels; while from scorpions Charybdotoxin and Kaliotoxin, target KCa channels.

Ion channels are important portals for a variety of toxins interacting with the plasma membrane [128]. Under normal conditions, the physiological role of these channels is to regulate with high selectivity, the transfer of cations or anions in order to maintain a resting membrane potential and to control action potentials in excitable tissues. There are many different types of ion channels, classified by their gating characteristics and by the type of ion passing through. These channels very effectively transport ions in a time frame period of milliseconds, often rising to 10<sup>6</sup> ions per second or greater. In view of the importance of ionic gradients for maintaining neuronal, cardiac, skeletal or smooth muscle tissue functions, numerous toxins, which modulate ion channel conductance and/or kinetics by serving as either channel openers or blockers with different selectivity, have been investigated. It has been well established that most Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and some Cl channels, are voltage-gated [129] but others including certain K<sup>+</sup> and Cl<sup>-</sup> channels, transient receptor potential (TRP) channels, ryanodine receptors and IP3 receptors, are relatively voltage-insensitive and are gated by second messengers and other intracellular and/or extracellular mediators [130].

### Enhancing Cytolytic Functions

Both ionic and peptide interactions assist in enhancement of cytotoxicity in cells. Melittin is an example that it from different conformational change in aqueous medium than mastoparan. In an aqueous solution, melittin went from a nonhelical form to an alpha helix when phosphate was added to the solution. The binding of melittin to the membrane results from electrostatic interactions, not hydrophobic interactions. Animal toxins act on several major portals consist of ion channels, pumps, transporters and ligand gated ionotropic receptors and disturbing the intracellular ion homeostasis. Scorpion toxins interact with group of portals consists of G-protein-coupled and tyrosine kinase receptors. After interaction with toxins, cell machinery alters second messengers towards pathological levels. Toxins also affect metabolic functions of subcellular organelles such as mitochondria, nucleus, protein and RNA-synthesis machineries, cytoskeletal networks and exocytic vesicles. A fundamental similarity occurs in different toxins with respect to the site of action and the secondary messengers and signaling cascades they trigger in the host; hence toxins are grouped according to their nature of action. But interaction with the initial portal is largely determined by the chemical nature of the toxin. After incorporation or import of toxin inside the cell, several ubiquitous second messengers and protein kinases/ phosphatases pathways are impaired, which also justify toxic effects? Therefore, toxins represent one of the most promising natural molecules for developing novel therapeutics

that selectively target the major cellular portals involved in human physiology and diseases.

Besides, cell cytoplasm, cytoskeleton, mitochondria play a major role in regulating cell death, which occurs upon permeabilization of their membranes. Once mitochondrial membrane permeabilization (MMP) occurs, cells die either by apoptosis or necrosis. MMP is regulated by levels of calcium, and cellular redox potential mainly levels of reactive oxygen species. Besides this mobilization and targeting to mitochondria of Bcl-2 family members are also important factors [131]. Melittin can induce apoptosis of human gastric cancer (GC) cells through the mitochondria pathways, and it may be a potent agent in the treatment of human GC [132]. BV treatment can be useful for protection of neurons against oxidative stress or neurotoxin-induced cell death. BV treatment inhibited the activation of JNK signaling and cleaved caspase-3 related to cell death and increased ERK phosphorylation involved in cell survival in rotenone-treated NSC34 motor neuron cells [133]. Melittin is one of the best-studied antimicrobial peptides, and many studies have focused on the membrane underlying its membrane-disruptive activity. melittin is involved in the mitochondria- and caspase-dependent apoptotic pathway in *C. albicans*. Our findings suggest that melittin possesses a dual antimicrobial mechanism, including membrane-disruptive and apoptotic actions [134].

From wasp and honey bee venom potent cytotoxic peptides can be derived that could used after surface modification and to give them target specificity to kill tumor cells. Both mastoparan and mitoparan are cell-penetrating peptides (CPPs) have potential pharmaceutical application in delivering macromolecules into cells [135]. CCPs also affect transport of doxorubicin encapsulating Tf-liposomes across BBB [136]. Transportan and some derivatives have the capacity to carry macromolecules [137-139]. Although mastoparan showed efficient translocation, the other CPPs, namely TAT peptide and penetratin, were more efficient. On the other hand, there is a chimeric galanin-mastoparan peptide called transportan, which contains the first 13 amino acids from the highly conserved amino-terminal part of galanin and the 14 amino acids sequence of mastoparan in the carboxyl terminus [140, 137].

For targeting can cancer cells recombinant molecules are being made which are used as targeted toxins, also known as immunotoxins or cytotoxins. These specifically bind to cell surface receptors which are over expressed in cancer and the toxin component kills the cell. These recombinant proteins consist of a specific antibody or ligand coupled to a protein toxin. The targeted toxins bind to a surface antigen or receptor over expressed in tumors, such as the epidermal growth factor receptor or interleukin-13 receptor. For lowering the toxic effect, the toxin part of the molecule is modified from bacterial or plant toxins, fused to an antibody or carrier ligand. Such molecules can be used to target malignant brain tumor in patients. By following similar approach However, non-toxic analog of apamin are made that bears two ornithine residues instead of arginines residues (Ap00) [141].

Bee venom can be used by acupuncture method to carry it to relevant sites in function of a specific disease or to acupoints. Melittin tagged with an endosomolytic agent linked to siRNA delivery system effectively stop hepatitis B virus infection [142]. Another molecule mitoparan, transports this potent cytotoxic peptide to the tumor and allows its accumulation in a controlled manner [143]. Another promising and feasible idea tested *in vivo* to combat cancer has been presented in the form of a patent, which discloses an ultra-small lipid nanoparticle carrying melittin with potential use in clinical practice [73]. Besides cancer treatment wasp venom kinins, polyamines are used in treatment of pain, inflammatory disease, and neurodegenerative diseases such as epilepsy and aversion [86].

Anoplin shows very high cytotoxicity efficacy, arrests MEL cells (murine erythroleukemia) in the G<sub>0</sub>/G<sub>1</sub> phase but it could not induce apoptosis in MEL cells [144]. It inhibits proliferation of MEL cells in a dose-dependent and time-dependent manner via disrupting the integrity of cell membrane. Similarly, MP-1, is short cationic  $\alpha$ -helical peptide isolated from the venom of the *Polybia paulista* wasp, is more toxic to human leukemic T-lymphocytes than to human primary lymphocytes. It shows higher content of anionic lipids increases the level of binding of the peptide to bilayers. Polybia-MPI exhibits excellent anticancer activity and remarkably suppresses the growth of sarcoma xenograft tumors [145]. Polybia- MPI /P takes a standard  $\alpha$ -helix conformation in the membrane which is important for its cytotoxicity on tumor cells [146]. The L-Pro substitution induces a significant reduction of antitumor activity. Polybia-MPI can be used for treatment of prostate cancer and bladder cancer, because of its lower cytotoxicity to healthy body cells [147].

### Scorpion Venom

The scorpion venom is a highly complex mixture of salts, nucleotides, biogenic amines, enzymes, mucoproteins, as well as peptides and proteins (e.g. neurotoxins). Scorpion venom is composed of water, salts, small molecules, biogenic amines, peptides, proteins and enzymes. It also contains variable amount of different toxins such as neurotoxins, cardiotoxins, nephrotoxins, and hemotoxins. It also contains some enzymes i.e. phosphodiesterases, phospholipases, hyaluronidase, glucosaminoglycans some other bio-chemicals such as histamine, serotonin, and tryptophan and cytokine releasers. The main functions of the abundant polypeptide toxins present in scorpion venoms are the debilitation of arthropod prey or defense against predators. Scorpion venoms are rich source of potential novel antitumor and anti-cancer therapeutic agents [148]. Scorpion venom fractions and toxin peptides can decrease cancer growth, induce apoptosis and inhibit cancer progression and metastasis *in vitro* and *in vivo* [149]. These toxins modulate the ion channels either by blocking the pore of the channel or by altering the voltage gating. Molecules which block the pores have been useful in deciphering the structure of the ion channels. Many scorpion toxins have already been used for probing the voltage gated sodium channels [148].

Based on their molecular size and pharmacological activity, toxin peptides are classified in to two main groups. The first group contains short toxins consisting of 30-40 amino acid residues with 3-4 disulfide bridges, which mainly affect the voltage dependent K<sup>+</sup> channels, and the large conductance calcium activated K<sup>+</sup> channels [150]. The second group includes long chain toxin peptides of 60-70 amino acids cross linked by 4 disulfide bridges, which mainly have an effect on voltage dependent Na<sup>+</sup> channels of excitable cells [151,152]. Insect toxins are  $\alpha$  insect toxins which are excitatory in nature and show depressant activity [153]. Several toxins, which specifically affect Na<sup>+</sup> and K<sup>+</sup> channels, have been exclusively studied with respect to their structure, mode of action and pharmacological properties [154,155]. In addition to it some toxins that specifically target Ca<sup>++</sup>, K<sup>+</sup> and Cl<sup>-</sup> channels have also been isolated [150,156] (Table 2). A 4.5 kD peptide toxin was isolated from the venom of *Palamneus gravimanus*, the Indian black scorpion, to block human Kv1.1 channels expressed in *Xenopus laevis* oocytes. This toxin peptide selectively blocks the human cloned voltage gated potassium channel at a very low concentration (10 nM). This blockage was found to be voltage dependent. Same peptide has shown typical hypertensive symptoms and showed a LD<sub>50</sub> value of 2-mg/kg mice (Table 1).

Venom peptides isolated from several scorpion species showed multiple biological activities such as cytotoxic, antiproliferative and apoptogenic effects on cancer cells [157]. Scorpion venom from *Androctonus australis hector* (Aah) and its toxic fractions (FtoxG-50 and F3) shows strong effect on NCI-H358 human lung cancer cells [158]. BmKn-2 exerts selective cytotoxic effects on human oral cancer cells by inducing apoptosis via a p53-dependent intrinsic apoptotic pathway [40]. Similarly, *A. bicolor* venom exert cytotoxic effects on MDA-MB-231 and HCT-8 cells in a dose- and duration-dependent manner and induced apoptotic cell death. *A. bicolor* venom arrests the cancer cells in G<sub>0</sub>/G<sub>1</sub> phase of cell cycle and induces apoptosis in breast and colorectal cancer cell lines [159] (Table 1).

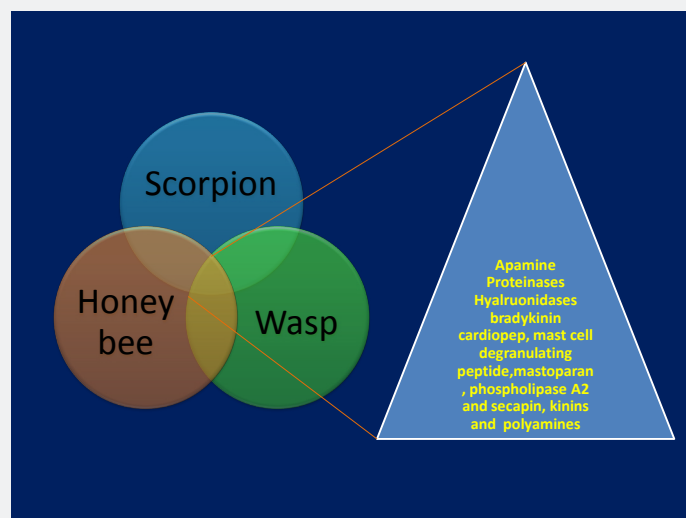
These venom peptides are highly active and specifically target all major ion channels such as Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, Ca<sup>++</sup> and ryanodine sensitive Ca<sup>++</sup> channels [160] and their subtypes both in vertebrates and invertebrates [161] (Table 2). The devastating potency of these toxins causes massive and recurring depolarization of nerve and muscle cells simultaneously makes prey disables or kills it. The most potent animal toxin is the neurotoxin, which occurs in form of both short and long chain low molecular weight peptides. Both are heat stable and cause cell impairment in nerves, muscles and alter ion channel permeability. The long chain polypeptides of neurotoxins cause stabilization of voltage dependent sodium channel and induce repetitive firing of the somatic, sympathetic and parasympathetic neurons. This repetitive firing results in autonomic neuromuscular excitation and prevents normal nerve impulse transmission. Further, it results in the release of excessive neurotransmitters such as acetylcholine, glutamate, aspartate, epinephrine and norepinephrine. The binding of neurotoxins to the host is reversible, but different neurotoxins have different affinities. The stability of these neurotoxins is due to presence of

four-disulfide bridges and compact three-dimensional structure. There are some chemicals, which can break the disulfide bridges and inactivate the toxin by causing it to unfold. However, the antigenicity of these toxins depends on the length and number of exposed regions that are striking out of the three-dimensional structure. Being heterogeneous mixture, scorpion venom accounts for the variable reactions in envenomated patients. Further, various constituents of the venom may act directly or indirectly and individually or synergistically to exert pharmacological effects. Though scorpion species show differences in amino acid sequences but have more similar immunological properties. Thus any modification in the amino acid sequence may result in the modification of structure, which ultimately alters immune function of the toxin.

After scorpion envenomation there occurs a heavy decrease in glycogen content of liver and cardiac and skeletal muscles in rabbits. Scorpion venom also induces diabetes destroying the islets of Langerhans or beta cells and increase triglyceride level in rabbits. It also causes hyperglycemia, hypercholesterolemia and increases the level of free fatty acids. Therefore, increased utilization of circulating FFA also enhances oxygen consumption. It also leads to aggravate the ischemic injury to myocardium, predisposing to arrhythmias and heart failure. Scorpion envenomation also alters insulin secretion and disturbs the insulin/glycogen ratio, which generates an anabolic state with more nutrient incorporation in peripheral tissues. Severe scorpion envenomation causes an autonomic storm

and induces release of catecholamines. Important symptoms and signs of scorpion envenomation are vomiting, profuse sweating, absence of pain to severe excruciating pain at the site of the sting, increased salivation, generalizing tingling and numbness, priapism, tachycardia, trachypnoea, transient hypertension followed by hypotension and pulmonary edema. Besides this, scorpion envenomation by *Mesobuthus tamulus concanensis* Pocock) envenomation causes cardiac sarcolemmal defects displayed by alterations in  $\text{Na}^+\text{K}^+$  ATPase and  $\text{Ca}^{2+}$  ATPase activities, initial hypertension followed by hypotension and shock, glycogenolysis in atria, ventricle, liver, skeletal muscle, hyperglycemia, increase free fatty acid levels and reduction in triacylglyceride levels. An altered erythrocyte  $\text{Na}^+\text{K}^+$  ATPase activity is also observed in scorpion-stung patients, which result in osmotic fragility of red blood cells, insulinemia and pancreatitis. It also enhances thyroid hormones, cortisol, and angiotensin II levels and also increases the clotting time. It also causes acute reduction in platelet counts and increased lactic acid and ketoacid levels. All above changes cause multiple organ system failures along with cardiogenic pulmonary edema and Adult Respiratory distress syndrome in patients. After administration of anti-venom all such changes could make reversal. Anti-venom resulted in reversal of ECG, reduction in LDH, SGOT, SGPT, CK MB and alpha HDBI enzyme levels. It also revert the blood pressure to normal and stop the hypotension. It also cause normal glucose level and do lipogenesis following anti-venom therapy. Anti-venom successfully neutralizes the venom in circulation and other body compartments.

### Development of Toxin Based Therapeutic Drug Delivery System



**Figure 7:** Showing biological composition of wasp, honey bee and scorpion peptides.

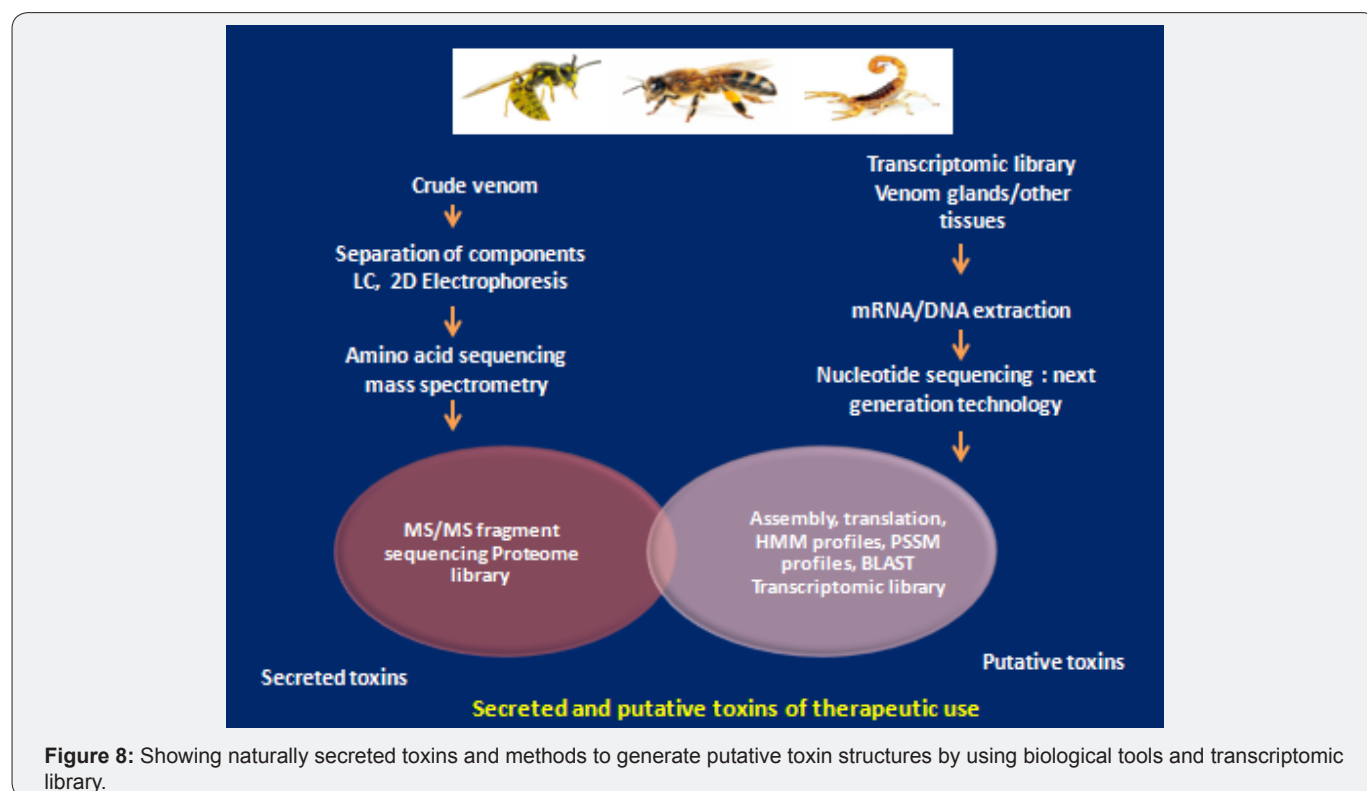
Animal toxins are natural weapons which display non-specific cytolytic activity, rapid degradation and excretion when injected in blood. Venom peptides present in wasp and honey bee showed immense cytolytic against cancer cells when conjugated as carrier. A pro-cytotoxic systems was made by using bee venom cytotoxic peptides and its conjugation with poly (l-glutamic acid) PGA polymer through specific cleavage sequences. These were found quite sensitive to over-expressed tumor proteases, such as the metalloproteinase-2 (MMP-2) or cathepsin B. After

their selective release cytolytic peptides inside tumor cell operates a spatiotemporal control over tumor cells and kill them successfully [143]. These nanoparticles (NPs) conjugated with toxin peptides showed high stability, uniform size, sufficient drug loading, targeting capability, and ability to overcome drug resistance [162]. NP formulation are used to target glioblastoma multiform (GBM). CTX loaded on NPs enhance the uptake of the NPs by GBM cells. It also improves the efficacy of PTX in killing both GBM and GBM drug-resistant cells. A nano complex IONP-



PTX-CTX-FL displayed great potential for brain cancer therapy [162,163] (Table 3). Besides natural toxin peptides synthetic analogues of each peptide, were engineered which also showed enhanced cationicity and amphipathicity. These also showed potent anti-proliferative activity against a range of human cancer cell lines [164]. Scorpion venom components are also used to make scaffolds for the development of drugs [165] (Figure 5). From available database on natural toxins new therapeutic molecules can be designed by using structure and activity related

information (Figure 6). Further, their target specificity can be improved by finding anticancer peptides by slight modifications in amino acids, lipid-protein and toxin receptor interactions and a common peptide could possible scorpion, honey bee, and wasp and spider bites due to their long evolutionary relationship and ontology (Figure 7). Thus new putative toxin structures can be developed from naturally secreted toxins by using bio-informatics tools and methods mainly proteomic and transcriptomic library (Figure 8).



Glioblastoma multiforme (GBM) is a most common malignant central nervous system tumor. This is highly malignant and aggressive and most lethal human cancers which show very high invasive capacity and lesser therapeutic options [166]. It is fast proliferating and destructive tumor has very low mean survival time (<24months) and develops very silently within the brain microenvironment. These malignant primary brain neoplasm showed mean survival of <24months. Drug chemotherapy of GBM shows low therapeutic efficiency and systemic side effects [167]. Only glioma-specific chloride ion channel represents a specific target for therapy. These effects are achieved mainly through the blocking of an array of ion channel types within the membranes of excitable cells. These ion channel-blocking toxins are tightly-folded by multiple disulphide bridges between cysteine residues [164]. Two nonadecapeptide FLFSLIPSVIAGLVSAIRN and FLFSLIPSAIAGLVSAIRN have shown very strong anti-tumor potential against GBM [167].

### Cisplatin-Based Therapy

Cisplatin-based therapy is one of the most important chemotherapy treatments for GBM, although its efficacy is limited

due to drug resistance and undesirable side effects. DMCa is a good candidate for clinical platinum-based therapy in GBM treatments and other cancer types [168]. DMCa exhibit strong anti-cancer efficacy compared to cisplatin, especially at low doses. By inducing intracellular oxidative stress, Pt-1-DMCa potentiated platinum-induced DNA damage and led to enhanced p53 phosphorylation, followed by increased activation of both mitochondrial and death receptor pathways. Decreased phosphorylated AKT and ERK levels were associated with the apoptosis induced by the novel synthesized cisplatin analogue. Thus, a chimera between platinum and a maurocalcine-derived CPP was found a highly successful anti-cancer compound that works by targeting the intracellular redox system [169] (Table 3).

### Chlorotoxin (CTX)-Targeted Nanoparticles Therapy

Chlorotoxin is a disulfide-rich stable peptide derived from Israeli scorpion *Leiurus quinquestriatus* venom. It shows wider therapeutic potential in treatment of cancer. It possesses compact shape and is convenient for intracranial delivery. It is specific and efficacious in blocking glioma Cl(-) channel activity [170]. CTX-conjugated NPs are preferentially bind to tumor cells and used

as a vehicle to deliver anti-cancer drugs specifically to cancer cells [171]. Chlorotoxin (CTX)-targeted nanoparticles (NPs) work as a targeting ligand that specifically binds to glioma. CTX was conjugated onto iron oxide NPs, quantum dots, and rare-earth upconversion NPs are used in magnetic resonance and fluorescence imaging of glioma (Table 3). CTX-conjugated NPs are also used as carrier system to load anticancer drugs or therapeutic genes for targeted chemotherapy or gene therapy of glioma, respectively [172]. It targets various gated channels i.e. voltage gated chloride channels (GCC), calcium-dependent phospholipid-binding protein Annexin-2, and an inducible extracellular matrix Metalloproteinase-2 (MMP-2). MMP-2 shows anti-neoplastic potential. Chlorotoxin possess tumor cell targeting domain and shows different effector functions [173]. Further, for enhancing its action on cancer cells its physical mixture (CTX + Onc) was prepared by conjugating it to an onconase. At high dose this CTX-Onc showed better anti-tumor effect than simple toxin [174]. Similar cytotoxic potential is also reported in *Centruroides tecomanus* chlorotoxin [175]. In addition, multifunctional nanocomposites are made by using polymeric nanoparticles (PNPs) containing two cytotoxic agents - the drug alisertib and silver nanoparticles. These PNPs have been conjugated with a chlorotoxin, an active targeting 36-amino acid-long peptide that specifically binds to MMP-2, a receptor over-expressed by brain cancer cells [176]. This silver/alisertib@PNPs-chlorotoxin combination does tumor reduction in vivo. Similar activity is also reported in peptides CA4 and CTX-23 which effectively reduce glioma cell proliferation. In addition, CTX, CA4 and CTX-23 impact on cell migration and spheroid migration. CA4 and CTX-23 are selective and show low toxicity against primary neurons and astrocytes. These also shorten cell extensions, increased nuclear sizes and reduce tumor volume. CTX and CA4 show anti-angiogenic properties with endothelial and angiogenic hotspots disrupting activities [167]. Recombinant AGAP (rAGAP) (Analgesic-antitumor peptide) also affect the migration and invasion of HepG2 cells via a voltage-gated sodium channel (VGSC)  $\beta$ 1 subunit and shows strong anti-tumor activity [177] (Table 3).

### Self-assembled Toxin based Polymeric Nano-Structures

Efforts have been made to self-assemble scorpion venom in polymeric nano-structures for controlled delivery of toxins towards tumorous sites [178]. For this purpose, polypeptide extract from scorpion venom (PESV) was combined to Rapamycin. It is taken up by H22 hepatoma cells in mice during autophagy [179]. This PESV combined Rapamycin inhibit the development of H22 hepatoma transplantation tumor in mice. It could be possible by inhibiting the activity of mTOR, enhancing expressions of ULK1, MAP1LC3A, and Beclin1 [179]. PESV also inhibit the angiogenesis of H22 hepatoma. The mechanisms might be associated with suppressing the expression of PI3K, P-Akt, HIF-1 alpha, and VEGF-A [180]. PESV (polypeptide extract from scorpion venom) successfully block cell cycle and inhibit angiogenesis directly to inhibit cell proliferation of non-small cell lung cancer cell line A549 mainly through reducing the expression of HIF-1alpha, VEGF and increasing the

expression of PTEN [181]. Mechanism of polypeptide extract from scorpion venom (PESV) on promoting anti-tumor effects of cyclophosphamide (CTX). In addition, polypeptide extracts from scorpion venom (PESV) combined to 5-fluorouracil (5-Fu) shows inhibition effects on vasculogenic mimicry (VM) of H2 hepatoma carcinoma cells in mice [182]. Similarly, TsAP-1 and TsAP-2 isolated from *Tityus serrulatus* showed broad spectrum anticancer potencies which can be significantly enhanced by increasing their cationicity [183]. PESV inhibit the expressions of VEGF and TGF-beta1, promote the maturation of DCs, recover its antigen uptake presentation function, and reverse the immune injury to the body by CTX. It also plays important role in inducing the tumor cell apoptosis [184]. Selectively targeting cancer tissue is one promising strategy. Similarly conjugation of toxins to platinum (IV) derivatives (Cisplatin), increase the cytotoxicity in cancer cells [185] (Table 3).

Similarly, The BmKn-2 peptide kills HSC-4 cells through induction of apoptosis [186]. This peptide displays specific membrane binding, growth inhibition and apoptogenic activity against human oral cancer cells [186]. *Buthus matensii* Karsch (BmK) scorpion venom extracts inhibit growth of human breast cancer MCF-7 cells by inducing apoptosis and blocking cell cycle in G0/G1 phase [187]. miRNA-based therapeutics is followed for specific killing of tumor and cancer cells in GBM. In this therapeutic approach intravenously-administered chlorotoxin (CTX) is coupled (targeted) with stable nucleic acid lipid particle (SNALP) to formulate anti-miR-21 oligonucleotides. These particles are send to approach towards tumor cells. These particles accumulate preferentially within brain tumors and promote efficient miR-21 silencing. It results in increased mRNA and protein levels of its target RhoB and showing no signs of systemic immunogenicity. This miRNA modulation by the targeted nanoparticles combined with anti-angiogenic chemotherapy shows high success rate in GBM treatment [166]. However, for modulation of tumor response to chemotherapy, a combination of treatment with small interference RNA (siRNA), chlorotoxin and conventional (temozolomide, TMZ) drugs is used. siRNA delivery by targeted nanoparticles resulted in modulating tumor response to chemotherapy in GBM. siRNA interrupt cell signaling, drug act as toxic agent, and CTX contributes apoptotic tumor cell death [188]. Similarly a chlorotoxin-conjugated graphene oxide (CTX-GO) drug delivery system shows better efficacy in glioma treatment. The method is developed by using graphene oxide and loading doxorubicin CTX-GO (CTX-GO/DOX) via noncovalent interactions [167]. The protein chlorotoxin (CTX) has been shown to preferentially target glioma cells. The combination of CTX-NO and chemotherapeutics also led to decreased cell invasion (Table 3). A new method MiRNA-21 silencing mediated by tumor-targeted nanoparticles combined with sunitinib: is developed for gene therapy of glioblastoma [167]. Further, immunotoxin molecules conjugated to some anticancer drug are to be prepared. For targeting can cancer cells recombinant molecules are being made which are used as targeted toxins, also known as immunotoxins or cytotoxins (Figure 9).

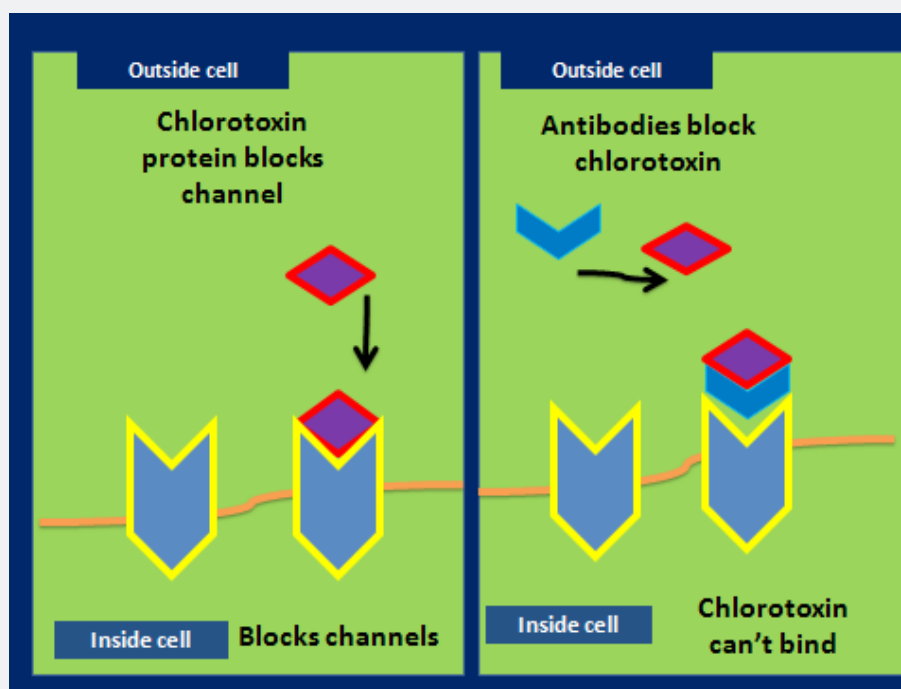


Figure 9: Showing chlorotoxin channel binding to receptors and its blockage by using antibodies.

Table 3: Animal Toxins and Nano-drug Delivery Systems Prepared for Cancer Therapeutics.

Drug Delivery System	Therapeutic Effect	Reference
Mastoparan and mitoparan	cell-penetrating peptides (CPPs)	[167]
Poly (l-glutamic acid) PGA polymer	NP formulation are used to target glioblastoma multiform (GBM).	[162]
CTX loaded on NPs	Enhance the uptake of the NPs	[162]
Cisplatin-based therapy	clinical platinum-based therapy GBM treatments	[168]
Maurocalcine-derived CPP	Anti-cancer therapy	[168]
Calcium-dependent phospholipid-binding protein Annexin-2, and an inducible extracellular enzyme matrix Metalloproteinase-2	Anti-neoplastic therapy	[172]
Conjugation of CTX + Onc.	CTX-Onc showed better anti-tumor effect than simple toxin	[173]
Nanocomposites (PNPs)	Contains two cytotoxic agents - the drug alisertib and silver nanoparticles	[174]
Polypeptide extract from scorpion venom (PESV) combined to Rapamycin.	Anti-angiogenesis therapy	[178]
Conjugation of toxins to platinum (IV) derivatives (Cisplatin),.	increase the cytotoxicity in cancer cells	[184]
siRNA delivery by targeted nanoparticles	siRNA interrupt cell signaling, drug act as toxic agent, and CTX contributes apoptotic tumor cell death	[187]

### Future Directions

So far researches have been done venom from several species of wasp, honey bee and scorpion have been isolated and characterized for their multiple biological activities. Being heterogeneous mixture, animal venom accounts for the variable biological activities. These show differences in amino acid sequences but have more similar immunological properties. Thus any modification in the amino acid sequence may result in the modification of structure, which ultimately alters immune function of the toxin. Efforts should be made to remove toxicity of venom peptides and targeted drug should conjugate with toxin.

Further, anticancer property of venom peptides can be enhanced by improving its cytolytic action on cancer cells. It could further be achieved by doing encapsulation of venom toxin peptides in ultra-small lipid nanoparticle. Development of strategies for effective delivery of venom peptide could speed up anticancer therapeutics. New additions of anti-angiogenic properties could be possible in many more dansylated analogue which bind to natural membranes and show cytolytic activity can be designed. Melittin produced a different conformational change than mastoparan. In an aqueous solution, melittin went from a nonhelical form to an alpha helix when phosphate was added to the solution. Further,

binding of toxins (melittin) to the membrane can be increased by improving electrostatic interactions or molecular surface charges. Besides hydrophobic interactions several other favorable electrostatic interactions are responsible for molecular modeling. By doing this toxins can bind to cell surface receptors mainly those which over-express in cancer and delivered toxin component may kill the cancerous cell.

To fulfill the therapeutic demand of novel drugs chemical and recombinant synthesis can be done for obtaining promising peptide toxins. Their target specificity can be decided after structural characterization through experimental and computational methods. Further bioactivity and molecular function can be extended through biological assays and computational modeling. By performing transcriptomic and genomic analysis try to identify genes expressed in venom glands, these could assist in production of nano-venins and nano-drug carriers for treatment of glioma, pancreatic, thymus, prostate, and liver cancers. There are many more avenues which can be investigated to explore new therapeutic uses of venom components. Development of pro-cytotoxic system based on mitoparan, which transports this potent cytotoxic peptide to the tumor and allows its accumulation in a controlled manner, emerges as a plausible approach to overcome off-target side effects of current cancer treatment. Therefore, after understanding of the molecular mechanisms and target specificity of animal-secreted toxins new drug molecules could be designed. Certainly these will reduce human health risks and opens new insights into therapies against bites, and pathologies ranging from neurological disorders to cancer, using toxinomimetic agents. Recently, molecular and cellular biology dissecting tools have provided a wealth of information on the action of these diverse toxins, yet, an integrated framework to explain their selective toxicity. Though there are several toxin molecules but different toxins showed fundamental mechanisms of toxicity at different biochemical, molecular and cellular- levels with particular consideration for the nervous system

### Conclusion

From researches it has been cleared that animal toxins from wasp, honey bee and scorpion possess several active molecules with anticancer activities, ranging from inhibition of proliferation and cell cycle arrest to induction of apoptosis and decreasing cell migration and invasion. These specially show cytotoxic effects in tumor and cancer cells and degrade/destroy. Scorpion venoms and toxins can decrease cancer growth, induce apoptosis and inhibit cancer progression and metastasis *in vitro* and *in vivo*. This activity depends on specific structure of peptides with biophysical properties (hydrophobicity, hydrogen bond donor and steric), to penetrate through membrane and block ion channels successfully and effect membrane environment due permeability alteration (sodium, potassium, chloride). These toxins showed heterogeneous distribution and interact with the lipid bilayer by using its hydrophobic side; and shift molecules within the lipid bilayer rather than on the surface. Upon conjugation with certain polymers and drugs these work as novel drug carriers and carry it to the targeted site. Venom peptides have potential application

as an alternative or complementary medicine for the treatment of human cancers. These toxin polypeptides not only work against cancer cells but also show good antimicrobial activity and can be used to treat autoimmune diseases and cardiovascular effects. By making few chemical modifications hydrophobic properties of toxins could be changed. Further, lower down of toxicity is also possible by making amino acid substitutions, and its expression in animal model systems and cell lines can assist in improvement of the maximum tolerated dose and the pharmacokinetic parameters of cytotoxic peptides *in vivo*.

For finding more appropriate drugs molecular modeling played important role in increasing molecular-level understanding of the activity and specificity of animal toxins. Further, partial modifications did in peptides after amino acid shifting, and replacement engineering made them more suitable for biotechnological and pharmaceutical applications. This review focuses on the biological effects of animal venom toxins on cancer cells mainly its interaction with membranes and ion channels. However, by exploration, characterization, and optimization of toxin peptides new integrative chemotherapeutic methods can be established. Further, taxonomic and phylogenetic analyses can assist in deciphering the molecular and ionic interactions of venom peptides from various animal groups that will alternatively help in unique modeling of peptides, its linkage for target specific drug delivery. New combination can be made by using radiation therapy and toxin therapy combinations for fast recovery of tumor patients. Clinical applications in GBM therapy, intraoperative imaging, nano-probes and nano-vectors based technology; targeted chemotherapy and immunotherapy are discussed as well. Chlorotoxin is likely to play a significant role in effective GBM immunotherapy in the future.

### Conflict of Interest

The author declares no conflict of interest, financial or otherwise.

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