

REVIEW

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Therapeutic potential of venom peptides: insights in the nanoparticle-mediated venom formulations

Aditya Vivek Joglekar¹, Deepa Dehari¹, Md. Meraj Anjum¹, Naveen Dulla¹, Aiswarya Chaudhuri¹, Sanjay Singh^{1,2} and Ashish Kumar Agrawal^{1*} 

Abstract

Background: Venoms are the secretions produced by animals, generally for the purpose of self-defense or catching a prey. Biochemically venoms are mainly composed of proteins, lipids, carbohydrates, ions, etc., and classified into three major classes, viz. neurotoxic, hemotoxic and cytotoxic based upon their mode of action. Venoms are composed of different specific peptides/toxins which are responsible for their unique biological actions. Though venoms are generally seen as a source of death, scientifically venom is a complex biochemical substance having a specific pharmacologic action which can be used as agents to diagnose and cure a variety of diseases in humans.

Main body: Many of these venoms have been used since centuries, and their specified therapies can also be found in ancient texts such as Charka Samhita. The modern-day example of such venom therapeutic is captopril, an antihypertensive drug developed from venom of *Bothrops jararaca*. Nanotechnology is a modern-day science of building materials on a nanoscale with advantages like target specificity, increased therapeutic response and diminished side effects. In the present review we have introduced the venom, sources and related constituents in brief, by highlighting the therapeutic potential of venom peptides and focusing more on the nanoformulations-based approaches. This review is an effort to compile all such report to have an idea about the future direction about the nanoplatforms which should be focused to have more clinically relevant formulations for difficult to treat diseases.

Conclusion: Venom peptides which are fatal in nature if used cautiously and effectively can save life. Several research findings suggested that many of the fatal diseases can be effectively treated with venom peptides. Nanotechnology has emerged as novel strategy in diagnosis, treatment and mitigation of diseases in more effective ways. A variety of nanoformulation approaches have been explored to enhance the therapeutic efficacy and reduce the toxicity and targeted delivery of the venom peptide conjugated with it. We concluded that venom peptides along with nanoparticles can evolve as the new era for potential treatments of ongoing and untreatable diseases.

Highlights

- Nature is an unending and the ultimate source of unique biological compounds which have different pharmacological actions and venom is one of them.

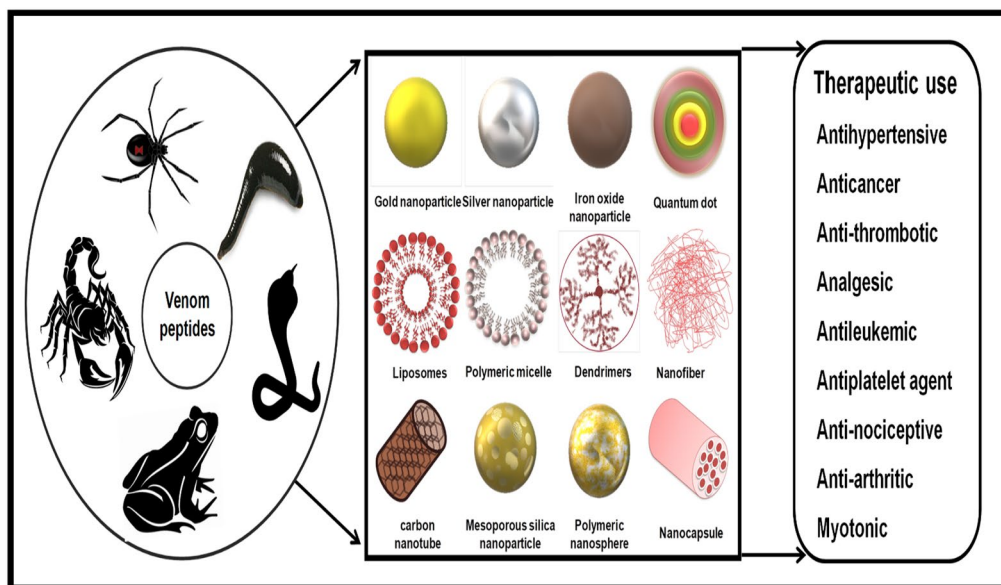
*Correspondence: ashish.phe@itbhu.ac.in

¹ Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (BHU), Varanasi 221005, India
Full list of author information is available at the end of the article

- Venoms are generally seen as a source of death; however, scientifically venom is a complex biochemical substance having a specific pharmacologic action which can also be used as agents to cure a variety of diseases in humans.
- Venom peptides along with nanoparticles can evolve as the new era for potential treatments of ongoing and untreatable diseases.

Keywords: Venom, Peptides, Pharmacology, Nanotechnology, Drug delivery, Therapeutics

Graphical Abstract



Background

Venom is a biochemical secreted by animals generally for their defense or catching a prey. A total of 5.4 million estimated snake bites with 100,000 deaths per year are reported per year. As per a report of WHO, India can be considered as snakebite capital with 2.8 million snake-bites and 45,900 estimated deaths per year. On the basis of their mode of action, venoms can be classified into three types, viz. neurotoxic, hemotoxic and cytotoxic [1]. Neurotoxic acts on the nervous system, hemotoxic acts on the blood clotting mechanism, while cytotoxic is responsible for cellular or tissue damage. Though venoms are generally seen as a source of death, scientifically venom is a complex biochemical mixture of proteins, lipids, carbohydrates, ions, etc., with specific pharmacological action which can be used as agents to diagnose and cure a variety of diseases in humans circling over mankind [2].

Paracelsus, a fifteenth-century philosopher, said “In all things there is poison; there is nothing without poison. It only depends upon the doses, whether a poison is a

poison or not” [3]. It clearly implies that whether it’s animal venom or drugs for the treatment, the toxicity will be determined by the amount of used substance. Specific dose of a substance can be therapeutic in action, while another dose of the same substance can be toxic. Venoms have been in use as a therapy since past sixty years. In Charka Samhita cobra venom is used for the treatment of ascites, while the Sushruta and Vaghabata have also mentioned similar use. Saranghara Samhita made use of cobra venom in ‘Sannipatik Jwara.’ Unani medicinal system has made use of cobra venom as aphrodisiac, hepatic stimulant, tonic and in collapsed condition. Homeopathic system also uses the venoms of Vipers, Cobras, Crotalus and Lacasis [4]. Cobra venoms have also been used in the treatment of stroke by Chinese physicians. Modern era drug Captopril was developed from venom of a pit-viper snake species found in Brazil called as *Bothrops jararaca* (Brazilian lancehead snake) [5]. The drug is quite a success in the treatment of hypertension and cardiovascular diseases and can be considered as first example of animal venoms in modern therapeutics [6]. Different

pharmacological and toxicological effects of venom peptides are demonstrated in Fig. 3.

In addition, several researches have been carried out on therapeutic use of animal venoms by conjugating venoms with nanoparticles as a promising treatment modality to further improve the target specificity, increased therapeutic response and reduce the side effects. Nanotechnology is a modern day science of building materials on a nanoscale having several advantages which have been explained in detail further in a section in this article. For example, ICD85 peptides were loaded into sodium alginate nanoparticles by Borumand, M.R. et al. It was observed that ICD-85 loaded nanoparticles in comparison to free ICD-85 exhibited more potent cytotoxicity and suppressed proliferation of HEp-2 cells with a sustained release profile [7]. Different types of nanoformulations that can be used to formulate venom peptides along with their possible therapeutic implications are depicted in Fig. 4.

Main text

Venom and its constituents

Venom is a biochemical compound which is mainly proteinaceous in nature. But that's not all about it, they are way more complex especially snake venoms as compared to venoms of spiders, scorpions, cone snails, etc. [8]. The venoms composition usually consists of Enzymes (e.g., proteases, phospholipases, etc.), non-enzymatic proteins (e.g., disintegrins) and peptides [1]. Oxidases and hydrolases are the two main enzymatic classes which are commonly found in venom. L-amino oxidase is the only oxidase, while phospholipase A₂ and hyaluronidases are the common oxidases which are found in snake venom. Non-enzymatic proteins include a diverse variety, e.g., three finger toxins, proteinase inhibitors, disintegrins, etc. [9]. Peptide toxins acting on nervous systems are found in scorpions, *elapidae* snakes, spiders and cone snails. Spider and Scorpion venom neurotoxins mostly attack the voltage-gated ion channels, while snake venom neurotoxins act ligand-gated ion channels (particularly nicotinic acetylcholine receptors). Cone snail toxins called conotoxins are much more diversified and act on both types of channels [10]. Disulfid bridged peptides are responsible for the activities in other organisms, while snake venoms are composed of a much more diverse array of peptides along with other biochemical which is responsible for a bigger variation in pharmacological and toxicological response [11]. The main components include phospholipases A₂ (PLA₂s), serine proteases, metalloproteinases and three finger peptides toxins (3FTX), while minor components include cysteine-rich proteins, kunitz peptides, C-type lectins, L-amino acid oxidases, disintegrins, natriuretic peptides, bradykinin

potentiating peptides, sarafotoxins (SRTXs), crostamine, waprin family of peptides and waglerins [12].

Phospholipase A₂s

PLA₂s are the major components of Elapidae and Viperidae venoms and have a molecular mass of 13-15 kDa. They are classified into major group I and II with an additional type called IIE [13]. PLA₂s are responsible for the neurotoxic and myotoxic effects [14]. Modulation of pre-synaptic terminals and sensory nerve endings is responsible for neurotoxic and some of the pro-inflammatory effects [14, 15]. Pre-synaptic effects of PLA₂s are characteristics of β -neurotoxins and attack the motor nerve terminals at the neuromuscular junction [15]. Though certain mechanisms involving PLA₂ effects on pre-synaptic terminals have no sufficient data, it was seen that PLA₂ β -bungarotoxin (structure in Fig. 2 (2)) acts by binding to potassium channels through an accessory Kunitz subunit [15, 16]. The overall pre-synaptic effect is that it causes vigorous exocytosis of neurotransmitters from the vesicular reserve which results into deficient release of neurotransmitter in the neuromuscular junction ultimately leading to muscle paralysis [15, 17]. The pain induction by PLA₂s is based on the inflammatory mechanisms and activation of sensory neuronal network. PLA₂s are key enzymes which are involved in production of lipid mediators. Bradykinin acts as an important mediator in inflammatory pain which is induced by PLA₂s [18, 19]. Mechanical hyperalgesia, induced by PLA₂s, is dependent on TNF- α , IL-1 β and prostaglandin production [20]. This therefore suggests that there is an increased production of arachidonic acid, which is processed by cyclooxygenase resulting in production of prostaglandins [21]. In support of this hypothesis, tests were carried out on rodents which showed that phospholipases isolated from different venoms induced hyperalgesia which were mediated through biogenic amines, sympathomimetic amines, cytokines, ATP, K⁺, purinergic receptor activation, glial cell activation and prostaglandins [22–24]. MitTx from *Micrurus tener*, which is a heteroatomic complex formed between PLA₂ and Kunitz subunit, activates somatosensory neurons. It induces robust pain behavior in mice through its agonistic action on ASIC channels (via activation of ASIC1 channels on capsaicin sensitive nerve fibers [24]). Also BomoTx, a Lys49 secreted PLA₂ from *Bothrops moojeni*, shows its action by exciting cohort of sensory neurons through promotion of ATP release from myotubes and consequently activating purinergic receptors, viz. P2X₂ and/or P2X₃ [22]. Some PLA₂s also have a hemotoxic action, acidic phospholipase A₂ (Asp49-PLA₂) named Bpir-PLA₂-I from *Bothrops pirajai* snake venom shows anti-platelet action [25]. Experimentally Bpir-PLA₂-I

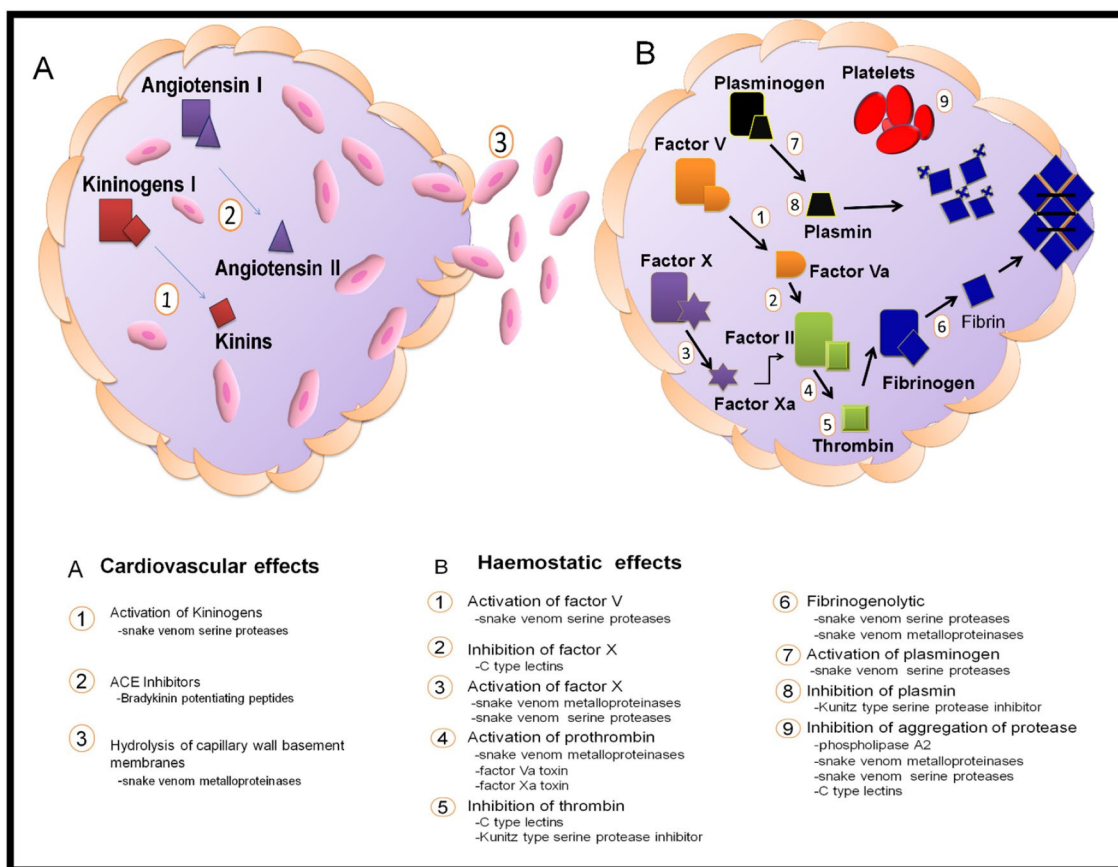


Fig. 1 Mechanism of action of different venom peptides acting on cardiovascular and hemostatic systems

shows hypotension in-vivo testing and inhibitory action on platelet aggregation in-vitro [25].

Metalloproteinases

Venom metalloproteinases are zinc-dependent proteinases ranging from 20 to 110 kDa in size. They are categorized into PI, PII and PIII based on their structural domain [26, 27]. Metalloproteinases present in venoms are responsible for developing symptoms such as hemorrhage, hypotension, edema, inflammation, necrosis and hypovolemia [28]. A family of proteinase homologous to zinc metalloproteinases have been discovered recently and named ADAMs for A disintegrin-like and metalloproteinase-containing protein [27]. Snake venom metalloproteinases have been developed from ADAM, more specifically from ADAM28 [29]. PIII is the most basal variant and it consists of metalloproteinase, disintegrin-like and cysteine-rich domains [30, 31]. PIII have molecular masses of 60–100 kDa and are the large sizes snake venom metalloproteinases (SVMPs) [32]. PII is diverged from PIII as it consists of metalloproteinase and disintegrin domains with latter commonly being a proteolytically

processed product [31]. PII are the medium size metalloproteinase with molecular masses ranging from 30–60 kDa [32], while PI are the small SVMPs consist of metalloproteinase domain only without having disintegrin encoding domain and have mass in the range of 20–30 kDa [31, 32]. This clearly indicates that snake venom metalloproteinases have evolved substantially over the history with proof of extensive gene duplications and bursts of accelerated molecular evolution [31]. Metalloproteinases have been known for their hemorrhagic activity and their ability to alter several steps in blood clotting cascade resulting in a lethal combination of systemic hemorrhage and incoagulable blood in victim [32]. In addition to this hemorrhagic activity metalloproteinases also show fibrin(ogen)olytic activity (Fig. 1), inhibit platelet aggregation (Fig. 1), prothrombin activation (Fig. 1), apoptotic activity, activate blood coagulation factor X (Fig. 1), pro-inflammatory and inhibition of blood serine proteinase [32]. SVMPs induced hemorrhage is a two-step mechanism [33, 34]. In the first step, the capillary wall is weakened by SVMPs through cleavage of basement membrane as the SVMP degrade components

of basement membrane including proteins like integrins and cadherins [33, 34] along with the cleavage of adhesion proteins of endothelial cell–matrix complex. In the second step, the endothelial cells detach from the basement membrane. The transmural pressure acting on the weakened capillaries make them distended [33]. The capillaries become extremely thin and become too fragile that leads to effusion of blood [33]. As a consequence of this, the capillary walls get disrupted as the weakened endothelial cell lining is susceptible to the shear stress of blood flow [33]. PIII type of SVMPs which comprise disintegrin-like, cysteine-rich and catalytic domains are more potent in hemorrhagic toxicity than PI type comprising only of metalloproteinase domain [34]. In addition to this proteinase activity, SVMPs also control the homeostasis and alter the blood coagulation contributing to toxic hemorrhagic effects [35]. Hyperalgesia and inflammation are the characteristics of pain induced by SVMPs. Research has shown that this pain is dependent on production of prostaglandins, cytokines, nitric oxide, leukotrienes, histamine, leukocytes migration, mast cell degranulation and activation of NF- κ B [36, 37]. This indicates that the SVMPs activate inflammatory mechanisms by inducing production of prostaglandins and sympathetic amines and activating cytokine-chemokine cascades to cause sensitization of nociceptors [37]. Neurotoxicity has also been reported by certain metalloproteinases, some of *Psammophis* propeptides inhibit α -7 nicotinic acetylcholine receptors and cause potential paralysis [38].

Serine proteases

Serine proteinases found in snakes are from S1 family, popularly called as Snake Venom Serine Proteinases (SVSPs). They display molecular masses in the range from 26 to 67 kDa. Studies indicate that these toxins have evolved from Kallikrein like serine proteases for functioning as venom. They have evolved through gene duplications and result into multiple isoforms [39]. SVSPs have been found in large variety of venoms; they are most abundant in viper venoms and relatively to lesser extent in elapid and colubrid venoms [40]. SVSPs rupture capillaries in their victims by altering mechanisms in homeostatic systems and inducing hyperalgesia and edema. It is a combination of blood coagulation, platelet aggregation, fibrinolysis, blood pressure, etc. [41]. Pro-coagulant action of SVSPs is through activation of coagulation factors V, VII, X and prothrombin [41]. The mechanism in brief includes production of thrombin through activation of prothrombin which further activates fibrinogen to form fibrin which are cross-linked to form fibrin clots. Thrombin also activates platelet aggregation, along with the activation of factor XIII to XIIIa which also activates

fibrin clot formation [42]. In addition to this, some SVSPs promote binding of platelets to fibrinogen by activating platelet receptors [43]. Anticoagulant SVSPs on the other side activate protein C and subsequently inactivate factors Va and VIIIa [44]. SVSPs act as thrombin-like enzymes and plasminogen activator and therefore eliminate blood clots as fibrin in the blood clot is eliminated and in total it all leads to coagulopathy [41]. These different mechanisms of venom peptides on homeostatic and cardiovascular system are demonstrated in Fig. 1. Activation/depletion and inactivation of blood clotting factors prevent clot formation and ultimately lead to internal and external bleeding. Batroxobin from venom of *Bothrops atrox* is an example of toxin-based drug used for the treatment of heart diseases which is sold under tradename Defibrase[®] [41]. It is an anticoagulant which acts by specific cleavage of Arg16–Gly17 bond in the alpha-chain of fibrinogen releasing fibrinopeptide A [41]. Hyperalgesia and inflammatory response induced by SVSPs are seen in venoms of *Bothrops jararaca* and *Bothrops pirajai* mainly through edema and leucocyte migration with neutrophils in majority, but the mediators are unknown and little is known about the actual mechanism [45, 46]. Inflammation is a feature of SVMP intoxication while having little role in pain [45, 46]. On the other hand, PLA2s and metalloproteinases have major roles in inflammatory responses as well as pain induction [45, 46].

Three-Finger Toxins (3FTXs)

As the name suggests, three-finger toxins (3FTXs) have a three-finger fold structure which is stabilized by disulfide bridges. They are non-enzymatic neurotoxins having 21 to 58 residues [47] mostly found in venoms of elapid and colubrid snake. The neurotoxins act on postsynaptic neuromuscular junctions and cause flaccid paralysis [48]. Three-finger toxins can be differentiated according to their lengths into short-chain and long-chain 3FTXs. Short-chain 3FTXs include α -neurotoxins, β -cardiotoxins, fasciculins, cytotoxins and mambalgins. They comprise 57–62 residues and 4 disulfide bridges [49]. Long-chain 3FTXs comprise 66–74 residues and 5 disulfide bridges. These include α -neurotoxins, κ -neurotoxins, γ -neurotoxins and hannalgesin [49]. In addition to this, 3FTXs have been found to exist as monomers and hetero or homodimers with covalent or non-covalent bonds. The long range of diversity of 3FTXs mentioned above is a result of continuous evolution and gene duplications from ancestral 3FTXs. This evolution can be explained with a paradigm; gene duplications have resulted in increase of 3FTX loci from non-venomous snakes like python having one to venomous elapid snakes like king cobra (*Ophiophagus hannah*)

Table 1 Different types of three-finger toxins and their mode of action

Toxins	Mode of action	References
α -neurotoxins	Inhibition of muscle acetylcholine receptors (nAChR)	[56]
κ -neurotoxins	Inhibition of neuronal acetylcholine receptors	[57]
Muscarinic toxins (MT7)	Potent and specific inhibition of muscarinic receptors through allosteric modulations (structure in Fig. 2 (10))	[58]
Fasciculins	Inhibition of acetylcholinesterase (AChE) through allosteric interactions	[59]
Calciseptine	Specific modulation of L-type calcium channels. Muscle relaxant and inhibits cardiovascular contractions	[60]
Cardiotoxins	Interaction with phospholipids, Induces concentration dependent insulin release and not dependent on presence and absence of glucose	[61, 62]
Mambin	Inhibition of ADP induced platelet aggregation, Inhibition of binding of platelet fibrinogen receptor GP IIb-IIIa to immobilized fibrinogen	[63]
Exactin	Selective inhibition of factor X (FX) activation by extrinsic tenase complex (ETC) and thus acts as an anticoagulant. It has selective preferential action on ETC-FX complex	[64]
β -cardiotoxins	Inhibition of β -adrenoreceptors (Binding affinity toward β -1 and β -2 subtypes)	[65]
MTa	Selective inhibition of α -2B-adrenoreceptors. Concentration-dependent decrease in intracellular calcium (Ca^{2+})	[66]
Mambaglins	Inhibition of nociception (abolish pain) by inhibition of acid-sensing ion channels (ASICs) particularly ASIC1a and ASIC2a in central neurons and ASIC1b in nociceptors	[67]
Tx7335	Activation of potassium channels through KcsA by allosteric interaction causing reduction in inactivation of the channels	[68]
Callitoxin	Activation of voltage-gated sodium channels (Na_v) by shifting voltage-dependence $Na_v1.4$ to more hyperpolarized potential, subsequently inhibiting inactivation and production of large increased currents	[69]

having nine [50]. The evolution is through strong selection of genes for adaption with changing environmental conditions, with the selection seen extensively acting on the surface exposed amino acid residues which have resulted in paralogous elapid 3FTX genes [51]. The collective effect of the evolution is that the 3FTXs now appear in different isoforms with different structures and various distinct biological functions. In elapid snakes a distinctive biological action of 3FTXs is seen for a specific taxon for example, genus *Naja* features cytotoxins and genus *Dendroaspis* feature neurotoxins [52]. In colubrid snakes taxon specific 3FTXs [53, 54] and pseudogenization in non-venomous species [55] have been detected which infer that catching a prey and its type is partially responsible for the variations in 3FTXs. Despite the fact that 3FTXs have a common threefold structure, they show distinct biological action and have diversity in their targets. Different 3FTXs and their action are listed in Table 1.

A point to be noted is that 3FTXs are not been known to have any hyperalgesia and inflammatory response as seen in other snake venoms [49]. The unique multifunctionality seen in 3FTXs is due to its resistance to degradation, tolerance to large deletions and mutations [70].

Disintegrins

Disintegrins are rich in cysteine and are produced through post-translational cleavage of snake venom metalloproteinases are related phylogenetically to ADAMS (a disintegrin and metalloproteinase [12]. Disintegrins are

seen to exist as monomers—short (49–51 amino acids, 4 disulfide bonds), medium (70 amino acids, 6 disulfide bonds), long (84 amino acids, 7 disulfide bonds); homodimers; heterodimers [71]. Disintegrins are found in *crocalidae* (17%) and *viperidae* (18%) snake venoms [40]. Disintegrins function as a support to other toxins and help in their distributions. The action is by binding to integrins present in prey and inhibits platelet aggregation and cell adhesion [72]. For example, Saxatilin (saxthrombin) (structure in Fig. 2 (6)) isolated from *Gloydius saxatilis* venom has shown thrombolytic effects when tested in mice model. It does not possess any fibrinolytic activity but, has ability to inhibit a number of integrins acting on platelets [73].

Bradykinin potentiating peptides (BPPs)

BPPs are small peptides and consist of 5–14 amino acids; they are rich in proline and have a hypotensive action on victim. BPPs have ability to inhibit angiotensin-converting enzyme (ACE) which is an important part of renin angiotensin system (RAS). It involves conversion of angiotensin 1 to angiotensin 2 by catalyzing degradation of bradykinin. Angiotensin is a vasoconstrictor while bradykinin is a vasodilator [12]. Marketed drug captopril which is an ACE inhibitor discovered from venom of *Bothrops jararaca* is the best example of potential of these peptides [5]. Hypotensive action shown by BPPs is also seen to be independent of ACE and other mechanisms involved include activation of arginosuccinate synthetase (ASS) in kidney, ASS increases arginine and nitric

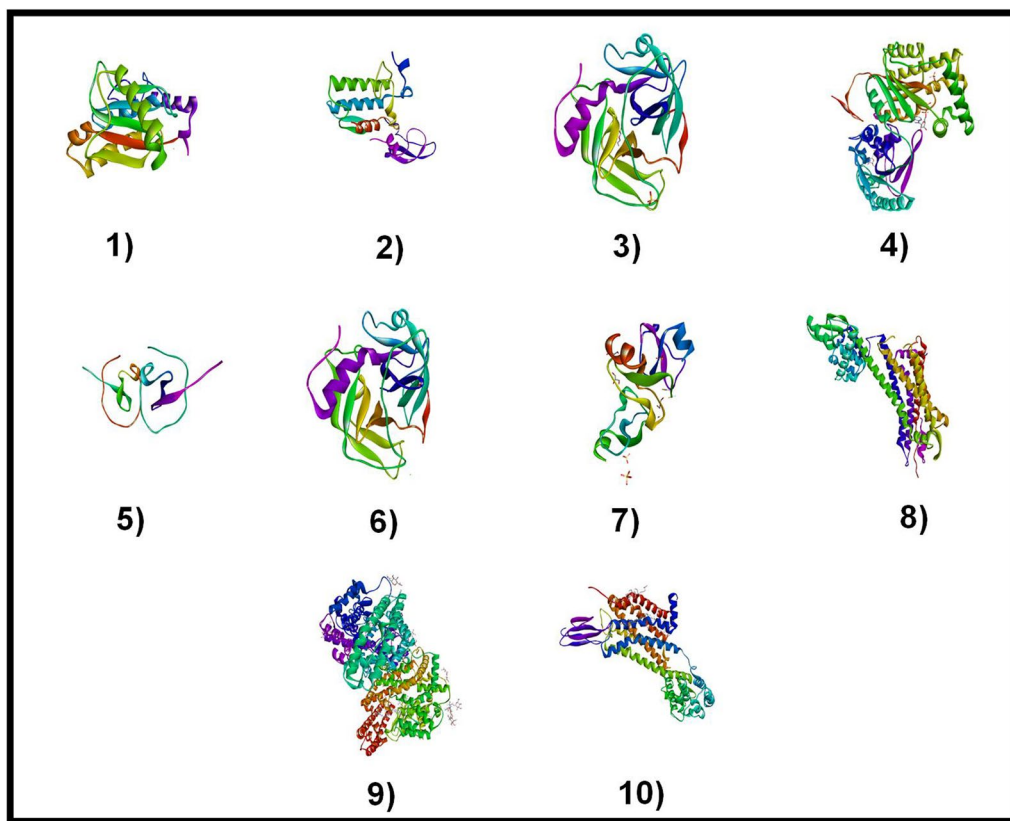


Fig. 2 Structures of different venom peptides obtained through X-ray diffraction. Description-1 Example of Tripeptides, Acutolysin A from venom of *Agkistrodon acutus*. 2 Example of Phospholipase A2, structure of beta2-bungarotoxin showing potassium channel binding by kunitz modules and targeted phospholipase action. 3 Example of Serine proteinase, crystal structure of AaV-SP-II, a glycosylated snake venom serine proteinase from *Agkistrodon acutus*. 4 Example of Metalloproteinase, crystal structure of VAP2 (macromolecule—catrocollastatin) from *Crotalus atrox* venom. 5 Example from Waprin family, structure of omwaprin from venom of *Oxyuranus microlepidotus*. 6 Example of Disintegrin, structure of saxthrombin (macromolecule—thrombin like enzyme defibrase) from venom of *Gloydius saxatilis*. 7 Example of Crotamine, structure of crotamine (macromolecule—crotamine Ile-19) from venom of Brazilian snake *Crotalus durissus terrificus*. 8 Example of Sarafotoxin, crystal structure of human endothelial ETB receptor in complex with sarafotoxin S6b from venom of *Atractaspis engaddensis*. 9 Example of Bradykinin potentiating peptides (BPPs), structure of human angiotensin-1 converting enzyme N-domain in complex with BPPb (Bradykinin potentiating peptide b) from venom of *Gloydius blomhoffii*. 10 Example of three-finger toxins, structure of muscarinic acetylcholine receptor-1 in complex with muscarinic toxin 7 (MT-7) from venom of *Dendroaspis angusticeps*

oxide and there blood pressure decreases [74]. A peptide named Bj-PRO-10 was seen to induce release of gamma-aminobutyric acid (GABA) and glutamate by modulating calcium fluxes in neurons. Bj-PRO-5a and Bj-PRO-10c were reported to be modulators of bradykinin-B2 and muscarinic M1 acetylcholine receptors. All these mechanisms show hypotensive action on blood pressure in vivo [74, 75].

Sarafotoxins (SRTXs)

SRTXs are structurally and functionally in similar to vertebrate endothelin (ETs). These are potent vasoconstrictors and their action is through interaction with endothelin receptors ET_A and ET_B [76]. The action of

SRT is modulation of cardiac and smooth muscle contraction [76]. SRTXs and its various isoforms exclusively feature in Genus *Atractaspis* [77]. These toxins contain amino acids in range 15 to 30 [78]. Sarafotoxins (structure in Fig. 2 (8)) showed coronary vasoconstriction in rat model and was reported to hydrolyze phosphoinositides with high affinity toward rat atrial and brain membrane.

Tripeptides

Tripeptides are small peptides and constitute three amino acids with pyroglutamate/glutamate featuring at its N-terminus while tryptophan at its C-terminus [79]. They are found only in *Viperidae* snake venoms and function as metalloproteinase inhibitors [80]. The function of these

peptides is to protect venom gland from auto digestion by its own proteinases and also preservation of venom proteins from hydrolysis during storage [81–83]. A synthetic tripeptide p-BTX-1 (Glu-Val-Trp) recently demonstrated its therapeutic implications in neurodegenerative disease (Parkinson) by demonstrating its ability to restore axonal connectivity in neurons undergoing degenerative processes and also enhanced neurogenesis [84]. Two tripeptides were extracted from venom of *Deinakistrodon acutus* named as Pt-A (Glu-Asn-Trp) and Pt-B (Glu-Gln-Trp); both these peptides were demonstrated to possess protective action against ADP induced paralysis in mice model. These peptides also exhibit antiplatelet aggregation and antithrombotic action when tested on human platelet-rich plasma and prevented pulmonary tissue thrombosis [85].

Crotamine

Crotamine also called as myoneurotoxin is found in the venom of *Crotalus durissus terrificus*, a South American snake and is a major constituent of its venom [86]. The composition of this polypeptide includes 42 amino acids, 3 disulfide bonds stabilizing the structure with 2 α -helices and 2 β -sheets [87]. The structure of crotamine (structure in Fig. 2 (7)) is unique having nearly every residue hydrophobic and charged exposed to solvent surfaces and thus able to show complex interactions [87]. Crotamine has this unique ability to show a variety of pharmacological actions. Due to a high positive charge, it is able to penetrate cells, bind with DNA and also has ability to cross the Blood–brain barrier (BBB) [87]. Since the surfaces of cancer cells have a negative charge, crotamine is attracted toward them strongly differentiating from normal cells [88]. When tested in mice model (C57Bl/6 J), crotamine has demonstrated anticancer activity against melanoma cells (B16-F10) [89]. Crotamine is a potent analgesic agent having potency 30 times more as compared to morphine which was evidenced by subcutaneous, intraperitoneal and oral dosing in mice model and measured with acetic acid-induced writhing method and the hot plate test [90]. Crotamine has antimicrobial activity, potential to cause mitochondrial dysfunction and ability to induce platelet aggregation [91]. Owing to its highly cationic nature, crotamine is able to cross cell membranes and accumulate inside cells without need of any specialized receptor, this ability can be used for targeting anticancer agents to cancer cells [92].

Waprin family

The first member from waprin family was isolated from venom of *Naja nigricolis* named as nawaprin (contains 51 amino acids) [93] and another member omwaprin-a (contains 50 amino acids) (structure in Fig. 2 (5)) was

isolated from venom of *Oxyuranus microlepidotus* [94] both having a similar structure and function. They have a flat disc like structure with whey acidic protein (WAP) domain, 4 disulfide bonds, a 3_{10} helix, β -sheets (short and antiparallel) and a spiral backbone [93, 95]. Nawaprin has structural similarity with human leukocyte elastase specific inhibitor (elafin) but due to variations in amino acid composition at important regions it does not act as an elastase specific inhibitor and rather has a different function [93]. Omwaprin has a selective and dose-dependent antimicrobial action on gram positive bacteria through membrane disruption. It was further reported that 3-D structure stabilized by 4 disulfide bonds and residues at N-terminal are essential for this activity. Omwaprin has specificity toward bacterial membrane and does not disrupt human erythrocytes (no hemolytic action) [94].

Waglerins

Waglerins are lethal toxins and are feature of *Tropidolaemus wagleri* venom [96]. They comprise a major portion, approximately 40% of total venom [97]. 22–44 amino acids are found in these peptides with 4 isoforms been identified. The isoforms show a high homology in sequence and differ only in few amino acids [96]. Walgerin-1 is a competitive antagonist of muscle nicotinic acetylcholine receptors. The intensity of action differs with species in human, mouse and rat with mouse being the prime target with highest toxicity imparted causing its death by respiratory failure [98]. Pentapharm Ltd. has developed an anti-wrinkle cream SYN-AKE™ which is a peptide mimic of Wtx-1, it is a prime example of waglerins use in therapeutics [99].

Therapeutic potential of venom peptides

It is very clear from the reports that venom peptides can be used as therapeutic moiety for the treatment of wide array of complications. The pharmacological potential of venom peptides can be utilized as medicaments in the treatment of several diseases which are in general very difficult to get cured or involves costly drugs with high toxicity. Cobra venom has been reported to be effective in treating tumors and also gives relief from the pain. Its pain relieving capacity was found better than morphine when tested on a 56 year old patient [100]. In a study involving ω -Conotoxin CVID from *Conus catus*, it was found that it is able to inhibit voltage sensitive calcium channel associated with neurotransmitter release in pre-ganglionic nerve terminals [101]. ω -Conotoxin has a potential in treating severe pain due to its selectivity toward N-type calcium channels. Among the four newly discovered ω -Conotoxins, CVID has highest affinity toward N-type channels [102]. α -Conotoxin MII is a toxin prepared by a marine predator snail *Conus magus*.

It is seen to inhibit nicotinic acetylcholine receptors of subtype $\alpha 3\beta 2$. In order to improve its bioavailability this peptide was coupled with 2-amino-D, L-dodecanoic acid (Laa) to the N terminus (LaaMII). It resulted in increased permeability across Caco-2 cells (continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells) with minimal toxicity [103]. Amphibian skins were investigated at large for biologically active arthropod alkaloids. Batrachotoxins were found in frogs of species *Phyllobates*, a potent irreversible sodium channel opener. It became an important tool for defining activity of anti-convulsant, analgesic, antiarrhythmic agents. Pumiliotoxins and allopumiliotoxins (7-hydroxypumiliotoxins) were found in *Dendrobates pumilio*, with potent myotonic and cardiotoxic activity through positive modulation of sodium channels [104]. Epibatidine extracted from Ecuadorian frog was found to be a powerful analgesic [104]. It is 200 times more potent than morphine. Its action is due to strong agonistic activity for nicotinic receptors. The amphibian skins of various poisonous species have proven to be a source of variety of biologically active compounds. Physalaemin, sauvagine, caerulein, magainin, dermorphin, biogenic amines, such as serotonins, tryptamines, histamines and tyramines, and also cardioactive steroidal bufadienolides, tetrodotoxins and samandarines, such as analgetic, vasoactive, cardiotoxic and antibiotic peptides, have been found in amphibian skin [104]. NN-32 protein isolated from Indian cobra (*Naja naja*) was tested on Ehrlich ascites tumors induced in BALB/c mice. It was observed that tumor decreased in weight and size significantly and the action was exhibited through apoptogenic and antioxidant property of NN-32 protein [105]. A protein isolated from venom of Indian monocellate cobra (*Naja kaouthia*) when tested on human leukemic cells K562 and U937 showed significant inhibition in proliferation of cell in a dose dependent manner, while the toxin showed less effect on normal leukocytes [106]. L-amino acid oxidase isolated from *Bothrops jararaca* venom showed anti-tumor activity. When tested on Ehrlich ascites tumor it showed significant inhibition of tumor growth and influx of polymorphonuclear cells was induced with spontaneous H_2O_2 liberation from peritoneal macrophages [107]. Venom peptides can be synthetically modified to improve anti-tumor effects. 13-mer synthetic peptide derived from hydrophobic/cationic C-terminal of Lys49 PLA2 homologue of BthTX-I from venom of *B. jararacussu* shows anti-tumor activity [108]. Phospholipases-A2 and their synthetic peptides derived from C-terminal have higher antitumor activity. The peptides derived from C-terminal of Lys49 phospholipase A2 from *Agkistrodon piscivorus piscivorus* and Lys49 myotoxin II from *bothrops asper* showed anti-tumor activity when tested on a number of different

tumor cell lines [109]. Peptides were synthesized from C-terminal of Myotoxic phospholipases A2 from venom of *Bothrops brazili* and then tested over various cancer cell lines and microbes, in results it demonstrated antitumor and antimicrobial activity of venom peptides synthesized from C-terminal [110]. drCT-I a heat stable protein toxin from venom of *Daboia russelli russelli* (Indian viper) was studied for its antitumor activity in Ehrlich ascites (EAC) cells in vivo in mice and on human leukemic cells in vitro. The drCT-I showed antiproliferative, apoptotic and cytotoxic activity against tumor cells. The effect of toxin on normal peripheral blood mononuclear cell (PBMNC) was found lesser as compared to cancer cells [111]. Sea snake (*Lapemis curtus*) venom was evaluated for antitumor activity by testing on Ehrlich ascites tumor in vivo in swiss albino mice and HeLa and Hep2 in vitro in cell cultures. It was found that a dose of 6.50 $\mu\text{g/ml}$ at 24 h effectively inhibited cell proliferation. The same dose when given by i.p. injection in EAC bearing mice a significant reduction in tumor was demonstrated and an increase in life span of mice by 205.25% was observed [112]. A peptide named Nubein6.8 was isolated from venom of Egyptian spitting cobra (*Naja nubiae*) and tested for anticancer activity. When tested on A375 cell line it showed a significant cytotoxic effect indicating its use against Melanoma. It also showed apoptosis in A2780 cell line showing its effectiveness in ovarian cancer. The mechanism of action were revealed and were through DNA damaging and activation of apoptosis [113]. Calciseptine a peptide isolated from venom of Black mamba specifically inhibits L-type calcium channels with no effect on other types such as N-type and T-type. Their action is similar to 1,4-dihydropyridines used in cardiovascular diseases [60]. Another peptide FS2 from mamba venom is a selective blocker of L-type calcium channel similar to Calciseptine and share site for binding on channels with 1,4-dihydropyridines [114]. These two peptides have shown to be more effective and sustainable in producing a hypotensive effect as compared to nifedipine [115]. Disintegrins are low molecular weight, cysteine rich proteins isolated initially from viperid venoms and contain an integrin binding site. Trigramin a fibrinogen inhibitor disintegrin isolated from *Trimeresurus gramineus*, binds to $\alpha_{IIb}\beta_3$ integrin receptor and thus prevents platelet aggregation [116]. Tirofiban (Aggrastat[®]) and Eptifibatide (Integrillin[®]) are the two drugs designed on basis of snake venom disintegrins and work as antiplatelet agents. Tirobafin was isolated from *Echis carinatus* venom. It binds to $\alpha_{IIb}\beta_3$ receptor by its RGD motif [117]. Eptifibatide was identified after screening about 62 snakes, which led to discovery of barbourin from *Sistrurus miliarius barbouri* venom, which binds specifically to $\alpha_{IIb}\beta_3$ through its

KGD motif [118]. Hemophilia and Thrombosis are caused due to imbalance in the blood coagulation process. Procoagulants need to be used in hemophilia while anti-coagulants in thrombosis. Animal venoms contain large varieties of peptides working on the hemostatic system. Examples of defibrinogenating agent are Ancrod from *Agkistrodon rhodostoma* [119] and Batroxobin from *Bothrops atrox* [120]. They belong to class A TLE and specifically cleave fibrinogen A α only. They act rapidly catalyzing the formation of soluble clots and reduce the level of circulating fibrinogen reducing formation of insoluble clots. Defibrinogenating agents therefore have a strong potential to be employed as anticoagulants as increased level of fibrinogen is a risk factor in acute arterial thrombosis. These two agents have shown many beneficial outcomes in clinically [35]. Cardiotoxin III (CTXIII) isolated from venom of *Naja naja atra* (Taiwan cobra) when exposed on MDA-MB-231 cells of breast cancer for checking the anticancer activity it was found that exposure with 0.03, 0.09 & 0.15 μ M of CTXIII for 18 h resulted in induction of apoptosis. Observation of cell indicated evidence accumulation of sub-G1 population, externalization of phosphatidylserine, mitochondrial membrane potential loss, release of cytochrome-c and activation of caspase-3 and caspase-9. The results all together indicated that CTXIII induces apoptosis in breast cancer cells MDA-MB-231 by inactivating JAK2, P13K, STAT3 and Akt signaling pathways [121]. ω -conopeptides SNX-111 and SNX-239 were checked for their nociception inhibiting ability. Acute (2 days) and chronic (7 days) studies were carried out on rat by infusing both the peptides for acute and chronic intervals and were compared with the effect caused by morphine. Formalin and Hot plate methods were employed to check the antinociception effect. Both peptides produced a significant reduction in response to hotplate and formalin as compared to morphine. Morphine was effective in acute treatment but seem to have reduced effect in chronic indicating tolerance. On the other hand, no tolerance was seen in both peptides SNX-111 and SNX-239 and their action was reversible in nature as response returned in 2 days after discontinuation in a 7-day course. ω -conopeptides act by blocking N-type voltage sensitive calcium channel and thus creating a powerful nociception [122]. *Daboia russelli* venom activates factor V through a serine protease enzyme present in the venom. Therefore it can be used to prepare a routine reagent for assay of factor V as it selectively activates factor V [123]. Russell's viper venom contains a potent activator of factor X. It has therefore been employed in assays especially for measurement of factor X [124], differentiating between factor VII & X deficiency [125] and in lupus anticoagulant assay [126]. Activated protein C (APC) functions as an

anticoagulant proteinase which functions as an inactivator of factor Va and VIIa and it has a key role in hemostasis. Moreover, Protein C (PC) is its inactive precursor. Assay of PC is difficult as activation by thrombin maybe incomplete and some thrombin may interfere in chromogenic assay. This difficulty has been solved by a fast acting activator of PC named Protac[®] obtained from venom of Southern copper head snake (*Agkistrodon c. contortrix*) [127, 128]. Voltage sensitive calcium channels play a crucial role in nervous system by mediating calcium entry into neurons. There are many types of calcium channels in mammalian brain but only N-type and L-type potent blockers are available. In order to find new channel blockers venom of funnel web spider was studied and a peptide ω -Aga-IVA was identified. ω -Aga-IVA is a blocker of both types of calcium channels in rat brain synaptosomes. P-type channel blocker in rat Purkinje neurons. ω -Aga-IVA will therefore help in characterization of calcium channels resistant to existing calcium channels blockers and will also be of assistance in designing new neuroprotective agents [129]. L-amino acid oxidases isolated from *Bothrops leucurus* venom shows inhibition of platelet aggregation dose-dependently when tested on both human PRP and washed platelets. The enzyme showed its cytotoxicity in vitro against *Leshmania* sp. and promastigotes with a very low EC₅₀ with dose of 0.07 μ M. Moreover, cytotoxicity was observed in stomach cancer cells MKN-45, colorectal RKO, adenocarcinoma HUTU and human fibroblast LL-24 cell lines. The enzyme action was dose dependent and the mechanism of action was through releasing excessive amounts of H₂O₂ which killed the cells [130]. Antitumor potential of a galactoside-binding lectin isolated from venom of *Bothrops leucurus* was studied. The lectin induced apoptosis in K562 cells, which was verified by externalization of phosphatidylserine and depolarization of mitochondrial membrane potential. The data also suggested that there was no induction of apoptosis on nontransformed human cells (normal cells). Therefore, in total the results suggests that the lectins from venom of *Bothrops leucurus* (BIL) has potential in cancer diagnostics and treatment [131]. The mechanism of action of BIL was studied on B16-F10 melanoma cells, and it was concluded that the selective death of B16-F10 melanoma cells was through dysregulation of calcium homeostasis in cells with increased levels of cytosolic calcium levels and MPT pore opening induced by calcium overload, resulting organelle failure and release of signaling factors in cytosol. Thus, followed by cell death [132], snake venom toxin (SVT) from *Vipera lebetine turanica* enhances the ability of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) to cause apoptosis in cancer cells. TRAIL is unable to cause apoptosis in resistant cells at even

higher concentrations. But when given in SVT there was a significant reduction growth of cells by inhibition by TRAIL even in resistant cells. The resultant is that SVT facilitates apoptosis induced by TRAIL in cancer cells by upregulation of TRAIL receptors, DR4 and DR5 through ROS and JNK pathway signals with subsequent downregulation of survival proteins [133]. The effects of TRAIL and SVT were studied on HCT116 and HepG2 cells and were found synergistic. Therefore, this combination can be used in treatment of colorectal cancer [133]. In an investigation on SVT it was found that it inhibits hormone-refractory human prostate cancer cell growth. The apoptosis is induced through inactivation of nuclear factor κ B [134]. Atroxylin-I a P-metalloproteinase isolated from venom of *Bothrops atrox* has ability to cause hemorrhages. It has specificity toward plasma proteins fibrinogen and FN collagens I and IV on the vessel walls, laminin-binding integrin $\alpha_7\beta_1$ and collagen binding integrin $\alpha_1\beta_1$. The enzyme possesses tissue degrading and digestive mechanisms. Proteolysis of substrate proteins involved in vessel integrity may be the reason behind hemorrhagic potential [135]. The novelty can be further study of interest for therapeutics in hemorrhagic disorders. Okinawa Habu apoxin protein-1 (OHAB-1) has been extracted from Okinawa Habu (*Trimeresurus flavoviridis*). When tested for apoptotic activity on malignant glioma cell lines rat C6 and human RBR 17 T, U251, it inhibited growth of all cell lines. The action was probably by promoting intracellular ROS generation and P53 protein expression. It suggests that OHAB-1 is a promising candidate in cancer therapy [136]. Snake venom cystatin (sv-systatin) was evaluated to check the possibility of use in cancer treatment. The evaluation was done on liver cancer cells (MHCC97H) in vitro and in vivo. cDNA of sv-cystatin was transfected in MHCC97H cells. The results suggested that there was a delay in invasion and metastasis exhibited by sv-cyst clone. The activity was through reduction in activity of proteinases (cathepsin B, MMP-2 and MMP-9) and epithelial-mesenchymal transmission (EMT) [137]. Ziconotide is a potent and reversible N-type calcium channel blocker, it is a synthetic derivative of ω -conotoxin peptides found in *Conus magus* (Cone snail). Nociception caused by ziconotide was studied in rats with morphine by administering both agents intrathecally. The results indicated that 1. acute intrathecal administration of morphine and ziconotide produced additive and synergistic effects, 2. chronic administration of morphine led to tolerance but no cross tolerance produced to ziconotide, 3. chronic administration of ziconotide showed no tolerance nor cross tolerance, and 4. intrathecal administration of ziconotide did not prevent or reverse morphine tolerance. Therefore ziconotide is a potential candidate for amelioration of severe pain [138].

Disintegrins from venoms can be effective in treating cancer. In order to test anticancer activity, rhodostomin from venom of *Calloselasma rhodostoma* Trigramin from venom of *T. gramineus* were isolated and tested on tumor cells. Rhodostamin inhibited adhesion, migration and invasion of cells in breast and prostate cancer cell lines. Trigramin was able to inhibit MDA-MB-231 which is an estrogen independent cell line which can metastasize to bone [139]. cDNA encoding batroxobin from venom of *Pichia pastoris* was cloned. The recombinant protein converted fibrinogen into fibrin clot in vitro and was able to shorten bleeding time and whole blood coagulation time in vivo. This study suggests that recombinant batroxobin is a potential candidate as a pro-coagulant agent [140]. ACTX-6 a cytotoxic peptide from *Agkistrodon acutus* snake venom is able to induce apoptosis when tested on A549 cells via Fas pathway. JNK pathway was involved in ACTX-6 apoptosis and the oxidative stress produced by ACTX-6 was responsible for activation of JNK pathway [141]. ACTX-8 another cytotoxic peptide from *Agkistrodon acutus* also shows apoptotic activity when tested on Hela cervical cancer cells. Study indicated that ACTX-8-induced apoptosis was through mitochondrial pathway and regulated by Bcl-2 family proteins. The study indicates ACTX-6 and ACTX-8 as potential anticancer agents [141]. Venom from *Agkistrodon acutus* has therefore caught an eye for further research on peptides isolated from it as a therapeutic utility. A brief summary of different venom peptides and their applications in treating various medical conditions is summarized in Table 2, whereas various peptides, their pharmacological and toxicological action are given in Fig. 3.

Nanoparticles as drug delivery agents for venom peptides
Nanotechnology is among the new technologies developing since decades. It is a nanoscale drug delivery system intended to alter kinetics, body distribution, and drug release factors associated with the drug [144]. Evolution of nanoparticle technology as a targeted delivery system has given quite some advantages over traditional therapy. Lowering the side effects, improving drug efficacy, increased patient compliance and reducing healthcare costs are among the advantages [145]. Moreover, they increase the half-life and the effect of drug at target site is enhanced eliminating the effect on non-target organs; also the dose required to achieve therapeutic concentration level is reduced [146, 147]. Nanoparticle inspired therapy is the trend of the era due to fascinating improvements in diagnosis, mitigation and care. Nanoparticles enhance the therapeutic efficacy of venom peptides conjugated with it. Systemic toxicity is certainly reduced, moreover targeted delivery

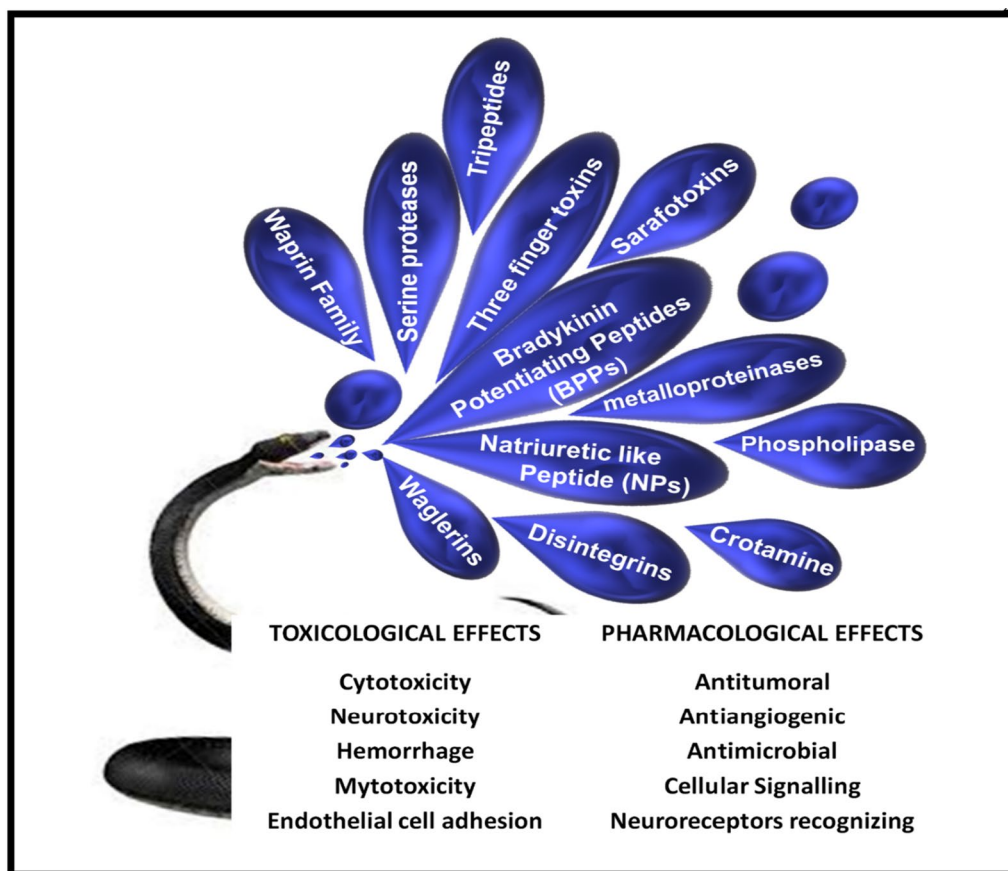


Fig. 3 Different types of venom peptides present in venom, their pharmacological and toxicological actions

increases potency. Stability, bioavailability, half-life can be altered as per the therapeutic needs [148, 149]. Various types of nanoparticles such as metallic, dendrimers, chitosan, super magnetic which are hydrophilic in nature have been studied for delivery of venom peptides [150]. Nanoparticles have unique properties; they can by themselves alter protein profile in vitro [151], also showing in vivo activity in cancer cells which make them invaluable in therapeutic and toxicology. Gold nanoparticles on their own are able to produce cytotoxicity as shown by *Patra et al.* Bare gold nanoparticles can arrest S phase of cell cycle. Therefore, gold nanoparticles can play a dual role of drug carrier as well as an anticancer agent [152].

In short, nanoparticles are able to take over the shortcomings of conventional deliveries. Nanoparticles can deliver vaccines, antibodies, peptides, vitamins, diagnostic agents along with the conventional medicine [144, 153–155]. There are diverse categories of nanoparticles as per their composition and role in drug delivery. Nanoparticles and their applications in drug delivery is described below (Fig. 4).

Polymeric nanoparticles

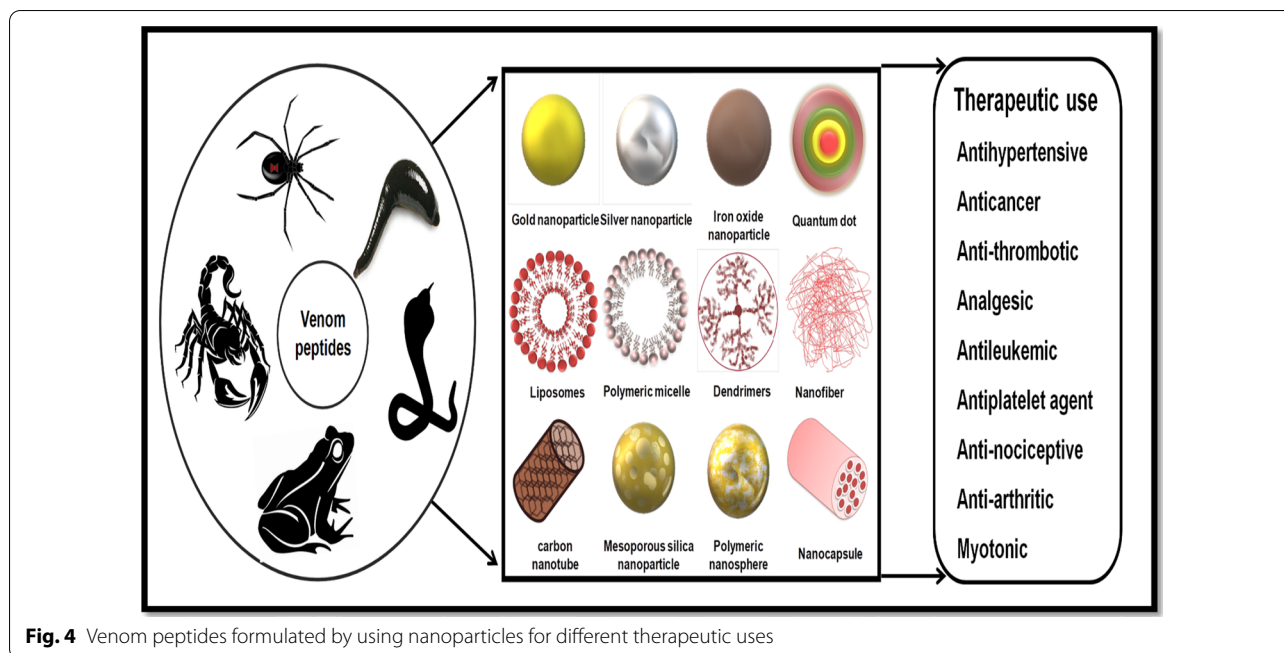
Polymeric nanoparticles are colloidal and in size range of 10 to 1000 nm, they are spherical, branched or core-shaped structures. They are prepared using biodegradable synthetic polymers (polylactide–polyglycolide copolymers, polyacrylates and polycaprolactones) [156] and natural polymers (albumin, alginate, gelatin, collagen and chitosan) [157–159]. Zinc (II) phthalocyanine (ZnPc) an agent for photodynamic therapy was formulated using PLGA and tested on mice tumors showed considerable reduction in tumor and increased life as compared to free ZnPc which indicates the increased photodynamic effect in target organ tissues [160]. ICD-85 is a venom derived peptide known to show anticancer activity. These peptides were loaded into Sodium alginate nanoparticles by *Borumand, M.R. et al.* It was observed that ICD-85 loaded nanoparticles in comparison to free ICD-85 exhibited more potent cytotoxicity and suppressed proliferation of HEP-2 cells with a sustained release profile [7]. *Echis carinatus* snake venom was formulated using chitosan nanoparticles into a novel drug delivery increasing over all efficacy compared to traditional delivery [161].

Table 2 Summary of venoms peptides and their uses

Source	Venom peptide	Potential use	References
<i>Bothrops jararaca</i>	Jarafirase	Antihypertensive	[5]
<i>Ophiopagus hannah</i>	L-amino acid oxidases	Antiproliferative/Anticancer	[142]
<i>Agkistrodon contortrix contortrix</i>	Fibrolase	Anti-thrombotic	[143]
<i>Crotalus atrox</i>	Atroxase	Anti-thrombotic	[143]
<i>Conus catus</i>	ω -conotoxin (CVID)	Severe pain relief	
<i>Conus magus</i>	α -conotoxin (MII)	Adenocarcinoma targeting agent	[103]
<i>Phllobates</i>	Batrachotoxins	Defining activity of anticonvulsant, analgesic, antiarrhythmic agents	[104]
<i>Dendrobates pumilio</i>	Pumiliotoxins and allopumiliotoxins	Myotonic and Cardiotonic	[104]
Ecuadorean frog	Epibatidine	Analgesic	[104]
<i>Naja naja</i>	NN-32	Ehrlich ascites tumor treatment	[105]
<i>Naja kaouthia</i>	α -cobratoxin/ disintegrins	antileukemic	[106]
<i>Agkistrodon piscivorus piscivorus</i>	Lys49 phospholipase A2	Antitumor	[109]
<i>Bothrops brazili</i>	Myotoxic phospholipases A2	Antitumor and Antimicrobial	[110]
<i>Daboia russelli russelli</i>	drCT-I	Ehrlich ascites tumor treatment	[111]
<i>Naja nubiae</i>	Nubein6.8	Treatment of Melanoma and Ovarian cell cancer	[113]
<i>Dendroaspis polylepis</i>	Calciseptine	Selective L-type calcium channel blocker (Antihypertensive)	[60]
<i>Trimeresurus gramineus</i>	Trigramin	Antiplatelet agent	[116]
<i>Echis carinatus</i>	Tirobafin	Antiplatelet agent	[117]
<i>Sistrurus miliarius barbouri</i>	Barbourin	Antiplatelet agent	[118]
<i>Agkistrodon rhodostoma</i>	Ancrod	Defibrinogenating agent	[119]
<i>Bothrops atrox</i>	Batroxobin	Defibrinogenating agent	[120]
<i>Naja naja atra</i>	Cardiotoxin III (CTXIII)	Treating breast cancer	[121]
Marine cone snail genus- <i>Conus</i>	ω -conoptides SNX-111 and SNX-239	Anti-nociceptive	[122]
<i>Daboia russelli</i>	a serine protease	Assay of factor-V	[124]
<i>Bothrops leucurus</i>	L-amino acid oxidases	Treating stomach cancer, colorectal cancer, adenocarcinoma, melanoma and action against Leshmania sp. And promastigotes	[130]
<i>Vipera lebetine turanica</i>	Venom toxin	Enhancement of ability of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) to cause apoptosis in cancer cells	[133]
<i>Bothrops atrox</i>	Atroxylsin-I	Therapeutics in hemorrhagic disorders	[135]
<i>Trimeresurus flavoviridis</i>	Okinawa Habu apoxin protein-1	Treating glioma malignancies	[136]
<i>Calloselasma rhodostoma</i>	Rhodostamin	Treating breast cancer	[139]
<i>Agkistrodon acutus</i>	ACTX-6	Treating Adenocarcinoma of lung	[141]

Naja naja oxiana venom was encapsulated in chitosan nanoparticles; stable, hydrophilic, with high loading capacity nanoparticles were formed. Venom from cobra *Walterinnesia aegyptia* (WEV) was isolated and investigated for apoptotic action on breast cancer cells. The study was performed on isolated human breast cancer cells from female patients and treated with WEV and also silica nanoparticle conjugated WEV (WEV-NP). Caspase activity and free radical count were monitored during investigation. Both WEV and WEV-NP showed ability to inhibit breast carcinoma cell proliferation in dose dependent manner, the WEV-NP showed more efficiency

and significantly enhanced the anticancer effect of WEV [162], showing the efficiency of nanoparticle delivery. Melittin isolated from bees' venom has been known for its anticancer activity. But also carries several disadvantages like unpredictable pharmacokinetics, toxicity to other cells and non-specificity. In order to overcome these problems, formulation was developed by incorporation of melittin in outer monolayer of perfluorocarbon nanoparticles [163]. This increased accumulation of melittin in cancer cells and also decreased the tumor cells significantly without any signs of envenomation. Moreover, its selectivity increased toward delivering melittin to cancer cells avoiding the normal cells [164].



Solid lipid nanoparticles

Solid lipid nanoparticles (SLNs) have been developed as an alternative to liposomes, emulsions and polymeric nanoparticles. They are made of solid lipids (highly purified triglycerides, complex glyceride mixtures or waxes) and are capable of controlled delivery [144, 154, 165]. SLN have use as a gene targeting agent, which has been seen in a study carried out by Yu et al. involving alveolar macrophages a in which DNA-loaded cationic SLN was surface modified by synthesizing mannan-based PE-grafted ligand which resulted in Man-SLN-DNA. Comparative study with non-modified SLN-DNA and Lipofectamine indicated Man-SLN-DNA has higher gene expression in vivo [166]. In a study, toad venom was formulated in solid lipid nanoparticles (TC-SLNs). The formulation was tested for its antitumor activity using Hela and SKOV-3 cells. Flow cytometry and MTT assay were performed to measure cell division and cell distribution. The results showed that in Hela cells G0/G1 phase decreased, S and G2/M phases increased which indicated that S and G2/M phases were blocked after incubation by TC-SLNs. On the other hand, in SKOV-3 cells G0/G1 phases increased, while S and G2/M phases decreased indicating that G0/G1 phases are blocked by TC-SLNs after incubation [167]. The overall results concluded that TV-SLNs inhibit fissiparism in a dose dependent manner in HeLa and SKOV-3 cells and thus an effective drug delivery systems for treatment of cervical and ovarian cancer [167].

Polymeric micelles

Polymeric micelles are formed by hydrophobic and hydrophilic monomer units of block co-polymers [168]. The core is formed of hydrophobic blocks, while it is stabilized by hydrophilic chains of outer corona. Generally, PEG blocks with weight 1 to 15 kDa are used for hydrophilic part, while the length of hydrophobic core is lesser or close to the hydrophilic part [169]. They enhance bioavailability, reduce side effects, control release possible and increase solubility; all this makes polymeric micelles an effective delivery system [168]. Adriamycin formulated in polymeric micelles shows much more efficiency in treating experimental murine solid tumor colon adenocarcinoma as compared to free drug [170]. Toxins have also been used as a targeting module, α conotoxin ImI isolated from cone snail *Conus imperialis* has target specificity toward $\alpha 7$ type ($\alpha 7$ -nAChR) receptors which among many are highly expressed in cancers [171], hence are subject to target specific treatments. Toxin conjugated polyethylene glycol-grafted distearoylphosphatidylethanolamine micelles (ImI-PMs) were prepared and tested by using drug paclitaxel for targeting efficiency [172]. It was observed that paclitaxel-loaded ImI-PMs showed greater cytotoxicity and apoptotic potential and also inhibited tumor growth in MCF-7 tumor-bearing mice. Thus, stating the potential of alpha-conotoxin ImI-modified nanocarrier for targeted delivery of drug to $\alpha 7$ -nAChR overexpressing tumor cells [164].

Liposomes

Liposomes are bilayer spherical self-assembled structures prepared from amphiphilic phospholipids. The core is aqueous while surrounded by a lipid bilayer. Liposomes are able to carry hydrophilic as well as hydrophobic drugs [173, 174]. Liposomes are the clinically most established systems and offer a great deal of advantages. They can prolong a drug effect by reducing clearance as well as able to reduce systemic toxicity [175]. Also possess potential for sustained release of drug [176]. Toxicity of doxorubicin an anticancer drug on heart and kidney is well known. A novel liposomal formulation of doxorubicin has resulted in reduced toxicity as lesser amount reaches heart and kidney, while the formulated drug targets tumors tissues [177]. Scorpion venoms loaded in liposomes were prepared by *Al-Asmari et al.*, and it was observed that venom loaded nanoparticles have much more pronounced anticancer activity as compared to free venom forms when checked on HCT-8 cell line. Furthermore, colorectal cancer cell line treated with venom-loaded nanoparticles showed statistically significant low cell survival, elevated ROS generation, high apoptosis and G0/G1 enrichment. It also states the vital role of liposomes in ever-developing field of nanomedicine [178].

Carbon nanotubes

Carbon nanotubes (CNTs), also known as bucky tubes, are consisting of hollow cylindrical tubes (molecules) containing carbon atoms organized in hexagonal lattice structure [179]. CNTs are broadly categorized as per number of graphic shells. However, two major categories are widely utilized now days. These are single-walled CNTs (SWNTs) and multi-walled CNTs (MWNTs). Carbon-based nanoparticles like carbon nanotubes, fullerene and nanodiamonds have been studied for their use as drug carriers [180]. Single-wall nanotubes conjugated with paclitaxel has shown more promise compared to other taxol in in vivo experiments [181]. A study was performed wherein *Crotalus durissus terrificus* (rattle snake) venom was developed into a fibrin sealant. It is a thrombin like enzyme with ability to convert fibrinogen into fibrin with qualities such as biodegradable, non-immunogenic, non-toxic, naturally gelling agent. The fibrin sealant was formulated with multi-walled carbon nanotubes (MWCNT) and nanohydroxyapatite and was proposed to have potential to accelerate bone regeneration [182].

Inorganic nanoparticles

Gold nanoparticles (AuNPs) are among the most synthesized metal based nanoparticles. As the gold nanoparticles carry a negative charge they are easily functionalized by various biomolecules [183]. Oxaliplatin formulated

with AuNPs shows an enhanced cytotoxicity when tested on cell lines; moreover, AuNPs showed an extraordinary ability to penetrate nucleus in lung cancer cells [184]. Ceramic nanoparticles are inorganic, porous, ultra-low sized particles which don't change their porosity and don't swell with changes in pH and temperature [185]. Ceramic nanoparticles can act as a nonviral vector for gene delivery by using Poly-L-lysine to modify silica nanoparticles (PMS-NPs) [186]. A peptide NKCT1 isolated from venom of *Naja kaouthia* was conjugated with PEGylated gold nanoparticles (GNP-NKCT1) for its anti-leukemic potential by *Bhowmik T.* et al. When tested on human leukemic lymphoma cell line and human myelogenous leukemic cell line it showed selective anticancer activity and apoptosis was induced probably through mitochondrial or lysosomal pathway [187]. There was lesser cardiotoxicity and neurotoxicity with conjugated NKCT1 as compared to free toxin. The histopathological reports suggested a reduction in toxicity of kidneys after NKCT1 conjugation [188]. Even twice the IC₅₀ dose of gold conjugated NKCT1 showed lesser toxicity as compared to unconjugated NKCT1 toward epithelial cells, fibroblasts and peripheral blood mononuclear lymphocytes [189]. Moreover, further study reported that gold conjugated NKCT1 has anticancer effect on Ehrlich ascites carcinoma, proved by in vitro and in vivo tests on EAC cell lines and induced EAC in mice, respectively. Also, caspase-dependent activity was demonstrated behind this action with upregulation of Bax and down-regulation of Bcl2 and ultimately increased expression of caspase 3/9 [190]. Gold nanoparticles also demonstrated their ability to increase the uptake of NKCT1 by cancer cells [189]. GNP-NKCT1 also showed anti-arthritis activity when tested in male albino rat model [191]. Hence, gold nanoparticle conjugated NKCT1 can be a good treatment against leukemia, EAC, arthritis [187, 190, 191]. [192]. From the skin of frog *Bombina bombina* a peptide called bombesin was isolated and found to have affinity for gastrin-releasing peptide (GRP) [193] which is highly expressed in prostate cancer, breast cancer and lung cancer. Now, this peptide was conjugated with gold nanoparticles, and tested on cancer cell lines in mice. It was found that intraperitoneal injection of bombesin-gold nanoparticles were able to avoid absorption by reticuloendothelial system and concomitant increase in selective uptake of drug by cancer cells was noted [194]. Chlorotoxin from scorpion *Leiurus quinquestriatus* venom has an ability to bind specifically to glioma cell surfaces as a chlorine channel and matrix metalloproteinase-2 blocker. This ability is useful in treating glioma [195]. Iron oxide nanoparticle-methotrexate-chlorotoxin conjugate has shown increased efficiency in treating tumor cells. In vitro experiments show increased

Table 3 Summary of nanoparticle-conjugated venom peptides

Venom/source	Nanoparticle	Use	References
ICD-85	Sodium alginate NPs	Anticancer	[7]
Scorpion venom	Liposomes	Colorectal cancer treatment	[178]
<i>Echis carinatus</i> snake venom	Chitosan NPs	Blood coagulant	[161]
<i>Naja naja oxiana</i> venom	Chitosan NPs	Anticancer	[162]
NKCT1	PEGylated gold NPs	Antileukemic, Anti-EAC, Anti-arthritic	[187]
Bombesin	Gold NPs	Selective drug targeting of GRP in cancer cells	[194]
<i>Walterinnesia aegyptia</i> venom	Silica NPs	Breast cancer treatment	[162]
Melittin	Perfluorocarbon nanoparticles	Anticancer	[163]
α -conotoxin Iml	Polyethylene glycol-grafted distearoylphosphatidylethanolamine micelles	Selective drug targeting to $\alpha 7$ type ($\alpha 7$ -nAChR) receptors in cancer cells	[172]
Chlorotoxin from <i>Leiurus quinquestriatus</i>	Iron oxide NPs	As a targeting agent for drugs in glioma and medulloblastoma	[196]
Chlorotoxin from <i>Leiurus quinquestriatus</i>	Polyamidoamine (PAMAM) dendrimers	As a targeting ligand for glioma-targeting gene delivery system	[201]
Fibrin sealant from <i>Crotalus durissus terrificus</i>	Multi-walled carbon nanotubes (MWCNTs)	Accelerating bone regeneration	[182]
Toad venom	Solid lipid nanoparticles	Anticancer activity against cervical and ovarian cancer	[167]

accumulation of NP-MTX-CTX in glioma and medulloblastoma cells, while in vivo studies on mice xenograft tumors showed increased delivery of drug preferentially to tumor cells and its retention for at least 2 weeks [196].

Dendrimers

Dendrimers, as the name suggests are tree like projections synthetically formed structures. These are highly branched 3-D synthetic polymeric structures. These are consisting of multiple branched monomers arising from central core and thereby producing star shaped architecture [197]. Dendrimers are mostly made of polyamidoamines (PAMAMs) [198]. Anticancer drug cisplatin was complexed with surface groups of G-4 carboxylate-terminated PAMAM dendrimer. In comparison to free drug, it exhibited slower rate of release, lower toxicity and increased accumulation of drug in solid tumors [199]. Chlorotoxin (CTX) obtained by purification from *Leiurus quinquestriatus* (giant Israeli scorpion) venom shows binding affinity toward membrane-bound matrix metalloproteinase-2 (MMP-2) endopeptidase which are highly expressed in glioma cells and hence possess binding affinity toward glioma tumors and other tumors of neuroectodermal origin [200]. Chlorotoxin can therefore be used as a targeting ligand for glioma-targeting gene delivery system [201]. In a study, nanoscopic high-branching dendrimer, polyamidoamine (PAMAM) was used as a vector, with targeting ligand being chlorotoxin and polyethylene glycol as a conjugating polymer and DNA as therapeutic agent together which formed PAMAM-PEG-CTX/DNA NPs [201]. When tested on

C6 glioma cells, it showed better uptake, distribution and gene expression as compared to formulations without CTX which clearly demonstrated the efficacy of CTX conjugated dendrimers (PAMAM-PEG-CTX/DNA) in glioma cell targeting [201]. Nanotechnology has certainly changed the traditional approaches in cancer therapy, with improved techniques of detection, diagnosis, tumor destruction, enhanced drug effects and targeted delivery [202]. Hence, in short nanotechnology has been a miracle in many aspects leading to improved therapy of many diseases and also taking in consideration the ability to formulate many new agents effectively.

Various venom peptides conjugated nanoparticles and their therapeutic applications have been demonstrated in Table 3.

Conclusions

Nature is the ultimate and essential source of endless biologically active substances that can supply treatments for a variety of unsolved health problems, with "venom-toxins" as real promising candidates whose power must be understood and disclosed. Several studies have suggested that venom peptides can successfully cure a variety of deadly illnesses. However, a variety of concerns related to delivery, pharmacokinetics, safety and stability must be addressed for therapeutic uses. The nanotechnology to nanodelivery provides an ideal foundation for tackling the venom delivery challenge. Targeted action, greater bioavailability, minimizing adverse effects and customizing drug pharmacokinetics as needed are all possible using

nanoparticle formulations of venom and associated peptides. Nanotechnology has a great potential to improve the current health care systems but should be used wisely. Venom peptides along with nanoparticles can evolve as the new era for potential treatments of ongoing and untreatable diseases.

Abbreviations

WHO: World Health Organization; PLA2s: Phospholipases A₂; 3FTX: Three-finger toxins; SRTXs: Sarafotoxins; ADAMs: A disintegrin-like and metalloproteinases; SVMPs: Snake venom metalloproteinases; NF- κ B: Nuclear factor kappa-light-chain-enhancer of activated B; BPPs: Bradykinin potentiating peptides; RAS: Renin angiotensin system; ACE: Angiotensin-converting enzyme; ASS: Arginosuccinate synthetase; GABA: Gamma-aminobutyric acid; BBB: Blood-brain barrier; WAP: Whey acidic protein; PBMC: Peripheral blood mononuclear cell; TNF: Tumor necrosis factor; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; EMT: Epithelial-mesenchymal transmission; ZnPC: Zinc (II) phthalocyanine; PLGA: Poly (lactic-co-glycolic acid); WEV-NP: *Walterinnesia aegyptia*-nanoparticle; SLNs: Solid lipid nanoparticles; PEG: Poly-ethylene glycol; ROS: Reactive oxygen species; CNTs: Carbon nanotubes; SWNTs: Single-walled CNTs; MWNTs: Multi-walled CNTs; AuNPs: Gold nanoparticles; PAMAMs: Polyamidoamines.

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Author contributions

Aditya Vivek Joglekar contributed to conceptualization and manuscript writing; Deepa Dehari contributed to graphical abstract and other images designing, tables and data curation; Md Meraj Anjum, Naveen Dulla and Aiswarya Chaudhuri performed data collection, validation and editing manuscript; Sanjay Singh performed reviewing; Ashish Kumar Agrawal read, reviewed, edited and approved the final manuscript. All authors read and approved the final manuscript.

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Declarations

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I authenticate that this manuscript is original, that it has never been published before, and that it will not be published elsewhere while Toxicon considers it. Furthermore, the study is not divided into multiple parts in order to increase the number of submissions to various journals or to a single journal over time. The submission has been received explicitly from all co-authors. This article does not contain any studies with human participants or animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

Consent for publication

All authors have the consent for publication.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work revised in this article.

Author details

¹Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (BHU), Varanasi 221005, India. ²Babasaheb Bhimrao Ambedkar University, Lucknow, India.

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References

1. Utkin YN (2015) Animal venom studies: Current benefits and future developments. *World J Biol Chem* 6(2):28
2. Augustyn A, Bauer P, Duignan B, Eldridge A, Gregersen E, McKenna A, Petruzzello M, Rafferty JP, Ray M, Rogers K, Tikkanen A, Wallenfeldt J, Zeidan A, Zelazko A (2019) Venom. In: *Encyclopædia Britannica T.E.o.E.* Britannica, Editor. Encyclopædia Britannica, Inc
3. Gantenbein UL (2017) Poison and its dose: paracelsus on toxicology. In: *Toxicology in the middle ages and renaissance.* Elsevier, pp 1–10
4. Pal SK, Gomes A, Dasgupta S et al (2002) Snake venom as therapeutic agents: from toxin to drug development. *Indian J Exp Biol* 40(12):1353–1358
5. Cushman DW, Ondetti MA (1999) Design of angiotensin converting enzyme inhibitors. *Nat Med* 5(10):1110–1112
6. Lewis RJ, Garcia ML (2003) Therapeutic potential of venom peptides. *Nat Rev Drug Discov* 2(10):790–802
7. Borumand MR (2013) Preparation and characterization of sodium inorganic nanoparticles containing ICD-85 (venom derived peptides). *Int J Innov Appl Stud* 4(3):534–542
8. Zelanis A, Tashima AK (2014) Unraveling snake venom complexity with 'omics' approaches: challenges and perspectives. *Toxicon* 87:131–134
9. McCleary RJ, Kini RM (2013) Non-enzymatic proteins from snake venoms: a gold mine of pharmacological tools and drug leads. *Toxicon* 62:56–74
10. Kalia J, Milescu M, Salvatierra J et al (2015) From foe to friend: using animal toxins to investigate ion channel function. *J Mol Biol* 427(1):158–175. <https://doi.org/10.1016/j.jmb.2014.07.027>
11. Zhang Y (2015) Why do we study animal toxins? *Zool Res* 36(4):183
12. Munawar A, Ali SA, Akrem A et al (2018) Snake venom peptides: tools of biodiscovery. *Toxins* 10(11):474
13. Six DA, Dennis EA (2000) The expanding superfamily of phospholipase A2 enzymes: classification and characterization. *Biochimica et Biophysica Acta (BBA) Mol Cell Biol Lipids* 1488(1–2):1–19
14. Harris JB, Scott-Davey T (2013) Secreted phospholipases A2 of snake venoms: effects on the peripheral neuromuscular system with comments on the role of phospholipases A2 in disorders of the CNS and their uses in industry. *Toxins* 5(12):2533–2571. <https://doi.org/10.3390/toxins5122533>
15. Šribar J, Oberčkal J, Križaj I (2014) Understanding the molecular mechanism underlying the presynaptic toxicity of secreted phospholipases A2: an update. *Toxicon* 89:9–16
16. Benishin CG (1990) Potassium channel blockade by the B subunit of beta-bungarotoxin. *Mol Pharmacol* 38(2):164–169
17. Harris J, Grubb B, Maltin C et al (2000) The neurotoxicity of the venom phospholipases A2, notexin and taipoxin. *Exp Neurol* 161(2):517–526
18. Zambelli VO, Picolo G, Fernandes CA et al (2017) Secreted phospholipases A2 from animal venoms in pain and analgesia. *Toxins* 9(12):406
19. Moreira V, Lomonte B, Vinolo MAR et al (2014) An Asp49 phospholipase A2 from snake venom induces cyclooxygenase-2 expression and prostaglandin E2 production via activation of NF- κ B, p38MAPK, and PKC in macrophages. *Mediators Inflamm* 2014:105879. <https://doi.org/10.1155/2014/105879>
20. Cunha F, Poole S, Lorenzetti B et al (1992) The pivotal role of tumour necrosis factor alpha in the development of inflammatory hyperalgesia. *Br J Pharmacol* 107(3):660
21. Verri WA Jr, Cunha TM, Parada CA et al (2006) Hypernociceptive role of cytokines and chemokines: targets for analgesic drug development? *Pharmacol Ther* 112(1):116–138
22. Zhang CC, Medzihradsky KF, Sanchez EE et al (2017) Lys49 myotoxin from the Brazilian lancehead pit viper elicits pain through regulated ATP release. *Proc Natl Acad Sci USA* 114(12):E2524–E2532. <https://doi.org/10.1073/pnas.1615484114>

23. Chacur M, Longo I, Picolo G et al (2003) Hyperalgesia induced by Asp49 and Lys49 phospholipases A2 from *Bothrops asper* snake venom: pharmacological mediation and molecular determinants. *Toxicon* 41(6):667–678
24. Bohlen CJ, Chesler AT, Sharif-Naeini R et al (2011) A heteromeric Texas coral snake toxin targets acid-sensing ion channels to produce pain. *Nature* 479(7373):410–414
25. Teixeira SS, Silveira LB, da Silva FM et al (2011) Molecular characterization of an acidic phospholipase A2 from *Bothrops pirajai* snake venom: synthetic C-terminal peptide identifies its antiplatelet region. *Arch Toxicol* 85(10):1219–1233
26. Hite LA, Jia L-G, Bjarnason JB et al (1994) cDNA sequences for four snake venom metalloproteinases: structure, classification, and their relationship to mammalian reproductive proteins. *Arch Biochem Biophys* 308(1):182–191
27. Jia L-G, Shimokawa K-I, Bjarnason JB et al (1996) Snake venom metalloproteinases: structure, function and relationship to the ADAMs family of proteins. *Toxicon* 34(11–12):1269–1276
28. Fox JW, Serrano SM (2005) Structural considerations of the snake venom metalloproteinases, key members of the M12 repolysin family of metalloproteinases. *Toxicon* 45(8):969–985
29. Casewell NR (2012) On the ancestral recruitment of metalloproteinases into the venom of snakes. *Toxicon* 60(4):449–454. <https://doi.org/10.1016/j.toxicon.2012.02.006>
30. Moura-da-Silva AM, Theakston RDG, Crampton JM (1996) Evolution of disintegrin cysteine-rich and mammalian matrix-degrading metalloproteinases: gene duplication and divergence of a common ancestor rather than convergent evolution. *J Mol Evol* 43(3):263–269
31. Casewell NR, Wagstaff SC, Harrison RA et al (2011) Domain loss facilitates accelerated evolution and neofunctionalization of duplicate snake venom metalloproteinase toxin genes. *Mol Biol Evol* 28(9):2637–2649
32. Markland FS Jr, Swenson S (2013) Snake venom metalloproteinases. *Toxicon* 62:3–18
33. Gutierrez JM, Rucavado A, Escalante T et al (2005) Hemorrhage induced by snake venom metalloproteinases: biochemical and biophysical mechanisms involved in microvessel damage. *Toxicon* 45(8):997–1011. <https://doi.org/10.1016/j.toxicon.2005.02.029>
34. Escalante Muñoz T, Rucavado Romero A, Fox JW et al (2011) Key events in microvascular damage induced by snake venom hemorrhagic metalloproteinases. *J Proteomics* 74(9):1781–94. <https://doi.org/10.1016/j.jprot.2011.03.026>
35. Markland FS (1998) Snake venoms and the hemostatic system. *Toxicon* 36(12):1749–1800
36. Bernardes CP, Menaldo DL, Mamede CC et al (2015) Evaluation of the local inflammatory events induced by BpirMP, a metalloproteinase from *Bothrops pirajai* venom. *Mol Immunol* 68(2):456–464
37. De Toni LG, Menaldo DL, Cintra AC et al (2015) Inflammatory mediators involved in the paw edema and hyperalgesia induced by Batroxase, a metalloproteinase isolated from *Bothrops atrox* snake venom. *Int Immunopharmacol* 28(1):199–207
38. Brust A, Sunagar K, Undheim EA et al (2013) Differential evolution and neofunctionalization of snake venom metalloprotease domains. *Mol Cell Proteomics* 12(3):651–663. <https://doi.org/10.1074/mcp.M112.023135>
39. Vaiyapuri S, Thiyagarajan N, Hutchinson EG et al (2012) Sequence and phylogenetic analysis of viper venom serine proteases. *Bioinformatics* 8(16):763
40. Tasoulis T, Isbister GK (2017) A review and database of snake venom proteomes. *Toxins* 9(9):290
41. Serrano SM (2013) The long road of research on snake venom serine proteinases. *Toxicon* 62:19–26
42. Murakami MT, Arni RK (2005) Thrombomodulin-independent activation of protein C and specificity of hemostatically active snake venom serine proteinases crystal structures of native and inhibited Agkistrodon contortrix contortrix protein c activator. *J Biol Chem* 280(47):39309–39315
43. Yip J, Shen Y, Berndt MC et al (2005) Primary platelet adhesion receptors. *IUBMB Life* 57(2):103–108
44. Kini RM (2006) Anticoagulant proteins from snake venoms: structure, function and mechanism. *Biochem J* 397(3):377–387
45. Menaldo DL, Bernardes CP, Pereira JC et al (2013) Effects of two serine proteases from *Bothrops pirajai* snake venom on the complement system and the inflammatory response. *Int Immunopharmacol* 15(4):764–771
46. Zychar BC, Dale CS, Demarchi DS et al (2010) Contribution of metalloproteinases, serine proteases and phospholipases A2 to the inflammatory reaction induced by *Bothrops jararaca* crude venom in mice. *Toxicon* 55(2–3):227–234
47. Kessler P, Marchot P, Silva M et al (2017) The three-finger toxin fold: a multifunctional structural scaffold able to modulate cholinergic functions. *J Neurochem* 142:7–18
48. Barber CM, Isbister GK, Hodgson WC (2013) Alpha neurotoxins. *Toxicon* 66:47–58
49. Ferraz CR, Arrahman A, Xie CF et al (2019) Multifunctional toxins in snake venoms and therapeutic implications: from pain to hemorrhage and necrosis. *Front Ecol Evol* 7:218 ARTN 21810.3389/fevo.2019.00218.
50. Vonk FJ, Casewell NR, Henkel CV et al (2013) The king cobra genome reveals dynamic gene evolution and adaptation in the snake venom system. *Proc Natl Acad Sci* 110(51):20651–20656
51. Sunagar K, Jackson TN, Undheim EA et al (2013) Three-fingered RAVERs: rapid accumulation of variations in exposed residues of snake venom toxins. *Toxins* 5(11):2172–2208
52. Lauridsen LP, Laustsen AH, Lomonte B et al (2017) Exploring the venom of the forest cobra snake: toxicovenomics and antivenom profiling of *Naja melanoleuca*. *J Proteomics* 150:98–108
53. Pawlak J, Mackessy SP, Fry BG et al (2006) Denmotoxin, a three-finger toxin from the colubrid snake *Boiga dendrophila* (Mangrove Catsnake) with bird-specific activity. *J Biol Chem* 281(39):29030–29041
54. Mackessy SP, Saviola AJ (2016) Understanding biological roles of venoms among the Caenophidia: the importance of rear-fanged snakes. Oxford University Press, Oxford
55. Li M, Fry B, Kini RM (2005) Eggs-only diet: its implications for the toxin profile changes and ecology of the marbled sea snake (*Aipysurus eydouxii*). *J Mol Evol* 60(1):81–89
56. Changeux J-P (1990) The TIPS lecture the nicotinic acetylcholine receptor: an allosteric protein prototype of ligand-gated ion channels. *Trends Pharmacol Sci* 11(12):485–492
57. Grant GA, Chiappinelli VA (1985) kappa-Bungarotoxin: complete amino acid sequence of a neuronal nicotinic receptor probe. *Biochemistry* 24(6):1532–1537
58. Marquer C, Fruchart-Gaillard C, Letellier G et al (2011) Structural model of ligand-G protein-coupled receptor (GPCR) complex based on experimental double mutant cycle data MT7 snake toxin bound to dimeric HM1 muscarinic receptor. *J Biol Chem* 286(36):31661–31675
59. Marchot P, Bourne Y, Prowse CN et al (1998) Inhibition of mouse acetylcholinesterase by fasciculin: crystal structure of the complex and mutagenesis of fasciculin. *Toxicon* 36(11):1613–1622
60. de Weille JR, Schweitz H, Maes P et al (1991) Calciseptine, a peptide isolated from black mamba venom, is a specific blocker of the L-type calcium channel. *Proc Natl Acad Sci* 88(6):2437–2440
61. Konshina AG, Krylov NA, Efremov RG (2017) Cardiotoxins: functional role of local conformational changes. *J Chem Inf Model* 57(11):2799–2810
62. Nguyen TTN, Folch B, Létourneau M et al (2012) Cardiotoxin-I: an unexpectedly potent insulinotropic agent. *ChemBioChem* 13(12):1805–1812
63. McDowell RS, Dennis MS, Louie A et al (1992) Mambin, a potent glycoprotein IIb-IIIa antagonist and platelet aggregation inhibitor structurally related to the short neurotoxins. *Biochemistry* 31(20):4766–4772
64. Girish VM, Kini RM (2016) Exactin: A specific inhibitor of Factor X activation by extrinsic tenase complex from the venom of *Hemachatus haemachatus*. *Sci Rep* 6:32036
65. Rajagopalan N, Pung YF, Zhu YZ et al (2007) β -Cardiotoxin: a new three-finger toxin from *Ophiophagus hannah* (king cobra) venom with beta-blocker activity. *FASEB J* 21(13):3685–3695
66. Koivula K, Rondinelli S, Näsman J (2010) The three-finger toxin MTa is a selective α 2B-adrenoceptor antagonist. *Toxicon* 56(3):440–447
67. Diochot S, Baron A, Salinas M et al (2012) Black mamba venom peptides target acid-sensing ion channels to abolish pain. *Nature* 490(7421):552–555
68. Rivera-Torres IO, Jin TB, Cadene M et al (2016) Discovery and characterization of a novel toxin from *Dendroaspis angusticeps*, named Tx7335, that activates the potassium channel KcsA. *Sci Rep* 6(1):1–11 ARTN 2390410.1038/srep23904.

69. Yang DC, Deuis JR, Dashevsky D et al (2016) The snake with the scorpion's sting: Novel three-finger toxin sodium channel activators from the venom of the long-glanded blue coral snake (*Calliophis bivirgatus*). *Toxins* 8(10):303
70. Kini RM, Doley R (2010) Structure, function and evolution of three-finger toxins: mini proteins with multiple targets. *Toxicon* 56(6):855–867
71. Bilgrami S, Yadav S, Kaur P et al (2005) Crystal structure of the disintegrin heterodimer from saw-scaled viper (*Echis carinatus*) at 1.9 Å resolution. *Biochemistry* 44(33):11058–11066
72. Saviola AJ, Modahl CM, Mackessy SP (2015) Disintegrins of *Crotalus simus tzabcan* venom: Isolation, characterization and evaluation of the cytotoxic and anti-adhesion activities of tzabcanin, a new RGD disintegrin. *Biochimie* 116:92–102
73. Kwon I, Hong S-Y, Kim YD et al (2013) Thrombolytic effects of the snake venom Disintegrin Saxatilin determined by novel assessment methods: a FeCl₃-induced thrombosis model in mice. *PLoS ONE* 8(11):e81165
74. Almeida J, Resende L, Watanabe RK et al (2017) Snake venom peptides and low mass proteins: molecular tools and therapeutic agents. *Curr Med Chem* 24(30):3254–3282
75. Morais KL, lanzer D, Miranda JRR et al (2013) Proline rich-oligopeptides: diverse mechanisms for antihypertensive action. *Peptides* 48:124–133
76. Kloog Y, Ambar I, Sokolovsky M et al (1988) Sarafotoxin, a novel vasoconstrictor peptide: phosphoinositide hydrolysis in rat heart and brain. *Science* 242(4876):268–270
77. Ducancel F (2005) Endothelin-like peptides. *Cell Mol Life Sci CMLS* 62(23):2828–2839
78. Quinton L, Le Caer JP, Phan G et al (2005) Characterization of toxins within crude venoms by combined use of Fourier transform mass spectrometry and cloning. *Anal Chem* 77(20):6630–6639. <https://doi.org/10.1021/ac050575k>
79. Munawar A, Trusch M, Georgieva D et al (2011) Venom peptide analysis of *Vipera ammodytes meridionalis* (Viperinae) and *Bothrops jararacussu* (Crotalinae) demonstrates subfamily-specificity of the peptidome in the family Viperidae. *Mol BioSyst* 7(12):3298–3307
80. Francis B, Kaiser II (1993) Inhibition of metalloproteinases in *Bothrops asper* venom by endogenous peptides. *Toxicon* 31(7):889–899
81. Huang K-F, Hung C-C, Wu S-H et al (1998) Characterization of three endogenous peptide inhibitors for multiple metalloproteinases with fibrinolytic activity from the venom of Taiwan habu (*Trimeresurus mucrosquamatus*). *Biochem Biophys Res Commun* 248(3):562–568
82. Wagstaff SC, Favreau P, Cheneval O et al (2008) Molecular characterization of endogenous snake venom metalloproteinase inhibitors. *Biochem Biophys Res Commun* 365(4):650–656
83. Yee KT, Pitts M, Tongyoo P et al (2017) Snake venom metalloproteinases and their peptide inhibitors from Myanmar Russell's viper venom. *Toxins* 9(1):15
84. Bernardes CP, Santos NA, Sisti FM et al (2018) A synthetic snake-venom-based tripeptide (Glu-Val-Trp) protects PC12 cells from MPP+ toxicity by activating the NGF-signaling pathway. *Peptides* 104:24–34
85. Ding B, Xu Z, Qian C et al (2015) Antiplatelet aggregation and antithrombosis efficiency of peptides in the snake venom of *Deinagkistrodon acutus*: Isolation, identification, and evaluation. *Evidence Based Complement Alternat Med* 2015:412841. <https://doi.org/10.1155/2015/412841>
86. Kerkis I, Hayashi MA, Prieto da Silva AR et al (2014) State of the art in the studies on crotamine, a cell penetrating peptide from South American rattlesnake. *BioMed Res Int* 2014:675985. <https://doi.org/10.1155/2014/675985>
87. Coronado MA, Gabdulkhakov A, Georgieva D et al (2013) Structure of the polypeptide crotamine from the Brazilian rattlesnake *Crotalus durissus terrificus*. *Acta Crystallogr D Biol Crystallogr* 69(10):1958–1964
88. Papo N, Shai Y (2005) Host defense peptides as new weapons in cancer treatment. *Cell Mol Life Sci* 62(7–8):784–790. <https://doi.org/10.1007/s00018-005-4560-2>
89. Pereira A, Kerkis A, Hayashi MA et al (2011) Crotamine toxicity and efficacy in mouse models of melanoma. *Expert Opin Investig Drugs* 20(9):1189–1200
90. Giorgi R, Bernardi M, Cury Y (1993) Analgesic effect evoked by low molecular weight substances extracted from *Crotalus durissus terrificus* venom. *Toxicon* 31(10):1257–1265
91. da Cunha DB, Silvestrini AVP, da Silva ACG et al (2018) Mechanistic insights into functional characteristics of native crotamine. *Toxicon* 146:1–12
92. Mambelli-Lisboa NC, Sciani JM, Brandao Prieto da Silva AR et al (2018) Co-localization of crotamine with internal membranes and accentuated accumulation in tumor cells. *Molecules* 23(4):968
93. Torres AM, Wong HY, Desai M et al (2003) Identification of a novel family of proteins in snake venoms purification and structural characterization of nawaprin from *naja nigricollis* snake venom. *J Biol Chem* 278(41):40097–40104
94. Nair DG, Fry BG, Alewood P et al (2007) Antimicrobial activity of omwaprin, a new member of the waprin family of snake venom proteins. *Biochem J* 402(1):93–104
95. Banigan JR, Mandal K, Sawaya MR et al (2010) Determination of the X-ray structure of the snake venom protein omwaprin by total chemical synthesis and racemic protein crystallography. *Protein Sci* 19(10):1840–1849
96. Schmidt JJ, Weinstein SA, Smith LA (1992) Molecular properties and structure-function relationships of lethal peptides from venom of Wagler's pit viper. *Trimeresurus wagleri* *Toxicon* 30(9):1027–1036
97. Tan CH, Tan KY, Yap MKK et al (2017) Venomics of *Tropidolaemus wagleri*, the sexually dimorphic temple pit viper: Unveiling a deeply conserved atypical toxin arsenal. *Sci Rep* 7:43237
98. Molles BE, Rezai P, Kline EF et al (2002) Identification of residues at the alpha and epsilon subunit interfaces mediating species selectivity of Waglerin-1 for nicotinic acetylcholine receptors. *J Biol Chem* 277(7):5433–5440. <https://doi.org/10.1074/jbc.M109232200>
99. Pennington MW, Czerwinski A, Norton RS (2018) Peptide therapeutics from venom: current status and potential. *Bioorg Med Chem* 26(10):2738–2758
100. Calmette A, Saenz A, Costil L (1933) Effects du venin de cobra sur les greffes cancéreuses et sur le cancer spontané (adeno-carcinome) de la souris. *CR Acad Sci* 197:205–210
101. Adams DJ, Smith AB, Schroeder CI et al (2003) ω -Conotoxin CVID inhibits a pharmacologically distinct voltage-sensitive calcium channel associated with transmitter release from preganglionic nerve terminals. *J Biol Chem* 278(6):4057–4062
102. Lewis RJ, Nielsen KJ, Craik DJ et al (2000) Novel ω -conotoxins from *Conus catus* discriminate among neuronal calcium channel subtypes. *J Biol Chem* 275(45):35335–35344
103. Blanchfield JT, Dutton JL, Hogg RC et al (2003) Synthesis, structure elucidation, in vitro biological activity, toxicity, and Caco-2 cell permeability of lipophilic analogues of α -conotoxin MII. *J Med Chem* 46(7):1266–1272
104. Daly JW (2003) Ernest Guenther award in chemistry of natural products. Amphibian skin: a remarkable source of biologically active arthropod alkaloids. *J Med Chem* 46(4):445–452
105. Das T, Bhattacharya S, Halder B et al (2011) Cytotoxic and antioxidant property of a purified fraction (NN-32) of Indian *Naja naja* venom on Ehrlich ascites carcinoma in BALB/c mice. *Toxicon* 57(7–8):1065–1072
106. Debnath A, Saha A, Gomes A et al (2010) A lethal cardiotoxic–cytotoxic protein from the Indian monocellate cobra (*Naja kaouthia*) venom. *Toxicon* 56(4):569–579
107. De Viegira Santos MM, Sant'Ana CD, Giglio JR et al (2008) Antitumoural effect of an L-amino acid oxidase isolated from *Bothrops jararaca* snake venom. *Basic Clin Pharmacol Toxicol* 102(6):533–542
108. Gebrim LC, Marcussi S, Menaldo DL et al (2009) Antitumor effects of snake venom chemically modified Lys49 phospholipase A2-like BthTX-I and a synthetic peptide derived from its C-terminal region. *Biologicals* 37(4):222–229
109. Araya C, Lomonte B (2007) Antitumor effects of cationic synthetic peptides derived from Lys49 phospholipase A2 homologues of snake venoms. *Cell Biol Int* 31(3):263–268
110. Costa TR, Menaldo DL, Oliveira CZ et al (2008) Myotoxic phospholipases A2 isolated from *Bothrops brazili* snake venom and synthetic peptides derived from their C-terminal region: cytotoxic effect on microorganism and tumor cells. *Peptides* 29(10):1645–1656

111. Gomes A, Choudhury SR, Saha A et al (2007) A heat stable protein toxin (d α CT-I) from the Indian Viper (*Daboia russelli russelli*) venom having antiproliferative, cytotoxic and apoptotic activities. *Toxicon* 49(1):46–56
112. Karthikeyan R, Karthigayan S, Balasubashini MS et al (2008) Inhibition of Hep2 and HeLa cell proliferation in vitro and EAC tumor growth in vivo by *Lapemis curtus* (Shaw 1802) venom. *Toxicon* 51(1):157–161
113. Khalid H, Mukhtar MM, Konstantakopoulos N (2015) Cytotoxicity of *Naja nubiae* (Serpentes: Elapidae) and *Echis ocellatus* (Serpentes: Viperidae) venoms from sudan. *J Toxins* 2015:7. <https://doi.org/10.1155/2015/167492>
114. Yasuda O, Morimoto S, Jiang B et al (1994) FS2, a mamba venom toxin, is a specific blocker of the L-type calcium channels. *Artery* 21(5):287–302
115. Watanabe TX, Itahara Y, Kuroda H et al (1995) Smooth muscle relaxing and hypotensive activities of synthetic calciseptine and the homologous snake venom peptide FS2. *Jpn J Pharmacol* 68(3):305–313
116. Huang T, Holt J, Lukasiewicz H et al (1987) Trigramin. A low molecular weight peptide inhibiting fibrinogen interaction with platelet receptors expressed on glycoprotein IIb-IIIa complex. *J Biol Chem* 262(33):16157–16163
117. Chen Y, Pitzemberger SM, Garsky VM et al (1991) Proton NMR assignments and secondary structure of the snake venom protein echistatin. *Biochemistry* 30(50):11625–11636
118. Scarborough RM, Rose JW, Hsu MA et al (1991) Barbourin. A GPIIb-IIIa-specific integrin antagonist from the venom of *Sistrurus m. barbouri*. *J Biol Chem* 266(15):9359–9362
119. Nolan C, Hall L, Barlow G (1976) [17] Ancrod, the coagulating enzyme from Malayan pit viper (*Agkistrodon rhodostoma*) venom. In: *Methods in enzymology*. Elsevier, pp 205–213
120. Stocker K, Barlow G (1976) [18] The coagulant enzyme from *Bothrops atrox* venom (batroxobin). In: *Methods in enzymology*. Elsevier, pp 214–223
121. Lin KL, Su JC, Chien CM et al (2010) Down-regulation of the JAK2/PI3K-mediated signaling activation is involved in Taiwan cobra cardiotoxin III-induced apoptosis of human breast MDA-MB-231 cancer cells. *Toxicon* 55(7):1263–1273. <https://doi.org/10.1016/j.toxicon.2010.01.017>
122. Malmberg AB, Yaksh TL (1995) Effect of continuous intrathecal infusion of ω -conopeptides, N-type calcium-channel blockers, on behavior and antinociception in the formalin and hot-plate tests in rats. *Pain* 60(1):83–90
123. Kisiel W, Canfield WM (1981) [22] Snake venom proteases that activate blood-coagulation factor V. In: *Methods in enzymology*. Elsevier, pp 275–285
124. Bachmann F, Duckert F, Koller F (1958) The Stuart-Prower factor assay and its clinical significance. *Thromb Haemost* 2(01):024–038
125. Quick AJ (1971) Thromboplastin generation: effect of the Bell-Alton reagent and Russell viper venom on prothrombin consumption. *Am J Clin Pathol* 55(5):555–560
126. Thiagarajan P, Pengo V, Shapiro SS (1986) The use of the dilute Russell viper venom time for the diagnosis of lupus anticoagulants. *Blood*. 68(4):869–74
127. Klein JD, Walker FJ (1986) Purification of a protein C activator from the venom of the southern copperhead snake (*Agkistrodon contortrix contortrix*). *Biochemistry* 25(15):4175–4179
128. Stocker K, Fischer H, Meier J (1988) Practical application of the protein C activator Protac from *Agkistrodon contortrix* venom. *Folia haematologica (Leipzig, Germany: 1928)* 115(3):260–264
129. Mintz IM, Venema VJ, Swiderek KM et al (1992) P-type calcium channels blocked by the spider toxin ω -Aga-IVA. *Nature* 355(6363):827–829
130. Naumann GB, Silva LF, Silva L et al (2011) Cytotoxicity and inhibition of platelet aggregation caused by an l-amino acid oxidase from *Bothrops leucurus* venom. *Biochimica et Biophysica Acta (BBA) General Subjects* 1810(7):683–694
131. Nunes ES, Souza MA, Vaz AF et al (2012) Cytotoxic effect and apoptosis induction by *Bothrops leucurus* venom lectin on tumor cell lines. *Toxicon* 59(7–8):667–671
132. Aranda-Souza MA, Rossato FA, Costa RA et al (2014) A lectin from *Bothrops leucurus* snake venom raises cytosolic calcium levels and promotes B16–F10 melanoma necrotic cell death via mitochondrial permeability transition. *Toxicon* 82:97–103
133. Park MH, Jo M, Won D et al (2012) Snake venom toxin from *Vipera lebetina turanica* sensitizes cancer cells to TRAIL through ROS- and JNK-mediated upregulation of death receptors and downregulation of survival proteins. *Apoptosis* 17(12):1316–1326. <https://doi.org/10.1007/s10495-012-0759-5>
134. Son DJ, Park MH, Chae SJ et al (2007) Inhibitory effect of snake venom toxin from *Vipera lebetina turanica* on hormone-refractory human prostate cancer cell growth: induction of apoptosis through inactivation of nuclear factor κ B. *Mol Cancer Ther* 6(2):675–683
135. Sanchez EF, Schneider FS, Yarleque A et al (2010) The novel metalloproteinase atroxlysin-I from Peruvian *Bothrops atrox* (Jergón) snake venom acts both on blood vessel ECM and platelets. *Arch Biochem Biophys* 496(1):9–20
136. Sun L-K, Yoshii Y, Hyodo A et al (2003) Apoptotic effect in the glioma cells induced by specific protein extracted from Okinawa Habu (*Trimeresurus flavoviridis*) venom in relation to oxidative stress. *Toxicol In Vitro* 17(2):169–177
137. Tang NH, Xie Q, Wang XQ et al (2011) Inhibition of invasion and metastasis of MHCC97H cells by expression of snake venom cystatin through reduction of proteinases activity and epithelial-mesenchymal transition. *Arch Pharmacol Res* 34(5):781–789. <https://doi.org/10.1007/s12272-011-0512-6>
138. Wang, Y.-X., D. Gao, M. Pettus, et al., (2000) Interactions of intrathecally administered ziconotide, a selective blocker of neuronal N-type voltage-sensitive calcium channels, with morphine on nociception in rats. *PAIN*, 84(2–3):271–281
139. Yang R-S, Tang C-H, Chuang W-J et al (2005) Inhibition of tumor formation by snake venom disintegrin. *Toxicon* 45(5):661–669
140. You W-K, Choi W-S, Koh Y-S et al (2004) Functional characterization of recombinant batroxobin, a snake venom thrombin-like enzyme, expressed from *Pichia pastoris*. *FEBS Lett* 571(1–3):67–73
141. Zhang L, Cui L (2007) A cytotoxin isolated from *Agkistrodon acutus* snake venom induces apoptosis via Fas pathway in A549 cells. *Toxicol In Vitro* 21(6):1095–1103
142. Ahn MY, Lee BM, Kim YS (1997) Characterization and cytotoxicity of L-amino acid oxidase from the venom of king cobra (*Ophiophagus hannah*). *Int J Biochem Cell Biol* 29(6):911–919
143. Siigur J, Siigur E (1992) The direct actinic α -fibrin (ogen)olytic enzymes from snake venoms. *J Toxicol Toxin Rev* 11(2):91–113
144. Parveen S, Misra R, Sahoo SK (2012) Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. *Nanomed Nanotechnol Biol Med* 8(2):147–166
145. Mohapatra D, Pratap R, Pandey V et al (2022) *Tinospora cordifolia* leaves derived carbon dots for cancer cell bioimaging, free radical scavenging, and Fe3+ sensing applications. *J Fluoresc* 32(1):275–292. <https://doi.org/10.1007/s10895-021-02846-6>
146. Lee HJ (2002) Protein drug oral delivery: the recent progress. *Arch Pharmacol Res* 25(5):572
147. Patel KK, Agrawal AK, Singh S (2020) Preformulation challenges: the concept behind the selection, design and preparation of nanoformulations. In: *Nanoformulations in human health*. Springer, pp 43–71
148. Karunanidhi P, Verma N, Kumar DN et al (2021) Triphenylphosphonium functionalized *Ficus religiosa* L. extract loaded nanoparticles improve the mitochondrial function in oxidative stress induced diabetes. *AAPS PharmSciTech* 22(5):158. <https://doi.org/10.1208/s12249-021-02016-8>
149. Kandimalla R, Aqil F, Alhakeem SS et al (2021) Targeted oral delivery of paclitaxel using colostrum-derived exosomes. *Cancers (Basel)* 13(15):3700
150. Biswas A, Gomes A, Sengupta J et al (2012) Nanoparticle-conjugated animal venom-toxins and their possible therapeutic potential. *J Venom Res* 3:15
151. Kumar DN, Chaudhuri A, Aqil F et al (2022) Exosomes as emerging drug delivery and diagnostic modality for breast cancer: recent advances in isolation and application. *Cancers* 14(6):1435
152. Negi S, Chaudhuri A, Kumar DN et al (2022) Nanotherapeutics in autophagy: a paradigm shift in cancer treatment. *Drug Deliv Transl Res*. <https://doi.org/10.1007/s13346-022-01125-6>
153. Jain S, Raza K, Agrawal AK et al (2021) Chapter 10—Cell-penetrating peptides in cancer targeting. In: Jain S et al (eds) *Nanotechnology applications for cancer chemotherapy*. Elsevier, New York, pp 201–220. <https://doi.org/10.1016/B978-0-12-817846-1.00010-2>

154. Patel KK, Surekha DB, Tripathi M et al (2019) Antibiofilm potential of silver sulfadiazine-loaded nanoparticle formulations: a study on the effect of DNase-I on microbial biofilm and wound healing activity. *Mol Pharm* 16(9):3916–3925. <https://doi.org/10.1021/acs.molpharmaceut.9b00527>
155. Aqil F, Munagala R, Jeyabalan J et al (2019) Milk exosomes—natural nanoparticles for siRNA delivery. *Cancer Lett* 449:186–195. <https://doi.org/10.1016/j.canlet.2019.02.011>
156. Jain S, Spandana G, Agrawal AK et al (2015) Enhanced antitumor efficacy and reduced toxicity of docetaxel loaded estradiol functionalized stealth polymeric nanoparticles. *Mol Pharm* 12(11):3871–3884. <https://doi.org/10.1021/acs.molpharmaceut.5b00281>
157. Panyam J, Labhasetwar V (2003) Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Adv Drug Deliv Rev* 55(3):329–347
158. Anjum MM, Patel KK, Dehari D et al (2021) Anacardic acid encapsulated solid lipid nanoparticles for *Staphylococcus aureus* biofilm therapy: chitosan and DNase coating improves antimicrobial activity. *Drug Deliv Transl Res* 11(1):305–317. <https://doi.org/10.1007/s13346-020-00795-4>
159. Patel KK, Agrawal AK, Anjum MM et al (2020) DNase-I functionalization of ciprofloxacin-loaded chitosan nanoparticles overcomes the biofilm-mediated resistance of *Pseudomonas aeruginosa*. *Appl Nanosci* 10(2):563–575. <https://doi.org/10.1007/s13204-019-01129-8>
160. Fadel M, Kassab K, Fadeel DA (2010) Zinc phthalocyanine-loaded PLGA biodegradable nanoparticles for photodynamic therapy in tumor-bearing mice. *Lasers Med Sci* 25(2):283–292
161. PoMohammadur Dounighi N, Mehrabi M, Avadi M et al (2015) Preparation, characterization and stability investigation of chitosan nanoparticles loaded with the *Echis carinatus* snake venom as a novel delivery system. *Arch Razi Inst* 70(4):269–277
162. Badr G, Sayed D, Maximous D et al (2014) Increased susceptibility to apoptosis and growth arrest of human breast cancer cells treated by a snake venom-loaded silica nanoparticles. *Cell Physiol Biochem* 34(5):1640–1651
163. Soman NR, Baldwin SL, Hu G et al (2009) Molecularly targeted nanocarriers deliver the cytolytic peptide melittin specifically to tumor cells in mice, reducing tumor growth. *J Clin Invest* 119(9):2830–2842
164. Utkin YN (2017) Modern trends in animal venom research-omics and nanomaterials. *World J Biol Chem* 8(1):4
165. Gade S, Patel KK, Gupta C et al (2019) An ex vivo evaluation of moxifloxacin nanostructured lipid carrier enriched in situ gel for transcorneal permeation on goat cornea. *J Pharm Sci* 108(9):2905–2916. <https://doi.org/10.1016/j.xphs.2019.04.005>
166. Yu W, Liu C, Liu Y et al (2010) Mannan-modified solid lipid nanoparticles for targeted gene delivery to alveolar macrophages. *Pharm Res* 27(8):1584–1596
167. Zhang S-J, Zhang Y-T, Zhao J-H et al (2013) Preparation and in vitro anti-tumor properties of toad venom extract-loaded solid lipid nanoparticles. *Die Pharmazie Int J Pharmaceut Sci* 68(8):653–660
168. Torchilin V (2004) Targeted polymeric micelles for delivery of poorly soluble drugs. *Cell Mol Life Sci CMLS* 61(19–20):2549–2559
169. Torchilin VP, Trubetskoy VS, Whiteman KR et al (1995) New synthetic amphiphilic polymers for steric protection of liposomes in vivo. *J Pharm Sci* 84(9):1049–1053
170. Matsumura Y, Yokoyama M, Kataoka KZ et al (1999) Reduction of the side effects of an antitumor agent, KR5500, by incorporation of the drug into polymeric micelles. *Jpn J Cancer Res* 90(1):122–128. <https://doi.org/10.1111/j.1349-7006.1999.tb00675.x>
171. Ellison M, Gao F, Wang H-L et al (2004) α -Conotoxins Iml and Imll target distinct regions of the human $\alpha 7$ nicotinic acetylcholine receptor and distinguish human nicotinic receptor subtypes. *Biochemistry* 43(51):16019–16026
172. Mei D, Lin Z, Fu J et al (2015) The use of α -conotoxin Iml to actualize the targeted delivery of paclitaxel micelles to $\alpha 7$ nAChR-overexpressing breast cancer. *Biomaterials* 42:52–65
173. Bamrungsap S, Zhao ZL, Chen T et al (2012) Nanotechnology in therapeutics: a focus on nanoparticles as a drug delivery system. *Nanomedicine* 7(8):1253–1271. <https://doi.org/10.2217/Nnm.12.87>
174. Jain S, Patil SR, Swarnakar NK et al (2012) Oral delivery of doxorubicin using novel polyelectrolyte-stabilized liposomes (Layersomes). *Mol Pharm* 9(9):2626–2635. <https://doi.org/10.1021/mp300202c>
175. Torchilin VP (2005) Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov* 4(2):145–160. <https://doi.org/10.1038/nrd1632>
176. Blume G, Cevc G (1990) Liposomes for the sustained drug release in vivo. *Biochimica et Biophysica Acta (BBA) Biomembranes* 1029(1):91–97
177. Safra T, Muggia F, Jeffers S et al (2000) Pegylated liposomal doxorubicin (doxil): reduced clinical cardiotoxicity in patients reaching or exceeding cumulative doses of 500 mg/m². *Ann Oncol* 11(8):1029–1033
178. Al-Asmari AK, Ullah Z, Al Balowi A et al (2017) In vitro determination of the efficacy of scorpion venoms as anti-cancer agents against colorectal cancer cells: a nano-liposomal delivery approach. *Int J Nanomed* 12:559
179. Dresselhaus MS, Dresselhaus G, Charlier JC et al (2004) Electronic, thermal and mechanical properties of carbon nanotubes. *Philos Trans A Math Phys Eng Sci* 362(1823):2065–2098. <https://doi.org/10.1098/rsta.2004.1430>
180. Lacerda L, Bianco A, Prato M et al (2006) Carbon nanotubes as nanomedicines: from toxicology to pharmacology. *Adv Drug Deliv Rev* 58(14):1460–1470. <https://doi.org/10.1016/j.addr.2006.09.015>
181. Liu Z, Chen K, Davis C et al (2008) Drug delivery with carbon nanotubes for in vivo cancer treatment. *Can Res* 68(16):6652–6660
182. Gouveia DM, Cogo JC, Kido HW et al (2020) Physical-chemical characterization of carbon nanotubes-hydroxyapatite nanocomposites with fibrin sealant derived from snake venom. *Toxicol* 177:S56–S56
183. Bhattacharya R, Patra CR, Earl A et al (2007) Attaching folic acid on gold nanoparticles using noncovalent interaction via different polyethylene glycol backbones and targeting of cancer cells. *Nanomed Nanotechnol Biol Med* 3(3):224–238. <https://doi.org/10.1016/j.nano.2007.07.001>
184. Brown SD, Nativo P, Smith J-A et al (2010) Gold nanoparticles for the improved anticancer drug delivery of the active component of oxaliplatin. *J Am Chem Soc* 132(13):4678–4684
185. Thomas SC, Kumar-Mishra P, Talegaonkar S (2015) Ceramic nanoparticles: fabrication methods and applications in drug delivery. *Curr Pharmaceut Des* 21(42):6165–6188
186. Li Z, Zhu S, Gan K et al (2005) Poly-L-lysine-modified silica nanoparticles: a potential oral gene delivery system. *J Nanosci Nanotechnol* 5(8):1199–1203
187. Bhowmik T, Saha PP, Dasgupta A et al (2013) Antileukemic potential of PEGylated gold nanoparticle conjugated with protein toxin (NKCT1) isolated from Indian cobra (*Naja kaouthia*) venom. *Cancer Nanotechnol* 4(1–3):39–55
188. Saha PP, Bhowmik T, Dasgupta AK et al (2014) In vivo and in vitro toxicity of nanogold conjugated snake venom protein toxin GNP-NKCT1. *Toxicol Rep* 1:74–84
189. Bhowmik T, Saha PP, Sarkar A et al (2017) Evaluation of cytotoxicity of a purified venom protein from *Naja kaouthia* (NKCT1) using gold nanoparticles for targeted delivery to cancer cell. *Chem Biol Interact* 261:35–49. <https://doi.org/10.1016/j.cbi.2016.11.007>
190. Bhowmik T, Pratim Saha P, Kumar DasGupta A et al (2014) Influence of gold nanoparticle tagged snake venom protein toxin NKCT1 on Ehrlich ascites carcinoma (EAC) and EAC induced solid tumor bearing male albino mice. *Curr Drug Deliv* 11(5):652–664
191. Saha PP, Bhowmik T, Dasgupta AK et al (2014) Nano gold conjugation, anti-arthritis potential and toxicity studies of snake *Naja kaouthia* (Lesson, 1831) venom protein toxin NKCT1 in male albino rats and mice. *Indian J Exp Biol* 52(8):763–772
192. Mohammadpourounighi N, Behfar A, Ezabadi A et al (2010) Preparation of chitosan nanoparticles containing *Naja naja oxiana* snake venom. *Nanomed Nanotechnol Biol Med* 6(1):137–143
193. DuVal J, Saffouri B, Weir G et al (1981) Stimulation of gastrin and somatostatin secretion from the isolated rat stomach by bombesin. *Am J Physiol Gastrointest Liver Physiol* 241(3):G242–G247
194. Chanda N, Kattumuri V, Shukla R et al (2010) Bombesin functionalized gold nanoparticles show in vitro and in vivo cancer receptor specificity. *Proc Natl Acad Sci USA* 107(19):8760–8765. <https://doi.org/10.1073/pnas.1002143107>
195. Fu YJ, An N, Li K et al (2012) Chlorotoxin-conjugated nanoparticles as potential glioma-targeted drugs. *J Neurooncol* 107(3):457–462. <https://doi.org/10.1007/s11060-011-0763-6>

196. Karthikeyan R, Karthigayan S, Sri Balasubashini M et al (2008) Inhibition of Hep2 and HeLa cell proliferation in vitro and EAC tumor growth in vivo by *Lapemis curtus* (Shaw 1802) venom. *Toxicon* 51(1):157–161. <https://doi.org/10.1016/j.toxicon.2007.08.012>
197. Tomalia DA, Baker H, Dewald J et al (1985) A new class of polymers: starburst-dendritic macromolecules. *Polym J* 17(1):117–132
198. Turnbull WB, Stoddart JF (2002) Design and synthesis of glycodendrimers. *Rev Mol Biotechnol* 90(3–4):231–255
199. Malik N, Evagorou EG, Duncan R (1999) Dendrimer-platinate: a novel approach to cancer chemotherapy. *Anticancer Drugs* 10(8):767–776
200. Lyons SA, O'Neal J, Sontheimer H (2002) Chlorotoxin, a scorpion-derived peptide, specifically binds to gliomas and tumors of neuroectodermal origin. *Glia* 39(2):162–173
201. Huang R, Ke W, Han L et al (2011) Targeted delivery of chlorotoxin-modified DNA-loaded nanoparticles to glioma via intravenous administration. *Biomaterials* 32(9):2399–2406
202. Pauwels EK, Kairemo K, Erba P et al (2008) Nanoparticles in cancer. *Curr Radiopharm* 1(1):30–36

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