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Minireview

Nanotechnology in snake venom research—an overview

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Nanotechnology has revolutionized the paradigm of today's upcoming biological sciences through its applications in the field of biomedical research. One such promising aspect is by interfacing this modern technology with snake venom research. Snake venom is a valuable resource of bioactive molecules, which has shown efficient and promising contributions in biomedical research. The potentiality of merging these two unique fields lies in the approach of interfacing active bioactive molecules derived from snake venoms, which would yield better therapeutic molecules for future applications in terms of drug delivery, enhanced stability, reduced toxicity, bioavailability and targeted drug delivery. Available literature on nanoconjugation of snake venom bioactive molecules have suggest that these molecules have better therapeutic advantage in several fields of biomedical research *viz*., arthritis, cancer, etc. Another perspective in snake venom research could be green synthesis or herbal based synthesis of nanoparticles, which has shown enhanced effect in snake venom neutralizing capacity. Therefore, in terms of snake venom therapeutic potential and development of snake venom antidote, nanotechnology is a prodigious tool to be taken into serious consideration by the researchers. In this review, a comprehensive overview has been given on bridging nanoparticles with active biomolecules derived from snake venoms/herbs, current scientific evidences and records in this field, present trends and developments in nanotechnology in venom research along with future prospects in this arena. This may open new domains in snake venom research using nanotechnology in the near future.

Keywords: Antidote, Ayurveda, Drug delivery, Herbal, Nano applications, Nanoconjugation, Snake envenomation, Venoms-toxins

Nanotechnology and snake venom

Since the birth of its concept, nanotechnology has been employed in various sectors including information and computing technology, energy, sensors, biotechnology, biomedicine, food industry and even agriculture owing to its unique physico-chemical properties which are completely different from their bulk form[1]. In fact, nanoscale materials, aggregates of atoms and molecules, gives rise to some unforeseen properties which needs analysis and further detailing since these properties are what which makes the nanotechnology— an unique technology. These unique yet highly potential properties of nanomaterials cannot be predicted by quantum mechanics (individual atoms) or classical physics (bulk materials). The novel properties of nanomaterial has opened the door to diverse applications of this technology in almost every field bridging different disciplines, such as biology, chemistry, physics, electronics, mechanics, optics, mathematics and others. Due to its rapid growth since its basic origin, it has climbed the steps of development and reached almost every sector of applications. It is a big shot commercial market today which has billions of investments in global perspectives, and is expected to be a giant market of future. Several nanoproducts are commercially available already and innumerable products are under research and development. Nanotechnology can therefore be considered as the next technological revolution which has direct impact on human health and environment.

With wide array of applications of nanotechnology in biomedical sciences, it is essential to know how these nanoparticles actually work within the biological system. Owing to their miniature size, these particles can translocate from their entry portals (routes exposed) into the circulatory and immune systems, and ultimately to body tissues and organs.

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Research in the field of cellular and animal interaction of nanoparticles is vital owing to its molecular chess game, which it plays within the system. This opens a new door to the use of nanomaterials in drug delivery and targeted therapy, and has been discussed in detail in this review. The way these particles behave greatly depends on their size, morphology, shape, pH, charge, dose, route of exposure, crystalinity, etc.[2]. Hence, these particles become favorite candidates to be manipulated for specific biomedical and research applications.

The modern trend in snake venom research includes the utilization of nanotechnology[3]. Research in nanotechnology and snake venom/toxin has gained two different dimensions: (i) Use of nanotechnology in the development of snake venom antidote; and (ii) Application of nanotechnology in the development of drug clue against pathophysiology (arthritis, cancer, HIV, etc.). Till date, antisnake venom serum (ASVS) has been the only clinically used antidote against snake envenomation developed in 1894 at Pasteur Institute, Paris, by Dr. Calmette. The limitations (cost factor, self life, no protection against local effects, hemorrhage and organ toxicity) and side effects (anaphylactic shock, pyrogenic reaction, serum sickness, etc.) in patients are well known. Therefore, worldwide, scientists are looking for a better antisnake venom antidote. The herbal resources of the world having antisnake venom potential have been known for a long time[4]. Folk and traditional medicinal system mentions about different herbs active against snake venom. The first scientific evaluation of herb as snake venom antidote was done by Knowles[5]. He screened several plants/plant constituents used by traditional practitioners, but failed to report their effectiveness against snake envenomation. Later, Mhaskar and Caius (1931) reported the ineffectiveness of herbs and herbal constituents against snake venom[6]. It was contradicted later by many reports on the effectiveness of herbal antidotes against snake envenomation. From the present laboratory many herbs and herbal constituents have been identified as an active antidote for snake venom[4]. Further studies with these herbs and herbal constituents established their disadvantages such as toxicities, low efficacy, etc[7], which confirmed the need to improve the activity of the herb and herbal constituent against snake envenomation.

India's traditional medicinal system Ayurveda mentioned the utilization of herbs, metals and minerals for medicinal purposes[8]. In the *Samhita* period (600-1000 BC) ayurvedic formulations used metals in powdered forms named *Ayaskrati*. The metals used in that period included mercury (*Parada*), gold (*Swarna*), silver (*Rajata*), copper (*Tamra*), iron (*Lauha*), tin (*Vanga*), lead (*Naga*), zinc (*Yasada*), etc. The metals were in micro forms and their internal use was limited because of their toxic effects. Development of *Rasashastra* of Ayurveda in 7th century AD introduced many new pharmaceutical techniques for metal formulations in Ayurvedic preparations including *Shodana*, *Jarana* and *Marana*[9], which converted the metals into very fine, absorbable, therapeutically most effective and least or non-toxic form of medicine known as *Bhasmas*. Literature shows that Ayurvedic processing of metallic formulations bring down its shape to nanometer scale[9]. Being too small, they are more powerful because the constituent metals and minerals do not react with the tissues of the body. The metals could combine with herbs which help in assimilation and delivery of the ingredients into the human cell/tissues[10]. There is a clear indication that metal-herb combination can work as medical wonders.

Nanotechnology in the development of snake venom antidote

Antisnake venom herbal compounds conjugated with nanoparticle have been a blooming area of current day research. Alam *et al*. (1994)[11] identified 2-hydroxy-4-methoxy-benzoic acid (HMBA) from the root extract of the Indian Sarsaparilla (*Hemidesmus indicus*) having viper venom neutralizing effects in animal models. However, HMBA posses low efficacy against viper venom and also had toxicity which was the major drawback of this herbal compound. But when conjugated with gold nanoparticle, it increased the viper venom neutralizing efficacy of HMBA and decreased the toxicity[12,13]. *Vitex negundo*, a woody, aromatic shrub found in Afghanistan, India, Pakistan, Thailand, Sri Lanka, eastern Africa and Madagascar has been used as antidote against cobra venom for many years[14]. Saha *et al*. (2015)[15] has synthesized gold nanoparticle from gold salt using the reducing property of *Vitex negundo* and examined its potency against viper venom induced acute stress and acute cytokine response in experimental animal model. Characterization of gold nanoparticle was done using dynamic light scattering (hydrodynamic diameter and zeta potential), fourier transform infrared spectroscopy and transmission electron microscopy. It

was found that the synthesized gold nanoparticles were of 25-40 nm size with nearly spherical shape. Treatment with *Vitex negundo*-reduced gold nanoparticle significantly restored viper venom-induced antioxidant parameters and cytokines responses. The particle was capable of reaching the snake venom target sites thereby significantly neutralizing the deleterious venom effects on the major organs (liver and kidney). Curcumin is an active compound found in well known spice turmeric (*Curcuma longa*). The anti-inflammatory and antioxidant potential of curcumin has been well established[16]. Curcumin was conjugated with gold nanoparticle by adsorption method, and its antiviper venom activity was assessed in experimental animals[17]. It was proposed that curcumin-conjugated gold nanoparticle may act by neutralizing viper venom-induced local damages at the vascular bed by inhibiting the pro-oxidant activity of the venom, by direct inhibition at the enzymatic level, and by interfering with cellular markers including inflammatory markers and antioxidants[17]. Some nanoparticles have pharmacokinetic and protein-binding properties that make them a low cost and easily available alternative for antivenom against systemically distributed snake venom toxins. Karain *et al*. (2016)[18] injected LD50 doses of southern Pacific rattlesnake (*Crotalus oreganus helleri*) venom into crickets (*Acheta domesticus*) and found the ability of C_{60} fullerene to bind venom toxins. Treatment with C_{60} fullerene increased the survival rate of cricket after envenomation, compared to control crickets. Survival analysis confirmed that C_{60} fullerene protected crickets up to 60 h, which was significantly longer than controls. It may further considered as a simple, low cost treatment for snakebite[18]. Oliveira *et al*. (2018)[19] showed the neutralization of silver nanoparticles with 50 nm diameter against *Bothrops jararacussu* snake venom induced neurotoxicity and myotoxicity in *ex vivo* and *in vitro* studies. Soares *et al*. (2018)[20] has produced antidote against *Bothrops jararaca* and *B. erythromelas* snake venom using chitosan polymer nanoparticle as an adjuvant. They have showed that chitosan polymer nanoparticle have potential immunoadjuvant properties, without profound inflammatory effect of the venom[20].

The molecular mechanism of action of these herbal-nanoparticle remain to be established and a big challenge for the toxinologists. The molecular targets of these herbo-nano-conjugated biomolecules involve the cellular signaling molecules, the cytokines, enzymes and pro-inflammatory markers. However, more detail research is needed in this area to validate the actions of hero-nano-conjugate as an effective alternative antidote against snake-bite.

Detection of snake venom type in victims is an essential step before snake venom therapeutics. Gold nanoparticle based lateral flow assay has been utilized for detection of Indian cobra venom and Russell's viper venom in mice model[21]. It is based on paper immunochromatography assay for detection of two snake venom species using polyvalent antisnake venom antibodies. The immunoassay was rapid, selective, and sensitive to detect venom concentrations upto 0.1 ng/mL. It has a potential to be a field diagnostic test to detect snake envenomation and assist in saving lives of snakebite victims.

Nanoparticle snake venom/fraction against pathophysiology

Over the years, snake venom molecules have been targeted as medical research tools or as therapeutic/diagnostic agent[22,23]. Calmette *et al*.[24] (1933) proposed that the physiologically active components of snake venom might have therapeutic potential. They showed that Cobra venom could kill cancer cells in animal model. Thereafter, many reports have established the anticancer potential of different species of Elapidae, Viperidae and Crotalidae snake venoms[25-29]. It was also noticed that these compounds are very toxic, producing neurological, cardiac, respiratory adverse effects[30]. Many attempts have been made to increase the therapeutic potential and to decrease the toxicity of venom-toxins in biological systems. One of such approach in modern research arena has been nanotechnology, which may provide increased efficacy and decreased toxicity (Table 1). A common approach is to attach the protein toxin to the nanoparticle via non-covalent electrostatic adsorption process[31]. However this non-covalent interaction is not at all stable at high salt concentrations and at pH values more than the isoelectric point of the protein. A covalent bond between venom protein toxin and nanoparticle is more stable and its attachment site to the nanoparticle may be controlled. Nanoparticle conjugation with snake venom/toxin may cause protein folding/unfolding[32]. It has been advised to verify venom/toxin structure-function after

Table 1 — Snake venom/toxin-nanoparticle used against experimental pathophysiology

Type of venom/toxin-nanoparticle	Effective against	Activity
Naja kaouthia cytotoxin 1 (NKCT1) conjugated gold nanoparticle (GNP-NKCT1)	Cancer	Significant inhibition of leukemic cell growth in dose and time dependent manner[36].
GNP-NKCT1	Cancer	Anticancer effect *in vivo* and *in vitro* in EAC cells and antitumor effect in EAC-induced mice[37].
GNP-NKCT1	Cancer	Caspase dependent apoptotic pathway and autophagy inducing potential in human leukemic U936 and K562 cell lines[38].
GNP-NKCT1	Cancer	Targeted delivery in cancer cells[39].
GNP-NKCT1	Cancer	Down regulation of cyclin dependent kinase-4 and MAP kinase in human breast cancer cell line[40]
Walterinnesia aegyptia venom combined with silica nanoparticle	Cancer	Combination with venom with nanoparticle strongly induced apoptosis in MDA-MB-231 and MCF-7 cancer cells[41]
Walterinnesia aegyptia venom combined with silica nanoparticle	Cancer	Inhibition of EGF-1 and IL-6-mediated multiple myoloma cell proliferation, change in cell cycle pattern and enhancement in induction of apoptosis in multiple myoloma cells[42].
Walterinnesia aegyptia venom combined with silica nanoparticle	Cancer	Decrease in the viability of multiple myoloma cells[43].
Walterinnesia aegyptia venom combined with silica nanoparticle	Cancer	Inhibition of multiple myoloma cell proliferation[44].
GNP-NKCT1	Arthritis	Decrease in rheumatoid arthritis induced paw/ankle swelling, urinary markers, serum markers and cytokines[47].
GNP-NKCT1	Arthritis	Change in physical parameter, urinary markers, serum and synovial membrane pro-inflammatory markers and metalloproteinase in collagenase induced osteoarthritic animals[48]
Echis carinatus venom-chitosan nanoparticles	Drug delivery	Noble drug delivery vehicle[49]
Agkistrodon halys venom-chitosan nanoparticles	Drug delivery	Noble drug delivery vehicle[35]

conjugation with nanoparticle. Co-functionalization of nanoparticle with polyethylene glycol may help in protein unfolding, and can be used in snake venom/toxin loaded nanoparticle formation.

Nanoparticles can be modified to carry drugs and imaging probes both and designed to specifically target molecules of diseased tissues. Nanoparticles for anticancer drug delivery had reached the first clinical trial in the mid-1980s, and the first nanoparticles (e.g. liposomal with encapsulated doxorubicin) had entered the pharmaceutical market in 1995. Since then, numerous new nanoparticles for cancer drug delivery have been approved and/or are currently under development due to their many advantages. Their advantages include enhancing solubility of hydrophobic drugs, prolonging circulation time, minimizing nonspecific uptake, preventing undesirable off-target and side effects, improving intracellular penetration, and allowing for specific cancer targeting. Mohammadpourdounighi *et al*. (2010)[33] encapsulated *Naja naja oxiana* venom in biodegradable and nontoxic chitosan nanoparticle. Chitosan nanoparticle was produced by ionic gelation process of tripolyphosphate. Optimum loading capacity of venom was achieved at 500 µg/mL concentration with chitosan at concentration 2 and 3 mg/mL. Zolfagharian and Mohammadpourdounighi (2009)[34] established a small scale double emulsion technique for incorporation of *Naja naja oxiana* venom into poly (lactide-co-glycolide) microspheres. They also showed that the antigenicity of venom was retained after incorporation into poly (lactide-co-glycolide) microspheres. *Agkistrodon halys* snake venom loaded chitosan nanoparticle was showed to be effective as adjuvant and antigen delivery system[35]. Bhowmik *et al*. (2013)[36] has reported the antileukemic potential of PEGylated gold nanoparticle conjugated with *Naja kaouthia* venom protein toxin NKCT1. Gold nanoparticle conjugated NKCT1 (GNP-NKCT1) was prepared by borohydrate reduction method. It significantly inhibited leukemic cell growth in by dose or time dependent manner by 2-3 folds more than NKCT1 alone. GNP-NKCT1 induced growth arrest of leukemic cells at G1 phase. Cell line migration was significantly low in GNP-NKCT1 treated cells. Bhowmik *et al*. (2014)[37] also reported the influence of GNP-NKCT1 on Ehrlich Ascites Carcinoma (EAC) and EAC induced solid tumor

bearing mice. The study established the characterization of GNP-NKCT1, where scanning electron microscopy showed the formation of gold nanoparticle and energy dispersive X ray spectroscopy and X ray powder diffraction confirmed 60-90% gold nanoparticles in the solution. GNP-NKCT1 showed anticancer effect *in vivo* and *in vitro* in EAC cells and antitumor effect in EAC-induced mice. It decreased the tumor volume and tumor weight in EAC induced tumor in male albino mice and induced caspase dependent apoptosis pathway in EAC cell. Bhowmik and Gomes has established the caspase dependent apoptotic pathway and autophagy inducing potential of GNP-NKCT1 in human leukemic U936 and K562 cell lines[38]. Induction of GNP-NKCT1 induced loss of mitochondrial membrane potential and high reactive oxygen species generation in both the leukemic cell lines. It downregulated PI3K/Akt and mTOR expression followed by autophagic cell death. Lysosomal staining confirmed lysosomal enzyme involvement in the autophagic response. Up regulation of Atg 3, Atg12, Beclin 1, LC3-II protein and BIF-1 and down regulation of Atg4B were also showed. Another study established the increased uptake and targeted delivery of GNP-tagged NKCT1 in cancer cells[39]. GNP-NKCT1 down-regulated cyclin dependent kinase-4 and MAP kinase through estrogen receptor mediated cell cycle arrest in human breast cancer cell line[40]. From the above findings, it is clear that tagging of gold nanoparticle with an anticancer snake venom toxin NKCT1 increased the efficacy of the venom toxin to kill the cancer cells.

Al-Sadoon *et al*. (2012)[41] has established the potential antiproliferative property of *Walterinnesia aegyptia* venom alone or combined with silica nanoparticle against mice immune cell and human breast carcinoma cell line (MDA-MB-231 and MCF-7). In this study, the IC_{50} value of venom and venom+silica nanoparticle were 50 and 20 ng/mL, respectively, which indicated the increase of anti-proliferative property of venom when combined with silica nanoparticle. At these concentrations, the venom did not affect the viability of normal breast epithelial cells (MCF-10). Combination with venom with nanoparticle strongly induced apoptosis in MDA-MB-231 and MCF-7 cancer cells without significant effect on normal MCF-10 cells. Venom-nanoparticle combination reduced the expression of Bcl_2 and increased the activation of caspase 3 in MDA-MB-231 and MCF-7 cells. The same venom-nanoparticle combination showed decrease in surface expression of the chemokine receptors CXCR3, CXCR4 and CXCR6 to a greater extent than venom alone in breast cancer cell line. Venom-nanoparticle combination also reduced migration in response to the cognate ligands CXCL10, CXCL12 and CXCL16. It strongly inhibited insulin-like growth factor 1 (EGF-1)- and IL-6-mediated multiple myeloma cell proliferation, changed the cell cycle pattern and enhanced the induction of apoptosis in multiple myeloma cells. The combination of venom with nanoparticle robustly decreased the expression of cyclin D1, Bcl_2 and the phosphorylation of AKT, increased the expression of cyclin B1, altered the mitochondrial membrane potential, increased the activity of caspase-3, -8 and -9; and sensitized multiple myeloma cells to growth arrest and apoptosis[42]. Sayed *et al*. (2012)[43] has demonstrated the antiproliferative and apoptotic effect of *Walterinnesia aegyptia* venom and *Walterinnesia aegyptia* venom + silica nanoparticle on multiple myeloma cells. In this study, venom alone and venom-nanoparticle both decreased the viability of multiple myeloma cells. Venom combined with nanoparticle strongly inhibited multiple myeloma cell proliferation. Cell cycle analysis of multiple myeloma cells indicated the altered cell cycle and increased apoptosis induction after venom-nanoparticle treatment. Badr *et al*. (2013)[44] has reported the therapeutic efficacy and molecular mechanisms of snake (*Walterinnesia aegyptia*) venom-loaded silica nanoparticles in the treatment of breast cancer- and prostate cancer-bearing experimental mouse model. In this study, xenograft breast and prostate tumor mice models were established. Treatment with venom alone and venom-loaded nanoparticle significantly decreased both breast and prostate tumor volumes compared to treatment with nanoparticle or vehicle alone. It was observed that venom-loaded nanoparticle strongly inhibited insulin-like growth factor 1 and epidermal growth factor mediated proliferation of breast and prostate cancer cells, respectively, and increased the apoptosis induction by inducing the activity of caspase-3,–8, and -9 in both breast and prostate cancer cells. Combination of venom with nanoparticle decreased the phosphorylation of AKT, ERK, and IκBα, decreased the expression of cyclin D1, surviving, and the antiapoptotic Bcl_2 family members Bcl_2, Bcl_{XL}, and

Mcl_1, markedly increased the expression of cyclin B1 and the proapoptotic Bcl_2 family members Bak, Bax, and Bim, changed the mitochondrial membrane potential and sensitized tumor cells to growth arrest. Badr *et al*. (2013)[45] also evaluated the impact of combination of *Walterinnesia aegyptia venom loaded silica nanoparticle on* the migration, invasion, proliferation and apoptosis of prostate cancer cells.

Snake venom/toxins have been identified as antiarthritic agent in animal models[22]. Gomes *et al*. (2010)[46] showed that Indian monocellate cobra (*Naja kaouthia*) venom had antiarthritic activity against Freund's complete adjuvant (FCA) induced arthritis in male albino rats. Antiarthritic potential of snake venom/toxin conjugated nanoparticle has been evaluated in animal model. Saha *et al*. (2014)[47] have conjugated NKCT1 with gold nanoparticle and examined its antiarthritic potential in animal model. Arthritis was induced in mice by FCA. In this study, NKCT1 conjugated gold nanoparticle significantly changed arthritis induced paw/ankle swelling, urinary markers, serum markers and cytokines. Gomes *et al*. (2016)[48] also reported the antiosteoarthritis potential of NKCT1 conjugated gold nanoparticle in collagenase induced experimental osteoarthritis. Physical parameter (ankle diameter), urinary markers (hydroxyproline, glucosamine, pyridoline, deoxy-pyridoline), serum and synovial membrane pro-inflammatory markers (TNF-α, IL-1β, IL-17, VEGF) and metalloproteinase (MMP1) were significantly restored in osteoarthritic animals.

Use of snake venom along with nanoparticle is now an emerging area of drug delivery research[49]. The feasibility of hydrophilic nanoparticles has been broadly investigated for use in drug delivery and therapeutic systems. In a study, *Agkistrodon halys* snake venom was loaded in chitosan nanoparticles in order to be used as an advanced adjuvant and antigen delivery system in antidote production industry[35]. Celen *et al*. (2018)[50] has encapsulated Vipera ammodytes transcaucasiana venom in PAMAM-G4 dendrimer nanoparticle by sol-gel method and showed its efficacy in drug delivery systems. The surface plasmon resonance property of nanoparticle has been utilized in the detection of snake venom protein using integrated biosensor, which can be used in detecting the concentration of venom proteins in the blood of snake bitten patients in the near future[51]. Phospholipase A_2 (PLA_2) is an important constituent of snake venom with enormous biopotential. In a study by Barros *et al*. (2016)[52], Asp-PLA_2 isolated from *Bothrops jararacussu* snake venom was incorporated into liposome nanoparticles and its efficacy against Leishmaniasis was shown experimentally. Liposomes were formed homogeneously using the extrusion method and physico-chemically characterized. The activity of Asp49-liposomes was evaluated *in vitro* against Leismaniasis and J774 macrophages. The results indicated that Asp49-liposome is a promising tool to enhance microbicidal activity of the infected macrophages in experimental leishmaniasis.

However, the major drawback of these bioactive toxin molecules is their toxicity, which limits their entry in clinical trials. In order to overcome the toxicity, several attempts have been made such as liposome encapsulation, silica coating, nanoconjugation, etc. Application of nanotechnology in current biomedical research revealed that nanoparticles have unusual properties to improve the pharmacological and therapeutic properties of drugs. Nano conjugation of therapeutically potent toxin molecules and pre-existing clinically used drugs, not only provides a media for better drug delivery but also enhance stability, bioavailability and targeted drug delivery. Saha *et al*. (2014)[30] showed that GNP conjugation with protein NKCT1 significantly decreased the minimal lethal dose of NKCT1, thus making the molecule less toxic. Subchronic toxicity study of NKCT1/GNP-NKCT1 showed significant reduction in food and water intake, which reflected in body weight loss and decreased fecal consistency in NKCT1 treated mice group. GNP conjugation with protein NKCT1 significantly decreased (1 fold) the minimal lethal dose of NKCT1, thus making the molecule less toxic.

Future directions of nanotechnology in snake venom research

Snake venom is a natural biological store of therapeutically active components including neurotoxic, cardiotoxic, cytotoxic, nerve growth factor, lectins, disintrigrins, haemorrhagins and many other different enzymes. These proteins not only cause death to animals and humans, but can also be used for the treatment of cancer, arthritis, thrombosis and many other diseases[22]. Application of nanotechnology along with therapeutically active snake venom/toxins may open a new domain of therapeutics. Possible snake venom/toxins that can be

Table 2 — Possible snake venom/toxins that may be used in nanotechnology research

Snake Venom/Toxins	Source	Therapeutic Use
NN_{32}	*Naja naja* venom	Anticancer, Antiarthritis, Anti-inflammatory, Antioxidant[53,54]
*dr*CT-I	*Daboia russellii* venom	Anticancer[55]
Labein	*Macrovipera lebetina* venom	Anticancer[56]
CC5 and CC8	*Cerastes cerastes* venom	Anticancer[57]
BnSP-6	*Bothrops pauloensis* venom	Anticancer[58]
BF-F47	*Bungarus fasciatus* venom	Antiarthritic[59]
Cobratoxin	Thailand cobra venom	Antiarthritic[60]
Whole venom	*Naja kaouthia*	Antiarthritic[61]
Viperatoxin-II	*Daboia russellii* venom	Antibacterial[62]
NN-XIb-PLA2	*Naja naja* venom	Antibacterial[63]
Bothropstoxin-I	*Bothrops sp*. Venom	Antibacterial[64]

utilized in pathophysiology with the help of nanotechnology are summarized in Table 2[53-64].

The questions may arise from the above research findings that (i) whether conjugate of venom/toxin with suitable nanoparticle will increase the efficacy for developing therapeutic agents against killer diseases (arthritis, cancer, HIV, etc.); (ii) whether conjugate of nanoparticle with venom/toxin shall decrease its toxicity to make it safe for drug trials; and (iii) whether we can conjugate venom/toxins to more than one nanoparticles, to make it more effective? Ultimately, the target for nanoparticle and snake venom/toxin research should be the development of drug clues against incurable diseases, such as cancer, arthritis, HIV, etc.

The need for an efficient technology/tool for the study of snake venom was felt for a long time. Nanotechnology is the upcoming technology that has started its journey in the field of snake venom research. Until now, very few approaches have been taken to explore nanotechnology in snake venom research. Not only in the field of supportive/alternate antidote of snake venom is required but also the need of therapeutic agents against incurable diseases was started using snake venom/toxin. The upcoming applications of nanotechnology in snake venom research remain to be explored are (i) Nanoparticle aided detection, distribution and targeting of snake venom/toxins; (ii) Nanoparticle aided interaction of snake venom/toxin in target organ; (iii) Nanoparticle aided repair/healing of major organs (liver, kidney, lung etc) affected in snake-bite; (iv) Nanoparticle conjugated herbal antidote (supported therapy) in the management of snake bite; (v) Nanoparticle conjugated snake venom/toxins in the management of communicable and non-communicable diseases; (vi) Nanoparticle conjugated snake venom/toxin as immunomodulator in immunodeficiency diseases; and (vii) Detail toxicity studies with the herbo-nanoparticle and nanoparticle conjugated snake venom/toxin are also required.

What is more important at this stage is that initiative should be taken by the Government and industries to take/support this emerging area of research so that snake bite problem in tropical countries may be tackled successfully. Further, ethical issues and biosafety measures in nanotechnology research should be followed according to guidelines.

Conflict of interests

No conflict of interest exists among the authors.

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