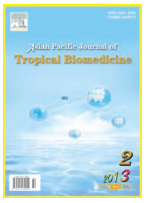
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(13)60042-8

Therapeutic potential of snake venom in cancer therapy: current perspectives

Vivek Kumar Vyas[1*], Keyur Brahmbhatt[1], Hardik Bhatt[1], Utsav Parmar[2]

[1]*Institute of Pharmacy, Nirma University, Ahmadabad 382481 Gujarat, India*

[2]*Arihant School of Pharmacy and Bio Research Institute, Adalaj, Gandhinagar 382421 Gujarat, India*

PEER REVIEW

Peer reviewer

Prof. Radheshyam Patidar, Department of Pharmacognocy and Natural Product, Mahakal Institute of Pharmaceutical Studies (MIPS), Rajiv Gandhi Technical University, Dewas Road, behind Air Strip, Datana Matana, Ujjain 456664 Madhya Pradesh, India.
Tel: +91 9669209181
E-mail: radhe1980@gmail.com

Comments

The therapeutic use of snake venom section provides an overview of different use of snake venom for different therapies. The actual review section is nicely written to provide information about the different components isolated, characterized and used on cancer cell line for their effectiveness to inhibit the growth of the tumor.
(Details on Page 160)

ABSTRACT

Many active secretions produced by animals have been employed in the development of new drugs to treat diseases such as hypertension and cancer. Snake venom toxins contributed significantly to the treatment of many medical conditions. There are many published studies describing and elucidating the anti-cancer potential of snake venom. Cancer therapy is one of the main areas for the use of protein peptides and enzymes originating from animals of different species. Some of these proteins or peptides and enzymes from snake venom when isolated and evaluated may bind specifically to cancer cell membranes, affecting the migration and proliferation of these cells. Some of substances found in the snake venom present a great potential as anti-tumor agent. In this review, we presented the main results of recent years of research involving the active compounds of snake venom that have anticancer activity.

KEYWORDS

Snake venom, Cytotoxin, Anticancer agents, Apoptosis inducer

1. Introduction

Snake venoms are the secretion of venomous snakes, which are synthesized and stored in specific areas of their body *i.e.* venom glands. Most of the venoms are complex mixture of a number of proteins, peptides, enzymes, toxins and non protein inclusions[1]. Many of them are harmless, but some can produce toxicity at certain degree. Snake venoms cause significant mortality and morbidity worldwide, and strike fear in most of us. Cytotoxic effects of snake venom have potential to degrade/destroy tumor cells[2]. It is a naturalistic approach available in the nature. Snake venoms are produced in the glands throughout life of the snake, so it subtracts the chances to kill the snake in the process of collection of venom, but it should be collected with scientific approaches and techniques[3]. Snake venom affects human body according to its potency and type. Different species have different types of venom, which depends upon its species, geographical location, its habitat, climate, age *etc.* (Table 1). It is very thick in

*Corresponding author: VK Vyas, Institute of Pharmacy, Nirma University, Ahmadabad 382 481 Gujarat, India.
Tel: +91 9624931060
Fax: +91 2717 241916
E-mail: vicky_1744@yahoo.com

Article history:
Received 4 Nov 2012
Received in revised form 8 Nov, 2nd revised form 13 Nov, 3rd revised form 17 Nov 2012
Accepted 28 Dec 2012
Available online 28 Feb 2012

Table 1
Species of some medicinally important snakes.

S. no.	Family	Scientific names	Common names
1	Crotalids	*Agkistrodon bilineatus*	Canti
2	Crotalids	*Agkistrodon contortrix*	Copperhead
3	Elapids	*Naja haje*	Egyptian or brown cobra
4	Crotalids	*Agkistrodon halys*	Mamushi
5	Crotalids	*Agkistrodon piscivorus*	Eastern cottonmouth
6	Elapids	*Naja atra*	Chinese cobra
7	Viperids	*Vipera russelli*	Saw scaled viper
8	Crotalids	*Calloselasma rhodostoma*	Malyan pit viper
9	Crotalids	Bothrops asper and/or atrox	Fer-de-lance
10	Crotalids	*Bothrops jararaca*	Jararaca
11	Crotalids	*Bothrops jararacussu*	Jararacussu
12	Elapids	*Naja naja*	Indian cobra
13	Crotalids	*Bothrops neuwiedi*	Jararaca pintada
14	Crotalids	*Crotalus adamanteus*	Eastern diamondback rattlesnake
15	Crotalids	*Crotalus atrox*	Western diamondback rattlesnake
16	Crotalids	*Crotalus basiliscus*	Mexican west-coast rattlesnake
17	Crotalids	*Crotalus scutulatus*	Mojave rattlesnake
18	Elapids	*Naja oxiana*	Central Asian Cobra
19	Crotalids	*Crotalus viridis helleri*	Southern Pacific rattlesnake
20	Elapids	*Ophiophagus*	Hannah-king cobra
21	Viperids	*Echis carinatus*	Russell's viper

winter hence less in amount while it gains more liquidity in summer and monsoon and has a more amount[4,5]. Snake venom is harmless if ingested in liquid or crystal form after drying through mouth and it will be excreted unchanged; it contains anti-clotting proteins. It produces toxicity only if contacted with blood. Snake venom is clear, viscous and transparent liquid, which can be dried to solid crystal form[6]. The constituents of snake venom can be preserved for longer period, if dried solid crystal form is stored properly. The crystal mass can dissolves readily in blood and water. There are basically three types of snake venom according to its effects[7]. Hemotoxic venoms, which affects cardiovascular system and blood functions, cytotoxic venoms targets specific cellular sites or muscles and neurotoxic venoms harms nervous system of human body. Snake venom has mainly two functions; 1) paralyzes the prey and 2) starts the digestive process. Enzymes present in snake venom hydrolyse proteins and membrane components, which lead to tissue necrosis and blood clotting. Components of venom are responsible for paralysis attack on nerve membrane and branches and neuro-muscular junctions. Based on mode of action, snake venom can be grouped into different categories. Some components of venom bind to cholinergic receptors without causing any biological activity. Prey usually dies as respiratory muscles no longer function. A group of toxins inhibits or merely increases release of acetylcholine, so the muscle cell can not react to nerve stimuli and results in spasm or relaxation of muscle. Some toxins are responsible for damage to the skin and connective tissues of the body, their precise mode of action are unknown. Cytotoxins and cardiotoxins in the venom causes damage to the cell membrane or interfere with the transport of substances or the transduction of signals across the membranes[8]. Nowadays, treatment of cancer is a major challenge to the medical world. Present methods of treatment are very costly and have numerous side effects. Patient has to suffer physically, mentally as well as economically. Some of the components of snake venom cause retardation of growth of cancerous cells. Due to its therapeutic activity, potency and availability, snake venom may be a vital nominee for the medicine in the future for many diseases and disorders[9]. Viewing and analysing with futuristic prospectus in pharmaceutical world, snake venom could open the doors for new era of medicines and research for treatment of cancer[10-13]. Snake venoms are frequently studied by scientists for its therapeutically use. Many excellent publications characterized use of venoms for the treatment of various therapeutic conditions such as cancer and inflammation[14,15]. The purpose of this article is to review recent literature regarding therapeutic potential of snake venom in an attempt to establish a scientific basis for use of snake venom for treatment of cancer.

2. Composition of snake venom

Snake venoms are complex mixtures; mainly it has proteins, which have enzymatic activities. Protein and peptides make 90 to 95 percent of the dry weight of venom. In addition to that snake venoms contain inorganic cations such as sodium, calcium, potassium, magnesium and small amounts of zinc, nickel, cobalt, iron, manganese. Zinc is necessary for anti-cholinesterase activity; calcium is required for activation of enzyme like phospholipase. Some snake venoms also contain carbohydrate, lipid, biogenic amines, and free amino acids[16]. Snake venoms contain at least 25 enzymes, but no single venom contains all of them. Enzymes are protein in nature, but few are depends on certain nonprotein prosthetic groups or cofactors.

2.1. Proteolytic enzymes

These enzymes catalyze the breakdown of tissue proteins and peptides. They are also known as peptide hydrolases, protease, endopeptidases and proteinases. They have molecular weights between 20000 and 95000 Da. They may sometimes inactivated by edetic acid and some reducing agents. Some metal ions help in catalysis and intrinsically involved in the activity of certain venom proteases and phospholipases[17].

2.2. Arginine ester hydrolase

This is one of the non-cholinesterase enzymes found in snake venoms. It causes hydrolysis of the ester or peptide linkage, to which an arginine residue contributes the carboxyl group. This activity was found in crotalid, viperid and some sea snake venoms but lacking in elapid venoms. Bradykinin-clotting activities of some venom related to esterase activities[18].

2.3. Thrombin

Thrombin releases fibrinopeptides A and B which are responsible for clotting of plasma.

2.4. Thrombin-like enzymes

They are glycoprotein in nature, and have molecular weights between the ranges of 29000 to 35000 Da. They act as defibrinating anticoagulants *in vivo*, whereas *in vitro* they clot plasma, citrated or heparinised plasma and also purified fibrinogen. Due to its action as defibrinating agent, more attention has been directed toward the characterization and study of the thrombin-like enzymes than toward those of the other venom pro-coagulant or anti-coagulant enzymes. Thrombin like enzymes such as crotalase, agkistrodon, ancrod and batroxobin can be purified from different snake venoms. They have been used clinically in animals for therapeutic and investigative studies. Treatment with ancrod before the formation of the experimentally induced thrombus in dog, prevented thrombosis and ensured vessel patency. However, ancrod has no thrombolytic effect after thrombus formation. Crotalase has been employed to evaluate the role of fibrin deposition in burns in the animals. The role of fibrin deposition has been evaluated in tumor metastasis, in which fibrinogen removed by treatment with ancrod and also by batroxobin[19].

2.5. Collagenase

Collagenase is a proteinase enzyme, which specifically digests collagen. Some snakes contain collagenase which digests mesenteric collagen fibers but not the other protein[20].

2.6. Hyaluronidase

This enzyme referred as the "spreading factor". It is thought to be related to the extent of edema produced by the venom. It acts upon connective tissues and decreases their viscosity, catalyzes the cleavage of internal glycoside bonds in certain acid mucopolysaccharides. Breakdown in the hyaluronic barrier allows other fractions of venom to penetrate the tissues.

2.7. Phospholipase

Many PLA_2 were found in snake venom. It has 120 amino acids and 14 cysteine residues forming 7 disulfide bonds. Venoms are the richest sources of PLA_2. It catalyzes the calcium dependent hydrolysis of the 2-acyl ester bond thereby producing free fatty acids and lysophospho lipid. PLA_2 can also cause hydrolysis of membrane phospholipids, and liberation of some bioactive products[21].

2.8. Phosphodiestsrase

It releases 5-mononucleotide from the polynucleotide chain and act as an exonucleotidase, thereby affecting DNA and RNA functions. It is found in all poisonous snakes[22].

2.9. Acetylcholinesterase

This is found in cobra and sea snake but absent in viperid and crotalid venoms. It catalyzes the hydrolysis of acetylcholine to choline and acetic acid.

2.10. RNase

This is present as the endopolynucleotidase RNase which has specificity toward pyrimidine containing pyrimidyladenyl bonds in DNA.

2.11. DNase

It gives oligonucleotides, which terminate 3′ monoesterified phosphate bond in DNA.

2.12. 5′-Nucleotidase

Nucleotidase is a most active phosphatase in snake venoms, which hydrolyzes phosphate monoesters linked with a 5′ position of RNA and DNA.

2.13. L-Amino acid oxidase (L-AAO)

L-AAO gives yellow color to venom. It catalyzes the oxidation of *L*-α-amino acid and α-hydroxy acid.

2.14. L-Actate dehydrogenase

It catalyzes the equilibrium between lactic acid and pyruvic acid and found in all animal tissues.

2.15. Polypeptides

These are a low molecular weight protein that lacks enzymatic activity. More than 80 polypeptides were isolated from snake venoms with different pharmacological activities[23].

3. Pharmacological actions of snake venom

Many toxins from snake venom are investigated and formulated into drugs for the treatment of conditions such as cancer, hypertension, and thrombosis. In general, the venoms of rattlesnakes and other new world crotalids produce alterations in resistance of blood vessels, changes in blood cells and coagulation mechanism, direct or indirect changes in cardiac and pulmonary dynamics. There may be alterations in nervous system and respiratory system[24-27]. The potency of venom and its effect on human depend on the type and amount of venom injected and the site where it is deposited. Other parameters such as sex, general health, size and age are also influencing factors. Clinical experiments and history show that the death may occur within less than 1 h to several days while the most deaths occurred between 18 to 32 h. Snake venoms significantly lower the blood pressure in human victims and experimental animals. Hypotension and shock are associated with snake venom poisoning[28]. Experimentally, it has been found that an intravenous bolus injection of a *Crotalus* venom causes an immediate fall in blood pressure and varying degree of shock, associated with initial heme concentration followed by a decrease in heamatocrit values[29]. Captopril was isolated from *Bothrops jararaca* venom is an example of a therapeutic derived from the snake venoms[30]. Increased blood volume in the lung and pulmonary artery pressure with a concomitant decrease in pulmonary artery flow and a relatively stable heart stroke volume is noticed. When *Crotalus* venom is given IV slowly for over a period of 30 min, there is hypovolume secondary to an increase in capillary permeability to proteins and RBCs. The experimental results showed initial haemoconcernatration, lactacidamiea and lipoprotienamia, respiration becomes labored and if period prolongs animal becomes oliguric, rales develop and the animal dies[31-36].

4. Recent advancements in the role of snake venom for cancer therapy

Cancer is characterized by uncontrolled cell division, cell transformation, and escape of apoptosis, invasion, angiogenesis and metastasis. Induction of apoptosis is the most important mechanism of many anticancer agents. Snake venom disintegrins are the low molecular weight molecules with different structure, potency and specificity initially isolated from viperid snake venoms, usually contain integrin, an agent for development of therapeutics for the treatment of cancer. Integrins are important in cell adhesion, cell migration, tissue organization, cell growth, hemostasis and inflammatory responses, so they are in the study for the development of drugs for the treatment of cancer[37]. Zhang *et al*. isolated ACTX-6 (98 kDa proteins containing two subunits) from *Agkistrodon acutus* snake venom[38]. The authors found that ACTX-6 could induce cell apoptosis. The authors reported that reactive oxygen species (ROS) was involved in apoptosis generated by oxidation of *L*-amino acid by ACTX-8. ACTX-8 has no activity on antiapoptopic/proapoptopic BCL2 family members. It works by mainly two mechanisms: firstly by translocation of Bax and Bad and second action was on Bad bound to Bcl-xL to substitute Bak. The activated Bax and Bak played an essential role in the release cytochrome C to mediate apoptosis. The induction of the apoptosis manifests the control on the tumour size and number of tumour cells hence establishing the application of apoptotis inducers as vital components in the treatment of cancer. Torii *et al*. purified an apoptosis-inducing factor[39], apoxin I from rattlesnake venom and amino-terminal sequences of the purified apoxin-I similar to *L*-amino acid oxidases (LAO). After creation of the primary structure of apoxin-I by using cloned c-DNA, the authors demonstrated that apoxin-I likely to bind FAD to catalyze oxidative deamination of *L*-amino acids and apoptosis inducing activity. Naumann *et al*. isolated and purified *L*-amino acid oxidases (LAAOs) from *Bothrops leucurus* (Bl-LAAO) and reported biochemical features of Bl-LAAO associated with its effect on platelet function and cytotoxicity[40]. Cytotoxicity of Bl-LAAO was observed in the stomach cancer MKN-45, adeno carcinoma HUTU, colorectal RKO and human fibroblast LL-24 cell lines. The authors concluded that *B. leucurus* venom is cytotoxin acting primarily via the generation of high amounts of H_2O_2 which kills the cells. Kim *et al*. purified venom of king cobra, *Ophiophagus hannah* and determined the cytotoxic components in purified venom[41]. The components were mainly consistent of *L*-amino acid oxidase. The authors observed cytotoxic effects of *L*-amino acid oxidase on stomach cancer, murine melanoma, fibrosarcoma, colorectal cancer and Chinese hamster ovary cell lines. It was observed that cytotoxic protein causes inhibition of cell proliferation by 74% according to [^{3}H]thymidine uptake assay. Mechanism of enzyme action may be related to the inhibition of thymidine incorporation and an interaction with DNA. Gebrim *et al*. evaluated both *in vitro* and *in vivo* antitumor activity of p-bromophenacyl bromide (BPB) modified bothropstoxin-I from *Bothrops jararacussu* venom (BthTX-I)[42]. Different tumor cell lines were found to susceptible from lytic action of BPB-BthTX-I and also from synthetic peptide. Guo *et al*. studied pharmacokinetics of cytotoxin from Chinese cobra (*Naja naja atra*) venom in rabbits[43]. Plasma levels of the cytotoxin were analyzed by a biotinavidin enzyme-linked immunosorbent assay. Gomes *et al*. purified a lethal cardiotoxic-cytotoxic protein from the Indian monocellate cobra (*Naja kaouthia*) venom by ion-exchange chromatography and HPLC[44]. Cytotoxicity studies on human leukemic U937 and K562 cells showed a significant inhibition of cell proliferation in a dose and time dependent manner. In another work, the authors purified venom from Indian Naja naja through ion exchange chromatography and found that fraction 32 produced cytotoxic-cardiotoxic properties[45]. NN-32 showed cytotoxicity on EAC cells, increased survival time of inoculated EAC mice, reduced solid tumor volume and weight. NN-32 induced anticancer activity in EAC mice mediated through its apoptogenic-antioxidant property. Markland *et al*. isolated and characterized a lectin (BJcuL) from the venom of the snake *Bothrops jararacussu*[46]. The authors examined *in vitro* effect of the BJcuL on adhesion of human ovarian and breast cancer carcinoma cells and viability of these cell lines, as well as of human glioblastoma, human bladder carcinoma, human leukemia and bovine brain endothelial cells. BJcuL was found as a potent inhibitor of

growth of some tumor cell lines and an endothelial cell line. Zhang *et al*. isolated ACTX-6 from *Agkistrodon acutus* snake venom and demonstrated cytotoxic activity to various cancer cells *in vitro*[47]. The authors investigated the exact mechanism (induce cell apoptosis) of ACTX-6. The authors reported that ACTX-6-induced cell death through production of ROS (hydrogen peroxide). Sun *et al*. extracted specific protein *Okinawa Habu* apoxin protein-1 (OHAP-1) from *Okinawa Habu* venom which is well known for its toxic effects[48]. In this study, it was investigated that OHAP-1 could induce apoptosis in some glioma cells and elucidated the possible mechanism involved. Induction of apoptosis was determined by using DNA gel electrophoresis, DNA flow cytometry and TUNEL assay. It was reported that apoptotic effect of OHAP-1 on malignant glioma cells could be through the generation of intracellular ROS and p53 protein expression. Karthikeyan *et al*. evaluated antitumor activity of the sea snake venom (*Lapemis curtus*) against Ehrlich's ascites carcinoma (EAC) in Swiss albino mice and HeLa and Hep2 tumor cell cultures[49]. Decrease in tumor volume and viable tumor cell count was observed these characteristics were considered as an important indicator of reduction of tumor burden. Fue *et al*. studied snake venom-derived arginine-glycine-aspartic acid (RGD)-containing disintegrins (*e.g.* rhodostomin)[50], which inhibited the adhesion of breast and prostate carcinoma cells to bone extracellular matrices, without affecting the viability of tumor cells. It was reported that co-administration of disintegrin with tumor cells inhibited tumor growth in bone through the decrease of cell adhesion, migration and osteolysis in bone. Gomes *et al*. purified and crystallized heat stable protein toxin (drCT-I) from Eastern Indian *Daboia russelli russelli* venom[51]. drCT-I was evaluated for anticancer activity against EAC cells *in vivo* and human leukemic cells (U937, K562) *in vitro*. drCT-I significantly decreased EAC cell count. The authors confirmed induction of apoptosis. It was found that drCT-I brought about apoptosis by G1 phase arrest of the cell cycle. Lin *et al*. isolated cardiotoxin III (CTX III), from *Naja naja atra* venom, and reported its anticancer activity[52]. It was evidenced by accumulation of sub-G1 population, externalization of phosphatidylserine, release of cytochrome C, and activation of both capases-9 and caspase-3 that CTX III-induced cell apoptosis. Study showed that CTX III suppressed phosphorylation of JAK2, STAT3, Akt, and activation of PI3K. It was suggested that CTX III suppressed JAK2- and PI3K-activation in parallel with the inhibition of STAT3 and Akt phosphorylation. Nunes *et al*. evaluated the anti-tumor potential as well as its cytotoxicity and hemolysis activity of BlL[53], a galactoside-binding lectin isolated from *Bothrops leucurus* venom. The authors verified induced apoptosis in K562 cells, by phosphatidylserine externalization analysis and mitochondrial membrane potential determination. Nolte *et al*. purified BjcuL, a lectin from *Bothrops jararacussu* venom by affinity chromatography and observed its cytotoxic effects to gastric carcinoma cells MKN45 and AGS[54]. BjcuL was examined on the cell morphology, cytoskeleton using fluorescence microscopy. The authors confirmed cytotoxicity of BJcuL on tumor cells mainly by altering cell adhesion and through induction of apoptosis.

5. Conclusions

Snake venoms are the complex mixtures of several biologically active proteins, peptides, enzymes, and organic and inorganic compounds. Venom from snakes is an important agent for curing many types of cancers. Many research publications discussed in this review showed a complete remission of tumor cells after treatment with molecules derived from snake venoms. It has been reviewed through many article, that snake venom acts by inhibiting cell proliferation and promoting cell death by different means: induction of apoptosis in cancer cell, increasing Ca^{2+} influx; inducing cytochrome C release; decreasing or increasing the expression of proteins that control cell cycle; causing damage to cell membranes. Snake venoms contain a vast array of components, the majority of which act on the peripheral nervous system for killing or immobilizing prey. We can anticipate the development of a new agent from snake venoms in the future which will be useful in cancer therapy.

Conflict of interest statement

The authors declare no conflicts of interests.

Acknowledgements

The authors would like to thank Director, Institute of Pharmacy, Nirma University, Ahmedabad, India.

Comments

Background

Snake venom is a complex mixture of number of proteins, peptides, enzymes, toxins and non protein inclusions. Cytotoxic effects of the snake venom have potential to degrade/destroy tumor cells. Cytotoxins and cardiotoxins in the venom causes damage to cell membrane or interfere with the transport of substances or the transduction of signals across the membranes. Viewing and analysing with futuristic prospectus in pharmaceutical world, snake venom could open the doors for new era of medicines and research for treatment of cancer.

Research frontiers

The purpose of this article is to review recent literature regarding therapeutic potential of snake venom in an attempt to establish a scientific basis for use of snake venom for treatment of cancer.

Related reports

Many excellent publications characterized use of venoms for the treatment of various therapeutic conditions like cancer and inflammation (Gomes A 2010).

Innovations and breakthroughs

Snake venom has many therapeutic uses. In this work, the authors are reviewed recent literature pertain to isolation, characterization and use of snake venom especially in anticancer therapy. This first of its kind, because many article has published for therapeutic use of snake venom in many therapy not with targeted one, so it is good.

Applications

Cancer is an uncontrolled cell growth and responsible for many deaths annually worldwide. Still the world is waiting for novel and effective treatment for cancer, due to the therapeutic activity of component of snake venom in cancer, it could open the doors for new era of medicines and research for treatment of cancer.

Peer review

Snake venoms are frequently studied by scientists for it's therapeutically use. The paper represent mostly successful efforts to complete information regarding current use of snake venom especially in anticancer. The paper is nicely organised with good introductory section preceding the actual information of snake venom. An overview of composition of snake venom ids also good. The therapeutic use of snake venom section provides an overview of different use of snake venom for different therapies. The actual review section if nicely written to provide information about the different components isolated, characterized and used on cancer cell line for their effectiveness to inhibit the growth of the tumor.

References

[1] Leon G, Sanchez L, Hernandez A, Villalta M, Herrera M, Segura A, et al. Immune response towards snake venoms. *Inflamm Allergy Drug Targets* 2011; **10**: 381-398.

[2] Marsh N, Williams V. Practical applications of snake venom toxins in haemostasis. *Toxicon* 2005; **45**: 1171-1181.

[3] Daltry JC, Wuster W, Thorpe RS. Diet and snake venom evolution. *Nature* 1996; **379**: 537-540.

[4] Sanchez EF, Schneider FS, Yarleque A, Borges MH, Richardson M, Figueiredo SG, et al. The novel metalloproteinase atroxlysin-I from Peruvian Bothrops atrox (Jergón) snake venom acts both on blood vessel ECM and platelets. *Arch Biochem Biophy* 2010; **496**: 9-20.

[5] Tashima AK, Sanz L, Camargo ACM, Serrano SMT, Calvete JJ. Snake venomics of the *Brazilian pitvipers, Bothrops cotiara* and *Bothrops fonsecai*, Identification of taxonomy markers. *J Proteo* 2008; **71**: 473-485.

[6] Guan HH, Goh KS, Davamani F, Wu PL, Huang YW, Jeyakanthan J, et al. Structures of two elapid snake venom metalloproteases with distinct activities highlight the disulfide patterns in the D domain of ADA Malysin family proteins. *J Stru Biol* 2010; **169**: 294-303.

[7] Jin H, Varner J. Integrins: roles in cancer development and as treatment targets. *Br J Cancer* 2004; **90**: 561-565.

[8] Yamazaki Y, Morita T. Snake venom components affecting blood coagulation and the vascular system: structural similarities and marked diversity. *Cur Pharma Des* 2007; **13**: 2872-2886.

[9] Debatin KM, Krammerr P. Death receptors in chemotherapy and cancer. *Oncogene* 2004; **23**: 2950-2966.

[10] Park MH, Choi MS, Kwak DH, Oh KW, Yoon DY, Han SB, et al. Anti-cancer effect of bee venom in prostate cancer cells through activation of caspase pathway via inactivation of NF-κB. *EINSTEIN* 2011; **71**: 801-812.

[11] Tang N, Xie Q, Wang X, Li X, Chen Y, Lin X, et al. Inhibition of invasion and metastasis of MHCC97H cells by expression of snake venom cystatin through reduction of proteinases activity and epithelial-mesenchymal transition. *Arch Pharm Res*. 2011; **34**: 781-789.

[12] Park MH, Jo M, Won D, Song HS, Song MJ, Hong JT. 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* 2012; **17**: 1316-1326.

[13] Heinen TE, Veiga ABG da. Arthropod venoms and cancer. *Toxicon* 2011; **57**: 497-511.

[14] Kalam Y, Isbister GK, Mirtschin P, Hodgson WC, Konstantakopoulos N. Validation of a cell-based assay to differentiate between the cytotoxic effects of elapid snake venoms. *J Pharmacol Toxicol Methods* 2011; **63**: 137-142.

[15] Gomes A, Bhattacharjee P, Mishra R, Biswas AK, Dasgupta SC, Giri B. Anticancer potential of animal venoms and toxins. *Indian J Exp Biol* 2010; **48**: 93-103.

[16] Santos MMDV, Santana CD, Giglio JR, Da Silva RJ, Sampaio SV, Soares AM, et al. antitumoural effect of an *L*-amino acid oxidase isolated from *Bothrops jararaca* snake venom. *Basic Clin Phramacol Toxicol* 2008; **102**: 533-542.

[17] Kitchens CS, Eskin TA. Fatality in a case of envenomation by *Crotalus adamanteus* initially successfully treated with polyvalent ovine antivenom followed by recurrence of defibrinogenation syndrome. *J Med Toxicol* 2008; **4**: 180-183.

[18] Biardi JE, Chien DC, Coss RG. California ground squirrel (*Spermophilus beecheyi*) defenses against rattlesnake venom digestive and hemostatic toxins. *J Chem Ecol* 2006; **32**: 137-154.

[19] Antunes TC, Yamashita KM, Barbaro KC, Saiki M, Marcelo L. Santoro. Comparative analysis of newborn and adult *Bothrops jararaca* snake venoms. *Toxicon* 2010; **56**: 1443-1458.

[20] Sales PBV, Santoro ML. Nucleotidase and DNase activities in Brazilian snake venoms. *Compr Biochem Physiol* 2008; **147**C:85-95.

[21] Chu CW, Tsai TS, Tsai IH, Lin YS, Tu MC. Prey envenomation does not improve digestive performance in Taiwanese pit vipers (*Trimeresurus gracilis* and *T. stejnegeri stejnegeri*). *Compr Biochem Physiol* 2009; **152**A: 579-585.

[22] Wuster W, Peppin L, Pook CE, Walker DE. A nesting of vipers: phylogeny, historical biogeography and patterns of diversification of the Viperidae (Squamata: Serpentes). *Mol Phylogenet Evol* 2008; **49**: 445-459.

[23] Tsai CH, Yang SH, Chien CM, Lu MC, Lo CS, Lin YH, et al. Mechanisms of cardiotoxin III-induced apoptosis in human colorectal cancer colo205 cells. *Clin Exp Pharmacol Physiol* 2006; **33**: 177-182.

[24] Gallacci M, Cavalcante WLG. Understanding the *in vitro* neuromuscular activity of snake venom Lys49 phospholipase A2 homologues. *Toxicon* 2010; **55**: 1-11.

[25] Marcussi S, Sant'Ana CD, Oliveira CZ, Rueda AQ, Menaldo DL, Beleboni RO, et al. Snake venom phospholipase A2 inhibitors: medicinal chemistry and therapeutic potential. *Cur Topics Med Chem* 2007; **7**: 743-756.

[26] Gutierrez JM, Alexandra R, Escalante T, Díaz C. Hemorrhage induced by snake venom metalloproteinases: biochemical and biophysical mechanisms involved in microvessel damage. *Toxicon* 2005; **45**: 997-1011.

[27] Marshall DM. Enzyme activities and biological functions of snake venoms. *Appl Herpetol* 2005; **2**: 109-123.

[28] Aguilar I, Guerrero B, Salazar AM, Giron ME, Perez JC, Sanchez EE, et al. Individual venom variability in the South American rattlesnake *Crotalus durissus* cumanensis. *Toxicon* 2007; **50**: 214-224.

[29] Gutierrez JM, Ownby CL. Skeletal muscle degeneration induced by venom phospholipases A2: insights into the mechanisms of local and systemic myotoxicity. *Toxicon* 2003; **42**: 915-931.

[30] Yamazaki Y, Takani K, Atoda H, Morita T. Snake venom vascular endothelial growth factors (VEGFs) exhibit potent activity through their specific recognition of KDR (VEGF receptor 2). *J Biol Chem* 2003; **278**: 51985-51988.

[31] Chippaux JP, Williams V, White J. Snake venom variability: methods of study, results and interpretation. *Toxicon* 1991; **29**: 1279-1303.

[32] Warrell DA. Snake venoms in science and clinical medicine. Russell's viper: biology, venom and treatment of bites. *Trans R Soc Trop Med Hyg* 1989; **83**: 732-740.

[33] Shashidharamurthy R, Jagadeesha DK, Girish KS, Kemparaju K. Variation in biochemical and pharmacological properties of Indian cobra (Naja naja) venom due to geographical distribution. *Mol Cell Biochem* 2002; **229**: 93-101.

[34] You WK, Choi WS, Koh YS, Shin HC, Jang Y, Chung KH. Functional characterization of recombinant batroxobin, a snake venom thrombin-like enzyme, expressed from Pichia pastoris. *FEBS Letters* 2004; **571**: 67-73.

[35] Mackessy SP. Biochemistry and pharmacology of colubrid snake venoms. *J Toxicol Toxin Rev* 2002; **21**: 43-83.

[36] Jin H, Varner J. Integrins: roles in cancer development and as treatment targets. *Br J Cancer* 2004; **90**: 561-565.

[37] Koh CY, Kini RM. From snake venom toxins to therapeutics - Cardiovascular examples. *Toxicon* 2012; **59**: 497-506.

[38] Zhang L, Wei LJ. ACTX-8, a cytotoxic *L*-amino acid oxidase isolated from *Agkistrodon acutus* snake venom, induces apoptosis in Hela cervical cancer cells. *Life Sci* 2007; **80**: 1189-1197.

[39] Torii S, Yamane K, Mashima T, Haga N, Yamamoto K, Fox JW, et al. Molecular cloning and functional analysis of apoxin I, a snake venom-derived apoptosis-inducing factor with *L*-amino acid oxidase activity. *Biochemistry* 2000; **39**: 3197-3205.

[40] Naumann GB, Silva LF, Silva L, Faria G, Richardson M, Evangelista K. Cytotoxicity and inhibition of platelet aggregation caused by an *L*-amino acid oxidase from *Bothrops leucurus* venom. *Biochim Biophys Acta* 2011; **1810**: 683-694.

[41] Ahn MY, Lee BM, Kim YS. Characterization and cytotoxicity of *L*-amino acid oxidase from the venom of king cobra (*Ophiophagus hannah*), *Int J Biochem Cell Biol* 1997; **29**:911-919.

[42] Gebrim LC, Marcussi S, Menaldo DL, De Menezes CSR, Nomizo A, Hamaguchi A. Antitumor effects of snake venom chemically modified Lys49 phospholipase A2-like BthTX-I and a synthetic peptide derived from its C-terminal region. *Biologicals* 2009; **37**: 222-229.

[43] Guo MP, Wang QC, Liu GF. Pharmacokinetics of cytotoxin from chinese cobra (*Naja naja atra*) venom. *Toxicon* 1993; **31**: 339-343.

[44] Debnath A, Saha A, Gomes A, Biswas S, Chakrabarti P, Giri B, et al. A lethal cardiotoxic-cytotoxic protein from the Indian monocellate cobra (*Naja kaouthia*) venom. *Toxicon* 2010; **56**: 569-579.

[45] Das T, Bhattacharya S, Halder B, Biswas A, Gupta SD, Gomes A. Cytotoxic and antioxidant property of a purified fraction (NN-32) of Indian Naja naja venom on Ehrlich ascites carcinoma in BALB/c mice. *Toxicon* 2011; **57**: 1065-1072.

[46] De Carvalho DD, Schmitmeier S, Novello JC, Markland FS. Effect of BJcuL (a lectin from the venom of the snake *Bothrops jararacussu*) on adhesion and growth of tumor and endothelial cells. *Toxicon* 2001; **39**: 1471-1476.

[47] Zhang L, Cui L. A cytotoxin isolated from *Agkistrodon acutus* snake venom induces apoptosis via Fas pathway in A549 cells. *Toxicol In Vitro* 2007; **21**: 1095-1103.

[48] Sun LK, Yoshii Y, Hyodo A, Tsurushima H, Saito A, Harakuni T, et al. 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* 2003; **17**: 169-177.

[49] Karthikeyan R, Karthigayan S, Sri Balasubashini M, Somasundaram ST, Balasubramanian T. Inhibition of Hep2 and HeLa cell proliferation *in vitro* and EAC tumor growth *in vivo* by *Lapemis curtus* (Shaw 1802) venom. *Toxicon* 2008; **51**: 157-161.

[50] Yang RS, Tang CH, Chuang WJ, Huang TH, Peng HC, Huang TF, et al. Inhibition of tumor formation by snake venom disintegrin. *Toxicon* 2005; **45**: 661-669.

[51] Gomes A, Choudhury SR, Saha A, Mishra R, Giri B, Biswas AK, et al. A heat stable protein toxin (drCT-I) from the Indian viper (*Daboia russelli russelli*) venom having antiproliferative, cytotoxic and apoptotic activities. *Toxicon* 2007; **49**: 46-56.

[52] Lin KL, Su JC, Chien CM, Chuang PW, Chang LS, Lin SR. 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* 2010; **55**: 1263-1273.

[53] Nunes ES, Souza MA, Vaz AF, Silva TG, Aguiar JS, Batista AM. Cytotoxic effect and apoptosis induction by *Bothrops leucurus* venom lectin on tumor cell lines. *Toxicon* 2012; **59**: 667-671.

[54] Nolte S, De Castro DD, Barea AC, Gomes J, Magalhães A, Mello Zischler LF. A lectin purified from *Bothrops jararacussu* venom, induces apoptosis in human gastric carcinoma cells accompanied by inhibition of cell adhesion and actin cytoskeleton disassembly. *Toxicon* 2012; **59**: 81-85.