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Snake Venoms-Based Compounds as Potential Anticancer Prodrug: Sand Viper Cerastes Cerastes as a Model

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Abstract

Cancer is a major public health issue that has recently been dubbed "the disease of the millennium." Tumor cell proliferation, angiogenesis, and the interaction of cancer cells with extracellular matrix components are all important events in carcinogenesis. Many of these routes are being studied as potential therapeutic targets for new antitumor drugs. On cancer cell lines from humans, several venoms and their venomous toxins have been demonstrated to have cancer-fighting abilities, opening up new avenues for creation of drugs. Snake venom, for example, is a mixture of proteins, peptides, carbohydrates, enzymes and other biologically active compounds that are either emitted by the snake in predation or in the face of danger. When compared to normal cells, snake venoms are significantly more cytotoxic to malignant cells. As a result, venoms have a variety of therapeutic actions, making them an appealing approach for cancer therapy. Here, we review the potential therapeutic value of venom isolated from the horned sand viper, Cerastes cerastes, endemic to the Egyptian desert.
Key words: Cancer; Anticancer therapy; Carcinogenesis; Snake venom; Egyptian sand viper, Cerastes cerastes; toxins

1. Introduction

One of the most common causes of deaths and morbidities worldwide is cancer. Surgical management and chemotherapy are still the mainstays of cancer treatment [1]. Targeted therapy which depends on interference targeting specific molecules while causing less toxicity to normal cells, are gaining popularity in chemotherapy [2, 3].

Purified and identified compounds from snake's venom have recently shown fantastic promise as efficient agents target cancer-specific [CC1] molecular pathways [4, 5]. Snake venoms are combinations of proteins, amino acids, as well as other bioactive substances emitted by the venom gland of the snake and injected with the snake's distinct fangs to weaken and ingest their prey. Despite the fact that envenomation from a snake's bite is a serious public health concern, snake's venom have recently been identified as a potential source of biologically active compounds used in the treatment of certain diseases, including cancer [6].

Several peptides have been isolated from the venom of elapidae and viperidae snakes targeting different types of cancer cells via specific molecular pathways (for example, causing cell membrane and DNA damage, increasing proapoptotic signals, inhibiting proliferation and metastasis, blocking DNA synthesis and promoting cell cycle arrest). The isolation and characterization of snake venom components began in the 1940s. Following that, different components were purified, sequenced, and structurally elucidated, including enzymes, non-enzyme proteins, and peptides. Because of their differential cytotoxicity toward tumor cells versus normal cells, these molecules are potential candidates for further investigation into their therapeutic potential [6].

Cerastes cerastes, also known as the desert-horned sand viper [7, 8], is known venomous snakes i n North Africa and the Middle Eat [9, 10, 11, 12]. In t his review, we focus on
(i)the anticancer effect of *C. cerastes* viper venom *in vitro* and *in vivo*, and (ii) the importance of its toxins

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as a good template for developing novel chemotherap eutic agents.

2. Snake venom

Snake venom is a venomous snake's secretion synthesized then stored in the venom gland [5]. Different snake species produce different types of venom based on their location, age, habitat, and other factors. It is primarily used to attack prey and contains components designed to immobilize the prey and facilitate digestion [13].

Snake's venom is a clear liquid which has a high viscosity that can be crystallized and dried [14]. It is made up of peptides, proteins, free amino acids, nucleotides, enzymes, inorganic cations (Ca, K, Mg, Na, and so on), carbohydrates, and lipids [15]. Proteins account for around ninety percent of the dry weight of snake's venom are the reason for the majority of the biological effects, and can be toxic or non-toxic in nature [16].

Toxins found in snake venom are classified as neurotoxins, hemotoxins, cardiotoxins, and cytotoxins based on their mode of action and effects.

- Neurotoxins are toxins that affect the central nervous system, resulting in breathing problems or failure of the heart. Moreover, such toxins negatively affect the ion movement through cell membranes and subsequently inhibits neuron communication [17].

  - Hemotoxins are toxins that cause red blood cell destruction and primarily have an impact on the circulatory system, blood flow, and muscles (resulting in scarring and gangrene) [18].
  - Cardiotoxins are toxins that cause damage to the heart muscle by binding to the cardiocytes preventing contraction of the muscle [19].
  - Cytotoxins are toxins that target specific cellular sites that affect the cell membranes or interferes with the signal transduction and substances transport through cellular membranes [20].

3. Pharmacological effects of snake's venom

Snakes have reported to fascinate humans, because of their lethal venom and its medicinal properties of this venom. New research approaches had identified appreciated number of bioactive molecules from snake venom, primarily peptides and proteins [21], which could be used as pharmaceutical agents [22, 23, 24]. Snake venom-derived compounds have been proven to have antiviral properties against certain viruses (yellow fever, dengue, and *herpes simplex* virus) [25, 26], antimicrobial properties against Gram-positive and some Gram-negative bacteria [27, 28], antifungal activities [29], and antiparasitic properties against *Plasmodium falciparum* [30] and *Leishmania sp.* [31]. Furthermore, they demonstrated thrombolytic effects, therefore they were proposed for usage in a number of cases of vascular diseases [32].

4. Anticancer activity of snake venom

Snake venom's cytotoxicity is linked to changes in cellular metabolism, which significantly affects cancer cells when compared with normal ones. These findings indicate that many scientists have attempted to create various drugs according to the cytotoxic ability of snake venom toxins, and provide a new direction and propose snake toxins as a potential cancer therapy [33, 34].

Snake's venom ability to act on tumor cells dates back over a century. The first study was carried out by DeWys et al. [35], who discovered that defibrination caused by Ancrod (*Agkistrodon rhodostoma-derived polypeptide*) and, cyclophosphamide, reduced the the tumor weight and activated fibrinolysis. Other systems of protection, such as aggregation of the platelets, were discovered to be participant in the reduction of dimensions of the tumor in the same study.

TU and Giltner [36] and their team had performed study and their data revealed that venoms from some snake families including Elapidae, Viperidae, and Crotalidae could induce Yoshida sarcoma lysis and the keratin-forming tumor cell line HeLa (KB cells) lysis too. Toxins from the snake *Naja nigricollis* have been tested for cytotoxicity against B16F10 melanoma and chondrosarcoma cell lines [37]. The venom toxins had the same cytotoxic activity on chondrosarcoma cells as they did on melanoma cells, inhibiting tumor development. Such results obtained with melanoma cells, *in vivo*, were completely consistent with the *in vitro results*, and the venom also exhibited similar cytotoxic activity on chondrosarcoma, inhibiting its development *in vivo*.

Omran [38] investigated the cytotoxic and antitumor activity of snake venom *Naja haje* on two established cell lines from human breast cancer (T47D and MDA-MB-468 cells). In the two cell lines, the IC50 values of such venom were 63 μg/ml and 18 μg/ml in MDA-MB-468 and T47D cells, respectively. He reported that after 24 hours incubation with 100 μg/ml of the venom, this venom induced significant cell death in breast cancer cell lines (MDA-MB-468 75% and T47D 98%). The dying cells exhibited fragmentation and condensation of their contents, as well as shrinkage and the appearance of vacuoles between the adherent cells.

In *in vivo* study, researchers used the crude venom of the Indian monocellate Cobra (*Naja kaouthia*) and Russell's viper (*Vipera russelli*) and proved their cytotoxic effect on Ehrlich ascites carcinoma (EAC) cells. The venoms increased the survival of EAC-bearing mice and improved the antioxidant system of these animals. The venoms were also found to have a strong apoptogenic and cytotoxic impact on human leukemea cells (U937/K562). They significantly reduced cellular division rates while the morphological changes supported the induction of apoptosis [39].

ACTX-8 (a protein fractionated from *Agkistrodon acutus* snake venom) induced apoptosis in cellular model of HeLa, according to Zhang and Wei [40]. The ACTX-8 was reported to induce cell death in a time and concentration dependent pattern. This was associated with caspase activation and production of reactive oxygen under ACTX-8 treatment validating the induction of apoptosis.

Karthikeyan et al. [41] had evaluated the antitumor activity of sea snake (*Lapemis curtus*) venom in albino mice and Hep2, HeLa and EAC cancer cells. From the various doses tested, they reported that 6.50 µg/ml at 24 h successfully inhibited the division of cancer cells. This dose was also administered intravenously to EAC-bearing mice and found to significantly reduced the tumor growth and increased the mice's life span by 201.25 percent.

Song et al. [42] investigated the apoptotic effect of *Vipera lebetina turanica* snake venom toxin in ovarian cancer, PA-1 and SK-OV3, cells. Their results revealed IC50 values of 6.5 µg/mL in SK-OV3 cells and 4.5 µg/mL in PA-1 cells. That toxin, significantly, inhibited the growth of ovarian cancer cells. This team proved that the toxin induced apoptosis in such ovarian cancer cells by inhibiting NF-kB and STAT3 signaling, as well as inhibiting p65 and p50 transfer to the nucleus. It also increased the expression of the pro-apoptotic proteins, Bax and Caspase-3, while decreasing the expression of the anti-apoptotic protein Bcl-2.

Khunsap et al. [43] investigated the cytotoxicity of venoms from *Cryptelytrops albolabris*, *Calloselasma rhodostoma*, and *Daboia siamensis* on a panel of cancer cell lines: BT474, SW620, KATO-III, , ChaGo and Hep-G2. Various concentrations of the three venoms demonstrated a time-dependent cytotoxicity against cancer cells. *C. albolabris* venom was most effective against cancer cells in the following concentrations: BT474 (2.96 ± 0.44 µg/ml), SW620 (3.32 ± 0.14 µg/ml), KATO-III (3.72 ± 0.11 µg/ml), and Hep-G2 (3.74 ± 0.43 µg/ml). The venom of *C. rhodostoma* was found to be cytotoxic to BT474 cells (3.16 ± 0.69 µg/ml), SW620 cells (3.5 ± 0.01 µg/ml), and KATO-2 III cells (3.74 ± 0.37 µg/ml), whereas *D. siamensis* venom was toxic only to ChaGo cells (0.48 ± 0.04 µg/ml).

Ebrahim et al. [44] investigated the cytotoxicity of the snake venom from Caspian Cobra (*Naja naja oxiana*) on HepG2, MCF7, and DU145 cell lines. The venom's IC50 values in HepG2, MCF7, and DU145 cell lines were 26.59, 28.85, and 21.17 µg/mL, respectively. This effect differed significantly from that of the MDCK normal cell line (IC50= 47.1 µg/mL). The venom induced mitochondrial and caspase-3 dependent apoptosis in cancer cell lines while having little effect on the normal cell line studied.

Several studies have been conducted in recent decades to demonstrate the antitumoral activity of toxins (cytotoxins & cardiotoxins) isolated from various snakes. Cytotoxins or cardiotoxins are polypeptides with 60-70 amino acid residues that are found in elapid family snakes and have various pharmacological effects such as muscle depolarization and hemolysis [45]. Cardiotoxin-III (CTX III), a polypeptide with 60 amino acid residues found in the venom of *Naja naja atra*, has been shown to have antitumor activity. It induced apoptosis in K562 cells that was validated by DNA fragmentation, as well as upregulation of both Bax and endonuclease G together with downregulation of Bcl-XL. CTX III treatment also resulted in mitochondrial membrane potential loss, mitochondrial cytochrome c release to the cytoplasm, and caspase-3 and -9 activation in K562 cells [46]. Another study on the same cancer cell line (K562 cells) suggested that Ca2+ influx triggered CTX III-induced apoptosis, which then activated caspase-12 and c-Jun N-terminal kinase (JNK) through a micro-calpain-independent cascade [47].

CTX III was used in two distinct researches on HL-60 (human leukemia cells). Its anti-proliferative action on HL-60 cells has been reported to be mediated by apoptotic cell death, as evidenced by an increase in DNA fragmentation, sub G1 population, and poly (ADP-ribose) polymerase cleavage. In addition to JNK activation, up-regulation of Bax, down-regulation of Bcl-2, release of mitochondrial cytochrome c to cytoplasm, and activation of capase-3 and -9 were observed [48]. Another study found that this toxin activated the endoplasmic reticulum pathway of apoptosis, as evidenced by increased levels of Ca2+ and glucose-related protein 78, induced apoptosis. Furthermore, it activated the intrinsic apoptotic pathway in HL-60 cells, as indicated by Bax/Bcl-2 ratio increase, cytochrome c release, and caspase 9 activation [49].

Lin et al. [50] discovered that CTX III can induce apoptosis in MDA-MB-231 (Human breast cancer cells), as evidenced by the sub-G1 population accumulation of the cells and loss the membrane potential of the mitochondria, as well as activation of both capases-9 and caspase-3. This was associated by increased levels of Bax and Bad, as well as decreased

levels of anti-apoptotic proteins such as Bcl-2, Bcl-XL and survivin in cells under CTX III treatment. CTX III also induced apoptotic cell death in MCF-7 breast cancer cells. Its effect on proliferation and apoptosis were linked to sub-G1 formation, phosphatidylserine externalisation, poly (ADP-ribose) polymerase cleavage, Bax upregulation, and Bcl-XL, Bcl-2, and XIAP downregulation. It also resulted in the release cytochrome c from the mitochondria to the cytoplasm, which activated capase-9. Furthermore, CTX III inhibited nuclear factor-kB activation by inhibiting IkappaB kinase activity [51].

CTX III was also discovered to induce apoptotic cell death in A549 cells by inhibiting the activation of the epidermal growth factor receptor (EGFR), phosphatidylinositol 3kinase (JAK) 2/signal transducer and activator of transcription (STAT) 3. It also caused an increase in the subG1 population, phosphatidylserine externalization, mitochondrial membrane potential loss with cytochrome c release, and caspase 3 and 9 activation. These actions were associated with increased levels of Bad and Bax and decreased levels of antiapoptotic proteins such as Bcl2, BclXL, Mcl1, pBad, and Xlinked inhibitor of apoptosis protein (XIAP) in the cells treated with the toxin.

drCT-I is a 7.2 kDa thermostable protein toxin obtained from the venom of Indian russell's viper (*Daboia russelli russelli*). It has been tested for its cancer-fighting properties against EAC cells *in vivo* and against human leukemic cells *in vitro* (K562, U937). drCT-I (125 g/kg, i.p/day for 10 days) decreased EAC cell count, cell viability and increased survival time in tumor bearing mice in a significant way, compared to untreated control mice. After 24 hours of treatment, drCT-I stifled the growth of K562 and U937 cells in a dose and time-dependent manner, with IC50 values of 6.7 and 8.9 µg/ml respectively. The lower MTT values after drCT-I treatment indicated that it was cytotoxic, which supported its anti-proliferative action. SEM and confocal microscopy revealed apoptosis-related features such as membrane blebbing, perforations, and nuclear fragmentation. Phosphatidylserine externalisation was observed using annexinV-FITC/PI staining and flow cytometric analysis, confirming the induction of apoptosis. As a result, both *in vitro* and *in vivo* experiments indicated that drCT-I had anticancer potential [53].

The cytotoxic effects of Nubein6.8 isolated from the venom of the Egyptian Spitting Cobra Naja nubiae on melanoma (A375) and ovarian carcinoma cell lines were investigated by Abdel-Ghani et al. [54]. The venom was highly cytotoxic to the A375 cell line and moderately cytotoxic to the A2780 cell line. In both tumor cell lines, Nubein6.8 was found to have DNA damaging properties as well as the ability to activate apoptosis.

Contortrostatin (CN) is a homodimeric disintegrin found in the venom of the southern copperhead snake. The anti-cancer activity was investigated in the ovarian cancer cells, OVCAR-5, cells. CN successfully inhibited cellular invasion via an artificial basement membrane by blocking OVCAR-5 cell adhesion to several extracellular matrix proteins. In a xenograft nude mouse model, OVCAR-5 cells were induced intraperitoneally and CN was injected intraperitoneally as therapy. According to the findings of this study, CN not only significantly inhibited ovarian cancer dissemination in treated mice, but it also significantly inhibited the recruitment of blood vessels to tumors at secondary sites [55].

Following that, other studies found that disintegrins can inhibit tumor behaviour both in vitro and in vivo [56, 57].

Snake venom containing cystatin (sv-cyst), a member of cysteine protease family's inhibitors, has been reported to play an important role in tumor invasion and metastasis. In a study carried out on MHCC97H (liver cancer) cells, sv-cyst has shown inhibition of tumor cell invasion and metastasis through the reduction of the proteinases activity and epithelial-mesenchymal transition with a decreased activity of cathespin B, MMP-2 and 9, and EMT change index. It also, increased the activity of Ecadherin, and decreased the activity of N-cadherin and twist activity [58].

Phospholipases A2 (PLA2) is an enzyme that hydrolyzes the sn-2 acyl ester bond in various phospholipids, resulting in free fatty acids and lysophospholipids [59]. Crotoxin is a neurotoxic PLA2 compound isolated from the venom of the South American snake *Crotalus durissus*. Crotoxin exhibited *in vitro* cytotoxic activity against a variety of murine and human tumor cell lines [60]. Crotoxin antitumor efficacy *in vivo* was investigated in Lewis lung carcinoma which inhibited the growth by 83% and MX-1 human mammary carcinoma by 69% using daily intramuscular administration of crotoxin. Moreover, Crotoxin activity was found to be lower in HL-60 leukemia cells, as it inhibited the growth by 44%, implying that crotoxin has a high specificity for solid tumors [61].

According to Bazaa et al. [62], the PLA2 from *Macrovipera lebentina* (MVL-PLA2) venom has activity against integrin (integrins are important protagonists of the complex multi-step process of angiogenesis which is now a significant target for the development of cancer fighting drugs). They discovered that MVL-PLA2 venom has potent anti-angiogenic properties in their study on HMEC-1 (human microvascular endothelial cells). Without

being cytotoxic, this PLA2 inhibited HMEC-1 cell adhesion and migration in a dose-dependent manner. They also discovered that MVL-PLA2 treatment disrupted the actin cytoskeleton and the distribution of alphav beta3 integrin (an important regulator of angiogenesis and a major component of focal adhesions).

LAAO (L-amino acid oxidase) is a dimeric flavoprotein with a non-covalently bound FAD as a cofactor [63]. Ahn et al. [64] purified Ophiophagus hannah venom and identified its cytotoxic components. The cytotoxic component's biochemical properties matched those of the l-amino acid oxidase. LAAOs were found to be cytotoxic in murine melanoma, fibrosarcoma, stomach cancer, colorectal cancer, and Chinese hamster ovary cell lines. Cytotoxicity caused loss of the ability to attach and the inhibition of cells division. According to the [3H] thymidine uptake assay, this cytotoxic protein reduced cell proliferation by 74%.

Furthermore, Lee et al. [65] reported that LAAOs isolated from *Ophiophagus hannah* venom (OH-LAAO) showed significant anti-proliferative activity against human lung and breast cancer cells. They also looked at its anti-tumor activity *in vitro* and *in vivo* in a human prostate adenocarcinoma (PC-3) model. OH-LAAO showed significant cytotoxicity against PC-3 cells after 72 hours of *in vitro* incubation, with an IC50 of 0.05µg/mL. This team demonstrated apoptosis induction through rise in caspase-3 and 7 cleavages and also rise in annexin V-stained population. They administered 1 µg/g OH-LAAO intraperitoneally to immunodeficient NU/NU (nude) mice with a PC-3 tumor xenograft implanted subcutaneously to test its anti-tumor activity *in vivo*. Following eight weeks of treatment, OH-LAAO-treated PC-3 tumors were significantly suppressed comparing with control animals. TUNEL staining of tumor sections revealed that the LAAO-treated animals had a significant increase in apoptotic cells. Importantly, the treatment had no effect on the weight of the mice during the course of the research.

Other LAAOs isolated and purified from *Bothrops leucurus* (Bl-LAAO) snake venom by Naumann et al. [66] were studied for their effect on platelet function and cytotoxicity. Bl-LAAO was tested for cytotoxicity in stomach cancer MKN-45, colorectal RKO, adenocarcinoma HUTU, and human fibroblast LL-24 cell lines. They discovered that the enzyme produced enough H2O2 in the culture medium to induce apoptosis in cell lines in a dose- and time-dependent manner.

The lectin is another important component of snake venom that has the capability of inhibiting cancer cells. Pereira–Bittencourt et al. [67] investigated the effect of a lectin isolated from the venom of Bothrops jararacussu (BJcuL) on the proliferation of eight human cancer cell lines. The BJcuL lectin was most effective as a growth inhibitor in pancreatic (CFPAC-1) and renal (Caki-1 and A-498) cancer cell lines, with IC50 values of 1 and 2 mM, respectively. The IC50 values for prostate (PC-3) and melanoma (Wm115) cancer cells were 8.5 and 7.9 mM, respectively, whereas breast (MCF7) and colon (Caco-2) cancer cell lines had no effect. Another study found that BJcuL is cytotoxic to gastric carcinoma cells (AGS and MKN45). This effect was caused by direct interaction with specific glycans on the cell surface and was manifested as actin filament disorganisation, decreased cell viability, and apoptosis [68].

The metalloproteinases are among the toxins isolated from the venom of snakes of the families Viperidae and Crotilidae. Toxin effects range from activation of coagulation factors, repression of platelet aggregation, and fibrinolytic activities to cancer fighting activates such as apoptotic and proinflammatory activities [69]. Jararhagin, a purified snake venom metalloproteinase from *Bothrops jararaca*, inhibited the adhesion of B16F10 murine melanoma cells, according to Maria et al. [70]. The authors came to the conclusion that Jararhagin's significant effect on melanoma cells was mediated by increased antiproliferative and caspase-3 activities. Another metalloproteinase (TSV-DM) purified from *Trimeresurus stejnegeri* snake venom induced morphological changes and inhibited the division of ECV304 cancer cells [71].

Owing to the high cytotoxicity of snake venom and its toxins, its effect on normal cell lines remains debatable, with some researchers claiming that it is not useful to normal cells while others claim that it has a cytotoxic effect on normal cell lines as well. As a result, researchers have discovered a remedy for this problem by fusing snake venom components with nanoparticles and allowing for targeted administration to the diseased location. As stated by Al-Sadoon et al. [72], snake venom extracted from *Walterinnesia aegyptia* (WEV) either by itself or in conjugation with silica nanoparticles (WEV+NP) inhibited proliferation of human breast cancer cell lines (MCF-7 and MDA-MB-231). In these cancer cell lines, the IC50 values for WEV alone and WEV+NP were 20 ng/ml and 50 ng/ml, respectively. Surprisingly, at the same concentrations, normal MCF-10 cells were not affected by the venom, also treatment with NP alone had no effect on any of these cell lines. WEV+NP increased caspase 3 activation while decreasing Bcl2 expression in MCF-7 and MDA-MB-231 cells.

Table 1 anticancer activity of snake venom-based compounds

Protein Family	Compounds	Snake's species	Cellular target/mechanism	Reference
L-Aminoacid oxidases (LAAOs)	LAAO	*Ophiophagus hannah*	Cytotoxicity in murine melanoma, cancer, fibrosarcoma stomach, ovary and colorectal cell lines	Ahn et al., [64]
	LAAO	*Agkistrodon halys*	Apoptosis induction	Suhr and Kim. [73] Suhr and Kim. [74]
	AHP-LAAO	*Agkistrodon halys pallas*	Induction of apoptosis of cultured Hela cells.	Zhang et al. [75]
	LAAO	*Vipera berus berus*	induced apoptosis in cultured HeLa and K562 cells	Samel et al. [76]
	OH-LAAO	*Ophiophagus hannah*	Apoptosis caused by the oxidative reaction of H2O2 causing gene expression changes in prostate adenocarcinoma (PC-3 cells)	Lee et al. [65]
	Rusvinoxidase	*Daboia russelii russelii*	MCF-7 cells undergo apoptosis with DNA fragmentation as a result of caspase-7 and caspase-8 activation.	Mukherjee et al. [77]
	LAAO	*Ophiophagus Hannah*	Reduces thymidine uptake and proliferation in murine fibrosarcoma, melanoma, and colorectal cancer cells.	Hiu and Yap [78]
Disintegrin	Leucurogin	*Bothrops leucurus*	antiangiogenesis	Higuchi et al. [79]
	Contortrostatin	*Agkistrodon contortrix*	antiangiogenesis of MDA-MB-435 cells	Zhou et al., 1999
	Lebein	*Macrovipera lebetina*	Inhibition of VEGF-induced neovascularization of human colon cancer cells	Zakraoui et al., [80]
	Obtustatin	*Vipera lebetina obtusa*	Inhibited the alpha1beta1 integrin and also inhibition of angiogenesis in the chicken chorioallantoic membrane assay, and in the Lewis lung syngeneic mouse model.	Marcinkiewicz et al. [81]
	Adinbitor	*Agkistrodon halys brevicaudus stejneger*	antiangiogenesis	Wang et al. [82]
	Salmosin	*Agkistrodon halys brevicaudus*	antiangiogenesis	Kang et al. [83]
	Lebestatin	*Macrovipera lebetina*	Interacts specifically with the α1β1 integrin found on leminin1 and collagen. The interaction of integrin and ECM is required for adhesion and migration of cancer cells	Olfa et al. [84]
	Rhodostomin	*Agkistrodon rhodostoma*	Interferes with the interaction of intergrins and ECM proteins, which is required for cancer cell proliferation.	Yang et al. [19]
	Eristostatin	*Eristicophis macmahoni*	Repression of the melanoma cells colonization in lung and liver	Tian et al. [85]
	Crotatroxin 2	*Crotalus atrox*	Brinogen binding to GP IIb/IIIa is inhibited, as is cell migration and tumor colonisation.	Galán et al. [86]
	Viperistatin	*Vipera paleastinae*	It prevents cancer cells from adhering and migrating.	Staniszewska et al. [87]
	Mojastin1	*Crotalus scutulatus*	Prevent platelet aggregation, cell migration, cell invasion, and tumor colonization caused by ADP.	Lucena et al. [88]
	Leucurogin	*Bothrops leucurus*	It has antiangiogenesis properties in cancer cells.	Higuchi et al. [79]
Cathelicidin-BF	Cathelicidin-BF	*Bungarus fasciatus*	Inhibits the proliferation of B16F10 and B16 cells	Marcinkiewicz et al. [89]
Lectin	Lebectin	*Macrovipera lebetina*	Adhesion, migration, and tumor cell invasion are all inhibited, as is angiogenesis.	Sarray et al. [90]
Batroxobin	Batroxobin	*Bothrops atrox*	Prevents the propagation and metastasis	Sahni et al. [91]

			of cancer cells	
phospholipase A2	Ammodytoxin	*Vipera ammodytes*	Activates autophagy and increases autophagosomes formation, as well as apoptosis.	Premzl et al. [92]
	MVL-PLA2	*Macrovipera lebetina*	it inhibits angiogenesis and causes transformations in the actin cytoskeleton. both *in vitro* and *in vivo*	Kessentini-Zouari et al. [93]
	Phospholipases A2	*Cerastes cerastes*	It has antiangiogenic and antitumor properties and acts primarily on integrins αβ and α5β1	Zouari-Kessentini et al. [94]
Bungarotoxin	Bungarotoxin	*Bungarus multicinctus*	It inhibits gastric cancer cell proliferation by causing G1 arrest and inhibiting nicotine NK-stimulated cell proliferation.	Shin et al. [95]
Dendrotoxin-κ	Dendrotoxin-κ	*Dendroaspis polylepis*	Suppresses tumor growth induced by human lung adenocarcinoma A549 cells in nude mice via the G1-S transition pathway. Increases the expression of the proteins p27Kip1, p21Waf1/Cip1, and p15INK4B, as well as the inactivation of cyclin dependent kinases, which regulate cell growth.	Jang et al. [96]

5. Anticancer activity of *Cerastes cerastes* snake venom

Cerastes cerastes viper is one of the Viperidae family members and is also a member of the genus Cerastes. This viper is found in North Africa and the Middle East, most notably in the Desert of the Upper Egypt [97]. Because they contain pharmacologically active molecules such as phospholipase A2 and proteinases, viperidae venoms are regarded as one of the most important bio-resources (metalloproteinases and serine proteinases). These molecules have the potential to be used as biomedicines or diagnostic tools in biotherapy [98]. The venom of *C. cerastes* has been described as a low complexity proteome composed of more than twenty five toxins from six protein families that primarily target the hemostatic system. The most abundant toxin family which measured 61% is Zn2+ metalloproteinases, followed by PLA2s which measured 19%, dimeric disintegrins which measured 8.5% and serine proteinases which measured 7%. Moreover, the cysteine-rich secretory proteins and C-type lectin-like molecules measured about less than 4% of all venom toxins [97].

According to Abu Sinna et al. [98], treating experimental mice bearing the Ehrlich ascites with two i.p. injections of the non-lethal fraction (F4) and the most lethal fraction (F3) of *Cerastes cerastes* snake venom resulted in significant antitumor activity, as evidenced by an inhibition of tumor growth (T/C percent 172 and 139, respectively) and an appreciated increase in tumor inhibition ratio of tumor growth. The administration of two i.p. injections of F4 or F3 to Swiss albino mice at the dose level used resulted in no adverse side effects, as evidenced by insignificant changes in serum and liver parameters.

Shebl et al. [99] investigated the ability of *C. cerastes* venom to cause apoptotic cell death in breast cancer, MCF-7, cell line. The venoms had a time and concentration-dependent cytotoxic impact on such breast cancer cells, according to this study. Data from flow cytometric analysis had proved that, after 24 hours of treatment, the percentage of apoptotic cells increased with increasing the venom concentrations. At the molecular level, that venom induced apoptosis in MCF-7 cells via increasing the expression of pro-apoptotic genes (p53 and Bax) and decreasing the expression of anti-apoptotic genes (Bcl-2). The data from this group suggested that the venom could act as an apoptotic stimulator, providing a novel and potentially cancer treatment strategy.

Ayman et al. [100] evaluated the cytotoxicity and the anti-cancer properties of *Cerastes cerastes* snake venom on prostate (PC-3) and colon (Caco-2) cancer cells. They reported that 24 h-treatment of cancer cells with *C. cerastes* venom induced a concentration-dependent cytotoxicity with IC_{50} values of 81 (PC-3 cells) and 60 (Caco-2 cells) μg/ml, in addition up regulation of pro-apoptotic and down regulation of anti-apoptotic genes were significantly enhanced indicating its potential efficacy in directing cancer cells towards apoptosis. Furthermore, Sarhan et al. [101] evaluated the antitumor efficacy of male and female *Cerastes cerastes* venoms in liver (HEPG-2), breast (MCF-7) and colon (HTC-116) cancer cells. Both male and female venoms reported anti-proliferative effects on tumor cell lines with varying potency. Female venom

had a higher cytotoxicity to colon cancer cells ($IC5_0$= 0.006 µg/ml) than male venom (IC_{50}= 0.019µg/ml. Male venom, on the other hand, was more toxic to breast cancer cells (IC50= 0.005 µg/ml) than female venom (IC50= 0.024µg/ml).

The effects of *Cerastes cerastes* snake venom and *Androctonus amoreuxi* scorpion venom and their mixture on prostate cancer cells (PC3) were evaluated by Akef et al. [102]. This group reported that venoms significantly reduced the viability of PC3 prostate cancer cells in a dose-dependent manner. Those venoms also significantly reduced the anti-apoptotic Bcl-2 gene expression and significantly increased the Bax/Bcl-2 ratio and the oxidative stress biomarker malondialdehyde (MDA). In addition, these venoms also increased the levels of antioxidant enzymes, catalase, glutathione reductase, glutathione peroxidase, superoxide dismutase and glutathione-S-transferase.

Ozverel and his colleagues used crude venoms to test the cytotoxicity of the same species' venom, *C. cerastes* and *C. purpureomaculatus*, on various human cancerous (SHSY5Y, MCF-7, HeLa, A-549, CaCo-2, MDA-MB-231, 253-JBV, U87MG) and non-cancerous (HEK-293) cell lines [103]. Both vipers' venoms had the most potent activity on the SHSY5Y cell line after 48 hours of treatment, with IC50 values of 0.12 µg/mL and 0.25 µg/mL, respectively. *C. cerastes* venom demonstrated higher activity levels on HeLa, CaCo-2, 253-JBV, U87MG, MDA-MB-231, and HEK-293 cell lines, but only a potency of moderate scale on MCF-7 and A-549 cell lines. The crude venom of *C. purpureomaculatus*, on the other hand, demonstrated significant potential activity on MDA-MB-231, U87MG, HeLa, CaCo-2, A-549, and HEK-293 cell lines, but not on MCF-7.

Finally, Abd El-Ghani and Amr [104] investigated the effect of *Cerastes cerastes* and *Naja hage* snake venom on a cancer cell line from the larynx (Hep2). The cancer cells treated with venom had low IC50 values, according to this team. The venom treatment also reduced the viability of the cancer cells, decreased the percentage of DNA content in G0-G1 and S phases, increased the percentage of DNA content in G2-M and pre G1 (total apoptosis) phases, increased the percentage of cells in early and late apoptotic phases, and increased the percentage of cells in early and late apoptotic phases in comparison to negative control cells.

6. Conclusions

To summarize, the use of snake venoms in cancer therapy has progressed from crude mixtures in the 1930s to the isolation of specific biologically active components targeting specific molecular pathways.

Many of the research papers discussed in this review demonstrated complete tumor cells remission after treatment with molecules derived from snake venoms. Snake venom contains a diverse set of components, the vast majority of which have an effect on the peripheral nervous system for the purpose of killing or immobilizing prey. We can predict the development of a new agent from snake venoms in the future which will be useful in cancer therapy.

Moreover, the combination of snake venoms with other technologies such as nanoparticles is still in its early stages for cancer therapy, and more combinational treatments are expected to emerge. Snake venoms are undeniably valuable resources for the development of cancer drugs.

7. List of abbreviations

EAC	Ehrlich ascites carcinoma
i.p.	Intraperitoneal
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
STAT3	Signal transducer and activator of transcription 3
CTX III	Cardiotoxin- III
JNK	c-Jun N-terminal kinase
ADP-ribose	Adenosine diphosphate ribose
XIAP	X-linked inhibitor of apoptosis protein
Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2
Bcl-XL	B-cell lymphoma-extra large
Bad	BCL2 associated agonist of cell death
EGFR	Epidermal growth factor receptor
JAK2	Janus kinase 2
(STAT) 3	Signal transducer and activator of transcription 3
Mcl- 1	Induced myeloid leukemia cell differentiation protein
CN	Contortrostatin
OVCAR-5 cells	Human epithelial carcinoma cell line of ovary
sv-cyst	Snake venom containing cystatin
MMP-2	matrix metalloproteinase-2
PLA2	Phospholipases A2
MVL-PLA2	PLA2 from Macrovipera lebentina
HMEC-1	Human microvascular endothelial cells
LAAO	L-amino acid oxidase
FAD	Flavin adenine dinucleotide
BJcuL	Lectin isolated from the venom of Bothrops jararacussu
GPIIb/IIIa	Glycoprotein IIb/IIIa
ADP	Adenosine diphosphate
p27Kip1	Cyclin-dependent kinase inhibitor 1B
p21Waf1/Cip1	cyclin-dependent kinase inhibitor 1

8. Conflicts of interest

There are no conflicts to declare

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10. References

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