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Review Article

Antineoplastic properties and pharmacological applications of *Crotalus durissus terrificus* snake venom

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ABSTRACT

Snake toxins are widely studied owing to their importance in snakebite accidents, a serious public health issue in tropical countries, and their broad therapeutic potential. Isolated fractions from venom produced by snakes of the genus *Crotalus sp.* present a wide variety of pharmacological uses such as antifungal, antiviral, antibacterial, and antitumor properties, among other therapeutic potentialities. Given the direct effect of this venom on tumor cells, isolation of its compounds is important for the characterization of its anticarcinogenic actions. *Crotalus durissus terrificus* venom and its toxins have been widely evaluated as potential candidates for the development of new antineoplastic therapies that are efficient against different tumor lines and cellular targets. This review highlights the venom toxins of this species, with a focus on their antineoplastic properties.

Keywords: Snake Venom. Cancer. Antitumor. Crotalid Venoms. Crotalus.

INTRODUCTION

Currently, approximately 11,341 reptile species are recognized worldwide[1], with 1,116 species found in Australia, 974 in Mexico, and 830 in Brazil. *Crotalus* comprises of the venomous *Viperidae* snakes[2-7] from the subfamily *Crotalinae*, also known as rattlesnakes. These are distributed across South America, mainly from Colombia to Argentina[5,7-9], with the following six subspecies found in Brazil: *Crotalus durissus cascavella, C. d. collilineatus, C. d. terrificus, C. d. marajoensis, C. d. ruruima,* and *C. d. durissus*[4,6,9].

These snakes are primarily nocturnal[5] and solenoglyphic dentition[5,10] presents loreal pits, a thermoreceptor organ of viperid species, visible as openings between the eye and the nostril of the animal head, which are of great importance for the detection of temperature variations, particularly of prey and predators[5]. The most striking characteristic of *Crotalus* snakes is the presence of a rattle at the end of their tails **(Figure 1)**[5,6].

Crotalus snakes cause frequent and severe accidents, represent a serious public health problem in tropical countries, and the snakebites are considered a neglected disease by the World Health Organization[6,11-13]. However, venom is an important biotechnological tool because of the specialization and efficiency of its components, which affect a large number of targets with high selectivity and affinity[14-16].

CROTALUS DURISSUS TERRIFICUS VENOM: COMPOSITION, GENERAL PHARMACOLOGICAL ACTIONS AND ANTINEOPLASTIC APPLICATIONS

Snake venom is one of the richest sources of bioactive substances in nature and is therefore of great interest for the development of new drugs[4,14-28]. Snake venoms are composed of a mixture of proteins, organic compounds, inorganic ions, carbohydrates, lipid fractions, and other substances[4,14,16,17,20,21,27,29,30].

Proteins account for approximately 90% of the dry weight of snake venom[4,21,29,31,32]. *C. d. terrificus* (Cdt) venom is mainly composed of phospholipase A_2 (PLA_2), serinoproteases, hyaluronidases, L-amino acid oxidases, peptides, low molecular weight organic compounds, inorganic ions, and enzyme inhibitors[4,33]. The main toxins found in Cdt venom are Crotoxin, Convulxin, Gyroxin, and

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FIGURE 1: (A) *Crotalus durissus terrificus*; **(B)** Rattle detail; **(C)** Loreal Pit (yellow circle).

Crotamine[4,6-8,34-38]. This venom also contains more than 60 different protein families[23]. Envenomation generates local manifestations of pain, edema, erythema, paresthesia, and systemic manifestations such as eyelid ptosis, facial muscle paralysis, and myoglobinuria, among other clinical signs[4,6,25,31,35,39], because of its neurotoxic, coagulant, and myotoxic actions[4,25,31,33,35].

There is a wide variety of pharmacological uses of the different fractions of *Crotalus sp*. venom, including antifungal, antiviral, antibacterial, antitumor, and antiprotozoal activities[4,15,26-28,37,40-43].

CROTOXIN

Crotoxin represents approximately 40%–60% of the dry weight of the Cdt venom[4,8,19,33,36,42-44] and is a potent neurotoxin formed by PLA_2 and crotapotin, forming a complex of high toxicity[4,8,32,38,42-54], and exhibits myotoxic, nephrotoxic, and cardiotoxic effects[4,37,38,43,44,46,48,50].

This neurotoxic action is mainly attributed to the inhibitory mechanism of acetylcholine release in presynaptic neurons[48,52,54]. Desensitization of postsynaptic nicotinic receptors is another mechanism that reduces the response to acetylcholine[48,52]. Thus, crotoxin acts by blocking potassium channels and prolonging the action potential at neuromuscular junctions, thereby increasing calcium influx into the channels, mainly due to the presence and high activity of PLA_2 in its composition[8,48,52].

Crotoxin has been widely studied for its immunomodulatory, anti-inflammatory, antitumor, antimicrobial, and analgesic actions[4,40,43,44,46,48,50-54]. *In vivo* studies have demonstrated its ability to inhibit the production of pro-inflammatory and anti-inflammatory cytokines from the injection of the toxin in rats, including IL-10, IL-4, IL-6, and tumor necrosis factor[36,43]. This immunomodulatory activity may be associated with the production of anti-inflammatory mediators via the lipoxygenase pathway, such as lipoxin A4 (LXA4), and the activation of formyl peptide receptors, in addition to its regulatory role in macrophages[36,43,44,51].

In vitro and *in vivo* studies have described activating mechanisms of cell apoptosis in different cancer cell lines[19,47-51,53,55] induced by cellular autophagy mechanisms[47,53]. Both cell death pathways activated by crotoxin (apoptosis and autophagy) can occur simultaneously or sequentially through mechanisms such as changes in mitochondrial membrane potential and release of intracellular cytochrome C. Another important factor related to the mechanism of action of crotoxin is its apparent selectivity for cells with high expression of epidermal growth factor receptors (EGFR) [19,21,47,50,56].

The cytotoxic action on glioblastoma and benign pituitary adenoma cells was partially attributed to crotoxin, which is also cytotoxic to human mammary duct carcinoma and human lung adenocarcinoma cell lines[4,19,47-51,55,57]. The application of portions of the toxin to murine erythroleukemia cells demonstrated the potential to reduce the viability of the strain[38]. To observe cytotoxicity, the B subunit of crotoxin was separated from PLA_2 and used alone[38].

The isolated crotoxin is cytotoxic to different cell lines, with different cell response[53]. The mechanisms evaluated involved changes in the mitochondrial membrane potential, release of cytochrome C, and activation of caspase-3, a protease essential for the process of cell apoptosis[47-50,52,53,55]. Furthermore, it was possible to conclude that the toxin did not interfere with the viability of keratinocytes, which are highly affected by current antineoplastic therapies[53].

Crotoxin provokes possible damage to the cellular DNA of PANC-1 cells, associated with pancreatic tumors, by upregulating protein expression[53]. DNA damage has also been observed in glioma cell lines, leading to an increase in the percentage of cells undergoing apoptosis. Some *in vitro* studies have also reported a higher percentage of apoptosis among SK-MES-1 cells, a lung cancer cell line, in addition to damage such as nuclear condensation and fragmentation[50,57].

When associated with tyrosine kinase inhibitors, crotoxin potentiates the antitumor effect of the drug against lung tumor cell lines[50,53,57]. In a dose-dependent manner, the toxin prevents DNA synthesis and interrupts the cell cycle in the S phase, suppressing the proliferation of SK-MES-1 cells both *in vitro* and *in vivo*[52,57]. One of the mechanisms identified was the increased expression and cleavage of caspase-3, which is responsible for inducing cell apoptosis[50,57]. Another mechanism observed was the induction of cytochrome C release, which increased the occurrence of cellular autophagy, a mechanism also observed in MCF-7 breast cancer lines[47,49,53].

Crotoxin also induces the release of LXA4, pro-inflammatory eicosanoid lipoxin, and its analogs through the induction of its synthesis in macrophages[36,44,46,48,51]. *In vivo* studies of Walker 256 carcinoma cells concluded that this mechanism is responsible for the antineoplastic action of crotoxin on the lineage, and the concentration of lipoxin was 74% higher in the plasma of animals treated with crotoxin than in those treated with saline solution[51]. Lipoxins have been shown to be antineoplastic owing to their ability to inhibit tumor growth by inhibiting endothelial cell proliferation and reducing the production of angiogenic growth factors[46,51].

Macrophages cultured *in vitro* in the presence of crotoxin secreted 47% less angiogenesis mediators than macrophages from a control group[46], confirming the role of the toxin in reducing tumor blood vessel neoformation.

The efficacy of crotoxin in dose-dependent inhibition of human esophageal carcinoma tumor growth (Eca-109 cells) was demonstrated *in vivo*[55,57]. The toxin causes cellular damage to the lineage, such as formation of pyknotic cell nuclei, cell lysis, and DNA damage[55]. Exposure of tumor cells to crotoxin also resulted in an increase in the number of stagnant cells in the G1 phase of cell division[53,55,57]. Increased expression of caspase 3, p17, and p15 proteins and reduced production of Bcl-25 protein can be envolved[55].

In vivo studies on the HL-60 leukemia cell line showed lower tumor growth inhibitory activity, suggesting that it acts preferentially on solid tumors[21,47,48,50]. The treatment of patients with solid tumors refractory to conventional antineoplastic therapies with the administration of different doses of crotoxin has demonstrated efficacy in reducing different types of carcinomas[21]. Mechanisms of mitochondrial collapse, cytochrome C release, and caspase 3 activation induced cell death in the human leukemia-associated K562 cell line, with the induction of apoptosis and autophagy observed[50,57].

Crotoxin has been shown to be more cytotoxic than standard chemotherapeutic agents for the treatment of glioma, pancreatic cancer, esophageal cancer, and cervical cancer. Therefore, novel antineoplastic therapies are of great interest, particularly against leukemia, lung cancer, colon cancer, renal cancer, ovarian cancer, esophageal carcinoma, breast carcinoma, melanoma, and brain tumors, whose proliferation is already known to be preventable by the toxin[19,53,57]. New drugs derived from the toxin, such as VRCTC-310 and CB24, have already been studied in murine and human cell lines[16,17,21,41,48].

PHOSPHOLIPASES A_2

PLA_2 are type 1 and type 2 enzymes associated with the induction of inflammatory processes, lipid membrane metabolism, and release of substances such as prostaglandins, prostacyclins, thromboxanes, and leukotrienes[16,21,58-60].

These enzymes represent the largest family of proteins contained in the venom[23,58], accounting for up to 80% of total proteins[24]. PLA_2 induces processes such as edema, blockage of neuromuscular junctions, platelet aggregation, and muscle necrosis[21,59]. It has a substantial pharmacological interest owing to a wide range of biological actions[60]. Some enzymes have anticoagulant activity through mechanisms of hydrolysis of procoagulant phospholipids, antagonistic effects with coagulant proteins, and interaction with factor X[25]. Cotrim et al. (2011) suggested that PLA_2 activity is attributable to its actions at different pharmacological sites, which are responsible for platelet aggregation, myotoxicity, and antibacterial activity, as well as anti-inflammatory and neurotoxic effects[58].

PLA_2 has shown anticancer properties by acting on epithelial growth factor receptors (EGFR), reducing the production of tumor necrosis factor, and inhibiting neoplastic growth in lung carcinoma, human breast carcinoma, and leukemia.

The *Cdt* crotoxin and *Naja naja atra* cardiotoxin association has been conducted to develop "VRCTC-310-Onco," which aims to interfere with the signaling of EGRFs, reduce the production of tumor necrosis factor, and exert cytotoxic action on tumor cells[16,48]. The development of EGFR receptor inhibitor drugs represents a new type of therapy against epithelial neoplasms[61,62] given that the receptors act in the signaling responsible for the formation of epithelial cell tumors[61].

Reduction in tumor necrosis factor production is also an important mechanism of anticancer action, since the presence of necrosis stimulates tumor phosphorescence mediators, favoring angiogenesis and tumor metastasis.

GYROXIN

Gyroxin, a member of the serinoprotease family, is a neurotoxic enzyme with coagulant action[4,6,25,45,63], including thrombin-like action[4,37,45,63,64], and represents the second most commonly found family of venoms[37]. Montoni et al. (2020) demonstrated that the toxin also has the ability to cross the blood-brain barrier[35].

In vitro studies have revealed that the enzyme generates clotting in human plasma samples with citrate, with the speed of clot formation being directly proportional to the amount of gyroxin[25], causing the breakdown of fibrinogen into fibrinopeptide A[25]. Gyroxin is the enzyme responsible for the coagulant activity of Cdt venom as it rapidly consumes circulating fibrinogen, making the blood incoagulable.

Brazilian researchers have used this activity to develop a biopolymer (Heterologous Fibrin Sealant, HFS), which consists of a fibrinogen-rich cryoprecipitate extracted from buffalo blood and a thrombin-like enzyme (gyroxin) purified from *Crotalus durissus terrificus* snake venom[27,63-65]. They successfully evaluated the safety and immunogenicity of HFS for the first time, estimated the optimum dose, and assessed its preliminary efficacy in the treatment of chronic venous ulcers (CVU) in a phase 2 clinical trial[27,63-65].

As gyroxin can cross the blood-brain barrier, it can be an important tool for studies of tumors of the brain and central nervous system.

CONVULXIN

Convulxin is a high-molecular-mass glycoprotein of the C-type lectin family, which has potent platelet activating and aggregating action[4,6,66,67], with high affinity for platelets[66]. However, its effect on human peripheral blood mononuclear cells (PBMCs) and the immune system remains unclear.

The mechanism of action of convulxin involves the activation of phospholipase C and its rapid phosphorylation, which is similar to the mechanism induced by collagens in mediating platelet aggregation[66].

In *in vitro* studies utilizing citrated human plasma samples, the protein generated clot formation without interfering with factors of the coagulation cascade[25].

CROTAMINE

Crotamine is a non-enzymatic polypeptide with myotoxic and neurotoxic actions, responsible for causing cell death in skeletal muscles due to alterations in their sodium channels[4,7,10,18,28,37,68-71].

A great curiosity is that this myotoxin is not present in all individuals of the species, being thus classified as crotamine-positive or crotamine-negative venom-producing animals[7,18,23,28,33,34,45,71]. In crotamine-positive venom-producing animals, the toxin comprises approximately 10%–15% of the venom[31,33,71,72].

This toxin induces skeletal muscle contraction through its action on sodium channels, interfering with ion permeability in the sarcolemma and reducing the resting potential of the membranes[18,28,69]. The changes in permeability cause a greater influx of sodium and calcium ions, which are responsible for depolarization, muscle contraction, vacuolization of sarcoplasmic reticulum, rupture of actin and myosin muscle filaments, and muscle necrosis[18,28,69,71].

Crotamine displays analgesic, antibacterial, antifungal, antiparasitic, and antitumor actions[4,7,18,26,28,71,73-76]. It can be classified as a cell-penetrating peptide, a protein transduction domain, a Trojan peptide, or a membrane translocation sequence[18,26,28,68,72].

Translocation across cell membranes occurs by binding between crotamine and cell surface heparan sulfate proteoglycans, endocytosis, and accumulation of the toxin in intracellular vesicles[18,28,69-72,75,76]. To reach the cytoplasm, crotamine induces changes in the permeability of vesicles, causing it to be released and dispersed in the cell[18,69,72,76]. In the cytoplasm, it can bind to centrosomes in the G1 phase of cell proliferation and enables the diagnosis of cell division phases by functioning as a molecular marker[18,68-71].

The antitumor and antimicrobial properties of crotamine are due to its ability to bind to surfaces and acidic cellular compartments such as lysosomes[28,74-76]. In tumor cells, the prevalence of negatively charged surface molecules, such as phospholipids and mucins, allows preferential binding with the toxin compared to that in healthy cells with electrically neutral surfaces[70].

To develop new drugs, synthetic analogs of crotamine were produced, composed of peptides with smaller chains, and were used to study their functions in comparison to natural crotamine, revealing the possibility of producing crotamine derivatives with important antimicrobial and antineoplastic functions[18,74].

Crotamine possesses preferential selectivity for proliferating cells and for certain phases of the cell cycle[18,69,71,72,74-77]. Both *in vitro* and *in vivo* studies have demonstrated specific and aggressive cytotoxicity against different tumor types[69].

The role of crotamine against murine melanoma cells, human melanoma cells, and primary human pancreatic adenocarcinoma cells has been extensively studied[26,68-71]. Although it is cytotoxic to normal cells when administered at high doses, it is non-toxic at low doses[18].

Crotamine administered via the intraperitoneal route at a concentration of one microgram per animal per day for 21 days demonstrated efficacy in reducing tumors in rats with subcutaneous melanoma[68-72].

Crotamine's action mechanisms to induce cell apoptosis include the activation of caspases, the reduction of mitochondrial membrane potential, and consequent alteration of organelle membrane permeability, inducing the release of intracellular calcium ions and the influx of extracellular calcium[28,68,70,71,73,76]. The activation of caspases is one of the mechanisms responsible for cell apoptosis signaling[78]. Its activation can occur by alterations in mitochondrial membrane permeability, which generates cytochrome C release that amplifies apoptosis signals[69] in HL-60 cells from human leukemia and urinary bladder tumors[69].

Owing to the ability of the toxin to penetrate cells, it is a potential delivery mechanism for other drugs and antitumor agents[18,28,68,69,71-74]. In addition to representing a possible antineoplastic or adjuvant therapy, crotamine can be used as a diagnostic marker for cancer[70,73,76]. Crotamine can be used as a diagnostic marker in human epithelial carcinoma (HeLa), human pancreatic adenocarcinoma (BxPC-3), human breast carcinoma (BT-474), and human colorectal carcinoma (Caco2) cells.

L-AMINO OXIDASES (LAAOS)

LAAOs are flavoenzymes responsible for catalyzing amino acids, which generate alpha-keto acids, ammonia, and hydrogen peroxide[14,16,17,21,32,79,80]. Members of this enzyme class are highly toxic and have great pharmacological importance[16,79], as they can cause platelet aggregation, hemorrhage, edema, cytotoxicity, and induction of apoptosis[14,16,17,37,79,80].

These enzymes induce apoptosis in human leukemia cells. Their toxicity is mainly attributed to the formation of hydrogen peroxide during oxidative reactions, among other mechanisms[16,21,30,79,80]. Although cytotoxic to tumor cells, LAAOs do not affect healthy cells[21,80].

The species-specific cytotoxicity of LAAOcdt was evaluated using nine human cancer cell lines, including pancreatic, esophageal, cervical, and glioblastoma tumors[80].

Purified LAAOs can act on different targets of cellular mechanisms such as DNA fragmentation, chromatin condensation, and nuclear fragmentation. Another mechanism is the induction of P53 protein expression, which is synthesized from a tumor suppressor gene that is functionally deficient in more than half of the human tumors[78,79,81]. Moreover, the induction of protein expression would be relevant to stimulate the monitoring of genome integrity, allowing the identification of damage and repair, resulting in the reduced proliferation of cells with genetic mutations.

LECTINS

Lectins belong to a family of proteins and glycoproteins that generate platelet aggregation[10,16,20,67]. C-type lectins are non-enzymatic calcium-dependent proteins that affect cell adhesion, endocytosis, and neutralization of pathogens[67]. These proteins may also interfere with tumor proliferation, which has been observed using lectins from venom of other species, offering potential for antineoplastic therapy[16].

METALLOPROTEASES

Metalloproteases present hemorrhagic action and induce coagulation alterations in the prey[16,21,82]. These proteins are copious in crotalid venom[82] but are present in low quantities in Cdt venom, thus conferring low proteolytic and hemorrhagic activity[33].

This class of enzymes is composed of endopeptidases that degrade extracellular matrix proteins, blood components, and endothelial cells[21]. In addition, metalloproteases play a fibrinolytic role and act as prothrombin activators, blood coagulation factor X activators, apoptosis inducers, platelet aggregation inhibitors, pro-inflammatory agents, and inactivators of serinoprotease inhibitors[82]. Different groups of metalloproteases found in viperid and crotalid venoms are involved in tumor proliferation and angiogenesis processes[16]. However, specific studies on Cdt venom metalloproteinases have not yet been conducted.

DISINTEGRINS

Disintegrins are also important for the inhibition of tumor cells, together with metalloproteases, by acting against angiogenesis and metastasis[16]. This group of non-enzymatic proteins of low molecular mass can interact with integrins expressed by different cells[16,17,20,83], important cell surface receptors that are involved in interactions between cells and between cells and the extracellular matrix[16,17,20,21,83].

Aggrastat® (Tirofiban, Merck & Co.) and Integrilin® (Eptifibatide, Cor Therapeutics, now part of Millennium Pharmaceuticals) were developed based on snake disintegrins such as echistatin from the saw-scaled viper *Echis carinatus* and barbourin from the southeastern pygmy rattlesnake *Sistrusus miliarius barbouiri*[14,20,84].

Integrins, one of the most important targets of antineoplastic action, are cell surface adhesion molecules that function as receptors and transmitters of cellular signals for migration, invasion and cell proliferation[16,83]. Inhibition of integrins is important because it affects cell proliferation, angiogenesis, and metastasis and is a widely studied antineoplastic treatment option[16,83].

Disintegrins isolated from Cdt venom inhibit the interaction between tumor cells, impairing their motility, and preventing the invasion of other tissues[21]. One of the mechanisms involved is the deposition of fibrin around the tumor, which limits its growth.

PHOSPHODIESTERASES

These enzymes are less abundant in the venom, representing only approximately 2% of its total[33]. Despite being present in the venom in low quantities, it is responsible for inducing important clinical signs of intoxication[33], and its antitumor activity has not yet been evaluated.

Figures 2, 3, and 4 summarize the main mechanisms of antineoplastic action for each component of *Crotalus durissus terrificus* venom.

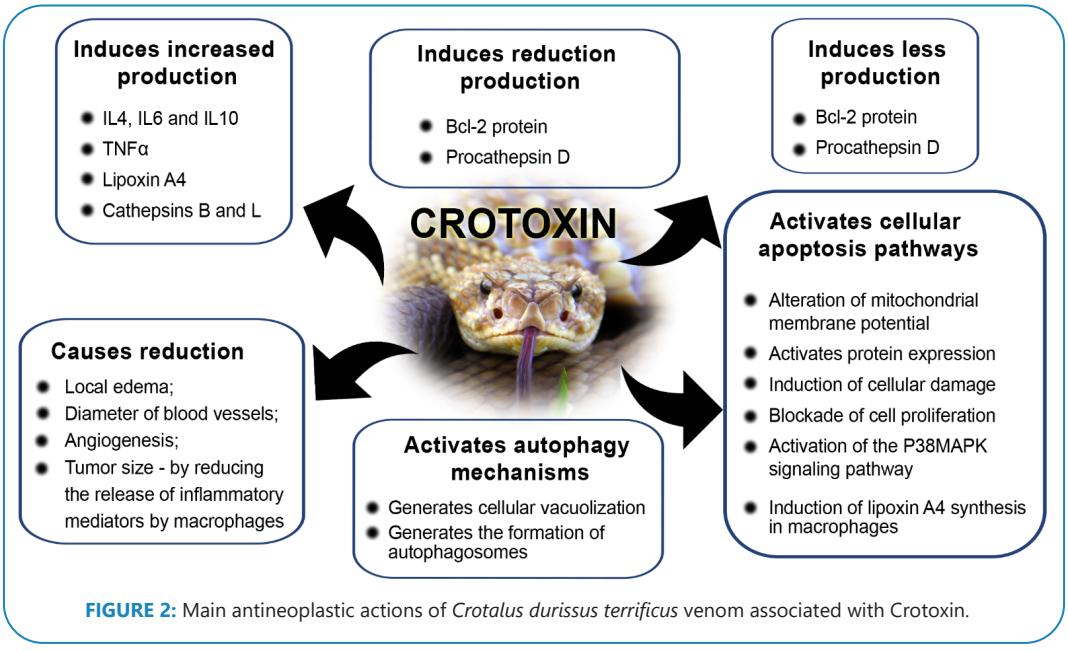

FIGURE 2: Main antineoplastic actions of *Crotalus durissus terrificus* venom associated with Crotoxin.

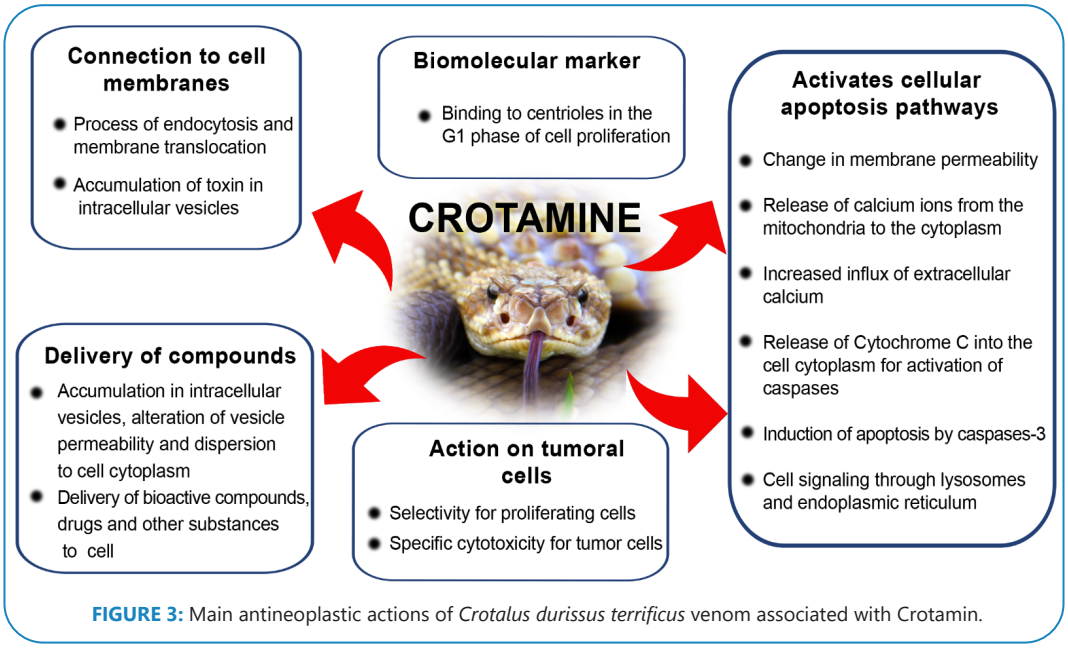

FIGURE 3: Main antineoplastic actions of *Crotalus durissus terrificus* venom associated with Crotamin.

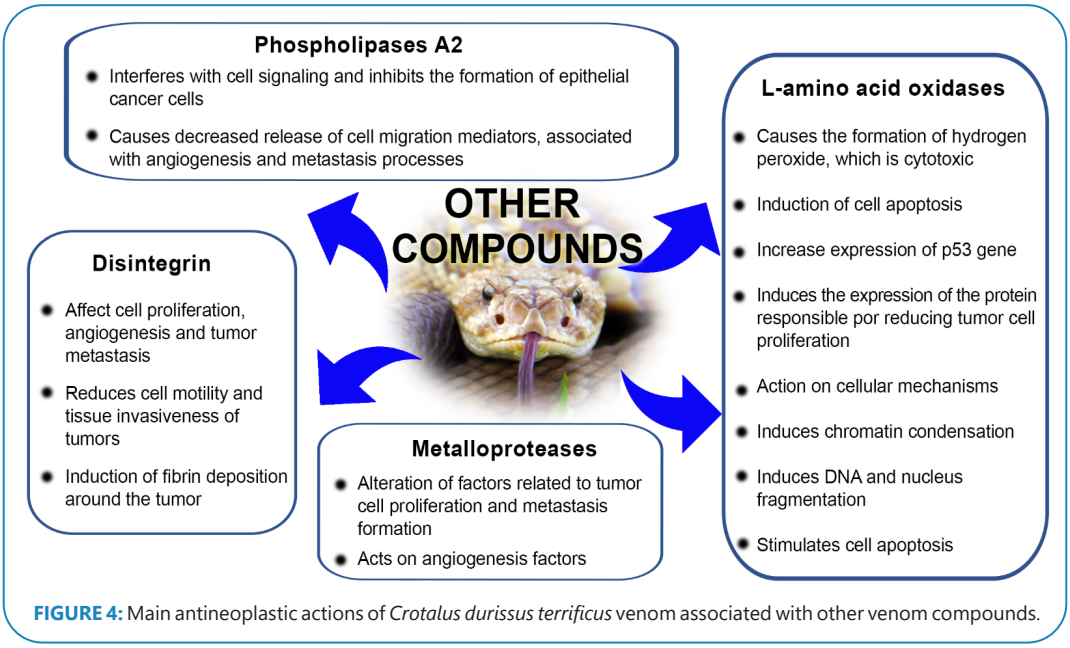

FIGURE 4: Main antineoplastic actions of *Crotalus durissus terrificus* venom associated with other venom compounds.

CLINICAL TRIALS

Crotoxin was administered to patients with solid tumors that were refractory to standard therapy in a phase 1 clinical trial that observed a partial response of more than 50% reduction in tumor mass and a complete response in three of the 23 evaluated patients[21,48,77]. The authors concluded that crotoxin is a new class of anticancer agents that acts through a novel mechanism of action and thought that neurotoxicity could be the principal toxic effect and appears to be manageable. They recommended 0.18 mg/m^2 a therapeutic dose for Phase II studies[77].

The same research team proposed an innovative design for a phase 1 trial with intra-patient dose escalation to study crotoxin[85]. As recorded on the clinical trial platform ClinicalTrial.gov, 18 patients were recruited for this study between 2015 and 2018. The researchers stated that the results would be published shortly[86].

CONCLUSIONS

After elucidating the various mechanisms of action of the *C. d. terrificus* venom, it may be stated that this venom is a potential candidate for the development of new antineoplastic therapies that are efficient against different tumor lines and act on different cellular targets.

Considering the selective cytotoxicity of venom components for tumor cells to the detriment of healthy cells, the development

of innovative therapies against cancer, based on the bioactive compounds of the rattlesnake, may present greater benefits compared to current therapeutic protocols, such as chemotherapy and radiotherapy, which are known to cause alterations in the normal cells of cancer patients.

The therapeutic use of compounds from *Crotalus durissus terrificus* snake venom also represents an alternative for the treatment of tumors resistant to drugs currently available on the market.

Therefore, one can conclude that the improvement of studies of the different fractions of ophidian venom is of great pharmacological interest, with potential for immense impact on the future of therapeutic medicine.

REFERENCES

1. Uetz P, Hošek J. The Reptile Database. [S.l.]: Peter Uetz; 2020 [updated 2021 may 22; cited 2020 october 27]. Available from: www.reptile-database.org.
2. Fraga R, Lima AP, Prudente ALC, Magnusson WE. Guide to the snakes of the Manaus region - Central Amazonia, 1st ed. Manaus: Editora Inpa, 2013. 303 p.
3. Mader DR. Reptile Medicine and Surgery, 2nd ed. [S. l.]: Elsevier; 2005. 1264 p.
4. Santos IGC, Fortes-Dias CL, Dos-Santos MC. Pharmacological applications of Brazilian snake venoms with emphasis in *Crotalus durissus terrificus* and *Crotalus durissus ruruima*. Sci Amazon. 2017;6(1):42–53.
5. Marques OAV, Medeiros CR. Our Incredible Serpents: Characterization, Biology, Accidents and Conservation. 1st ed. Cotia: Ponto A; 2018. 80 p.
6. Frare BT, Resende YKS, Dornelas BC, Jorge MT, Ricarte VAS, Alves LM, et al. Clinical, Laboratory, and Therapeutic Aspects of *Crotalus durissus* (South American Rattlesnake) Victims: A Literature Review. Biomed Res Int. 2019; Article ID 1345923:1-7. Available from: https://doi.org/10.1155/2019/1345923
7. Tasima LJ, Serino-Silva C, Hatakeyama DM, Nishiduka ES, Tashima AK, Sant'Anna SS, et al. Crotamine in Crotalus durissus: distribution according to subspecies and geographic origin, in captivity or nature. J Venom Anim Toxins Incl Trop Dis. 2020;26:e20190053. Available from: https://doi.org/10.1590/1678-9199-jvatitd-2019-0053
8. Rangel-Santos A, Dos-Santos EC, Lopes-Ferreira M, Lima C, Cardoso DF, Mota I. A comparative study of biological activities of crotoxin and CB fraction of venoms from *Crotalus durissus terrificus, Crotalus durissus cascavella* and *Crotalus durissus collilineatus*. Toxicon. 2004;43(7):801-10. Available from: https://doi.org/10.1016/j.toxicon.2004.03.011.
9. Wüster W, Ferguson JE, Quijada-Mascareñas JA, Pook CE, Salomão MDG, Thorpe RS. Tracing an invasion: Landbridges, refugia, and the phylogeography of the Neotropical rattlesnake (Serpentes: Viperidae: *Crotalus durissus*). Mol Ecol. 2005;14(4):1095-80. Available from: https://doi.org/10.1111/j.1365-294X.2005.02471.x.
10. Fry BG, Sunagar K, Casewell NR, Kochva E, Roelants K, Scheib H, et al. The origin and evolution of the toxicofera reptile venom system. In: Fry BG. Venomous reptiles & their toxins: Evolution, pathophysiology & biodiscovery. New York: Oxford University Press; 2015. p. 1-31.
11. WHO - World Health Organization. Report of the Tenth Meeting of the WHO Strategic and Technical Advisory Group for Neglected Tropical Diseases. Geneva: WHO; 2017 [cited 2020 Oct 27]. Available from: http://www.who.int/neglected_diseases/NTD_STAG_report_2017.pdf?ua=1
12. Grego KF, Vieira SEM, Vidueiros JP, Serapicos EO, Barbarini CC, Silveira GPM, et al. Maintenance of venomous snakes in captivity for venom production at Butantan Institute from 1908 to the present: a scoping history. J Venom Anim Toxins Incl Trop Dis. 2021;27:e20200068. Available from: https://dx.doi.org/10.1590/1678-9199-jvatitd-2020-0068
13. Chippaux JP. Snakebite envenomation turns again into a neglected tropical disease. J Venom Anim Toxins Incl Trop Dis. 2017;8;23:38. Available from: https://doi.org/10.1186/s40409-017-0127-6
14. El-Aziz TMA, Soares AG, Stockand JD. Snake Venoms in Drug Discovery: Valuable Therapeutic Tools for Life Saving. Toxins. 2019;11(10):1-25. Available from: https://doi.org/10.3390/toxins11100564
15. Almeida JR, Resende LM, Watanabe RK, Carregari VC, Huancahuire-Vega S, Caldeira CAS, et al. Snake Venom Peptides and Low Mass Proteins: Molecular Tools and Therapeutic Agents. Curr Med Chem. 2017;24(30):3254-82. Available from: https://doi.org/10.2174/0929867324666161028155611
16. Calderon LA, Sobrinho JC, Zaqueo KD, Moura AA, Grabner NA, Mazzi MV, et al. Antitumoral activity of snake venom proteins: New trends in cancer therapy. BioMed Res Int. 2014;2014:203639. Available from: https://doi.org/10.1155/2014/203639
17. 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(2):93-103.
18. Kerkis I, Silva F, Pereira A, Kerkis A, Rádis-Baptista G. Biological versatility of crotamine--a cationic peptide from the venom of a South American rattlesnake. Expert Opin Investig Drugs. 2010;19(12):1515–25. Available from: https://doi.org/10.1517/13543784.2010.534457
19. Soares MA, Pujatti PB, Fortes-Dias CI, Antonelli L, Santos RG. Crotalus durissus terrificus venom as a source of antitumoral agents. J Venom Anim Toxins incl Trop Dis. 2010;16(3):480-92. Available from: https://doi.org/10.1590/S1678-91992010000300015
20. Vonk FJ, Jackson K, Doley R, Madaras F, Mirtschin PJ, Vidal N. Snake venom: From fieldwork to the clinic: Recent insights into snake biology, together with new technology allowing high-throughput screening of venom, bring new hope for drug discovery. Bioessays. 2011;33(4):269-79. Available from: http://dx.doi.org/10.1002/bies.201000117
21. Jain D, Kumar S. Snake venom: a potent anticancer agent. Asian Pac J Cancer Prev. 2012;13(10):4855-60. Available from: http://dx.doi.org/10.7314/APJCP.2012.13.10.4855
22. Kollipara PS, Won DH, Hwang CJ, Jung YY, Yoon HS, Park MH, et al. Enhanced Anti-Cancer Effect of Snake Venom Activated NK Cells on Lung Cancer Cells by Inactivation of NF-κB. Biomol Ther. 2014;22(2):106-13. Available from: http://dx.doi.org/10.4062/biomolther.2013.103
23. Melani RD, Araujo GDT, Carvalho PC, Goto L, Nogueira FCS, Junqueira M, et al. Seeing beyond the tip of the icebeg: A deep analysis of the venome of the Brazilian Rattlesnake, *Crotalus durissus terrificus*. EuPA Open Proteom. 2015;8:144-56. Available from: https://doi.org/10.1016/j.euprot.2015.05.006
24. Boldrini-França J, Cologna CT, Pucca MB, Bordon KCF, Amorim FG, Anjolette FAP, et al. Minor snake venom proteins: Structure, function and potential applications. Biochim Biophys Acta Gen Subj. 2017;1861(4):824-38. Available from: http://dx.doi.org/10.1016/j.bbagen.2016.12.022
25. Sousa IDL, Barbosa AR, Salvador GHM, Frihling BEF, Santa-Rita PH, Soares AM, et al. Secondary homeostasis studies of crude venom and isolated proteins from the snake Crotalus durissus terrificus. Int J Biol Macromol. 2019;131:127–33. Available from: https://doi.org/10.1016/j.ijbiomac.2019.03.059

26. Pérez-Peinado C, Defaus S, Andreu D. Hitchhiking with Nature: Snake Venom Peptides to Fight Cancer and Superbugs. Toxins. 2020;12(4):1-23. Available from: https://doi.org/10.3390/toxins12040255

27. Santos L, Oliveira C, Vasconcelos BM, Vilela D, Melo L, Ambrósio L, et al. Good management practices of venomous snakes in captivity to produce biological venom-based medicines: achieving replicability and contributing to pharmaceutical industry. J Toxicol Environ Health B Crit Rev. 2021;24(1):30-50. Available from: http://dx.doi.org/10.1080/10937404.2020.1855279

28. Cunha DB, Silvestrini AVP, Silva ACG, Estevam DMP, Pollettini FL, Navarro JO, et al. Mechanistic insights into functional characteristics of native crotamine. Toxicon. 2018;146:1-12. Available from: https://doi.org/10.1016/j.toxicon.2018.03.007

29. Vyas VK, Brahmbhatt K, Bhatt H, Parmar U. Therapeutic potential of snake venom in cancer therapy: current perspectives. Asian Pac J Trop Biomed. 2013;3(2):156-62. Available from: http://dx.doi.org/10.1016/S2221-1691(13)60042-8

30. Shanbhag VKL. Applications of snake venoms in treatment of cancer. Asian Pac J Trop Biomed. 2015;5(4):275–6. Available from: http://dx.doi.org/10.1016/S2221-1691(15)30344-0

31. Devi A. The Protein and Nonprotein Constituents of Snake Venoms. In: Bucherl W, Buckley EE, Deulofeu V, editors. Venomous Animals and their Venoms. 1st ed. New York: Academic Press; 1968. p. 119-65. Available from: https://doi.org/10.1016/B978-1-4832-2949-2.50014-X

32. Sarkar NK, Devi A. Enzymes in Snake Venoms. In: Bucherl W, Buckley EE, Deulofeu V, editors. Venomous Animals and their Venoms. 1st ed. New York: Academic Press; 1968. p. 167-216. Available from: https://doi.org/10.1016/B978-1-4832-2949-2.50015-1

33. Fusco LS, Neto EB, Francisco AF, Alfonso J, Soares A, Pimenta DC, et al. Fast venomic analysis of *Crotalus durissus terrificus* from northeastern Argentina. Toxicon X. 2020;7:100047. Available from: https://doi.org/10.1016/j.toxcx.2020.100047

34. Lourenço Jr A, Creste CFZ, Barros LC, dos Santos LC, Pimenta DC, Barraviera B, et al. Individual venom profiling of *Crotalus durissus terrificus* specimens from a geographically limited region: Crotamine assessment and captivity evaluation on the biological activities. Toxicon. 2013;69:75-81. Available from: https://doi.org/10.1016/j.toxicon.2013.01.006

35. Montoni F, Andreotti DZ, Eichler RAS, Santos WS, Kisaki CY, Arcos SSS, et al. The impact of rattlesnake venom on mice cerebellum proteomics points to synaptic inhibition and tissue damage. J Proteomics. 2020;221:103779. Available from: https://doi.org/10.1016/j.jprot.2020.103779

36. Lima TS, Neves CL, Zambelli VO, Lopes F, Sampaio SC, Cirillo MC. Crotoxin, a rattlesnake toxin, down-modulates functions of bone marrow neutrophils and impairs the Syk-GTPase pathway. Toxicon. 2017;136:44–55. Available from: https://doi.org/10.1016/j.toxicon.2017.07.002

37. Tasima LJ, Hatakeyama DM, Serino-Silva C, Rodrigues C, de Lima E, Sant'Anna SS, et al. Comparative proteomic profiling and functional characterization of venom pooled from captive *Crotalus durissus terrificus* specimens and the Brazilian crotalic reference venom. Toxicon. 2020;185:26–35. Available from: https://doi.org/10.1016/j.toxicon.2020.07.001

38. Corin RE, Viskatis LJ, Vidal JC, Etcheverry MA. Cytotoxicity of crotoxin on murine erythroleukemia cells in vitro. Invest New Drugs. 1993;11(1):11–5. Available from: https://doi.org/10.1007/BF00873905

39. Bordon KCF, Perino MG, Giglio JR, Arantes EC. Isolation, enzymatic characterization and antiedematogenic activity of the first reported rattlesnake hyaluronidase from *Crotalus durissus terrificus* venom. Biochimie, 2012;94(12):2740–8. Available from: https://doi.org/10.1016/j.biochi.2012.08.014.

40. Faure G, Bakouh N, Lourdel S, Odolczyk N, Premchandar A, Servel N, et al. Rattlesnake Phospholipase A2 increases CFTR-chloride channel current and corrects F508CFTR dysfunction: impact in Cystic Fibrosis. J Mol Biol. 2016;428(14):2898-915. Available from: http://dx.doi.org/10.1016/j.jmb.2016.05.016

41. Marcussi S, Santos PRS, Menaldo DL, Silveira LB, Santos-Filho NA, Mazzi MV, et al. Evaluation of the genotoxicity of Crotalus durissus terrificus snake venom and its isolated toxins on human lymphocytes. Mutat Res. 2011;724(1-2):59–63. Available from: https://doi.org/10.1016/j.mrgentox.2011.06.004

42. Muller VDM, Russo RR, Cintra ACO, Sartim MA, Alves-Paiva RM, Figueiredo LTM, et al. Crotoxin and phospholipases A_2 from Crotalus durissus terrificus showed antiviral activity against dengue and yellow fever viruses. Toxicon. 2012;59(4):507–15. Available from: https://doi.org/10.1016/j.toxicon.2011.05.021

43. Sartim MA, Menaldo DL, Sampaio SV. Immunotherapeutic potential of crotoxin: anti-inflammatory and immunosuppressive properties. J Venom Anim Toxins incl Trop Dis. 2018;24:1-13. Available from: https://doi.org/10.1186/s40409-018-0178-3.

44. Pimenta LA, Almeida MES, Bretones ML, Cirillo MC, Curi R, Sampaio SC. Crotoxin promotes macrophage reprogramming towards an antiangiogenic phenotype. Sci Rep. 2019;9(1):1-15. Available from: https://doi.org/10.1038/s41598-019-40903-0

45. Silva-Júnior LN, Abreu LS, Rodrigues CFB, Galizio NC, Aguiar WS, Serino-Silva C, et al. Geographic variation of individual venom profile of *Crotalus durissus* snakes. J Venom Anim Toxins Incl Trop Dis. 2020;26:e20200016. Available from: https://doi.org/10.1590/1678-9199-jvatitd-2020-0016

46. Sampaio SC, Brigatte P, Sousa-e-Silva MCC, dos-Santos EC, Rangel-Santos AC, Curi R, et al. Contribution of crotoxin for the inhibitory effect of *Crotalus durissus terrificus* snake venom on macrophage function. Toxicon. 2003;41(7):899–907. Available from: https://doi.org/10.1016/S0041-0101(03)00069-2.

47. Yang C, Yang Y, Qin Z, Gu Z, Reid P, Liang Z. Autophagy is involved in cytotoxic effects of crotoxin in human breast cancer cell line MCF-7 cells. Acta Pharmacol Sin. 2007;28(4):540–8. Available from: http://dx.doi.org/10.1111/j.1745-7254.2007.00530.x

48. Sampaio SC, Hyslop S, Fontes MRM, Prado-Francechi J, Zambelli VO, Magro AJ, et al. Crotoxin: Novel activities for a classic β-neurotoxin. Toxicon. 2010;55(6):1045–60. Available from: http://dx.doi.org/10.1016/j.toxicon.2010.01.011

49. Ye B, Xie Y, Qin ZH, Wu JC, Han R, He JK. Anti-tumor activity of CrTX in human lung adenocarcinoma cell line A549. Acta Pharmacol Sinica. 2011;32(11):1397–401. Available from: https://doi.org/10.1038/aps.2011.116

50. Wang J, Xie Y, Wu J, Han R, Reid PF, Qin Z, et al. Crotoxin enhances the antitumor activity of gefinitib (Iressa) in SK-MES-1 human lung squamous carcinoma cells. Oncol Rep. 2012;27(5):1341-7. Available from: https://doi.org/10.3892/or.2012.1677

51. Brigatte P, Faiad OJ, Nocelli RCF, Landgraf RG, Palma MS, Cury Y, et al. Walker 256 Tumor Growth Suppression by Crotoxin Involves Formyl Peptide Receptors and Lipoxin A_4. Mediators Inflamm. 2016;2016:2457532. Available from: http://dx.doi.org/10.1155/2016/2457532

52. Cavalcante WLG, Matos JBN, Timoteo MA, Fontes MRM, Gallacci M, de Sa PC. Neuromuscular paralysis by the basic phospholipase A2 subunit crotoxin from Crotalus durissus terrificus snake venom needs its acid chaperone to con-currently inhibit acetylcholine release and produce muscle blockage. Toxicol Appl Pharmacol. 2017;334:8–17. Available from: https://doi.org/10.1016/j.taap.2017.08.021

53. Muller SP, Silva V, Silvestrini A, Macedo LH, Caetano GF, Reis RM, et al. Crotoxin from Crotalus durissus terrificus venom: In vitro cytotoxic activity of a heterodimeric phospholipase A2 on human cancer-derived cell lines. Toxicon. 2018;156:13–22. Available from: https://doi.org/10.1016/j.toxicon.2018.10.306

54. Andrade CM, Rey FM, Cintra A, Sampaio SV, Torqueti MR. Effects of crotoxin, a neurotoxin from Crotalus durissus terrificus snake venom, on human endothelial cells. Int J Biol Macromol. 2019;134:613–21. Available from: https://doi.org/10.1016/j.ijbiomac.2019.05.019

55. He J, Wu X, Wang Y, Han R, Qin Z, Xie Y. Growth inhibitory effects and molecular mechanisms of crotoxin treatment in esophageal Eca-109 cells and transplanted tumors in nude mice. Acta pharmacol Sin. 2013;34(2):295–300. Available from: https://doi.org/10.1038/aps.2012.156

56. Donato NJ, Martin CA, Perez M, Newman RA, Vidal JC, Etcheverry M. Regulation of epidermal growth factor receptor activity by crotoxin, a snake venom phospholipase A2 toxin. A novel growth inhibitory mechanism. Biochem pharmacol. 1996;51(11):1535-43. Available from: https://doi.org/10.1016/0006-2952(96)00097-4

57. Han R, Liang H, Qin ZH, Liu CY. Crotoxin induces apoptosis and autophagy in human lung carcinoma cells in vitro via activation of the p38MAPK signaling pathway. Acta Pharmacol Sin. 2014;35(10):1323–32. Available from: https://doi.org/10.1038/aps.2014.62

58. Cotrim CA, Oliveira SCB, Filho EBSD, Fonseca FV, Jr LB, Antunes E, et al. Quercetin as an inhibitor of snake venom secretory phospholipase A2. Chem Biol Interact. 2011;189(1-2):9–16. Available from: https://doi.org/10.1016/j.cbi.2010.10.016

59. Sunagar K, Jackson TNW, Reeks T, Fry BG. Group I phospholipase A_2 enzymes. In: Fry BG. Venomous reptiles & their toxins: Evolution, pathophysiology & biodiscovery. New York: Oxford University Press; 2015. p. 327–34.

60. Sunagar K, Tsai IH, Lomonte B, Jackson TNW, Fry BG. Group II phospholipase A_2 enzymes. In: Fry BG. Venomous reptiles & their toxins: Evolution, pathophysiology & biodiscovery. New York: Oxford University Press; 2015. p. 335–40.

61. Herbst RS. Review of epidermal growth factor receptor biology. Int J Radiat Oncol Biol Phys. 2004;59(2):2–26. Available from: http://dx.doi.org/10.1016/j.ijrobp.2003.11.041

62. Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. Nat Rev Cancer. 2007;7:169–81. Available from: https://doi.org/10.1038/nrc2088

63. Ferreira RS, de Barros LC, Abbade LPF, Barraviera SRCS, Silvares MRC, de Pontes LG, et al. Heterologous fibrin sealant derived from snake venom: from bench to bedside - an overview. J Venom Anim Toxins Incl Trop Dis. 2017;23:21. Available from: http://dx.doi.org/10.1186/s40409-017-0109-8

64. Abbade LPF, Barraviera SRCS, Silvares MRC, Lima ABBCO, Haddad GR, Gatti MAN, et al. Treatment of Chronic Venous Ulcers with Heterologous Fibrin Sealant: A Phase I/II Clinical Trial. Front Immunol. 2021;12:627541. Available from: http://dx.doi.org/10.3389/fimmu.2021.627541

65. Abbade LPF, Ferreira Jr RS, dos Santos LD, Barraviera B. Chronic venous ulcers: a review on treatment with fibrin sealant and prognostic advances using proteomic strategies. J Venom Anim Toxins Incl Trop Dis. 2020;26:e20190101. Available from: http://dx.doi.org/10.1590/1678-9199-jvatitd-2019-0101

66. Francischetti IMB, Ghazaleh FA, Reis RAM, Carlini CR, Guimarães JA. Convulxin induces platelet activation by a tyrosine-kinase-dependent pathway and stimulates tyrosine phosphorylation of platelet proteins, including PLCγ2, independently of integrin $\alpha_{IIb\beta3}$. Arch biochem biophys. 1998;353(2):239–50.

67. Arlinghaus FT, Fry BG, Sunagar K, Jackson TNW, Eble JA, Reeks T, et al. Lectin proteins. In: Fry BG. Venomous reptiles & their toxins: Evolution, pathophysiology & biodiscovery. New York: Oxford University Press; 2015. p. 299–311.

68. Kerkis I, Hayashi MAF, da Silva ARBP, Pereira A, Júnior PLS, Zaharenko AJ, et al. State of the art in the studies on crotamine, a cell penetrating peptide from South American rattlesnake. Biomed Res Int. 2014;2014:675985. Available from: https://doi.org/10.1155/2014/675985

69. Rádis-Baptista G, Kerkis I. Crotamine, a small basic polypeptide myotoxin from rattlesnake venom with cell-penetrating properties. Curr Pharm Des. 2011; 17(38):4351–61.

70. Falcao CB, Rádis-Baptista G. Crotamine and crotalicidin, membrane active peptides from *Crotalus durissus terrificus* rattlesnake venom, and their structurally-minimized fragments for applications in medicine and biotechnology. Peptides. 2020;126:170234. Available from: https://doi.org/10.1016/j.peptides.2019.170234.

71. Silva ARBP, Fry BG, Sunagar K, Scheib H, Jackson TNW, Rádis-Baptista G, Zaharenko AJ, Jr PLS, Pereira A, Oguiura N, Hayashi MAF, Kerkis A, Yamane T, Kerkis I. Beta-defensins. In: Fry BG. Venomous reptiles & their toxins: Evolution, pathophysiology & biodiscovery. New York: Oxford University Press; 2015. p. 228-38.

72. Campeiro JD, Marinovic MP, Carapeto FC, Mas CD, Monte GG, Porta LC, et al. Oral treatment with a rattlesnake native polypeptide crotamine efficiently inhibits the tumor growth with no potential toxicity for the host animal and with suggestive positive effects on animal metabolic profile. Amino Acids. 2018;50(2):267-78. Available from: http://dx.doi.org/10.1007/s00726-017-2513-3

73. Vu TTT, Jeong B, Yu J, Koo B, Jo S, Robinson RC, et al. Soluble prokaryotic expression and purification of crotamine using an N-terminal maltose-binding protein tag. Toxicon. 2014;92:157–65. Available from: http://dx.doi.org/10.1016/j.toxicon.2014.10.017

74. Dal Mas C, Pinheiro DA, Campeiro JD, Mattei B, Oliveira V, Oliveira EB, et al. Biophysical and biological properties of small linear peptides derived from crotamine, a cationic antimicrobial/antitumoral toxin with cell penetrating and cargo delivery abilities. Biochim Biophys Acta Biomembr. 2017;1859(12):2340-9. Available from: https://doi.org/10.1016/j.bbamem.2017.09.006

75. Yamane ES, Bizerra FC, Oliveira EB, Moreira JT, Rajabi M, Nunes GL, et al. Unraveling the antifungal activity of a South American rattlesnake toxin crotamine. Biochimie. 2013;95(2):231–40. Available from: https://doi.org/10.1016/j.biochi.2012.09.019

76. Nascimento FD, Sancey L, Pereira A, Rome C, Oliveira V, Oliveira EB, et al. The natural cell-penetrating peptide crotamine targets tumor tissue in vivo and triggers a lethal calcium-dependent pathway in cultured cells. Mol Pharm. 2012;9(2):211-21. Available from: https://doi.org/10.1021/mp2000605

77. Cura JE, Blanzaco DP, Brisson C, Cura MA, Cabrol R, Larrateguy L, et al. Phase I and pharmacokinetics study of crotoxin (cytotoxic PLA(2), NSC-624244) in patients with advanced cancer. Clin Cancer Res. 2002;8(4):1033-41. PMID: 11948110.

78. Anazetti MC, Melo PS. Apoptosis Cell Death: biochemistry and molecular aspects. Metrocamp Pesquisa. 2007;1(1):37–58.

79. Tan NH, Fry BG, Sunagar K, Jackson TNW, Reeks T, Fung SY. L-Amino acid oxidase enzymes. In: Fry BG. Venomous reptiles & their toxins: Evolution, pathophysiology & biodiscovery. New York: Oxford University Press; 2015. p. 291-8

80. Teixeira TL, Silva VAO, Cunha DB, Polettini FL, Thomaz CD, Pianca AA, et al. Isolation, characterization and screening of the in vitro cytotoxic activity of a novel L-amino acid oxidase (LAAOcdt) from *Crotalus durissus terrificus* venom on human cancer cell lines. Toxicon. 2016;119:203–17. Available from: https://doi.org/10.1016/j.toxicon.2016.06.009

81. Fett-Conte AC, Salles ABCF. The importance of the p53 gene in human carcinogenesis. Rev Bras Hematol Hemoter. 2002;24(2):85–9. Available from: https://doi.org/10.1590/S1516-84842002000200004

82. Markland JR, Francis S, Swenson S. Snake venom metalloproteinases. Toxicon. 2013;62:3–18. Available from: https://doi.org/10.1016/j.toxicon.2012.09.004

83. Galán JA, Sánchez EE, Rodríguez-Acosta A, Soto JG, Bashir S, McLane MA, et al. Inhibition of lung tumor colonization and cell migration with the disintegrin crotatroxin 2 isolated from the venom of *Crotalus atrox*. Toxicon. 2008;51(7):1186–96. Available from: https://doi.org/10.1016/j.toxicon.2008.02.004

84. Pennington MW, Czerwinski A, Norton RS. Peptide therapeutics from venom: Current status and potential. Bioorg Med Chem. 2018;26(10):2738–58. Available from: https://doi.org/10.1016/j.bmc.2017.09.029

85. Medioni J, Brizard M, Elaidi R, Reid PF, Benlhassan K, Bray D. Innovative design for a phase 1 trial with intra-patient dose escalation: The Crotoxin study. Contemp Clin Trials Commun. 2017;7:186-8. Available from: http://dx.doi.org/10.1016/j.conctc.2017.07.008

86. ClinicalTrials.gov [Internet]. Gil-Delgado MA (MD): National Library of Medicine (US). 2011 Nov 28. Identifier NCT01481532, Open Label Clinical Trial of Intravenous Crotoxin; 2018 Jan 31 [cited 2022 Sep 21]. Available from: https://clinicaltrials.gov/ct2/history/NCT01481532?V_5=View#StudyPageTop