The Dose Makes the Cure: Harnessing the Therapeutic Potential of Peruvian Snake Venoms

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Abstract

For decades, snake venom has been studied for its diverse array of biological properties. From the isolation of various biomolecules, it has been possible to obtain resources for the development of therapeutic tools for a wide variety of medical conditions. This work compiles findings from multiple studies conducted on the subject, focusing specifically on the biomolecules from the four genera of venomous snakes found in Peru—*Bothrops*, *Crotalus*, *Lachesis*, and *Micrurus*—to highlight their importance as resources for biotechnological research. It presents information on metalloproteases, disintegrins, phospholipases A2, L-amino acid oxidases, serine proteases, and other biomolecules isolated from different species of these genera, describing their characteristics and potential applications in the development of therapeutic tools to treat a wide range of ailments, such as microbial and viral infections, various types of pain, and cancer, among others. Lastly, it briefly outlines several suggestions for the conservation and protection of these species, in hopes of expressing gratitude toward the natural sources that provide these valuable resources.

1. Introduction

Biomolecules extracted from snake venom have been used for decades in research aimed at developing new drugs to treat a wide variety of diseases and medical conditions. These range from coagulation disorders and bacterial, viral, or parasitic diseases—such as hepatitis and leishmaniasis—to severe illnesses such as cancer.

In Peru, several species of venomous snakes can be found, whose venoms have given rise to biomolecules that show potential for the development of new therapies.

Among these are *Bothrops* (lanceheads) and *Micrurus* (coral snakes), the most prolific and abundant genera in Peru's diverse ecosystems, which have recently drawn attention due to the ability of their venom proteins to inhibit platelet aggregation or induce cytotoxicity in tumor cells—through molecules such as metalloproteases (SVMPs), L-amino acid oxidases (LAAOs), and phospholipases A_2 (PLA_2s).

Lachesis (bushmasters) is a less well-known genus of venomous snakes also found in Peru. Its venom has been studied and shown to contain biomolecules shared with the other genera—such as the aforementioned SVMPs, LAAOs, and PLA_2s.

Finally, the better-known *Crotalus* (rattlesnakes) produce toxins such as crotoxin, crotamine, and gyrotoxin. These proteins have long been recognized and studied for their anticancer, analgesic, antiplatelet, antiviral, and other bioactive properties.

The lack of studies concerning the biomolecules of several venomous snake species found in Peru—specifically those belonging to the genera *Bothrops* and *Micrurus*—led to the inclusion of studies on non-Peruvian species, as a point of comparison and as examples of what the closely related Peruvian species could potentially provide.

2. *Bothrops*

Bothrops pit vipers, commonly known as lanceheads, represent one of the two most diverse and widely distributed genera of venomous snakes in Peru, occurring across the three main ecological regions: the coast, the highlands, and the rainforest. They are generally medium-sized vipers, with the terrestrial species being the largest. The arboreal species, on the other hand, tend to be smaller and less robust.

The venoms of snakes belonging to this genus have been extensively studied, leading to the isolation of several proteins — primarily disintegrins, but also enzymes such as L-amino acid oxidases (LAAOs). Their potential medical applications include cytotoxic and anticancer effects, which may help prevent tumor cell proliferation and metastasis.

Although some studies like that conducted by Sanchez et al. (2009) have focused on *Bothrops* species that are not native to Peru, these snakes are closely related to the Peruvian species of the same genus. This strongly suggests that Peruvian *Bothrops* species may exhibit similar protein characteristics in their venoms. Consequently, research on these species could open new possibilities in the fields of biotechnology and bioengineering.

2.1. Bothropoidin

Bothropoidin is a snake venom metalloprotease (SVMP) isolated for the first time from the venom of *Bothrops pauloensis*. A study conducted by Guimarães et al. (2017) evaluated its potential use as an anticancer and anti-angiogenic agent. It exhibited both properties, reducing the viability of endothelial cells in vitro and showing moderate cytotoxicity in breast cancer cell lines.

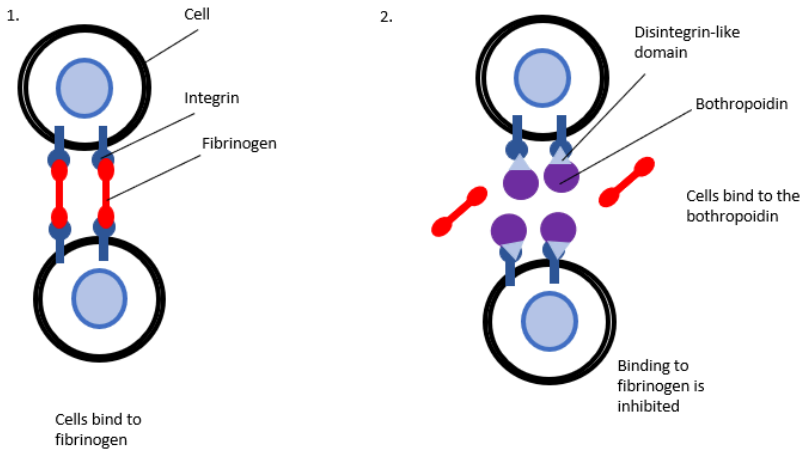
Snake venom disintegrins and metalloproteases can interact with integrins—key cell-surface receptors responsible for regulating a variety of cellular processes—in multiple cell types, making them promising candidates for anticancer therapies. Bothropoidin can inhibit platelet

aggregation and interfere with the viability and adhesion of endothelial cells. In this way, it suppresses the formation of new blood vessels, hindering tumor growth by depriving cancer cells of nutrients, while also preventing metastasis by reducing the blood supply to and from the tumor.

According to the study, bothropoidin exhibited approximately 30% cytotoxicity at a dose of 100 µg/mL after 24 hours of treatment in MDA-MB-231 breast cancer cells. Another assay was performed with MCF10A, a non-tumorigenic breast epithelial cell line. Bothropoidin showed reduced cytotoxicity, around 10%, indicating a preferential effect against cancer cells. Moreover, it did not reduce the viability of non-tumor cells. Bothropoidin significantly increased both early and late apoptotic processes in MDA-MB-231 cells; however, the exact mechanism remains to be elucidated.

Since MDA-MB-231 is a highly metastatic cancer cell line, the effect of bothropoidin on cell adhesion and migration was also assessed. Bothropoidin inhibited cell adhesion in a dose-dependent manner. At 10 µg/mL, it reduced cell migration by approximately 45%. At concentrations ≥20 µg/mL, bothropoidin decreased endothelial cell viability. It also inhibited new capillary formation in Matrigel previously stimulated with bFGF. Its anti-angiogenic capacity may involve binding to cell-surface integrins in addition to degrading extracellular matrix proteins.

Figure 1. *Inhibition mechanism of bothropoidin*



This antiangiogenic property, as is the case with proteins from other snake venoms, makes bothropoidin a molecule of interest for biotechnological research. The inhibition of capillary network formation can be useful in the treatment of cancer as well as other conditions or pathologies that depend on this process, such as certain inflammatory diseases. An antiangiogenic therapy could limit tumor growth and expansion by depriving it of the nutrients and pathways necessary for its propagation. Moreover, when combined with another compound

designed to destroy cancer cells directly, it could enhance the overall effectiveness of the treatment.

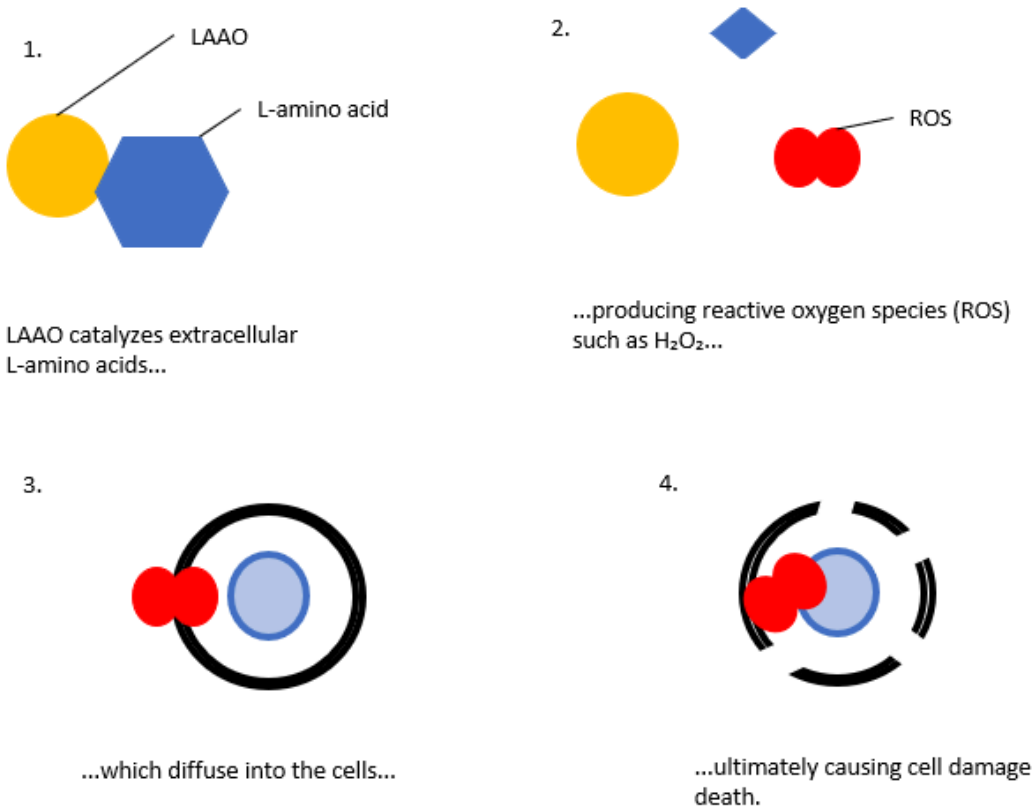
2.2. LAAOs from *Bothrops*

L-amino acid oxidases (LAAOs) are enzymes present in the venoms of various snake species. They are FAD-containing enzymes (Flavin Adenine Dinucleotide), an important enzymatic cofactor in metabolic processes, which contribute to the oxidative deamination of L-amino acids, generating α-keto acid, hydrogen peroxide, and ammonium as reaction products.

Present in the venoms of viperids and elapids, and in large amounts in crotalines, they can account for up to 25% of the total protein content of venom.

Although other biological characteristics (effects on platelet aggregation, hemorrhage, necrosis, or edema) have been attributed to snake venom LAAOs (svLAAOs), they are mainly studied for their cytotoxic effects. In vitro tests show that svLAAOs use L-amino acids present in a cell culture to produce hydrogen peroxide, which induces oxidative stress in cancer cells and disrupts their natural physiology. The most common assumption is that hydrogen peroxide, a type of reactive oxygen species (ROS), is the main cytotoxic mechanism, since a study conducted by Lazo et al. (2017) showed that cells treated with catalase—an enzyme protective against hydrogen peroxide—did not die when exposed to svLAAOs.

Figure 2. *Mechanism of the cytotoxicity of LAAOs*



ROS—such as hydrogen peroxide—are byproducts of oxygen reduction during metabolic processes. These molecules are highly reactive, stealing electrons from any other molecule, bond, or structure they encounter, thereby causing damage to DNA, lipids, or proteins. Although they are normally neutralized by the antioxidant system—which is enhanced in cancer cells—there are circumstances in which the accumulation of ROS can induce apoptosis through oxidative stress.

Hydrogen peroxide release is considered a major factor in svLAAO cytotoxicity, along with other mechanisms. Studies have shown that svLAAO-induced apoptosis can proceed via a fast pathway (through hydrogen peroxide) or a slow pathway (through L-amino acid depletion). Both reactions release cytochrome c into the cytosol, which binds to another protein to form the apoptosome. This activates caspase-9, which degrades cellular components, causing programmed cell death.

A study led by Tan and colleagues (2018) listed several properties of this type of enzyme in different species. Among these, it was found that this protein family shows a preference for binding to the cell membranes of tumor cells, potentially reducing its cytotoxicity toward healthy tissue. The same study reports a series of LAAOs from different species (*Bothrops* spp., *Cerastes cerastes*, *Crotalus durissus*, *Ophiophagus hannah*, among others) capable of inducing apoptosis in various tumor cell lines, and explains several of the pathways through which this effect is achieved.

2.2.2. Bpic-LAAO from *Bothrops pictus*

Bpic-LAAO is a L-amino acid oxidase (LAAO) isolated from the venom of *Bothrops pictus*, commonly known in Peru as the “jergón costero.”

The same study conducted by Lazo et al. (2017), mentioned above, showed that Bpic-LAAO has antibacterial activity against both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Vibrio cholerae*. The inhibition of bacterial growth was shown to be mediated by the enzymatic activity of the protein, since catalase abolished this effect.

The bactericidal properties of this LAAO demonstrate significant potential for the development of future therapies against cholera, bacterial pneumonia, and other infectious diseases. Moreover, its high homology with LAAOs from other *Bothrops* species suggests that these enzymes may exhibit similar or closely related capabilities. This underscores the need for further research to

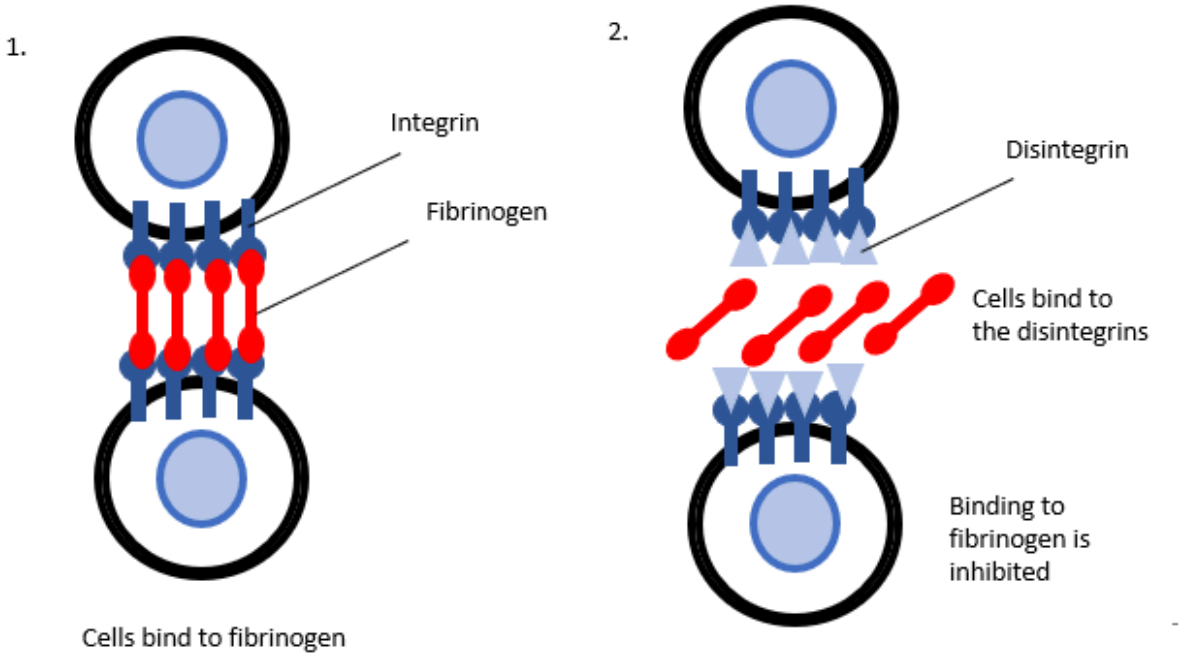
characterize the functions of each LAAO individually, with the aim of identifying those that are most effective against specific bacterial targets.

2.3. Disintegrins from *Bothrops*

Stefan Niewiarowski and Tur-Fu Huang isolated for the first time a non-enzymatic protein from the venom of the snake *Trimeresurus gramineus* (currently reclassified as *Craspedocephalus gramineus*), called trigramin. This protein was capable of blocking the binding of fibrinogen to platelet GPIIb/IIIa receptors, thereby inhibiting platelet aggregation.

They were described as a new class of cysteine-rich snake venom peptides possessing an RGD (Arg-Gly-Asp) motif in their structure. About ten years after their discovery, non-RGD disintegrins were identified, which led to the discovery of integrins recognizing alternative binding motifs.

***Figure 3**. Inhibition mechanism of disintegrins.*



Other biological activities investigated in disintegrins include interference with neutrophil chemotaxis, antiparasitic and antiviral properties, and antitumor effects mediated by apoptosis, cytotoxicity, and inhibition of tumor cell development.

Integrins are transmembrane receptors responsible for mediating and regulating different cellular processes by binding to specific extracellular ligands. They are heterodimeric proteins

composed of one α (alpha) and one β (beta) subunit. Integrins recognize ligands from the extracellular matrix, cell surfaces, and soluble ligands. The specific combination of α and β subunits determines their ligand specificity.

Structurally, each integrin subunit consists of an extended multidomain extracellular region, with each domain performing a specific function. The α subunits contain up to 1104 amino acid residues, while the β subunits contain up to 778. The remainder of the integrin consists of a single transmembrane helix and a short cytoplasmic tail (20–70 amino acids long). The globular head, responsible for ligand binding, is formed by the N-terminal regions of both subunits located in the extracellular domain.

Integrins are expressed on the surface of many cell types. They mediate interactions between cells and extracellular matrix proteins such as collagen, fibronectin, and laminin-1. They are involved in a wide range of biological functions, including cell aggregation, wound healing, neuronal connectivity, and synapse regulation. Integrins are also associated with several pathological processes, such as inflammation, fibrosis, atherosclerotic plaque formation, and cancer development.

Snake venom disintegrins can be classified according to their structure as monomeric or dimeric. Monomeric disintegrins can be further divided by length: short (41–51 amino acids), medium (around 70 amino acids), and long (approximately 84 amino acids). Dimeric disintegrins can be either homodimeric or heterodimeric, each subunit containing about 67 amino acids with ten cysteine residues forming four intrachain and two interchain disulfide bonds. Disintegrins are highly homologous proteins, suggesting a common evolutionary origin.

During envenomation, disintegrins participate in several biological functions. One is to bind platelet receptors and prevent adhesion, disrupting hemostatic balance. The ability of disintegrins to bind specific integrins is determined by their binding motifs.

The potential applications of disintegrins include the inhibition of bone resorption —since it has been shown to inhibit it in vitro— to prevent bone loss, fractures and osteoporosis; as diagnostic tools —such as bistatin, that has potential for molecular imaging of thromboembolic diseases; interference with neutrophil chemostasis in inflamed or damaged tissues, antiparasitic activity, particularly shown against *Leishmania infantum*; and antitumoral properties, by preventing cell adhesion and inhibiting angiogenesis, depriving the tumor of blood and nutrients.

There are already drugs based on disintegrins. For example, tirofiban is a synthetic drug derived from the RGD domain of echistatin from *Echis carinatus*. It interacts with platelet GPIIb/IIIa glycoprotein receptors, inhibiting platelet aggregation by competitively displacing fibrinogen. It was approved by the FDA for the treatment of acute coronary syndrome (ACS), a group of

conditions in which blood flow to the heart is suddenly reduced, such as through plaque formation or thrombosis in the coronary artery.

The existence of drugs based on disintegrins provides strong evidence of their potential for the development of therapeutic compounds. Therefore, further research appears necessary in order to identify disintegrins that are more effective as treatments.

2.3.1. DisBa-01

DisBa-01 is a recombinant disintegrin derived from the snake *Bothrops alternatus*. It has shown high affinity for integrins αvβ3 and αIIbβ3, producing strong antiplatelet and antithrombotic effects. In human microvascular endothelial cells (HMECs), this disintegrin reduced both migration speed and directional persistence. In *in vivo* assays, it inhibited angiogenesis in two distinct animal models.

Angiogenesis is a key process in the regulation of various physiological and pathological events. An imbalance between pro- and anti-angiogenic factors can lead to multiple disorders. In cancer, it can promote tumor growth and metastasis. Additionally, several non-neoplastic diseases depend on angiogenesis, such as rheumatoid arthritis, diabetic retinopathy, atherosclerosis, and other inflammatory conditions.

Tumor responses to antiangiogenic therapy are diverse. The angiogenic programming process is regulated by tumor and stromal cells, along with their bioactive products. Examples include cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs). Bioactive products involved comprise cytokines, growth factors, extracellular matrix components, and their ligands. Tumors exhibit altered properties and patterns in newly formed blood vessels.

Angiogenesis in solid tumors is mainly driven by vascular endothelial growth factor (VEGF) through the VEGFR2 tyrosine kinase receptor on endothelial cells. Signaling between VEGFA and VEGFR2 activates several intracellular pathways, such as phosphatidylinositol 3-kinase (PI3K), extracellular signal-regulated kinase (ERK) pathway, focal adhesion kinase (FAK), Src family kinases and paxillin.

These pathways induce vascular permeability, vessel remodeling, endothelial proliferation, prostanoid synthesis, migration, tubulogenesis, and secretion of matrix metalloproteinases (MMPs) and nitric oxide. This signaling network, which is thought to cross-interact with integrin receptors, is essential for vascular development and nutrient supply.

Recent studies have shown that the onset of angiogenesis (the “angiogenic switch”) depends on a series of receptors and accessory proteins, including integrin αvβ3, vascular endothelial

cadherin (VE-cadherin) y syndecan-1 (SDC-1).

Blocking any of these components, in addition to VEGF inhibition, can impair angiogenesis. Furthermore, an association has been demonstrated between VEGFR2 and the cytoplasmic tails of the β3 integrin subunit.

Endothelial cells express integrins such as α2β1, α5β1, αvβ3, and αvβ5. These are activated during vascular growth and remodeling associated with inflammation, wound healing, ischemic injury, tumor growth, and metastasis. Integrin αvβ3 is the most abundant proangiogenic regulatory receptor on endothelial cells due to its cross-talk with VEGFR2. VEGFR2 phosphorylation increases when endothelial cells are seeded on extracellular matrix (ECM) proteins such as vitronectin and fibrinogen, which are αvβ3 ligands. Moreover, both receptors can form functional interconnections that enhance ligand-induced tyrosine kinase receptor activity through integrin activation.

Experiments using *in vitro* microvascular endothelial cells demonstrated that integrin αvβ3 is activated by VEGF-A. These integrins are also more abundantly expressed at sites of ongoing angiogenesis. Understanding this cross-talk may improve current pro- and antiangiogenic therapeutic strategies.

Antibodies and cyclic RGD peptide inhibitors targeting integrins effectively inhibit angiogenesis by blocking adhesion. Disintegrins possess this same capacity.

Human umbilical vein endothelial cells (HUVECs) were stimulated with VEGF, treated with DisBa-01, and analyzed for changes in proliferation, viability, migration, invasion, and adhesion to ECM components.

After 24 hours, cell number increased by 34.5% due to VEGF stimulation. DisBa-01 alone did not affect cell viability but inhibited proliferation by 61%. Matrigel invasion was reduced by 58% after 18 hours, even in the presence of VEGF.

Cells exposed only to DisBa-01, at 100 and 1000 nM, showed a 13% and 19% reduction in migration, respectively. VEGF alone did not alter migration but enhanced the inhibitory effect of DisBa-01 up to 69%.

Integrin αvβ3 is a multifunctional receptor that binds at least four RGD-domain proteins, including fibrinogen and vitronectin. Adhesion to fibrinogen was not affected by treatment, but adhesion to vitronectin increased. HUVEC adhesion decreased by 41% when simultaneously treated with both proteins. This indicates that DisBa-01 interaction with endothelial surface receptors prevents VEGF-induced proliferation and adhesion to vitronectin.

The effect of the disintegrin on VEGF-induced tube formation in HUVECs was also evaluated. In Matrigel, cells formed stable capillary-like structures, whether treated or untreated with VEGF. In VEGF-treated cells, values increased as follows: length (17%), meshes (67%), nodes (47%), and major junctions (60%), raising the angiogenesis score.

Subsequently, the effect of DisBa-01 on VEGF-induced angiogenesis was tested. Tubes formed by disintegrin-treated cells differed from those in untreated or VEGF-only groups.

DisBa-01 inhibited total structure length by percentages depending on the dose of 23% at 1 nM, 38% at 100 nM and 26% at 1000 nM. At the highest concentration, DisBa-01 completely suppressed VEGF's angiogenic effect, yielding values similar to control. Similar results were obtained for all analyzed parameters. According to the analysis, DisBa-01 inhibited tube formation by 56% and the VEGF-stimulated effect by 40%. Therefore, at least at 1000 nM, the disintegrin inhibited the angiogenic effect of VEGF.

Expression of VEGF receptors and β3 integrin subunits was analyzed to clarify the mechanisms underlying angiogenesis inhibition in human vascular endothelial cells treated with VEGF and DisBa-01.

β3 subunit levels were unaffected by DisBa-01, as were the mRNA levels encoding these subunits, in both VEGF-treated and untreated cells. The disintegrin also reduced VEGFR2 levels in cell lysates after one hour of exposure compared with both VEGF-treated and untreated groups. These levels returned to baseline after 24 hours. Treatment with VEGF alone did not affect VEGFR2 protein expression.

2.3.2. Batroxostatin

Batroxostatin is a disintegrin isolated from the venom of the snake *Bothrops atrox*. A single active protein fraction was obtained from the complete HPLC profile of *Bothrops atrox* venom, representing approximately 0.5–1% of the total venom proteins. To analyze and stabilize the protein for amino acid sequencing, S-pyridylethylated batroxostatin was used.

An automated sequencing of the N-terminal region was performed, yielding three overlapping sequences, which may have resulted from partial protein degradation prior to sequencing. Subsequently, the protein was digested with the endoproteases Asp-N, Lys-C, and Arg-C to obtain smaller, well-defined peptides. These fragments were separated by reverse-phase HPLC for individual analysis. The complete amino acid sequence of batroxostatin was then reconstructed by comparing the peptide fragments and identifying overlapping regions. The presence of phenylalanine as the C-terminal amino acid was confirmed by amino acid release following digestion with carboxypeptidase Y.

The inhibition of ADP-induced platelet aggregation in platelet-rich plasma by batroxostatin was compared to that of trigramin. Fifty percent inhibition of platelet aggregation was achieved at 204 nM for trigramin and 133 nM for batroxostatin. To achieve the same inhibitory effect under identical conditions, 110 μM of RGDS peptide was required.

In B16-F10 mouse melanoma cells, inhibition of cell adhesion to fibronectin by batroxostatin and trigramin was dose-dependent. In C32 human melanoma cells, inhibition of fibronectin-mediated adhesion by batroxostatin was similar to that previously reported for trigramin. At a concentration of 150 nM, trigramin inhibited cell adhesion by 49%, whereas batroxostatin inhibited it by 56%. A comparable degree of inhibition was observed with GRGDS peptide at 60 μM. At 670 nM, batroxostatin inhibited the adhesion of chick fibroblasts to fibronectin by approximately 50%.

In B16 mouse melanoma cells, cell adhesion was tested on both fibronectin and batroxostatin immobilized on Co-Bind™ plates. Similar to fibronectin, batroxostatin promoted B16 cell adhesion, and comparable results were observed in C32 cells. It is suggested that the adsorption of batroxostatin onto the plastic surface results from its binding to integrin receptors, as this adsorption was inhibited by an anti-integrin antiserum.

2.3.3. Colombistatin

Colombistatin is another disintegrin, this time isolated from the venom of *Bothrops colombiensis*. The venom of this species contains thrombin-like enzymes capable of cleaving fibrinogen to generate fibrin. Colombistatin was obtained from the crude venom through proteolytic processing of P-II or P-III class metalloproteases. Two main analytical procedures were performed: first, a multidimensional liquid chromatography (MDLC) system was used, followed by mass spectrometry (MS) for characterization.

As previously mentioned, snake venom disintegrins can be applied to the development of new treatments for cardiovascular and cerebrovascular accidents, diabetes, osteoporosis, and cancer. The differences in ligand-binding affinity among disintegrins (to fibronectin, vitronectin, fibrinogen, and other ligands) depend on the specific type of binding motif each disintegrin possesses. These affinities are also influenced by the amino acids adjacent to the binding motif, which affect the folding and conformation of the protein.

A study led by Sánchez et al. (2009) confirmed that colombistatin strongly inhibits the adhesion of melanoma cells to fibronectin. Its biological activity was tested in several assays: inhibition of ADP-induced platelet aggregation in human whole blood; inhibition of adhesion of human bladder carcinoma cells (T24) and skin melanoma cells (SK-Mel-28) to fibronectin; and inhibition of cell migration in T24 cells.

Using reverse-phase C18 chromatography, 36 fractions of the crude venom were obtained. Each fraction was tested for proteolytic activity and for inhibition of ADP-induced platelet aggregation in human whole blood. The absence of proteolytic activity in all fractions was attributed not to the absence of proteolytic enzymes, but rather to denaturation of high–molecular weight proteins caused by the solvents used in reverse-phase chromatography.

Fraction 12 inhibited both platelet aggregation and the adhesion of T24 cells to fibronectin. This fraction was further separated by size-exclusion chromatography, yielding five new fractions, among which Fraction 2 exhibited the same inhibitory properties. Capillary electrophoresis confirmed a high degree of purity. Colombistatin has a molecular mass of 7.78 kDa (determined by MS) and consists of 72 amino acids.

The enzyme showed neither hemorrhagic nor fibrinolytic activity. However, it displayed concentration-dependent inhibitory activity on ADP-induced platelet aggregation in human whole blood, with an IC_{50} of 210 nM. It also inhibited the adhesion of T24 and SK-Mel-28 cells to fibronectin, with IC_{50} values of 4.4 μM and 33 nM, respectively. Colombistatin additionally inhibited the migration of T24 cells: in a scratch-wound assay, inhibition of wound closure indicated a decrease in cell migration, with an IC_{50} of 1.8 μM.

The group of snakes belonging to the genus *Bothrops* represents several opportunities for the development of new drugs. These include multiple approaches to cancer therapy —such as the use of disintegrins, metalloproteases, and L-amino acid oxidases (LAAOs)— as well as the inhibition of both Gram-positive and Gram-negative bacteria. It is worth noting that all *Bothrops* species show a high degree of similarity in the composition of their venoms. This makes it reasonable to assume that other species within the genus, including those found in Peru, possess similar or potentially more promising pharmacological properties. Therefore, further research is needed to fully explore the possibilities these animals offer for the development of novel therapeutic tools.

Table 1. *Biomolecules from Bothrops and their potential uses*

Potential uses for biomolecules from *Bothrops*	
Biomolecule	**Possible uses**
Metalloproteases (e.g. bothropoidin)	• Antitumoral effects • Antiangiogenic effects
LAAOs (e.g. Bpic-LAAO, BA-LAAO, BP-LAAO)	• Antiparasitic effects • Antibacterial effects • Antitumoral effects
Disintegrins (e.g. DisBa-01, Colombistatin, Batroxostatin	• Inhibition of platelet aggregation • Leishmanicidal effects • Antiangiogenic effects • Therapies for inflammatory conditions • Inhibition of bone resorption • Inhibition of cell adhesion in cancer cells

3. *Crotalus*

Within the genus *Crotalus*, commonly known as rattlesnakes, only one subspecies is found in Peru: *Crotalus durissus terrificus*. It is distributed in the southeasternmost region of the country, particularly in the rainforests of the departments of Puno and Madre de Dios. This species is of medium size, reaching approximately 1.5 meters in length, with the largest individuals attaining up to 2 meters.

It is a venomous snake whose venom exhibits mainly neurotoxic and myotoxic activity, although it also shows cytotoxic effects and can alter blood coagulation and pressure.

The proteins contained in the venom of *C. d. terrificus* are among the most extensively studied of all snake-derived biomolecules. From crotoxin, with its diverse biological functions ranging from antitumor to antimicrobial activity, to gyroxin, proposed as a biological sealant, the venom components of this species display remarkable biochemical versatility.

These diverse molecules make this venom a promising source for biotechnological and therapeutic development, with potential applications in the treatment of various diseases.

Therefore, further research and experimental testing are necessary to refine current knowledge and enable the development of practical biomedical tools based on the toxins of this rattlesnake species.

3.1. Crotoxin

Crotoxin, derived from the venom of *Crotalus durissus terrificus* (South American tropical rattlesnake), was first studied, analyzed, and crystallized in 1938 at the Butantan Institute in São Paulo. Initially, it was attributed with the typical toxic effects of rattlesnake venom: neurotoxicity, myotoxicity, nephrotoxicity, and cardiotoxicity. However, more recent studies have suggested additional properties, including immunomodulatory, antimicrobial, antitumor, anti-inflammatory, and analgesic activities.

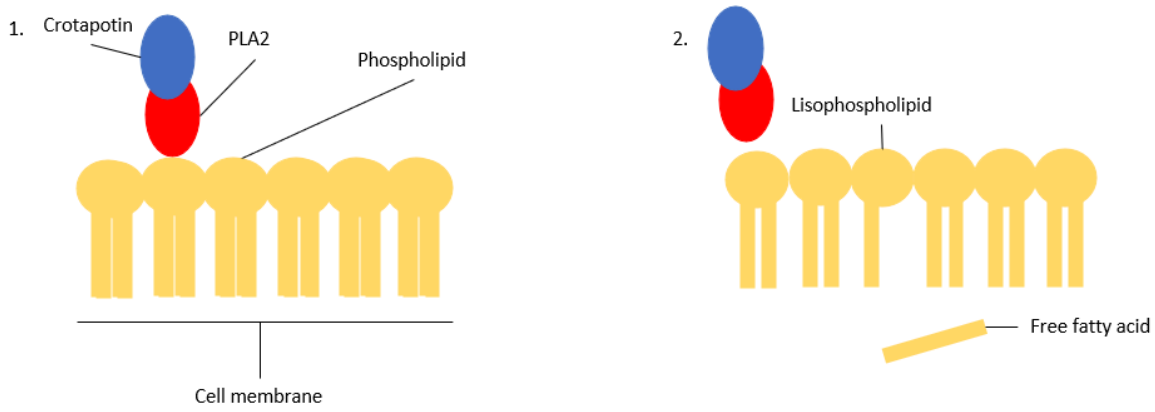
The original work isolating and crystallizing crotoxin from rattlesnake venom was carried out by the German chemist Karl Heinrich Slotta (1895–1987) at the Butantan Institute, marking a milestone in toxicology and opening the way for the study and isolation of animal venoms.

At first, crotoxin was believed to be a single pure protein, based on ultracentrifugation and electrophoresis assays that, under the criteria of the time, indicated homogeneity. Subsequent studies raised doubts about its purity, and between the 1950s and 1970s it was demonstrated that crotoxin is a heterodimeric complex composed of two proteins:

- **Phospholipase A_2 (PLA_2):** an enzyme with relatively low toxicity on its own. It hydrolyzes ester bonds in phospholipids, releasing free fatty acids—most notably arachidonic acid, a precursor of several bioactive compounds involved in envenomation symptoms—and lysophospholipids, which disrupt cell membranes, activate the immune system, and contribute to pain.

- **Crotapotin (CA):** an acidic, non-enzymatic protein that acts as a “chaperone” for PLA_2. Although non-toxic on its own, it stabilizes and guides PLA_2, preventing its premature degradation in the organism until it reaches its site of action.

Figure 4. *Mechanism of action (MoA) for crotoxin on phospholipids.*



W. P. Neumann and E. Habermann (1955–56) were the first to identify crotoxin as a two-component system. Later, Hendon and Fraenkel-Conrat (1971) identified the CB subunit as the toxic and hemolytic component, while CA lacked these effects. Jen and Fraenkel-Conrat (1978) demonstrated that CA shields CB, highlighting the importance of the complex's structure. Marlas and Bon (1982) later confirmed that CA prevents non-specific binding of CB to membrane phospholipids. That same year, Bon and Radvanyi concluded that CB's toxic action depended on forming a dimeric structure with another CB. Radvanyi et al. further showed that CB's hydrophobic domain is not exposed when bound to CA.

These findings demonstrated that crotoxin possesses distinct structural domains responsible for different pharmacological functions, and that the two parts of CB function as complementary elements of the same functional complex. The CB subunit binds to presynaptic membranes, inhibiting acetylcholine release and inducing neuromuscular blockade.

The pharmacological properties of crotoxin were first presented in 1966 at the Animal Venoms Symposium at the Butantan Institute. Studies reported its toxicity, neuromuscular activity, cardiovascular effects, and nephrotoxicity in several mammalian species and pigeons, using different routes of administration.

In mice and pigeons, flaccid paralysis was observed, limited to the hind limb muscles in mice. In unanesthetized cats and dogs, symptoms included defecation, salivation, vomiting, and renal damage. Paralysis was reversible in all species except rabbits.

Consciousness remained unaffected. Intracerebroventricular injection in cats induced clonic seizures, tachypnea, salivation, vomiting, and death. In anesthetized cats and dogs, neural blockade lasted 24–48 hours.

Early studies suggested that crotoxin's neurotoxic activity was primarily postsynaptic, as it binds to acetylcholine receptors, keeping them inactive. However, later evidence clarified that the main mechanism is presynaptic, involving a triphasic effect on neurotransmitter release: an initial rapid decrease, followed by a transient increase, and then a slow, sustained decrease. Repeated injections induced resistance to neural blockade.

In anesthetized dogs, crotoxin induced hypotension that developed gradually within about three minutes of injection. Respiratory effects were generally absent, though some animals exhibited transient increases in respiratory rate and amplitude, followed by apnea and subsequent tachypnea.

Together, these findings laid the foundation for subsequent studies on the pharmacology and mechanisms of action of crotoxin.

Crotoxin has a broad spectrum of pharmacological activities. One is its immunoregulatory functions. Crotoxin modulates circulating lymphocyte and leukocyte levels. It promotes leukocyte adhesion to endothelial cells. Additionally, crotapotin exhibits anti-edematogenic and anti-inflammatory effects.

Crotoxin also displays antibacterial activity against a broad range of bacteria, thought to be closely related to PLA_2 activity.

The crotoxin induces antinociception. Intraperitoneal (IP) and intracerebroventricular (ICV) injections inhibited pain-induced neuronal firing, indicating central nervous system involvement. It has also been shown to inhibit neuropathic pain in rats following sciatic nerve transection, when administered subcutaneously. The effect was potent and long-lasting, up to 65 days, without impairing locomotor function. Serotonergic and noradrenergic systems were also shown to participate, suggesting a complex interplay in crotoxin-mediated analgesia.

Crotoxin also exhibits antitumor properties. In Phase I clinical trials on patients with solid tumors, it reduced both pain and tumor size. Tumor growth inhibition was also confirmed in animal models. Crotoxin interferes with tumor progression by altering specific cellular processes. Alone or combined with cardiotoxin, crotoxin is cytotoxic to various human and murine tumor cell lines, both in vitro and in vivo. The CB subunit plays a central role in this activity. While effects against leukemia were less pronounced, likely due to its specificity for solid tumors, crotoxin has been shown to inhibit tumor neovascularization. In a Phase I clinical study, 30 days of crotoxin treatment led to >50% tumor mass reduction, with complete regression of the primary tumor in one patient.

Among the various uses proposed by research for the complex crotoxin from the venom of *Crotalus durissus terrificus*, we find its antiviral use as an agent for the treatment of infection by the Hepatitis C virus (HCV). The tests were carried out in a culture of Huh-7.5 cells (a human liver cell line permissive to HCV), suitable for viral infection. Its capacities as a preventive agent for viral entry, as a treatment for already infected cells, as a virucide, and as an inhibitor of viral replication were studied.

The virus and the toxin were added at the same time and incubated for 4 hours, after which the toxins and virus that had not entered the cells by that point were removed. Subsequently, the virus was allowed to replicate for 48 hours and replication was measured by a focus formation assay. The crotoxin complex inhibited viral entry into the cells by around 85%, and the PLA2-CB subunit inhibited viral entry by approximately 97.3%. On the other hand, crotapotin did not inhibit focus formation.

The difference in the percentage of inhibitions leads one to think that the inhibition caused by the crotoxin complex is due to the presence of the PLA2-CB subunit, the enzyme with the highest inhibition among the three.

The virucidal effect of the toxins was also tested by leaving them in a cell-free environment together with the virus for one hour, and then using the toxin-virus mixture to infect Huh-7.5 cells for 4 hours. Crotoxin showed inhibition of around 75.5%, while PLA2-CB showed inhibition of approximately 93%.

It was also assessed whether the antiviral action of the toxins was due to their direct effect on the cells. Cell cultures were treated with toxins one hour before and then exposed to the virus for 4 hours. PLA2-CB reduced HCV infectivity by around 73.5%, but neither CX nor crotapotin showed a reduction in infectivity, suggesting that PLA2-CB loses its antiviral effectiveness when bound with crotapotin in the crotoxin complex. The tests suggested that the antiviral activity of the toxins is related to CD-81 receptors.

The inhibitory effect of the toxins on HCV replication was also tested. The virus was exposed to the toxins under non-cytotoxic conditions. While PLA2-CB considerably reduced replication and crotoxin showed no changes in replication, crotapotin significantly increased HCV replication levels. PLA2-CB exhibited a dose-dependent antiviral effect and increased the viability of the infected cell culture, showing a more specific effect rather than simply killing the cells. It was tested in both genotypes 2a and 1b, showing a similar effect in both, and in the complete virus. When bound with crotapotin in the crotoxin complex, PLA2-CB lost its antiviral capacity, indicating that crotapotin must interfere in some way with the antiviral action of the PLA2-CB subunit.

In the study led by Shimizu et al. (2017) of the mechanism, PLA2-CB showed the ability to bind to RNA, preventing it from being used by the virus for replication. PLA2-CB was shown to reduce the signal of double-stranded RNA (a product of HCV replication), indicating that it reduced viral replication.

When compared with Sofosbuvir (a viral polymerase inhibitor), it was shown that although the latter inhibited more strongly than PLA2-CB, their combined effect was greater than that of each one individually, suggesting an additive effect between them.

Although crotapotin seems less useful so far, or even detrimental for the proposed treatments, research has been carried out to explore its potential clinical utility. For this, the effect of the toxins on HCV release was measured. Infected cells were treated with the toxins for 24 hours, then harvested, and traces of intracellular and extracellular virus were measured.

The results showed that, while PLA2-CB reduced the amount of virus released, this was due to the inhibition of replication. There was no difference between the amount of virus produced and the amount released. Crotoxin and crotapotin showed reduced levels of intracellular and extracellular RNA, indicating that not all of the virus produced was released. The reduction

caused by crotoxin was around 50%, and by crotapotin around 78%, indicating that the action of crotoxin in this area is due to the presence of crotapotin.

The fact that crotapotin can bind to PLA_2-CB to form crotoxin suggests possible applications for this particular subunit. If the part of crotapotin structure that determines which proteins it can bind to could be modified, it might be used as a delivery factor for other animal proteins. Since it reduces the systemic toxicity of crotoxin as a complex by increasing its specificity, it could perform the same function with other proteins, such as LAAOs or SVMPs. At the very least, it could serve this role with PLA_2s from other snake species.

Finally, crotoxin also exhibits documented anticoagulant activity. In a study conducted by Gimenez et al. (2020), peripheral blood mononuclear cells (PBMCs) were treated with lipopolysaccharide (LPS) to recreate a procoagulant environment. Under these conditions, the cells displayed a reduced plasma clotting time.

It was shown that crotoxin did not decrease PBMC viability. Prior treatment with crotoxin at a concentration of 0.04 µg/mL mitigated the LPS-induced procoagulant effect. It is also noteworthy that crotoxin by itself did not induce inflammation, and that it reduced the release of pro-inflammatory cytokines triggered by LPS.

Crotoxin demonstrated a binding preference for monocytes over lymphocytes. This feature may be relevant for the management of certain disorders. For example, in sepsis, monocytes release large amounts of tissue factor (TF), which can lead to microthrombosis and multiple organ failure. Thus, based on crotoxin's effects in in vitro PBMC models, it is plausible to propose that it might reduce clot formation by lowering levels of both pro-inflammatory cytokines and TF.

The diverse effects of crotoxin make it a valuable resource for biomedical research. Even more noteworthy are the results of phase I clinical trials, which show promising progress regarding its potential therapeutic use. However, further research is still needed to fully evaluate its application in cancer therapy and to continue investigating its other properties before beginning patient trials. Additional studies are required before a crotoxin-based compound can be approved for commercial use, although the evidence available so far remains encouraging.

3.2. Crotalphine

Crotalphine is a peptide extracted from the crude venom of *Crotalus durissus terrificus* (South American rattlesnake), to which long-lasting antinociceptive effects have been attributed through the activation of kappa opioid receptors (KOR) and delta opioid receptors (DOR).

When administered orally, crotalphine has been shown to produce antinociception in the two test models. The first one is *Mechanical hyperalgesia in rats*, involving acute local pain caused by touch or pressure. It can be induced by prostaglandin, a pro-inflammatory chemical that increases pain sensitivity; or by carrageenan, a seaweed-derived substance that, when injected,

causes inflammation and localized pain, in a manner similar to human inflammatory pain. The analgesic effect lasted for 5 days, acting on kappa and delta opioid receptors. The second model is the *Hot plate test in mice*, in which a previously anesthetized mouse is placed on a heated surface in order to measure the response time. The longer it takes for the animal to react to the stimulus, the greater the efficacy of the compound.

Crotalphine has shown to be biologically stable, active at low doses, and capable of producing a long-lasting pain-relieving effect. Its effects have also been studied in models of neuropathic pain in rats.

Chronic neuropathic pain is a disease resulting from damage to the nervous system. The main clinical symptoms are hyperalgesia —a state of increased sensitivity to pain, in which mild or moderate stimuli cause extreme pain—, allodynia —a condition in which normally painless stimuli cause pain, such as skin contact— and continuous and spontaneous pain, without an apparent cause.

Treatment is not always effective, which often results in patient disability and decreased quality of life. Treatments involve the use of analgesics (such as morphine or gabapentin) and anti-inflammatory drugs.

Although the mechanisms of chronic neuropathic pain in humans are not fully understood, some understanding has been achieved through the use of animal models, in which symptoms similar to those of neuropathic pain can be induced. These include:

- **Chronic constriction injury:** the sciatic nerve is tied without being completely severed, creating constant pressure.
- **Spinal nerve ligation:** ligation of specific spinal nerves.
- **Partial sciatic ligation:** a portion of the sciatic nerve is tied, leaving another portion intact.
- **Sural and tibial transection:** the sural and tibial nerves are cut, leading to loss of function of the affected limb in addition to pain.
- **Complete sciatic transection:** the sciatic nerve is completely severed.

The individual nerves tied or cut in the tests conducted by Gutierrez et al. (2008) are as follows:

- **Sciatic nerve:** the largest peripheral nerve in the body, running from the lumbar region on each side of the spine down to the leg. It controls almost all sensation and movement of the hind limb. It is formed in the lumbar and sacral regions, from the nerve roots L4, L5, S1, S2, and S3.
- **Tibial nerve:** the main branch of the sciatic nerve, innervating the posterior leg and the foot.

- **Sural nerve:** the main branch of the tibial nerve, providing sensation to the outer part of the ankle and the foot.
- **Spinal nerves:** nerves that originate directly from the spinal cord. In the spinal nerve ligation model, nerves L5 and/or L6 from the lumbar region are typically tied.

The study results showed that crotalphine was more effective than morphine or gabapentin, both of which are used in the treatment of chronic neuropathic pain in humans. The peptide increased the pain threshold of operated rats: it reduced the frequency of licking or lifting of the affected limb, without affecting the number of times the animal moved or bore weight on its hind legs.

As part of the study on crotalphine's mechanism of action, rats were injected intraplantarly with naloxone. Naloxone is an opioid receptor antagonist and abolished the antinociceptive activity of crotalphine. This demonstrated that its mechanism of action, as well as that of the crude snake venom that was also tested, is related to opioid receptors.

To test its efficacy in long-term treatment, rats were administered either crotalphine or crude venom every 3 days for 75 days, starting on day 14 after surgery that induced neuropathic pain symptoms. The rats did not develop tolerance to the antinociceptive effect of crotalphine. These results show that crotalphine may be used for long-term treatments without causing tolerance, unlike other opioids such as morphine. The study also reported no changes in breathing patterns or withdrawal symptoms, both of which are effects seen with morphine.

The results indicate that crotalphine may represent a superior option for the treatment of neuropathic pain compared to currently available medications such as morphine. Although clinical trials have not yet been conducted, the existing evidence points to a promising potential for this biomolecule to open avenues for advances that could improve the quality of life of patients suffering from chronic pain.

3.4. Crotamine

Crotamine is a basic polypeptide isolated from the venom of *Crotalus durissus terrificus*. It is an amphipathic, non-enzymatic molecule composed of 42 amino acid residues, including 11 basic residues and 6 cysteines forming three disulfide bonds that stabilize its three-dimensional structure. The myotoxic effect of this protein was initially considered its main contribution to envenomation, associated with its action on intracellular calcium homeostasis, swelling of the sarcoplasmic reticulum, and modulation of voltage-gated sodium and/or potassium channels.

More recently, crotamine has been recognized as a cell-penetrating peptide (CPP) due to its ability to penetrate cells both *in vitro* and *in vivo*, and to electrostatically interact with centrioles and chromosomes. Crotamine is rapidly internalized during the S/G2 and G2/M phases of the

cell cycle, being detected in the cytoplasm or bound to centrioles and chromosomes. At low incubation temperatures (e.g., 4 °C), it remains on the cell surface. Crotamine can also interact with both single- and double-stranded DNA. This interaction is influenced by salt concentration but can only be reversed by detergents. Its electrostatic affinity for nucleic acids supports its potential use as a gene delivery vector.

Selective internalization of native crotamine has been demonstrated *in vitro* in mouse blastocysts, embryonic stem cells, and several cancer cell lines, and *in vivo* in brain, kidney, lung, peritoneal, and bone marrow cells. Because crotamine shows lower toxicity toward non-tumoral cells than toward tumoral ones, it has been proposed as a theranostic agent, a cell cycle marker, and/or a carrier of bioactive molecules.

In addition to intraperitoneal injection, which remains the preferred route of administration, crotamine has also been shown to be effective when administered orally. Moreover, crotamine can be immobilized on gold nanoparticles and successfully delivered into the cytoplasm of cells, according to a work by Hayashi et al. (2022).

Crotamine shares several structural and functional features with β-defensins, one of the two major groups of antimicrobial peptides (AMPs). Accordingly, it has been classified as an AMP. It exhibits inhibitory activity against various *Escherichia coli* strains, with minimal inhibitory concentrations (MICs) ranging from 25 to 100 μg/mL. However, its antimicrobial spectrum appears to be narrow, as it shows little or no activity against other Gram-negative and Gram-positive bacteria, except for *Micrococcus luteus*, with MICs between 1 and 2 μg/mL.

Intramolecular disulfide bonds seem dispensable for the antibacterial function of the native full-length crotamine, whereas cysteine residues are essential for the antimicrobial activity of smaller crotamine-derived peptides. Crotamine permeabilizes bacterial membranes and kills the cells within one hour, with maximal activity observed after two hours of incubation. Its preference for negatively charged membranes likely explains this mechanism.

Crotamine also exhibits antifungal activity against *Candida* spp., *Trichosporon* spp., and *Cryptococcus neoformans*, with MICs ranging from 12.5 to 50 μg/mL. More recently, it has been reported to be active against the multidrug-resistant human pathogen *Candida auris*, highlighting its potential as a valuable AMP with candidicidal and anti-yeast properties.

Additionally, crotamine displays antiparasitic activity, particularly against *Plasmodium falciparum*, one of the etiological agents of human malaria, by inhibiting parasite growth ($IC_{50} \approx 1.87$ μM). This occurs through the internalization of crotamine by red blood cells infected with the parasite. Furthermore, crotamine reduces the fluorescence of parasite organelles, indicating disruption of their normal function, as under physiological conditions these structures accumulate the lysosomotropic fluorochrome acridine orange and appear fluorescent under microscopy. Since

crotamine lacks hemolytic activity, it represents a promising molecule for developing new antimalarial drugs, especially due to its affinity for acidic organelles of the parasite.

The same affinity underlies its anthelmintic activity against the nematode *Caenorhabditis elegans*, a model organism capable of developing resistance to conventional anthelmintic drugs. This suggests that crotamine could serve as a therapeutic agent for infections caused by parasitic nematodes, including drug-resistant species.

Because cancer cells display a higher density of negatively charged molecules on their surface, crotamine exhibits greater affinity for these cells than for normal ones. Once internalized, crotamine accumulates in acidic vesicles, leading to tumor cell death. This cytotoxicity is mediated not only by lysosomal lysis but also by mitochondrial depolarization and intracellular calcium overload. The preferential uptake of crotamine by highly proliferative cells is consistent with its relatively low toxicity toward normal mammalian cells, reinforcing its potential as a tumor-selective marker or theranostic agent.

In experiments using C57BL/6 mice bearing subcutaneous melanoma tumors, daily administration of 1 μg of crotamine per animal effectively inhibited tumor growth and increased survival after 21 days of treatment. Oral administration of 10 μg per day for the same period reduced tumor size by approximately 80%. Oral delivery thus appears to be an effective method that may reduce crotamine-associated toxicity typically observed with higher parenteral doses.

In healthy mice, crotamine predominantly accumulates in the kidneys without causing observable toxicity. After 21 days of treatment, no significant changes were found in body weight or major organ weights, and biochemical markers of kidney and liver function remained unaltered. Crotamine was internalized by proximal tubular epithelial cells (PTECs) in the kidney, successfully delivering a plasmid DNA vector and enabling the expression of the green fluorescent protein (GFP) reporter. This shows crotamine's potential for targeted delivery of therapeutic molecules to specific renal tissues.

Crotamine also exhibits antinociceptive effects in adult male Swiss mice, with an efficacy approximately 30 times greater than that of morphine via intraperitoneal or subcutaneous routes at doses around 0.4% of the LD_{50}. The inhibition of its analgesic effect by naloxone indicates a mechanism similar to that of opioids. Importantly, crotamine did not induce hyperalgesia.

Its antinociceptive and anti-inflammatory properties have also been demonstrated following oral administration. In acetic acid-induced abdominal pain and formalin-induced pain tests, crotamine at 0.16 mg/kg reduced the nociceptive response by 45% in the neurogenic phase and by 60% in the inflammatory phase, without signs of toxicity up to the highest tested dose (11 mg/kg). Crotamine also reduced croton oil-induced ear edema by 77% and decreased

carrageenan-induced pleurisy. Leukocyte migration was reduced by 52%, neutrophils by 46%, and mononuclear cells by 59%.

It should be noted, however, that the native crotamine used in this particular study was purified from the venom of *C. d. collilineatus*, a different subspecies, using a purification method that may not ensure complete protein purity. Such methodological differences should be considered when interpreting the results.

3.5. Gyroxine

Gyroxine is another peptide isolated from the venom of the snake *Crotalus durissus terrificus*. It is a serine protease with a thrombin-like function, exhibiting procoagulant properties and potentially participating in hemostasis.
Its function is based on the hydrolysis of peptide bonds within fibrinogen, allowing it to polymerize into fibrin. Fibrin then forms networks that stabilize the clot. Gyroxine recognizes the amino acid arginine (Arg) at the P1 position of substrates, specifically cleaving those peptide bonds.

Fibrin sealants play an important role in hemostasis. They promote angiogenesis and collagen synthesis, and contribute to accelerated re-epithelialization. They have also been used as adhesives in reconstructive and plastic surgeries.

Fibrinogen is cleaved into two fibrinopeptides, A (FPA) and B (FPB). Thrombin cleaves both peptides to form fibrin and activates factor XIII, whereas some snake venom thrombin-like enzymes (SVTLEs) cleave only one of them, thus failing to activate factor XIII and producing unstable clots that are rapidly degraded by plasmin.

A heterologous fibrin sealant has been proposed, composed of fibrinogen-rich cryoprecipitate derived from *Bubalus bubalis* (water buffalo) blood, incubated with gyroxine from *C. d. terrificus*. This sealant mimics the final steps of the coagulation cascade, converting fibrin monomers into a stable clot.

Intravenous injection of this protein has been shown to induce barrel rotation syndrome in mice, a condition in which the animal repeatedly rotates along its longitudinal axis involuntarily. This indicates that the toxin acts on the central nervous system. Although the exact mechanism remains unknown, the procoagulant activity of gyroxine is thought to involve protease-activated receptors (PARs), which are also believed to be associated with this syndrome.

Five distinct gyroxine isoforms have been identified in the *C. d. terrificus* cDNA, although their biological activities have not yet been characterized. Nonetheless, these isoforms offer new therapeutic possibilities and warrant further study.

Serine proteases such as gyroxine may be useful for the early detection of certain cancers, since altered levels of coagulation factors or prostate-specific antigen (PSA) can serve as indicators of thrombosis or prostate cancer.
PARs are also involved in the proliferation, growth, and invasiveness of several cancer types, mainly PAR-1 and PAR-2. Consequently, the development of protease inhibitors and receptor active-site blockers has been investigated. Since gyroxine can prevent the activation of PAR-1 in cultured cells, it may potentially help modulate PAR function in cancer cells, thereby influencing their proliferation, growth, and invasiveness.

3.6. Bradykinin-Potentiating Peptides from *Crotalus durissus*

Bradykinin-potentiating peptides (BPPs) from snake venom are short linear peptides rich in proline residues. They were first isolated from *Bothrops jararaca* venom but are also found in other species such as *Crotalus durissus terrificus*.

Reported effects include smooth muscle relaxation and antihypertensive activity, which may result from a combination of the reduction of circulating angiotensin II levels through inhibition of angiotensin-converting enzyme (ACE) and the activation of argininosuccinate synthase, a key enzyme in L-arginine synthesis, which is the precursor of nitric oxide (NO), a potent vasodilator.

The main application of BPPs has been the development of the first active-site–targeted antihypertensive drug, captopril. Their potential use has also been suggested in the development of anti–SARS-CoV-2 drugs and the management of mental disorders, but current research has not produced conclusive results, leaving this as a topic for future investigation.

The wide variety of unique proteins found in the venom of a single snake species makes *Crotalus durissus terrificus* one of the most valuable resources in Peru in terms of animal-derived biomolecules, particularly for the development of new therapeutic and treatment tools. Further studies are still required to determine the viability and safety of proteins obtained from this species' venom.

Supporting such research is essential, as it may contribute to improving the quality of life of patients suffering from hard-to-treat conditions such as chronic neuropathic pain, as well as infectious diseases like malaria, which can cause localized epidemics in developing regions during periods of increased vector populations.

Moreover, the proteins in *C. d. terrificus* venom hold great potential for the development of therapeutic tools aimed at treating cancer and combating bacterial, parasitic, and viral diseases, including hepatitis C.

Table 2. *Biomolecules from Crotalus and their potential uses*

Potential uses for biomolecules from *Crotalus*	
Biomolecule	**Possible use**
Crotoxin	• Antimicrobial activity • Immunomodulatory activity • Antitumor activity • Anti-inflammatory activity • Analgesic activity • Antiviral activity (Hepatitis C)
Crotalphine	• Potent and long-lasting analgesic effect
Crotamine	• Gene delivery vector • Theranostic agent • Antimicrobial activity, although with a narrow spectrum • Antifungal activity • Antiparasitic activity (Plasmodium falciparum – malaria) • Antitumor activity • Inhibition of tumor growth • Renal targeting (affinity for the kidneys) • Analgesic effects • Anti-inflammatory activity • Gyroxina
Gyroxin	• Procoagulant activity
Bradikinin-potentiating peptides	• Antihypertensive activity

4. *Lachesis*

Lachesis muta, commonly known as the *shushupe* or South American bushmaster, is the only species of the genus *Lachesis* found in Peruvian territory. It is a large venomous snake, with the largest individuals reaching around 3.5 meters in length.

Its venom contains a variety of biomolecules, which, although as diverse as those of other snake groups, have been less extensively studied. Some biomolecules present in the venom of

L. muta that remain unexplored include a disintegrin named lachesin, three-finger toxins (3FTx), serine proteases, and C-type lectins (CTLs). It is also important to mention that, although there have been studies on the many proteins from *L. muta* venom, some of these papers are not open-access, so I could not obtain that information for this research. Most existing research has focused primarily on the activity of its L-amino acid oxidases, a class of enzymes shared with other venomous snake groups.

This suggests that there are still potentially therapeutic properties yet to be discovered, making *L. muta* venom a promising subject for the study of new biotechnological tools and treatments for various pathologies. So far, it is known that some of the proteins in this snake's venom exhibit antitumor and antiparasitic activities. However, further studies—similar to those conducted on other snake venom biomolecules—are still needed to assess their therapeutic viability and safety for human use.

4.1. LAAO from *Lachesis muta*

The L-amino acid oxidase isolated from *Lachesis muta* venom (LmLAAO) shows a catalytic preference for hydrophobic amino acids, similar to the LAAOs of other venomous snake species. This is due to the fact that the substrate-binding sites of LAAOs contain three hydrophobic subsites. Snakes in general appear to conserve this structural configuration in their catalytic sites.

The enzymatic activity of LmLAAO was tested at pH values ranging from 7.0 to 9.0, showing optimal hydrolysis of L-leucine at pH 8.0. A pH higher or lower than this value can cause structural changes in the enzyme that may interfere with its catalytic function. Regarding temperature, LmLAAO retained only 5.9% of its enzymatic activity after undergoing a freeze–thaw process at −70 °C. The ideal storage temperature for the enzyme is 4 °C.

When the enzymatic kinetics of LmLAAO were tested using L-leucine as a substrate, the enzyme exhibited a strong affinity for this amino acid:

- **Vmax (maximum velocity):** The maximum reaction rate when all enzyme active sites are saturated. It indicates the maximal catalytic capacity of the enzyme under these conditions. LmLAAO showed a Vmax of 0.063 ± 0.002 mmol/min.

- **Km (Michaelis constant):** The substrate concentration at which the enzyme reaches half of its maximum velocity. It reflects the affinity between the enzyme and the substrate; a lower Km indicates higher affinity. LmLAAO exhibited a Km of 0.97 ± 0.07 mmol/L.

LmLAAO showed a similar amino acid sequence to other snake venom LAAOs. *Lachesis muta* venom contains a single LAAO. The final section of the structural model remains uncharacterized due to insufficient data.
In general, SV-LAAOs (snake venom LAAOs) possess three domains: one FAD-binding domain, one substrate-binding domain, and one α-helical domain. The FAD cofactor is located in a cavity formed between the cofactor-binding and substrate-binding domains. The structure of all SV-LAAOs is highly conserved, showing approximately 80% sequence identity.

Minor sequence differences between LmLAAO and other SV-LAAOs do not appear to significantly affect the charge or spatial distribution of the protein. Comparison of the predicted structure with its template (LAAO from *Gloydius halys*) revealed that the cofactor- and substrate-binding domains, as well as the glycosylation sites, remained intact—indicating that the conservation of these sites is crucial for enzymatic function.

Intradermal injection of 50 µg of the enzyme into the abdominal region of mice caused no hemorrhage. Similarly, injection of 10 µg into the paws did not induce edema. Twenty-four hours after intravenous administration of 100 µg of LmLAAO, no morphological changes were observed in the cardiac, pulmonary, or renal tissues of the mice.

Two batches of LmLAAO were injected into the quadriceps of two groups of mice. An increase in serum creatine kinase levels—a marker of muscle damage—was detected, indicating that although mild, LmLAAO exhibits myonecrotic activity. Comparable results between both test groups demonstrated the reliability of the purification method.

Like LAAOs from other snake species, LmLAAO exhibits cytotoxic effects against certain human tumor cell lines. In the study led by Bregge-Silva et al. (2012), the enzyme was cytotoxic to human gastric adenocarcinoma (AGS) and human breast cancer (MCF-7) cells. This effect is associated with hydrogen peroxide release into the medium, as catalase treatment inhibits the cytotoxic activity of LmLAAO.

Other cell lines in which LAAO has been shown to inhibit tumor growth include HL-60 (acute promyelocytic leukemia), HeLa (human cervical adenocarcinoma), Glioma (human brain tumor), A2780 (human ovarian epithelial carcinoma), HUVECs (human umbilical vein endothelial cells), NR-3 (murine non-tumor endothelial cells), EL-4 (murine lymphoma), SKBR-3 (human breast adenocarcinoma), Jurkat (human T-cell leukemia), EAT (murine mammary tumor), AGS (human gastric adenocarcinoma) and MCF-7 (human breast carcinoma).

Additionally, LmLAAO was tested as an antiparasitic agent against *Leishmania braziliensis*. Similar to its cytotoxic effect, this antiparasitic activity was also linked to hydrogen peroxide generation, as catalase administration abolished the leishmanicidal effect of the enzyme. LAAO

from *Bothrops moojeni* was used as a comparison. The *Lachesis muta* enzyme was slightly less effective, requiring a somewhat higher dose to achieve the same antiparasitic effect.

The enzyme was also tested against *Trypanosoma cruzi*, the causative agent of Chagas disease. The IC_{50} value could not be determined, as the highest concentration of LmLAAO tested did not kill 50% of the parasites. *T. cruzi* has previously been shown to display resistance to LAAOs, particularly that from *Bothrops atrox*.

4.2. Phospholipases A_2 from *Lachesis muta*

Sharing several protein families with other viperid venoms, Lachesis muta also contains phospholipases A_2 (PLA_2s) in its venom. These enzymes are being investigated for their potential in the development of novel drugs. In particular, an acidic PLA_2 isolated from whole venom ($LAPLA_2$) is being studied for its potential role in promoting skin regeneration.

The process of skin regeneration is complex, dependent on multiple interrelated mechanisms, and prone to disruption by external agents, such as metabolic diseases—like diabetes—or microbial infections caused by bacteria, fungi, or parasites. Although the body possesses mechanisms to protect itself, skin wounds serve as entry points for opportunistic infections, which can lead to complications or, in severe cases, death.

In the United States alone, approximately 28 billion dollars are spent annually on wound treatment. This high cost is partly due to chronic wounds—injuries whose full recovery takes more than three months, often because of one or more interfering factors that hinder processes related to tissue repair. Treating such wounds is expensive, time-consuming, and sometimes not entirely effective. This highlights the need for new therapeutic strategies that broaden the options available to both professionals and patients.

While animal-derived biomolecules are currently being studied for their therapeutic properties in various biomedical fields, regenerative medicine largely remains beyond the reach of such investigations.

To explore a novel alternative for the development of regenerative therapies, the proliferative and healing properties of $LAPLA_2$ isolated from L. muta venom were evaluated. The venom of this snake is known to be complex, containing numerous enzymatic and non-enzymatic compounds. Antimicrobial properties have already been reported, but current research seeks to explore new medical applications for its proteins—particularly within regenerative medicine. The study led by García et al. (2020) aimed to determine whether this specific enzyme could assist in the wound-healing process.

The proliferative activity of $LAPLA_2$ was tested in fibroblast and keratinocyte cultures. A marked, time-dependent stimulation of cell proliferation was observed. The highest concentration of

crude venom to which HaCaT keratinocytes were exposed (20 µg/mL) resulted in a 59% reduction in cell viability, whereas cultures treated with purified $LAPLA_2$ remained fully viable.

Cell proliferation in HaCaT keratinocytes increased significantly when treated with 10 µg/mL of $LAPLA_2$ compared to those treated with crude venom. Doubling the dose did not further enhance the proliferative effect. In both cases, cell viability remained at 100%.

The antioxidant and regenerative activities of $LAPLA_2$ were assessed in a murine model consisting of four groups: a negative control group (C) treated with physiological saline solution (0.9% NaCl); a positive control group (SS) treated with 1% silver sulfadiazine; group L1, treated with 0.5% $LAPLA_2$; and group L2, treated with 0.25% $LAPLA_2$.

Wounds were made under aseptic conditions and treated topically once per day for 12 days. Male Swiss-Webster mice were used. The animals were anesthetized, the dorsal area was shaved, and a circular incision was made with a scalpel down to the dorsal muscle fascia. Wounds were cleaned with saline solution before applying the treatments. In group L1, the wound area was significantly reduced compared to group C.

Tissue samples were collected every four days. Animals treated with $LAPLA_2$ showed increased tissue cellularity relative to group SS on day 4. On day 8, group L2 exhibited greater cellularity than the other groups. By day 12, treated groups showed reduced cellularity compared to the control, with L2 values lower than those of group SS.

After 4 days, group L1 displayed greater blood vessel density compared to the control, SS, and L2 groups. By day 8, vessel density in groups L1, L2, and SS had increased relative to the control. Group L2 showed a higher mast cell count than the control on day 4, while both L1 and L2 had higher counts than SS on day 8. Treated groups maintained higher mast cell numbers on day 12 compared to the control.

On day 4, type III collagen levels were higher in groups SS, L1, and L2 compared to the control. On day 8, greater deposition of type III collagen was observed. By day 12, this parameter had increased in groups L1 and SS relative to the control and L2. On day 8, groups L1 and L2 exhibited higher type I collagen deposition compared to control and SS groups. The SS and control groups showed predominance of type III collagen, while $LAPLA_2$-treated groups displayed higher deposition of type I collagen.

In vivo, 0.5% $LAPLA_2$ increased catalase (CAT) and superoxide dismutase (SOD) activities while decreasing malondialdehyde (MDA) and carbonylated protein (CP) levels in scar tissue samples. SOD activity was higher in group L1 than in the other groups on day 4, and the control group also showed significantly higher SOD activity than L2 and SS. On day 8, SOD activity in group L2 surpassed that of the other groups, and CAT activity also increased in L2 compared to

the others. CAT activity in the control was higher than in L1. On day 12, CAT levels in L2 remained elevated compared to SS and the control.

On day 4, MDA levels decreased in LAPLA$_2$-treated groups compared to SS. By day 12, MDA levels in L1, L2, and SS groups were lower than in the control. CP levels decreased on days 4 and 8 in groups L1, L2, and SS relative to the control, and by day 12, CP levels in L2 were lower than in SS and control groups.

The peptide effectively stimulated cell proliferation, neoangiogenesis, and deposition and maturation of type I and III collagen in a time-dependent manner, thereby reducing wound closure time.

The results indicated that LAPLA$_2$ has remarkable therapeutic potential, as it improved oxidative balance and the structural reorganization of scar tissue. This occurred through stimulation of cellularity, angiogenesis, collagenogenesis, and wound contraction. These findings suggest that this enzyme from Lachesis muta venom could be a promising candidate for the development of a new wound-healing drug.

In addition to LAPLA2, other PLA$_2$ isoforms have been isolated from the venom of *Lachesis muta*. One of them is found in the venom of the subspecies *L. m. rhombeata*. Although this subspecies is not present in Peru, its PLA$_2$ is highly homologous to those from *L. m. muta*, such as LmTX-I and LmTX-II. This suggests that the characteristics observed in one may also be present in the others. Therefore, it is reasonable to assume that the isoforms from *L. m. muta* could play the same or a similar role in research as the isoform obtained from the venom of *L. m. rhombeata*.

Since PLA$_2$s constitute a broad and diverse family of proteins capable of altering homeostasis, LmrTX was evaluated for its ability to influence thrombus formation. In other words, the study conducted by Damico et al. (2012) aimed to assess a possible effect of the protein on blood coagulation.

To this end, a photochemically induced arterial thrombosis model was used in mice. The parameter measured was the time required for arterial occlusion to occur. A longer occlusion time indicates a greater delay in coagulation caused by the protein. The control group, which did not receive the protein, showed a normal occlusion time of 57 ± 7.8 minutes.

Mice received doses of 3.25, 7.5, and 15 µg/mouse. The lowest dose did not produce a marked difference (62.6 ± 10 minutes). In contrast, the 7.5 and 15 µg/mouse doses resulted in significant increases in occlusion time, with 99 ± 10 minutes and 94 ± 11.5 minutes, respectively. This indicates that the effect occurs at medium and high doses.

The enzyme also exhibited anticoagulant activity in vitro. Under these conditions, LmrTX prolonged the coagulation time of platelet-poor plasma, as assessed by the activated partial thromboplastin time (APTT).

In addition, LmrTX was administered to mice at different intervals prior to APTT determination. The enzyme showed significant activity, with a maximal response 15 minutes after injection, which persisted for approximately one hour. No effect was observed on PT, either in vitro or ex vivo.

The effect of LmrTX on platelet aggregation was also examined. Animals received 15 µg of the enzyme. A 43% inhibition of ADP-induced aggregation and a 44% inhibition of thrombin-induced aggregation were observed. Platelets were obtained from the animals' blood and subsequently washed.

It has been shown that modification of the His48 residue in snake venom PLA_2s suppresses their enzymatic activity by nearly 90%. This residue plays a crucial role in the function of these proteins.

Taken together, these findings reveal another enzyme with potential utility in the treatment of thromboembolic diseases, as it delays clot formation and partially inhibits platelet aggregation.

One final PLA2 from *Lachesis muta* that we would like to mention is LM-PLA2-I. This protein has been evaluated for its possible role in physiological processes involving neuronal cells. This interest arises from the fact that, in the nervous system, PLA2 enzymes participate in several physiological responses, such as neurotransmitter release, neurite outgrowth, membrane repair, and cell growth and differentiation.

With this objective, the effect of LM-PLA2-I on the survival of axotomized retinal ganglion cells—cells that have lost their axon—was analyzed. Retinal ganglion cells that undergo axotomy normally activate a series of events that lead to apoptosis. However, some are capable of surviving, and certain treatments can promote survival and even stimulate axonal regeneration.

Different concentrations of the enzyme, ranging from 2.5 to 12.5 µg/mL, were added to the culture medium. Cultures were maintained for 48 hours. After this period, the greatest increase in cell survival was observed at 5 µg/mL, which produced a 50% increase in the survival of retinal ganglion cells. Higher concentrations produced a smaller effect on survival, although neuronal outgrowth was observed—a surprising result.

To investigate the mechanism, it was determined that the effect depends on the enzymatic activity of LM-PLA2-I. This was confirmed by treating the enzyme with p-BPB, an inhibitor that binds to the catalytic site of PLA2 enzymes and blocks their activity. This treatment abolished

the effects of LM-PLA2-I on the cells, indicating that the observed activity depends on the release of lysophosphatidylcholine (LPC) generated by the enzyme.

These findings open an important window for research, as they suggest a potential therapeutic approach for neurological diseases. The neuronal protection induced by LM-PLA2-I could contribute to therapies for optic nerve injury, glaucoma, neuropathies, and even neurodegenerative diseases. The observed induction of neuronal outgrowth is particularly noteworthy.

4.3. Bradykinin-Potentiating Peptides from *Lachesis muta*

As with other viperid venoms, *Lachesis muta* venom contains bradykinin-potentiating peptides (BPPs). These peptides are responsible for the venom's hypotensive action and may serve as models for the development of new antihypertensive drugs in humans.

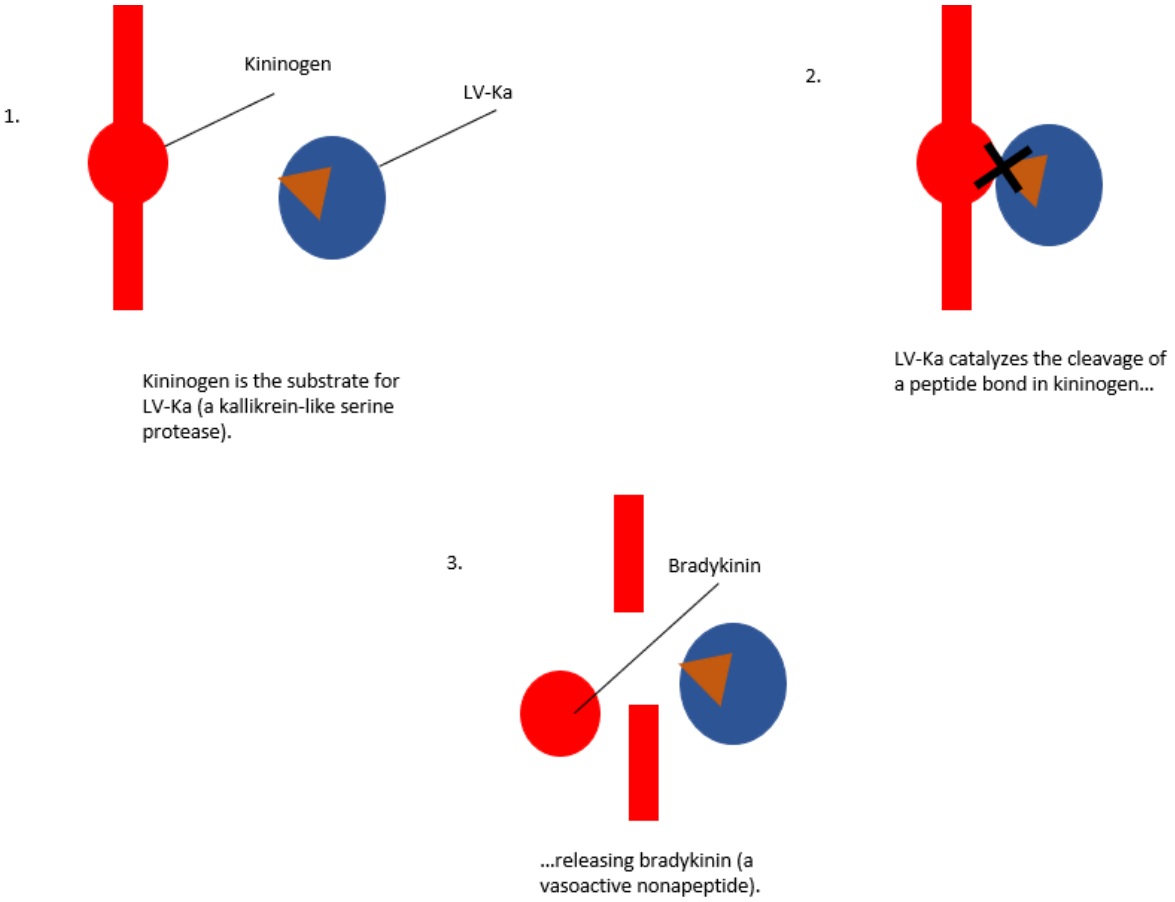
Although the presence of these peptides in *L. muta venom* is well established, it should be noted that, based on the study by Pinheiro-Júnior et al. (2018), the sub-species from which BPPs have been isolated and synthesized is L. m. rhombeata. The Peruvian subspecies, L. m. muta, has not yet been studied—or at least no studies were found during the preparation of this text—regarding its BPPs as potential antihypertensive agents.

The specific BPP extracted from the crude venom of *L. m. rhombeata* (LmrBPP9) has been synthesized and tested as antihypertensive compounds in hypertensive rats, showing effective inhibition of angiotensin-converting enzyme (ACE). Although the venom of *L. m. rhombeata* is known to exhibit a stronger hypotensive effect than that of *L. m. muta*, it would still be valuable to investigate which structural or compositional features account for this difference in efficacy—or whether *L. m.* muta's enzymes possess other properties that could complement or enhance those of the former subspecies.

4.4. Serine protease from *Lachesis muta*

Another protein from *Lachesis muta* venom has also been identified that contributes to vasodilatory action, this time through the direct generation of bradykinin. LV-Ka is a serine protease isolated from crude *L. m. muta* venom, with activity similar to kallikrein.

Figure 5. *Mechanism through which LV-Ka cleaves kininogen*



An experiment was conducted to compare the action of LV-Ka with that of bradykinin, another potent vasodilator. 600 µL of bovine kininogen was incubated with 3.8 µg of LV-Ka at 37 °C for 20 minutes in a 20 mM Hepes buffer, pH 8.0. Aliquots ranging from 5 µL to 300 µL were then added to three different organ baths. A fourth bath received bradykinin at 100 µM as a positive control. This setup allowed evaluation of kinin production induced by LV-Ka.

The tissue tested consisted of mesenteric arterial rings from spontaneously hypertensive rats (SHR).

When the mixture was applied to both endothelium-intact and endothelium-denuded rings, both contracted, with no appreciable difference between them.

Responses to the LV-Ka/bovine kininogen mixture and to bradykinin were reproducible when appropriate intervals between consecutive challenges were maintained. Challenges with bradykinin produced similar responses at 20-minute intervals, whereas the LV-Ka/kininogen mixture required 60-minute intervals for reproducible responses.

At 300 µL of the incubated mixture, contractions reached 95 ± 5% of those induced by 60 mM KCl, and were comparable to responses elicited by 1 µM bradykinin. Responses to smaller volumes of the mixture were proportionally smaller, indicating a dose-dependent effect.

Administration of LV-Ka or kininogen alone did not induce contractions. Furthermore, both the action of 300 µL of the mixture and 1 µM bradykinin were completely inhibited by 10-minute preincubation with 1 µM indomethacin.

Both indomethacin and HOE-140, a B2 bradykinin receptor antagonist, blocked the contractile effects of the mixture and bradykinin. Despite this, the tissue still responded to norepinephrine and KCl, demonstrating preserved vascular contractility.

These results indicate that the LV-Ka/bovine kininogen mixture effectively generates bradykinin or similar peptides, providing a potential new avenue for the development of antihypertensive drugs, in addition to previously described BPPs.

Another serine protease from *L. m. muta* venom that has shown medical potential is the enzyme known as LV-PA. This enzyme is a plasminogen activator: it acts by cleaving this precursor to generate plasmin. This protease interacts with a wide range of substrates, but its main function is to degrade fibrin in blood clots, thereby breaking them down into soluble fragments.

An enzyme capable of enhancing plasmin activity could be useful for the treatment of thromboembolic diseases, since its ability to facilitate clot degradation directly counteracts the main pathological feature of these disorders: the uncontrolled formation of clots throughout the circulatory system. For this reason, the activity of LV-PA was evaluated in order to determine its effectiveness in activating plasminogen.

The enzyme was incubated with human plasminogen for extended periods (30 to 240 minutes) at 37 °C. The substrate–enzyme ratio was 50:1. A dose-dependent lysis of fibrin clots was observed. In addition, the plasmin generated by the mixture of LV-PA and human plasminogen was tested using the substrates S-2251 and Tos-Gly-Pro-Lys-pNA. The resulting protease showed a higher affinity for S-2251.

Although plasminogen activation was slow and of moderate potency, these results indicate that the enzyme indeed has potential as a clot-lysing agent. Further studies are needed to determine which portion of the enzyme limits its functional efficiency, and in what ways this serine protease could play a role in the development of new therapies. Tests could also be carried out to evaluate the synergy between LV-PA and other plasmin-activating enzymes, allowing it to act in a complementary manner with other compounds to achieve a better therapeutic effect.

4.5. Metalloproteases from *Lachesis muta*

A study conducted by Estevão-Costa et al. (2008) isolated two metalloproteases from the crude venom of Lachesis muta muta. These proteins, named mutalysin I and mutalysin II, belong to the group of endopeptidases; that is, enzymes capable of breaking peptide bonds within a protein.

Both mutalysins display biochemical characteristics that make them interesting candidates for the development of antithrombotic drugs. In particular, they exhibit fibrinogenolytic and fibrinolytic activity.

The mutalysins degrade fibrinogen in such a way that it can no longer be coagulated by thrombin, effectively inhibiting its function. This occurs at substrate/enzyme molar ratios of 10:1 for mutalysin I and 29:1 for mutalysin II. The enzymes act mainly on the Aα chain of fibrinogen, while the Bβ chain is also degraded, although more slowly. In contrast, the γ chain remains essentially unaffected.

In addition to degrading fibrinogen, both mutalysins can hydrolyze preformed fibrin clots, although with differences in efficiency. When the fibrin clot was incubated with mutalysin I at a molar ratio of 11:1, the Aα chain was the most affected. In contrast, mutalysin II—at a ratio of 45:1—completely degraded the Aα chains and the γ–γ dimer within 15 minutes, while the Bβ chain showed partial degradation after two hours of incubation.

The combined ability of the mutalysins to inhibit clot formation and promote the lysis of already polymerized fibrin makes them potential tools for the management of thrombotic diseases. In theory, they could contribute to the prevention or treatment of myocardial and cerebral infarction, deep vein thrombosis, pulmonary embolism, and the prevention of further growth of preexisting thrombi.

Finally, the mutalysins can also inhibit the interaction of platelets with collagen due to their ability to degrade type I collagen. This anti-adhesive effect could further enhance their value as starting points for the development of new antithrombotic drugs.

The venom of *Lachesis muta*, being a complex mixture containing several families of proteins with different biological functions, still presents many avenues for research. Other proteins remain to be further explored. Together with the previously mentioned components, these molecules may prove valuable for biomedical research, both in the development of novel cancer therapies—similarly to LAAOs, PLA_2s, and disintegrins from other snake species—and in the treatment of various diseases, such as the potential use of SVMPs for thrombotic disorders, BPPs for hypertension, and topical regenerative therapies.

Table 3. *Biomolecules from Lachesis and their potential uses*

Potential uses for biomolecules from *Lachesis*	
Biomolecule	**Possible uses**
LmLAAO	• Antitumor activity • Inhibition of tumor growth • Antiparasitic activity (Leishmania and Trypanosoma)
PLA_2s	• Skin regeneration • Anticoagulant activity • Neuronal protection
BPPs (e.g. LmrBPP9)	• Antihypertensive activity
Serine proteases (e.g. LV-Ka, LV-PA)	• Antihypertensive activity • Anticoagulant activity
Metalloproteases (e.g. mutalysin I and II)	• Anticoagulant activity

5. *Micrurus*

Micrurus species, commonly known as coral snakes, represent the other major group of venomous snakes found in Peru. They are distributed mainly throughout the Amazon rainforest and, to a lesser extent, in Andean regions. These snakes are elusive, possess small fixed front fangs, and their venom exhibits predominantly neurotoxic activity.

The difficulty of maintaining coral snakes in captivity, combined with the small amount of venom they produce and its relative instability during storage, has historically limited the study of their protein components. Nevertheless, the analyses conducted so far have revealed remarkable therapeutic potential, for instance in the development of anticancer treatments, due to the cytotoxic activity of certain enzymes.

Nevertheless, the studies conducted so far show promising potential. The whole venoms of different species have demonstrated cytotoxic activity against cancer cell lines, as well as cytotoxic activity against parasites such as *Plasmodium falciparum* y *Leishmania amazonensis*. Although some of these venoms exhibit adverse effects, such as edema or nociception, the key lies in understanding the mechanisms underlying these responses and finding a way to isolate the beneficial compounds, while either discarding the harmful ones or identifying new applications for them.

Further research is still required, which, together with emerging analytical methodologies, could enable a more comprehensive understanding and lead to the discovery of new biomedical applications for the venoms of these snakes.

5.1. PLA_2s from *Micrurus*

The phospholipases A_2 (PLA_2s) present in elapid venoms are associated with neurotoxicity and myotoxicity. In several *Micrurus* species, both presynaptic neurotoxicity and systemic myotoxicity have already been described. In addition, PLA_2s are known to exert other venom-related effects. For instance, PLA_2s from *Micrurus fulvius* venom induce hemolysis in mice, and similar effects have been reported in dogs. Interestingly, this hemolytic activity is not observed in red blood cells from other mammals, including humans, and it remains unknown whether this effect occurs in the natural prey of *M. fulvius*.

A PLA_2 from *M. frontalis* was shown to cause pulmonary hemorrhage in mice, despite not producing hemorrhage in the mouse skin test. The specific biological mechanisms underlying these effects are still unclear, but they illustrate the versatility of PLA_2 actions in coral snake venoms.

Although myotoxicity is not a prominent symptom in human envenomation by *Micrurus*, experimental studies in rats, mice, and colubrids (natural prey of coral snakes) have shown muscle degeneration and myonecrosis. This discrepancy may be due to the relatively small doses injected into humans during bites.

The myotoxic action of the venom has been detected 1–3 hours after intramuscular injection, by measuring creatine kinase activity in plasma. This has been confirmed by direct observation of necrosis in skeletal muscle fibers.

Both myotoxic and non-myotoxic PLA_2 isoforms coexist in *Micrurus* venoms, making it of interest to determine which structural features dictate their toxic activity. Myotoxic venom activity has been proposed as an adaptation for early digestion, making prey muscle tissue more susceptible to breakdown by gastric enzymes.

Although the neurotoxic PLA2s of *Micrurus* generally exhibit moderate or low cytotoxicity, this should not lead to dismissing a potential cytotoxic role against microbes or tumor cells. For example, the neurotoxin from *Crotalus durissus terrificus* is a neurotoxic PLA2, yet it still shows cytotoxicity toward tumor cell lines. In the case of crotoxin, this effect is due to its association with crotapotin, which enables the toxin to reach its site of action; without crotapotin, crotoxin B would not be as effective. Therefore, it is a plausible hypothesis that a similar delivery mechanism could potentially enhance the cytotoxic capabilities of the neurotoxic PLA2s from *Micrurus*, opening the possibility of using them as tools for treating microbial infections and cancer.

5.2. 3FTx from *Micrurus*

Proteomic studies of viperid and elapid venoms have shown that they are composed of a relatively limited set of protein families. However, these families are expressed in many different proteoforms, increasing venom complexity. In *Micrurus* venoms, the number of protein families ranges from as few as 3 to as many as 11, generally between 6 and 8. Only two protein families are universal across the genus: three-finger toxins (3FTx) and phospholipases A_2 (PLA_2). Other common but not universal protein families in *Micrurus* include metalloprotease (MP), L-amino acid oxidases (LAAO), kunitz-type serine protease inhibitors (KUN), serine proteases (SP) and C-type lectin-like proteins (CTL)

Other protein families are present in only some species and are considered cellular contaminants with no apparent toxic role.

Relative protein abundance in venoms is typically determined in three steps: (1) chromatographic separation of protein fractions; (2) SDS-PAGE to resolve protein bands and estimate their relative abundance; (3) mass spectrometry to identify proteins when multiple species are present in the same band.

The two ubiquitous components (3FTx and PLA_2) are by far the most abundant. The others vary in abundance from 1% to 10%, with Kunitz inhibitors sometimes reaching ~10%, ranking as the third most abundant family. By contrast, some families are much less abundant (0.1–1%), such as: cysteine-rich secretory proteins (CRISP), acetylcholinesterase (AChE), phospholipase B (PLB), phosphodiesterase (PDE), 5′-nucleotidase (5′-Nuc), hyaluronidase (HYA), nerve growth factor (NGF) and ohanin-like proteins (OHA)

Coral snake venoms can be broadly divided into two types according to their predominant toxins: PLA_2-rich venoms and 3FTx-rich venoms. PLA_2-predominant venoms exhibit stronger myotoxic effects. Several isolated PLA_2s from *Micrurus* venoms have been shown to be myotoxic. In contrast, venoms dominated by 3FTx show little or no skeletal muscle damage. Nevertheless, the predominance of one toxin type does not exclude the presence of the other, and 3FTx-dominant venoms may still retain some myotoxicity.

Unlike Old World elapids, *Micrurus* species lack cytolytic 3FTx, so muscle damage in their venoms is limited to the effects of PLA_2s.

Although the 3FTx from *Micrurus* are mostly not cytotoxic, they can still fulfill certain functional roles. Their small and stable structure makes them attractive for drug development, as their characteristics allow them to be modified with relatively low risk of destabilization compared to larger biomolecules. Furthermore, due to their high binding specificity, if their target receptor can be redesigned, it is plausible that they could be used as vectors to deliver therapeutic molecules to specific cells.

5.3. Complex MitTx from Micrurus venom

Coral snake venoms appear to have been shaped not only by prey capture needs but also by predator pressures. Although little is known about their natural predators—mainly due to the difficulty of observing coral snakes—raptors and even toucans have been reported preying on them.

This may have driven the evolution of the MitTx complex, first isolated from *Micrurus tener* venom. MitTx is a heterodimer composed of a Kunitz-like subunit (MitTx-α) non-covalently linked to an inactive PLA_2 homolog (MitTx-β). This complex activates ASIC1 somatosensory receptors, producing pain, a clinical symptom of envenomation by some *Micrurus* species.
MitTx has also been identified in three other PLA_2-dominant species (*M. mosquitensis, M. nigrocinctus,* and *M. dumerilii*), but not in the closely related *M. fulvius*.

The presence of pain-inducing venom components could serve dual purposes: immobilizing prey and deterring predators. Notably, the four coral species in which MitTx has been found share similar color patterns. The presence of MitTx may also explain why some nonvenomous "false coral snakes" mimic the coloration of true corals, as this would discourage predator attacks.

Although MitTx is a harmful compound for organisms to which it is administered, due to the intense pain it induces, it may still play an important role in biotechnological research. Since MitTx acts through the activation of ASIC1, it is reasonable to assume that an analgesic capable of inhibiting the pain caused by this toxin could also inhibit pain produced by other conditions that share the same mechanism. MitTx can thus be used as a model to test drugs aimed at

suppressing inflammatory, neuropathic, ischemic, visceral, postoperative, migraine-related, or cancer-induced pain, among others.

5. 4. LAAO from *Micrurus*

Although they do not constitute a major fraction of the venom, L-amino acid oxidases (LAAOs) still play important roles. As with LAAOs from other snake venoms, they possess cytotoxic activity, making them promising candidates for the development of new research tools.

A notable example is an LAAO isolated from *M. mipartitus* venom, known as MipLAAO, which has been tested for its antibacterial properties. This enzyme exhibited activity against *Staphylococcus aureus*, with a minimum inhibitory concentration (MIC) of 2 µg/mL. Against *Escherichia coli*, it inhibited growth, producing an inhibition halo of 19.8 ± 0.6 mm, whereas chloramphenicol used as a control produced a halo of only 12 ± 1 mm.

Furthermore, LAAOs from other snakes have demonstrated cytotoxic activity against tumor cell lines. Since crude *Micrurus* venom has shown antitumor properties, it is reasonable to attribute part of this activity to its LAAOs. This is because the phospholipases A_2 found in *Micrurus* venom are primarily neurotoxic and generally do not exhibit strong cytotoxicity toward tumor cells.

Therefore, LAAOs from *Micrurus* represent another potential tool for biotechnological research aimed at cancer study and treatment. Further studies and experiments are still required, but their potential is significant.

Given the aforementioned reasons that have hindered the study of coral snake toxins, much remains to be investigated regarding the potential use of their proteins. Nevertheless, a foundation of understanding already exists. For instance, snake venom PLA_2s could be employed, as previously noted for other species, in the treatment of cancer and microbial infections — that is, in applications that rely on their cytotoxic activity. It would also be worthwhile to examine a potential analgesic effect, similar to that observed in rattlesnakes. Further studies are needed, both to explore new possibilities and to deepen the current knowledge.

Table 4. *Biomolecules from Micrurus and their potential uses*

Potential uses for biomolecules from *Micrurus*	
Biomolecule	**Possible uses**

PLA_2s	• Possible cytotoxicity
3FTx	• Drug development • Delivery vector
MitTx	• Development of models for study of pain
LAAOs	• Cytotoxicity • Leishmanicidal activity • Plasmodicidal activity

6. Conclusions and Future Directions

To conclude this overview of the various biomolecules identified in snake venoms, as well as the research conducted to explore their therapeutic potential, it is worth noting that most of these studies remain at the experimental stage. Scientific progress requires continuous support to achieve plausible results within the shortest feasible time, without compromising either patient safety or product effectiveness.
From the author's perspective, such support constitutes a moral imperative, given the vast opportunities it presents for improving the quality of life of millions of patients worldwide.

Globally, it is estimated that between 7% and 8% of adults suffer from chronic pain with neuropathic characteristics, and approximately 20% of cancer patients develop neuropathic pain, either as a consequence of the disease itself or its treatment. This syndrome markedly diminishes patients' quality of life, as hypersensitivity to mild stimuli leads to reduced mobility, lower productivity, and sleep disorders, alongside social and psychological consequences such as isolation, depression, irritability, or anxiety. Drugs developed from studies on crotoxin and crotalphin could help improve the quality of life of these patients, since neuropathic pain typically responds poorly to conventional therapies.

In addition, research also highlights the potential use of snake venom biomolecules as tools in cancer therapy. In 2020, cancer was the leading cause of death worldwide, accounting for roughly 10 million fatalities. The most frequently diagnosed types include breast cancer (2.26 million new cases), lung cancer (2.21 million), and colorectal cancer (1.93 million), followed by prostate, non-melanoma skin, and gastric cancers. The highest mortality rates correspond to lung, colorectal, and liver cancers, followed by gastric and breast cancer. The high and still increasing global burden of cancer underscores the importance of developing more effective and diverse treatments, whether as novel therapeutic options or as complements to existing ones. Studies involving disintegrins, L-amino acid oxidases (LAAOs), phospholipases A_2 (PLA_2),

and other venom-derived proteins offer promising avenues to save lives and enhance patients' well-being.

Furthermore, venom-based research provides new opportunities for combating diseases transmitted by hematophagous insects, particularly in Latin America, where ecological and social factors contribute to high incidence rates, especially in rural or economically disadvantaged populations. Viral diseases such as dengue (which rose from just over half a million cases in 2000 to 14.6 million in 2024), Zika (with 5–15% of infants born to infected mothers presenting infection-related complications), chikungunya (400,000 cases and around 400 deaths in 2023 in the Americas), and yellow fever (212 cases and 85 deaths by April 2025), as well as parasitic diseases such as leishmaniasis (700,000 to 1 million new cases per year) and Chagas disease (7 million infections and roughly 10,000 deaths annually), remain major public health concerns. Studies involving PLA_2s, disintegrins, LAAOs, and other venom biomolecules present promising strategies for addressing these outbreaks, once again contributing to saving and improving countless lives.

Finally, although some of these studies focus on species not native to Peru, they belong to the same genera and are closely related to those found in the country. Therefore, Peruvian venomous snakes should be regarded as a potential source of valuable proteins and compounds for the treatment of various diseases. To ensure the availability of this biological resource for future research, the conservation of these species and their ecosystems is essential.

Given that biodiversity loss is a recognized and widespread issue in Peru, it is in the national and scientific interest that institutions involved in snake venom research and product development actively participate in protecting the natural environments that sustain these species—safeguarding both scientific progress and the biological heritage that may hold the key to future discoveries.

Some measures to ensure the conservation of the country's natural resources involve strengthening the fight against illegal logging. Of the approximately 2,800 km^2 of Peruvian forests cleared each year, nearly 80% is lost due to illegal activities. These activities include small-scale agriculture, commercial logging, and, most notably, mining, especially gold extraction.

Possible solutions include implementing measures inspired by REDD+, such as reforestation, satellite monitoring, and—above all, to address the root of the problem—a firm crackdown on illegal mining and logging. In addition to creating new laws, it is necessary to revise and strengthen existing ones, as well as ensure that the competent authorities increase the rigor of their enforcement. In this way, perpetrators can be stopped, and others are discouraged from engaging in the same activities.

These and other actions offer alternatives for safeguarding Peru's natural resources. Protecting the continuity of species and ecosystems keeps the door open for new discoveries. Each species lost as a result of human activities represents a scientific and ecological opportunity that disappears. This is why it is essential that everyone take part in the effort to protect natural resources, both in this country and around the world.

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