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Lachesis Muta Muta: State of the Art

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Abstract: This article was carried out to gather a collection of data extracted from several studies about the snake *Lachesis muta muta* (surucucu), encompassing in this information from the effects and properties of its venom, case report, treatment and even diagnosis. *L. m. muta* was addressed throughout the study by referring mainly to their venom, which in isolated fractions, as LHFI, LHFII, LmTX-I, among others, present several activities, among which stand out are: coagulant, hemorrhagic, proteolytic and neurotoxic. Besides, other studies involving its diagnosis, treatment, cytotoxicity, edema, inflammation, genotoxicity and mutagenicity have also been carried out. The bibliographic survey initially made, followed by the selection of collected data, was properly organized in the text using the narrative method, so that the findings could be chronologically distributed in a timeline. Therefore, it was possible to conclude that all these studies were and are of paramount importance to help understand the pathophysiology of envenoming by this snake, in addition to pointing out the *Bothrops-Lachesis* antivenom, opening a wide therapeutic window for numerous applications.

Key words: *Lachesis muta muta*, snake venom, state of the art.

1. Introduction

In Brazil, the *Lachesis* genus is represented only by the species *Lachesis muta* (Linnaeus, 1766) and the ophidian accidents caused by these snakes are the second most lethal (mortality/number of accidents) in this country [1-3]. *Lachesis muta muta*, the largest snake in Latin America (reaches 3 meters long) [4], occupies, in Brazil, the third place in the ranking of the most venomous snakes in the country, behind only the true coral (*Micrurus* genus) and the rattlesnake (*Crotalus* genus) and also in occurrence, behind *Bothrops* and *Crotalus* genera [5]. In this study, the main findings of *L. m. muta* venom collected from the literature were analyzed to establish the state of the art knowledge about this venom.

2. Signs and Symptoms Caused by *Lachesis Muta Muta* Envenomation

The venom of *Lachesis muta muta* has 4 types of actions in the victim's body: coagulant, hemorrhagic, proteolytic (necrotizing), and neurotoxic. Besides this, the signs and symptoms caused by envenoming are local pain, edema, ecchymosis (which may progress to every affected member), blistering, gingival bleeding, epistaxis, bleeding of the eyes and ear, absence or presence of vague manifestations such as diarrhea, vomiting, bradycardia, abdominal colic, hypotension or shock. In addition to these symptoms, the venom may cause hemoglobinuria, due to its hemolytic activity, causing the victim to present acute renal failure, which can lead to death [1, 5-7].

3. Timeline of *Lachesis Muta Muta* Studies

Due to its high degree of dangerousness, research on *L. m. muta* has been carried out since 1987 for the knowledge of the snake and its venom and, although research on this reptile is scarce, due to the difficult capture and maintenance in captivity, the venom is widely studied and explored to provide greater knowledge about its cytotoxicity, properties, and components [8].

Among several studies carried out on *L. m. muta* the first article found on this snake was by Sánchez et al.

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in 1987 [9] and treated purification of a hemorrhagic factor (LHF-I) of their venom. Performed by a five-step procedure, LHF-I (glycoprotein) showed caseinolytic action that was associated with hemorrhagic activity throughout the purification procedure.

Two years later, Silveira et al. in 1989 [10] purified and characterized a thrombin-like enzyme (F1) of the *L. m. muta* venom, resulting in the knowledge that it only cleaves fibrinopeptide A from fibrinogen, does not activate the coagulation factor XIII and is devoid of activity similar to kallikrein. In the same year, Da Silva et al. [11] suggested that the *Lachesis* venom would contain a crotoxin-like molecule, observing its ancestry with other crotalids (family reptiles of the order of ophidians or scaly), and the possible neurotoxic activity of their venom. This work concluded that non-toxic phospholipases were present in venom, but no crotoxin homolog. Besides, the induction of gyrotoxin through the isolation and purification of a toxic protein promoted rapid rolling motions in the tested rats.

Then, Sanchez et al. in 1991 [12] chose to study and analyze the hemorrhagic factor LHFII of venom, the metalloproteinase isolated from the *L. m. muta* venom. The research in question aimed at sequencing the amino acids LHFII hemorrhagic agent, a Ca^{2+}-containing Zn+, and isolated snake venom metalloproteinase Bushmaster *L. m. muta* which was determined by automated DABITC and PITC microsequencing. Finally, it was pointed out the sequence of LHFII, consisting of 200 residues beyond the considerable homology display sequence with hemorrhagic toxins from several other snake venoms and some metalloproteinases in the region of the supposed Zn^{+} binding sites.

Two years later, in 1993, Chavez-Olortegui et al. [13] published an article listing the snakes of *Bothrops atrox* and *L. m. muta*, to identify (through a developed ELISA assay) which antigen among the two venoms was circulating through the body of an individual bitten by a snake, due to the similar clinical symptoms of both. The results were satisfactory because the ELISA assay was able to successfully identify which of the two antigens was circulating in mice experimentally poisoned with one of the two venoms. Besides, it succeeded in identifying the circulating antigen in people accidentally bitten by one of these two snakes.

In the following year, another study was carried out relating the two species of snakes, *Bothrops* and *Lachesis*. Bard et al. in 1994 [14] aimed at investigating the efficiency of *Bothrops* antivenom handled in *Lachesis* accidents and pointed out its inefficiency in neutralizing the coagulant activity for the *L. m. muta* venom, concluding that the use of *Bothrops* antivenom is not recommended in the treatment of accidents with *Lachesis*.

Later, Sanchez et al. [15] correlated the two LHF-I and LHF-II previously mentioned. This work was based on the investigation of the proteolytic activities of these two hemorrhagic metalloproteinases (LHF-I and LHF-II) using as substrate the oxidized B-chain of bovine insulin. They observed that both hemorrhagic factors cleaved the Leu-Leu bonds in the fluorogenic peptide. Besides, the hemorrhagic activity and casein digestion remained unchanged. Finally, the inhibition of hemorrhagic and proteolytic activities was observed when crude venom was treated with ethylenediaminetetraacetic acid (EDTA), confirming that only metalloproteinases are responsible for these activities.

The next publication took place two years ago by Jorge et al. in 1997 [6], in which the authors referred to a report of a man bitten by an *L. m. muta* and who developed as symptoms: pain and edema at the site of the bite, nausea, vomiting, abdominal colic, diarrhea, and sweating in addition to presenting peripheral neutrophilic leukocytosis and evidence of consumption of fibrinogen with secondary activation of the fibrinolytic system. There was also the comparison and confirmation of the symptoms of this

case with those that occurred in other countries also due to *Lachesis* because these symptoms were not seen in victims of other American crotaline snakes, emphasizing, finally, the importance of developing more potent specific antivenom and treating the effects dramatic and vital cardiovascular diseases threatening the *L. m. muta* venom.

In the same year, Rodrigues et al. [16] published an article based on the isolation and analysis of immunoglobulins from the blood plasma of hyperimmune horses against the *L. m. muta* venom. It was observed that the incubation of the venom in these isolated antibodies resulted in a decrease in the caseinolytic and hemorrhagic activities of the venom. Furthermore, tests involving osmolytes and isolated immunoglobulins were made, wherein an osmolyte (sorbitol) proved to be able of stabilizing antibody at high temperatures, without significant disruption of the secondary structure or affinity in the *L. m. muta* venom. These results pointed to the possibility of using sorbitol, or other osmolytes, as stabilizers of immunoglobulin preparations.

The last study of the 20s found in the literature on *Lachesis* was of Fortes-Dias et al. [17], which was carried out by isolating, purifying and cloning a phospholipase A2 inhibitor (called CNF for the *Crotalus* neutralizing factor), from the plasma of the South American rattlesnake. This inhibitor interacted with crotoxin, the main neurotoxin in the *C. d. terrificus* venom, abolishing phospholipase A2 activity. After the tests, it was concluded that CNF can inhibit the PLA2 activity of all bushmaster including *L. m. muta* snake venom.

The beginning of the 21st century was marked by the publication of Estevão-Costa et al. in 2000 [18]. In this study, the zinc endopeptidases mutalysin I and mutalysin II were isolated from the *Lachesis* venom and subsequently observed their hemorrhagic activities and their hydrolyzed actions before the Aalpha > Bbeta fibrinogen chain without clot formation. These enzymes act directly on fibrin and are not inhibited by serine proteinase inhibitors (SERPINS). It was obtained as a result of the investigation: (a) Mutalysin I selectively hydrolyzed the alpha fibrin chain, leaving the beta and gamma-gamma chains unchanged; showed a selective inhibitory effect on the collagen-induced aggregation of human PRP; its proteolytic effect against dimethyl casein and fibrin was not significantly inhibited by α2-M; (b) Mutalysin II completely digested the alpha and gamma and gamma chains and partially the beta chain; did not inhibit platelet aggregation in human PRP induced by collagen or ADP; its proteolytic activity against dimethyl casein and fibrin has been completely abolished by α2-macroglobulin; (c) Both mutalysins do not activate plasminogen and had no effect on the activation of protein C. Therefore, the suggestion made was that the disintegrin-like domain also participates in the inhibitory effect (in addition to these enzymes in question). In conclusion, the data obtained explained why mutalysin I contributed significantly not only to local bleeding but also to systemic bleeding associated with the pathological effects observed in venom.

A year later, Colombini et al. [19] published an article by evaluation of the antigenic cross-reactivity between *L. m. muta* and *B. atrox* venoms using their polyclonal and monoclonal antibodies. Besides the effectiveness of their respective experimental antivenoms in cross-neutralizing, the main toxic activities of these venoms were also evaluated. By immunizing mice, several species — specific monoclonal antibodies were obtained and used as important tools for the development of an immunoassay capable of discriminating patients bitten by these two snakes. The experiments that involved the cross-neutralization of the main activities of the venoms, on the other hand, showed that the hemorrhage and blood incoagulability induced by the *B. atrox* venom was also neutralized by the *B. atrox* and *L. m. muta* antivenoms. However, the *B. atrox* antivenom partially neutralized the bleeding and failed

to neutralize the coagulopathy induced by the *L. m. muta* venom. Therefore, the antigenic variation that occurred between these two venoms suggested the use of specific antivenom for patients bitten by *Lachesis* snakes.

Spent 4 years, Felicori et al. [20] in 2005 dealt with the development of immunosorbent assays linked to sandwich-type enzymes (ELISA) that would detect the *L. m. muta* venom using antibodies against the plasminogen activating enzyme (LV-PA). They concluded at the end, that the immunosorbent assay could correctly discriminate among the antigens circulating in mice that were experimentally inoculated with *L. m. muta* venom of mice inoculated with *Bothrops* venoms.

Five months after the publication of Felicori, Damico et al. [21] addressed the biochemical and enzymatic characterization of two basic isoforms of phospholipase A2 Asp49 from the venom of *L. m. muta*. This study involved two basic isoforms of phospholipase A2 (PLA2) that were isolated from the snake venom *L. m. muta* and consecutively analyzed. Initially, by two-dimensional electrophoresis, they obtained the isoelectric point (pI) estimated at 8.7 and 8.6 for LmTX-I and LmTX-II, respectively. These two proteins were subsequently sequenced and differentiated from each other by a single amino acid substitution, Arg (LmTX-II)→Pro 65 (LmTX-II). The amino acid sequence showed a high degree of homology between the PLA2 isoforms of the *L. m. muta* venom and other snake venoms, such as *Crotalus durissus cascavella*. This occurred because the enzymatic activity of LmTX-I was inhibited by the crotapotin of the rattlesnake venom *C. d. cascavella*. This suggested that the crotapotin binding site in this PLA2 was similar to another in the basic PLA 2 of the crotoxin complex of the *C. d. cascavella* venom.

Then, Hermogenes et al. in 2006 [22] exposed their study of respect to the plasminogen activator (LV-PA) isolated in the *Lachesis* venom, which is activated by the fibrinolytic system *in vitro*. The inhibitory effect of plasma alpha2-macroglobulin plasma proteinase on LV-PA and its comparison with the effect on tissue-type plasminogen activator were examined. Proteolytic activity of LV-PA, isolated or incubated with human plasminogen, was completely inhibited by human alpha2-M. Meanwhile, the synthetic peptides Tos-Gly-Pro-Lys-pNA and HD-Pro-Phe-Arg-pNA have been hydrolyzed with almost no rate reduction. The proteinase also interacted with alfa2-M, and each mole of alfa2-M bound 2 moles of the enzyme in which it was shown through sodium dodecyl sulfate gel electrophoresis of reduced samples that the interaction of alfa2-M with LV-PA or t-PA pre-incubated with Plg resulted in the formation of fragments of approximately 90 kDa and complexes of high molecular mass, generated by the incubation mixture LV-PA and Plg. Therefore, the data suggested that LV-PA is a direct type of PA and its fibrinolytic effect can be reduced by alfa2-M *in vivo*.

In the following finding, Damico et al. [23] studied the cytotoxicity of the *L. m. muta*'s venom and its LmTX-I isoform of PLA (2) purified in Madin-Darby cultured canine kidney (MDCK) and in skeletal muscle cell lines (C2C12). As a result, they obtained that the venom induced a significant decrease in the cellular viability of MDCK cells. Furthermore, LmTX-I did not show cytotoxicity in the MDCK and C2C12 cell lines. As a result, an increase in the picnosis process was observed, an apparent reduction in the number of mitotic nuclei and nuclear fragmentation of some MDCK cells after incubation with the *Lachesis* venom. The authors concluded that the venom of this snake exerts toxic effects on cultured MDCK cells and that probably LmTX-I does not contribute to the direct cytotoxicity of the venom because it did not affect the morphologies of the nuclei and cells, nor on the organization of the actin cells stress fibers actin.

As a continuation of the previous article, Damico et al. in 2008 [24] again addressed the toxin LmTX-I, comparing to the pharmacological effects of the

venom, after intramuscular injection in the anterior tibial (TA) and after sub-plantar injection in the hind legs of mice. During the tests, a considerable increase in the activity of plasma creatine kinase and strong production of myonecrosis and inflammatory reactions in the TA muscle caused by LmTX-1 were observed, as well as induction of intense local hemorrhage by the venom. Pretreatment of the venom and LmTX-1 respectively with EDTA and indomethacin significantly inhibited the edema-forming activity and the bleeding of both.

A year later, the toxin LmTX-1 was addressed once again by Ferreira et al. [25] about the ability of crude venom and a basic phospholipase A (2) (LmTX-I) from *L. m. muta* venom to increase the microvascular permeability in rat paw and skin. The study in question evaluated rat paw edema and the leakage of dorsal plasma from the skin and Histamine release from rat peritoneal mast cell. Experiments carried out showed that skin leakage induced by LmTX-I was successfully inhibited by cyproheptadine, mepyramine, indomethacin, and PCA4248. In the end, they concluded that the venom of *L. m. muta* and LmTX-I increased microvascular permeability by mechanisms that involved the *in vivo* activation of mast cells and arachidonic acid metabolites. Besides, the responses induced by crude venom also involved the release of substance P, nitric oxide, and bradykinin, regardless of whether the responses induced by LmTX-I involve the platelet activating factor (PAF).

After a long pause in publications, Lima and Haddad Junior [26], in 2015, reported a confirmed case involving a child bite by *L. m. muta* snake in the State of Pernambuco, Brazil. The clinical manifestations caused by the venom were observed and reported, as well as the treatment with the administration of ten ampoules of bothropic-lachetic antivenom therapy that helped to normalize the patient's condition. He was discharged 5 days after the poisoning.

In 2016, Coriolano de Oliveira et al. [27] studied the ability of extracts, fractions, or isolated products of *Erythroxylum ovalifolium* and *Erythroxylum subsessile* to neutralize some toxic effects of the *L. m. muta* venom through *in vivo* and *in vitro* tests. The research was addressed aiming at alternative treatments for lachetic accidents due to the failure of antivenom to neutralize the local effects, leading to deficiencies in the victims. The extracts of crude fractions of *Erythroxylum* spp. inhibited 20% to 100% of the toxic effects of the venom, but *in vivo* tests in which the venom was injected before the plant extracts, the toxic effects of the venom were not inhibited. On the other hand, inhibition of 5% to 40% was obtained when extracts or products were administered before the injection of venom, concluding that the products of *Erythroxylum* spp. are a promising source of molecules capable of treating the local toxic effects of poisoning by *L. muta* venom, helping to develop new strategies for antivenom treatment.

A year later, publication of Nielsen and Matika [28] dealt with hypofibrinogenemia (an important clinical consequence after poisoning by this snake) that occurs through the catalysis of a fibrinogenase metalloproteinase enzyme and a thrombin-like serine protease (found in the *L. m. muta* venom). The publication in question demonstrated a decrease in the catalytic activity of these two enzymes by exposing fibrinogen to iron (Fe) and carbon monoxide (CO). It was observed that the pretreatment of plasma with Fe and CO markedly attenuated the effects mediated by the venom. Thus, after experiments, it was concluded that the addition of Fe and/or CO proved effective in protecting human plasma coagulation of the fibrinogenase activity, but not the effects of thrombin-like activity in the venom of *L. m. muta* (because the venom exposed to CO alone and then placed in the plasma, did not inhibit the thrombin-like enzyme).

In early 2018, Diniz-Sousa et al. [29] aimed to isolate a Lys49 PLA2 counterpart from the *L. m. muta*

venom using two chromatographic steps: size exclusion and reverse phase. Further analysis of the isolate identified, through its primary structure, a protein called LmutTX. Analyses made from synthetic peptides projected from LmutTX allowed to evaluate its cytotoxic and antimicrobial activities. Such analyses showed that LmutTX was cytotoxic against C2C12 myotubes in concentrations of at least 200 μg/mL while the peptides showed a low cytolytic effect. Besides, LmutTX showed antibacterial activity against Gram-positive and Gram-negative bacteria. Therefore, this article first described the isolation of a Lys49 PLA2 from the snake venom *Lachesis* and shows that peptides from specific regions of the sequence may constitute new sources of molecules with biotechnological potential.

The penultimate work to be published about *L. m. muta* was authored by Stransky et al. [30], about the cellular mechanisms related to cell death and tissue destruction, triggered by the *L. m. muta* venom as well as the investigation of the cytotoxic effect of this poison on the human body and human keratinocytes. The events evaluated were: apoptosis, necrosis, changes in the potential of the mitochondrial membrane as well as the induction of autophagy. Morphologically, the incubation with *L. m. muta* led to significant cell retraction and formation of cell aggregates that were cytotoxic to normal human keratinocytes and other cell lines. The authors concluded that this toxicity involved the integration of different modes of cell death, with cell autophagy useful for unraveling cell pathways and the mechanisms triggered by the venom.

The last research published, until the due moment, was by Cardoso Trento et al. in 2019 [31] who exposed the protective effect exerted by ascorbic acid on DNA fragmentation of human leukocytes induced by *L. m. muta* venom. This study had been as the main focus to evaluate the genotoxic and mutagenic effects of the toxins present in the *L. m. muta* venom in the leukocytes of human peripheral blood and the protective potential of ascorbic acid in DNA fragmentation. This research showed that, at the evaluated concentrations, the venom induced genotoxicity and mutagenicity, but were not cytotoxic as they did not alter the rate of cell proliferation after blocking cytokinesis with cytochalasin. Herein, ascorbic acid was also tested and proved effective in inhibiting the cytotoxicity induced venom. Consequently, future studies will be needed to elucidate the protective mechanisms of ascorbic acid on the genotoxic effects induced by toxins present in snake venoms.

Thus, as many as studies of *L. m. muta* and its venom are scarce, between the period of 1987 until 2019, 24 articles were published specifically on this snake (Fig. 1) to clarify about cytotoxicity, properties and components of its venom, being analyzed alone or concerning other poisons and even plant extracts.

As this venom shares several effects as those shown by *Bothrops* venoms, but the treatment only using the *Bothrops* antivenom is not enough against the lethality of *L. m. muta* [14], is the great medical importance to know the constituents of the venom. Table 1 shows the identified components collected from the literature and from the Medical Subject Headings (MeSH) database.

All the fractions isolated from the *L. m. muta* venom justify the main effects exhibited by the venom such as coagulant (fibrinogen clotting enzyme; thrombin-like enzyme; LV-PA), hemorrhagic (LHFI; LHFII), proteolytic (mutalysin I and II, mut IIa and mut IIb proteins), and neurotoxic (LMTX-I; LMTX-II; LM-PLA2-I and II), as expressed in Table 1.

4. Treatment of *L. m. muta* Snakebite

The successful treatment against snakebite depends on the time between the bite and the adequate treatment [26], which can help especially the identification of snake. In the function of the unimpressive therapeutic efficacy of non-specific *Bothrops* antivenom [14], *Bothrops/Crotalus* polyvalent antivenoms [6],

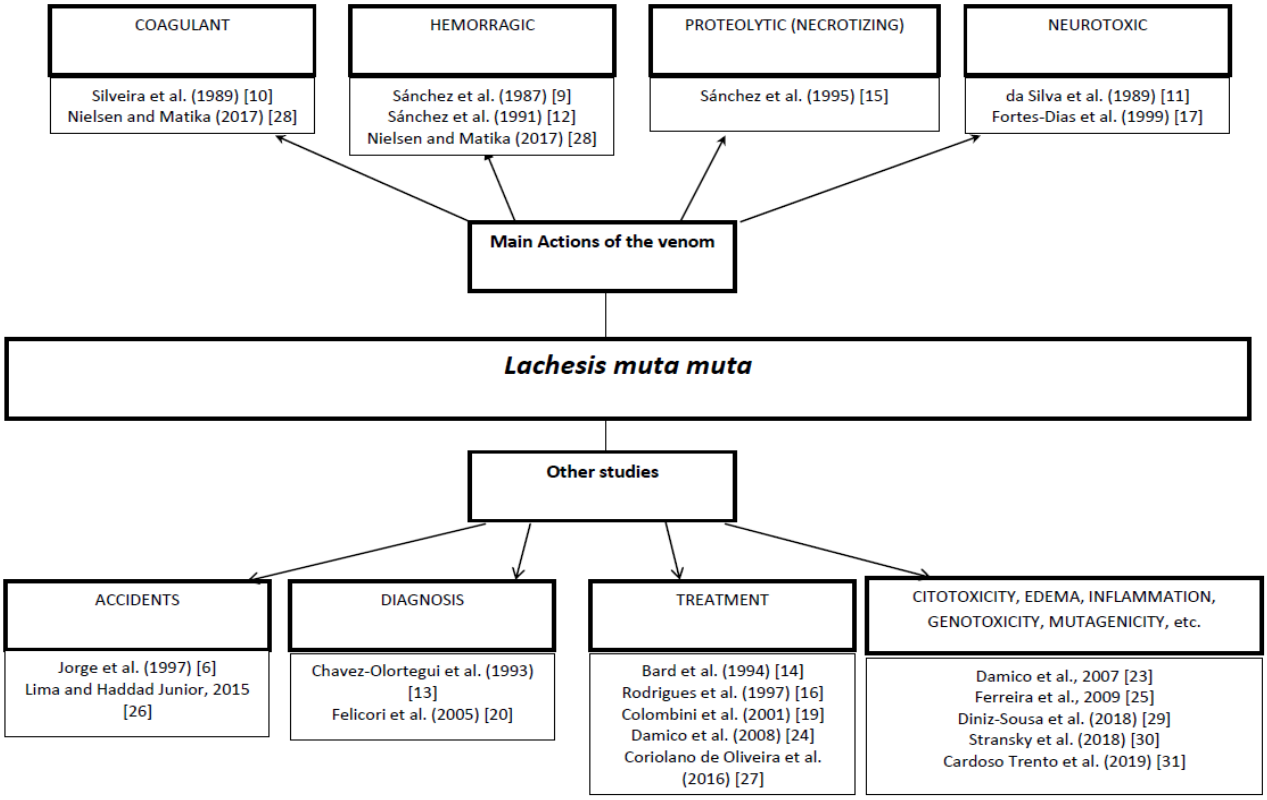

Fig. 1 Timeline of studies with *Lachesis muta muta* venom.

Table 1 *Lachesis muta muta* venom fractionation.

Obtained fraction	Characteristic	Activity	Authors
Fibrinogen clotting enzyme (thrombin-like enzyme)	Serine endopeptidase	Fibrinolytic	MeSH [32]
LHFI	Glycoprotein	Caseinolytic associated with hemorrhagic	Sánchez et al. [9]
	Metalloproteinase	Hemorrhagic	Sanchez et al. [15]
LHFII	Metalloproteinase	Hemorrhagic	Sanchez et al. [12]
LmTX-I	A basic PLA2	Edematous activity	Ferreira et al. [25]
Thrombin-Like Enzyme	Protease	Fibrinolytic	Silveira et al. [10]
LmTX-II isoform	A basic PLA2	Phospholipasic	Damico et al. [21]
LM-PLA2-I and II	An acid PLA2	Phospholipasic	Fuly et al. [33]
LV-PA	Serine protease	Fibrinolytic	Hermogenes et al. [22]
Mutalysin I protein	Metalloendopeptidase	Proteolytic	MeSH [34]
Mutalysin I and II	Metalloendopeptidase	Proteolytic	Estevão-Costa et al. [18]
Mut IIa and mut IIb proteins	Glycoprotein	Proteolytic	MeSH [35, 36]

Serine protease or endopeptidases are enzymes able to hydrolyze peptide bonds in the inner regions of peptide chains. Metalloendopeptidases are also endopeptidases, in which metal ions are required for catalysis. *Lachesis* venom was introduced at MeSH in 1992 [37], with the amino acid sequence determined and characterized by hemorrhagic and proteolytic activities.

Bothrops atrox antivenom [19], and due to the difficult capture and maintenance in captivity — or because such bites are rare or not reported — the production of specific *Lachesis* antivenom is not viable, a reason by which a mixture of *Bothrops-Lachesis* venoms is used (each mL of this antivenom neutralizes at least 5.0 mg of *Bothrops* sp. reference-venom and 3.0 mg of *Lachesis muta*

reference-venom). Besides, it is known that the snake venoms can share both one antigenic [38] and the other antibody cross-reactivities [39].

5. Conclusion

For a while, the isolated fractions from *L. m. muta* venom have helped to understand the physiopathology of envenomation and pointed out to *Bothrops-Lachesis* antivenom, but open a wide therapeutic window for countless applications.

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