rfm
Revista *de la* Facultad *de* Medicina

REVIEW ARTICLE
DOI: http://dx.doi.org/10.15446/revfacmed.v68n3.75992
Received: 06/11/2018. **Accepted:** 01/03/2019

Neurotoxical activity of *Micrurus* snake venom and methods for its analysis. A literature review

Actividad neurotóxica del veneno de serpientes del género Micrurus y métodos para su análisis. Revisión de la literatura

Janeth Alejandra Bolívar-Barbosa[1,2] **Ariadna Lorena Rodríguez-Vargas**[2]

[1] Universidad Nacional de Colombia - Bogotá Campus - Faculty of Medicine - Department of Toxicology - Master's Degree in Toxicology - Bogotá D.C. - Colombia.
[2] Universidad Nacional de Colombia - Bogotá Campus - Faculty of Sciences - Department of Chemistry - Protein Research Group - Bogotá D.C. - Colombia.
Corresponding author: Janeth Alejandra Bolívar-Barbosa. Departamento de Toxicología, Facultad de Medicina, Universidad Nacional de Colombia. Carrera 30 No 45-03, building: 471, office: 203. Telephone number: +57 1 3165000, ext.: 15120. Bogotá D.C. Colombia.
Email: jbolivarb@unal.edu.co.

Abstract

Introduction: Snakes of the genus *Micrurus* have fossorial habits, passive temperament and scarce production of powerful venom with neurotoxic characteristics that block the synaptic transmission at the neuromuscular junction.
Objective: To present an overview of the neurotoxicity of the *Micrurus* snake venom, and its functional characterization by *ex vivo* analysis methods.
Materials and methods: A literature review was conducted in MedLine and ScienceDirect using specific terms and their combinations. Search strategy: type of studies: articles on the neurotoxicity of *Micrurus* snake venom and techniques to determine its neurotoxic activity by *in vitro, in vivo* and *ex vivo* models; publication period: articles published until June 2018; publication language: English and Spanish.
Results: Out of 88 studies identified in the initial search, 28 were excluded because they did not meet the inclusion criteria (based on reading their titles and abstracts). 8 additional articles (books and reports) were included, since, according to the authors' opinion, they complemented the information reported by the selected studies. The studies included in the review (n=68) were original research papers (n=44), review articles (n=16), and book chapters, reports, guides and online consultations (n=8).
Conclusions: Studies performed using *ex vivo* muscle and nerve preparations to evaluate the effect of neurotoxins provide a good model for the characterization of the pre-synaptic and post-synaptic effect of the venom produced by snakes of the genus *Micrurus.*
Keywords: *Elapidae; Micrurus;* Phospholipases A2; Neuromuscular Junction (MeSH).

Bolívar-Barbosa JA, Rodríguez-Vargas AL. Neurotoxical activity of *Micrurus* snakes venom and methods for its analysis. A literature review. Rev. Fac. Med. 2020;68(3):453-62. English. doi: http://dx.doi.org/10.15446/revfacmed.v68n3.75992.

Resumen

Introducción. Las serpientes del género *Micrurus* son animales de hábitos fosoriales, de temperamento pasivo y escasa producción de un potente veneno con características neurotóxicas que bloquean la transmisión sináptica en la placa neuromuscular.
Objetivo. Presentar un panorama general de la neurotoxicidad del veneno de las serpientes *Micrurus* y su caracterización funcional mediante métodos de análisis *ex vivo.*
Materiales y métodos. Se realizó una revisión de la literatura en MedLine y ScienceDirect usando términos específicos y sus combinaciones. Estrategia de búsqueda: tipo de estudios: artículos sobre la neurotoxicidad del veneno de serpientes *Micrurus* y técnicas para determinar su actividad neurotóxica mediante modelos *in vitro, in vivo* y *ex vivo;* periodo de publicación: sin límite inicial a junio de 2018; idiomas: inglés y español.
Resultados. De los 88 estudios identificados en la búsqueda inicial, se excluyeron 28 por no cumplir los criterios de inclusión (basándose en la lectura de títulos y resúmenes); además, se incluyeron 8 documentos adicionales (libros e informes), que, a criterio de los autores, complementaban la información reportada por las referencias seleccionadas. Los estudios incluidos en la revisión (n=68) correspondieron a las siguientes tipologías: investigaciones originales (n=44), artículos de revisión (n=16) y capítulos de libros, informes, guías y consultas en internet (n=8).
Conclusiones. Los estudios que describen el uso de preparaciones *ex vivo* de músculo y nervio para evaluar el efecto de neurotoxinas ofrecen un buen modelo para la caracterización del efecto presináptico y postsináptico del veneno producido por las serpientes *Micrurus*.
Palabras clave: *Elapidae; Micrurus*; Fosfolipasas A2; Unión neuromuscular (DeCS).

Bolívar-Barbosa JA, Rodríguez-Vargas AL. [Actividad neurotóxica del veneno de serpientes del género *Micrurus* y métodos para su análisis. Revisión de la literatura]. Rev. Fac. Med. 2020;68(3):453-62. English. doi: http://dx.doi.org/10.15446/revfacmed.v68n3.75992.

Introduction

Ophidian accidents are events caused by the bite of a snake and are of public health interest worldwide. Specifically, in Central and South America, about 300 000 bites of these animals are reported each year, of which 12 000 generate sequelae and 4 000 lead to death.[1] In Colombia, according to the *Instituto Nacional de Salud* (National Health Institute), 4 978 cases of snakebites were reported in 2017, of which 66 were caused by snakes of the genus *Micrurus*,[2] the most diverse and representative of the family *Elapidae*.[3-6]

Snakes of the genus *Micrurus*, also known as coral snakes, are docile animals that do not attack humans unless provoked. These reptiles have coloration patterns that serve as a defense mechanism and repel their predators. They also possess a powerful venom with a neurotoxic effect that they only use to defend themselves[7] and proteroglyph dentition, that is, their venom inoculating fang is located at the front end of the upper jaw. In this type of dentition, the groove through which the venom passes in the fang is not completely closed and, for this reason, the snake must hold onto their prey for a few seconds to ensure the entry of the venom.[8]

These species have a venom gland situated on each side of the head, which is made up of a main gland, a primary duct, and an accessory mucous gland. Moreover, the main gland is surrounded by branches of the pterygoid muscles and external jaw adductors.[8-11]

Since the production of venom in snakes is a slow process, they store it mainly in intracellular form in seromucous cells or in the central lumen of the venom gland (to a lesser extent).[8,12] In addition, the production of toxins in this gland is stimulated by biochemical and morphological changes in the secretory epithelial cells; this production process is carried out asynchronously after the extraction or inoculation of the venom, so its concentration is altered.

The amount and composition of the venom produced by snakes depends on epigenetic variations between individuals, the species, the site of origin, the ontogenetic stages, the phylogenetic changes and the feeding habits of each individual, as well as on the environmental conditions where they develop and live.[10,12-16] Costa-Cardoso *et al.*[10] state that the venom of coral snakes contains 25% total solids, 70-90% proteins and polypeptides and 10-30% low molecular weight substances such as amines, carbohydrates, amino acids, ions and inorganic compounds. Lomont *et al.*[15] identified 22 protein families in the venom of *Micrurus* snakes using analytical techniques; the most abundant and representative are three-finger toxins (3FTx) and phospholipases A2 (PLA2), which contribute to the neurotoxic effect of these substances.

The proportion of PLA2 and 3FTx toxins in coral snake venoms is a key element for identifying the main neurotoxic effects of the venoms of all species of the genus *Micrurus*.[6,9,15-17] Different researches on this subject have established that neurotoxins act through two mechanisms: on the one hand, presynaptic neurotoxins block the release of acetylcholine from the presynaptic neuron and, on the other, the postsynaptic neurotoxins competitively bind to the nicotinic receptor at the neuromuscular junction.[10,18,19] Both situations lead to respiratory failure and death of the patient, if adequate treatment with antivenom is not provided timely.

The presence of these presynaptic and post-synaptic neurotoxins makes *Micrurus* venom lethal at low doses,[20-22] making the study of the effects of this venom on the neuromuscular transmission of electrical impulses highly relevant. To this end, *ex vivo* preparations of striated muscle tissue and nerve are used.[23,24]

Based on the abovementioned, two types of tests are used to evaluate the neurotoxic and myotoxic effects of snake venom: *ex vivo* assessment of neurotoxicity and *ex vivo* assessment of myotoxicity. The neurotoxicity assessment of these venoms should be performed in both muscle and nerve preparations due to the responses of the models, as explained below.[25-27]

Ex vivo techniques for the assessment of neurotoxicity

The neurotoxic activity of the venom produced by *Micrurus* species is determined by applying a stimulus through the electrodes that are in contact with the biventer cervicis muscle of 4- to 8-day-old male chicks, or with the diaphragm and the phrenic nerve of male mice with body weight between 25g and 35g, which results in muscle contraction under normal conditions.[24] To perform this analysis, electrical impulses (0.1Hz for 0.2ms) are applied using a low-frequency stimulator and muscle contractions are recorded with a force displacement transducer that is coupled to recording equipment located in the muscle tissue of an isolated organ perfusion system. After a stabilization period of 20 minutes, a single concentration (0.1, 0.5, 1, 5 or 10 µg/mL) of the venom under study is added to the organ bath. To confirm the complete block of muscle contractions,[8,10] and to establish the concentration of the venom that caused such an effect,[27] it is necessary to apply new electrical stimuli and verify the records using a dose-response curve.

Ex vivo techniques for the assessment of myotoxicity

Using muscle preparations similar to the biventer cervix muscle of male chicks, it is also possible to evaluate the ability of the venom to induce muscle damage.[26]

To achieve a selective stimulation of the muscle by suppressing neuromuscular activity, the preparations are placed in an organ bath in 10µM d-tubocurarine and the muscle is directly stimulated with electrical impulses of 0.1Hz at a maximum voltage of 0.2ms. The poison is then added to the preparation and left in contact until contraction is blocked or after 3 hours, after which time the tissues are immersed in 10% formaldehyde for histological examination to confirm myotoxicity.[19,23,25,27]

Electrophysiological alterations

Micrurus snake venom, or some of its specific toxins, can alter the transmission of the normal electrical pulse in the neuromuscular junction. This is reflected, on the one hand, in a decrease or blockage of the response to direct electrical stimulation on the muscle and, on the other, in fluctuations in resting membrane potential, such as changes in amplitude, form and frequency, and Wedensky inhibition in some cases; these effects are caused by both prolonged and short exposures to the venom or one of its toxins.[24,28]

Similarly, by evaluating muscle contractility after applying electrical stimulation in the presence of acetylcholine

(ACh) and the venom under study, it is possible to determine if there are effects on the post-synaptic response to ACh. When there are no alterations in muscle contractility, the effect is considered presynaptic.[24,25]

Myotoxicity can be evaluated in an observational way and without the need for a pathological study, assessing the response of the striated muscle when exposed to the venom and the blocking action of the toxins on muscle contracture in the presence of a direct electrical impulse and high concentrations of K^+ in the organ bath.[26,27]

Since there is new information on *Micrurus* snake venoms and considering the large number of this genus species in Colombia, it is necessary to encourage research that characterizes these substances biochemically and biologically and promotes the development of more specific and useful antivenins to treat snakebite accidents.

In this context, the objective of this review was to present an overview of the neurotoxicity of *Micrurus* snake venom and its functional characterization using *ex vivo* analysis methods.

Materials and methods

A literature review was conducted in the MedLine and Science Direct databases using the following search strategy: type of studies: research articles, reviews and specialized book chapters addressing neurotoxicity of *Micrurus* snake venom and techniques to determine their neurotoxic activity by means of *in vitro*, *in vivo* and *ex vivo models;* publication period: no initial limit until June 2018; languages: English and Spanish; search terms: "*Micrurus*", "*Elapidae*", "actividad neuromuscular", "neurotoxicidad", "miotoxicidad", "veneno de *Micrurus*", "fosfolipasas A2" and "Toxinas de tres dedos", which were combined with the "AND" and "OR" connectors to establish the search equations.

The review started with the search of the basic concepts of venom, mechanisms of neurotoxicity, myotoxicity and characterization, and determination *in vitro.* References that made a functional characterization of the venom using *ex vivo* preparations of the biventer cervicis muscle of chicks or the phrenic nerve of mice were included, and those that did not meet the search criteria were excluded.

A total of 151 publications were retrieved, of which 63 were eliminated because they were duplicated; the remaining 88 were reviewed for title, abstract and methodology, and 28 were excluded because they were not relevant to the topic of interest or did not meet the selection criteria. The study also included 8 additional records (books and reports) identified through other sources and which, in the authors' opinion, complemented the information reported by the selected references; finally, 68 publications were included in the review (Figure 1).

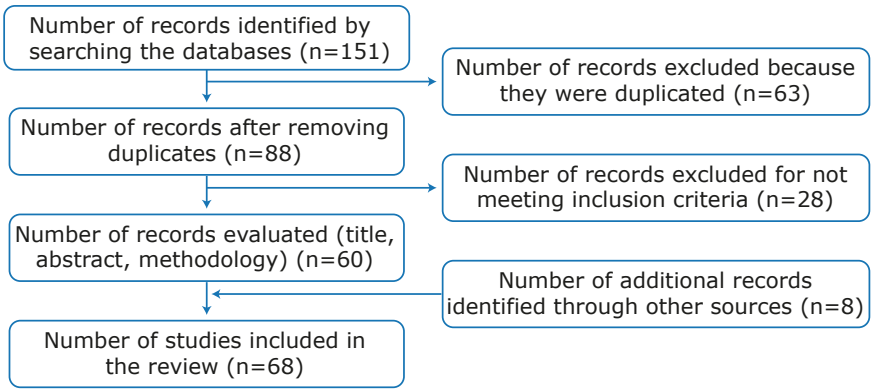

Figure 1. Review flowchart.
Source: Own elaboration.

Results

Most of the studies (n=28) included in the review were published between 2010 and 2018, with a significant increase in the number of publications since 2000 (Figure 1). Except for 3 works developed in Colombia by regulatory bodies, all the literature found was published in English.

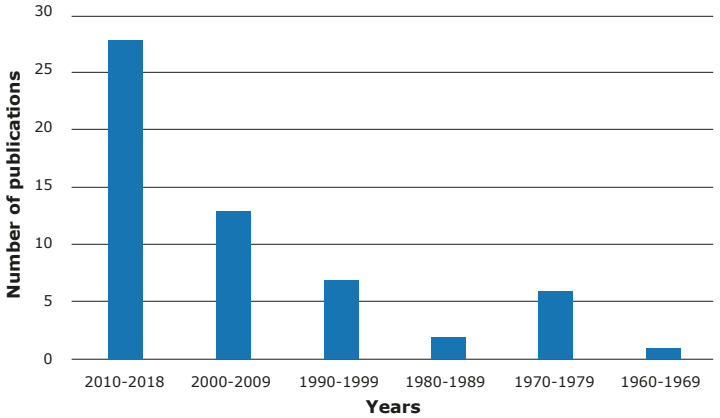

Figure 2. Number of publications on neurotoxicity of *Micrurus* snake venom and methods for its analysis.
Source: Own elaboration.

Of the 68 documents included, 16 were review articles, 5 were book chapters, 2 were guidelines and reports, and 1 was an Internet search; the remaining 44 publications were original articles and 18 of them used venoms of snakes of the genus *Micrurus* as samples (Table 1). Of the articles that specifically studied coral snake venom, only 6 were on Colombian species, including *Micrurus dumerilii*, *Micrurus mipartitus*, *Micrurus dissoleucus*, *Micrurus lemniscatus*, *Micrurus spixii and Micrurus surinamensis*, that is, only about 16% of the snake venoms of the genus *Micrurus* found in the country have been studied. Out of these species, only the proteome of the venom of *M. dumerilii* and *M. mipartitus* have been characterized[16,21,29] since they are the main varieties involved in snakebites.

One of the studies found[19] assessed the neurotoxic activity of the venom of *M. mipartitus* and *M. dissoleucus* by means of isolated muscle preparations and established the functional characterization of each one. Another study[20] described the multiple enzymatic activities of the venom of *M. lemniscatus*, *M. spixii* and *M. surinamensis* and evaluated the toxicity of each venom on different prey animals.

The study developed by Rey-Suarez *et al.*[29] showed that the venom proteome of the species *M. mipartitus* found in Colombia has a higher proportion of 3FTx, which is a different phenotype from that of the venom of *M. dumerilii*, a species that has higher levels of PLA2.[16,21] Moreover, the study by Renjifo *et al.*[19] found that the venom of *M. mipartitus* species has post-synaptic activity associated with the inhibition of muscle contraction caused by the presence of ACh, which is related to the large amount of 3FTx.

The use of *ex vivo* models for the assessment of snake venom neurotoxicity began with studies on elapid species, especially in the Old World, as evidenced in 7 of the articles included in the review.[24,26,28,30-33] Table 1 contains the studies found that were conducted on snakes of the genus *Micrurus*, as well as the articles that assessed neurotoxic activity of elapid venoms.

Table 1. Articles that assess neurotoxic activity included in the review.

Authors	Type	Title	Year	Snakes of the genus *Micrurus*	Venom from species found in Colombia
Chang *et al.*[30]	Original research	The presynaptic neuromuscular blocking action of Taipoxin. A comparison with Beta-Bungarotoxin and Crotoxin	1977	No	No
Su *et al.*[24]	Original research	The presynaptic neuromuscular blocking effect and phospholipase A2 activity of textilotoxin, a potent toxin isolated from the venom of the Australian brown snake, *Pseudonaja textilis.*	1983	No	No
Su & Chang[31]	Original research	Presynaptic effects of snake venom toxins which have phospholipase A2 activity (beta-bungarotoxin, taipoxin, crotoxin)	1984	No	No
Rowan *et al.*[28]	Original research	On the blockade of acetylcholine release at mouse motor nerve terminals by beta-bungarotoxin and crotoxin	1990	No	No
da Silva *et al.*[34]	Original research	Comparative chromatography of Brazilian coral snake *(Micrurus)* venoms	1991	Yes	No
Harvey *et al.*[26]	Original research	Screening of snake venoms for neurotoxic and myotoxic effects using simple *in vitro* preparations from rodents and chicks	1994	No	No
Wilson *et al.*[32]	Original research	Induction of giant miniature end-plate potentials during blockade of neuromuscular transmission by textilotoxin	1995	No	No
Da Silva & Aird[20]	Original research	Prey specificity, comparative lethality, and compositional differences of coral snake venoms	2001	Yes	Yes
Hodgson & Wickramaratna[23]	Review	*In vitro* neuromuscular activity of snake venom	2002	No	No
Hodgson *et al.*[33]	Original research*	The neuromuscular activity of paradoxin: a presynaptic neurotoxin from the venom of the inland taipan *(Oxyuranus microlepidotus)*	2007	No	No
Olamendi-Portugal *et al.*[17]	Original research	Proteomic analysis of the venom from the fish-eating coral snake *Micrurus* surinamensis: novel toxins, their function and phylogeny	2008	Yes	No
Moreira *et al.*[35]	Original research	Frontoxins, three-finger toxins from *Micrurus* frontalis venom, decrease miniature endplate potential amplitude at frog neuromuscular junction	2010	Yes	No
Fernández *et al.*[36]	Original research	Venomic and Antivenomic Analyses of the Central American Coral Snake, *Micrurus nigrocinctus (Elapidae)*	2011	Yes	No

Table 1. Articles that assess neurotoxic activity included in the review. (continued)

Authors	Type	Title	Year	Snakes of the genus *Micrurus*	Venom from species found in Colombia
Rey-Suárez *et al.*[29]	Original research	Proteomic and biological characterization of the venom of the redtail coral snake, *Micrurus mipartitus (Elapidae),* from Colombia and Costa Rica	2011	Yes	Yes
Corrêa-Netto *et al.*[37]	Original research	Snake venomics and venom gland transcriptomic analysis of Brazilian coral snakes, *Micrurus altirostris* and *M. corallinus.*	2011	Yes	No
Ciscotto *et al.*[38]	Original research	Venomic analysis and evaluation of antivenom cross-reactivity of South American *Micrurus* species	2011	Yes	No
Renjifo *et al.*[19]	Original research	Neuromuscular activity of the venoms of the Colombian coral snakes *Micrurus dissoleucus* and *Micrurus mipartitus:* an evolutionary perspective	2012	Yes	Yes
Vergara *et al.*[39]	Original research	Eastern coral snake *Micrurus fulvius* venom toxicity in mice is mainly determined by neurotoxic phospholipases A2	2014	Yes	No
Bérnard-Valle *et al.*[40]	Original research	Biochemical characterization of the venom of the coral snake Micrurus tener and comparative biological activities in the mouse and a reptile model	2014	Yes	No
Fernández *et al.*[41]	Original research	Snake venomics of *Micrurus* alleni and *Micrurus mosquitensis* from the Caribbean region of Costa Rica reveals two divergent compositional patterns in New World elapids	2015	Yes	No
Rey-Suárez *et al.*[21]	Original research	Integrative characterization of the venom of the coral snake *Micrurus dumerilii (Elapidae)* from Colombia: Proteome, toxicity, and cross-neutralization by antivenom	2016	Yes	Yes
Henao-Duque & Núñez-Rangel[14]	Original research	Maintenance of red-tail coral snake *(Micrurus mipartitus)* in captivity and evaluation of individual venom variability	2016	Yes	Yes
Casais-E-Silva *et al.*[42]	Original research	Lemnitoxin, the major component of *Micrurus lemniscatus* coral snake venom, is a myotoxic and pro-inflammatory phospholipase A2.	2016	Yes	No
Lomonte *et al.*[43]	Original research	Venom of the Coral Snake *Micrurus clarki:* Proteomic Profile, Toxicity, Immunological Cross-Neutralization, and Characterization of a Three-Finger Toxin	2016	Yes	No
Sanz *et al.*[22]	Original research	Venomic analysis of the poorly studied desert coral snake, *Micrurus tschudii tschudii,* supports the 3FTx/PLA2 dichotomy across *Micrurus* venoms	2016	Yes	No
Rey-Suárez *et al.*[16]	Original research	Primary structures and partial toxicological characterization of two phospholipases A2 from *Micrurus mipartitus* and *Micrurus dumerilii* coral snake venoms	2017	Yes	Yes

Source: Own elaboration.

Discussion

Although the number of publications on snake venoms of the genus *Micrurus* has increased worldwide since 2007, studies in Colombia are still scarce. This increase in research may be explained by the development of proteomic techniques that allow analyzing the protein composition of venoms; thus, techniques such as reversed-phase high-performance liquid chromatography, electrophoresis and mass spectrometry have been used to identify the proteins present in venoms and determine their relative abundance and sequencing.[32,44-46] However, it should be noted that this type of study may have limitations in terms of accessibility, cost and operation of some of the equipment used.

The neurotoxicity of coral snake venom is associated with PLA2 and 3FTX, which lead to flaccid paralysis of the respiratory muscles. For this reason, the development of neurotoxicity models that allow verifying the influence of full venom and these toxins on the synaptic transmission at the neuromuscular junction can be key for its functional characterization, as it has been evidenced in studies developed with elapid venoms of the genus *Bungarus*,[28,30,31,47] *Oxyuranus*,[24,30,31,34,48] *Pseudonaja*,[23,33] and *Notechis*.[49]

The following are the findings on *Micrurus* snake venom, its neurotoxicity, and its effects on the neuromuscular junction.

Mechanism of action of PLA2 and 3FTx

The venom of snakes from the genus *Micrurus* has neurotoxic components. Lomonte *et al.*[15] identified the following families of proteins in its proteome: 3FTx (α-neurotoxins), PLA2 (β-neurotoxins), metalloproteases, L-amino-acid oxidases, Kunitz-type serine protease, C-type lectin-like proteins, acetylcholinesterase and hyaluronidases, being the first two the ones with more involvement. The proportion of PLA2 and 3FTx toxins in these venoms is a key element for identifying their main neurotoxic and myotoxic effects.[15,16,18]

Table 2 presents some of the neurotoxins identified in snakes of the family *Elapidae*. It also includes the characteristics of the snake venom of the genus *Crotalus durissus* due to its neurotoxic and myotoxic behavior.[22]

Table 2. Neurotoxins identified in snake venom.

Species	Toxin	Type	Reference
Notechis scutaus	Notexin	β-neurotoxins	49
Oxyuranus scutellatus	Taipoxin	β-neurotoxins	30,31,48
Oxyuranus microlepidotus	Paradoxin	β-neurotoxins	34,50
Crotalus durissus terrificus	Crotoxin	β-neurotoxins	28,30,31,51,52
Pseudonaja textiles	Textilotoxin	β-neurotoxins	23,33
Bungarus multicinctus	β-bungarotoxin	β-neurotoxins	28,31
Micrurus frontalis	Frontoxin	α-neurotoxins- 3FTX	35
Micrurus lemniscatus	Lemnitoxin	β-neurotoxins	42
Micrurus dumerilli	MdumPLA2	β-neurotoxins	16
Micrurus mipartitus	MmipPLA2	β-neurotoxins	16

Source: Elaborated based on Hodgson & Wickramaratna.[23]

Recent studies in proteomics have demonstrated the predominance of PLA2 and 3FTx toxins in the venom phenotype in species of the genus *Micrurus* and have shown that their proportions vary widely across the American continent.[14,16,21,22,41-43] Similarly, Lomonte *et al.*[15] made a projection of the behavior of these two toxins, finding that PLA2 is more abundant in the southern cone and that 3FTx predominates in Central and North America. However, it is worth mentioning that this projection should be corroborated by a detailed analysis of each of the venoms since the evolutionary and ecological processes of each species are factors that determine the greater proportion of one of these 2 toxins.

PLA2 toxins

PLA2 is a superfamily of enzymes composed of 16 groups that are classified according to their type into: secreted PLA2 (sPLA2), cytosolic PLA2 (cPLA2), calcium-independent PLA2 (iPLA2), platelet activating factor (PAF), lipoprotein-associated PLA2 (LpPLA2s), adipose PLA2 (AdPLA2s) and lysosomal PLA2 (LPLA2s). The first two are especially important because they are involved in the inflammatory and degenerative response of the central nervous system.[53] sPLA2 are present in different classes of venoms and are classified in four main subtypes, and types 1 and 2 are found in snake venoms, mainly in elapids, vipers and crotalids; these enzymes are single-chain polypeptides and have a molecular mass of 13-15 kDa, with approximately 7 disulfide bonds. Finally, cPLA2 has a molecular mass of 40-100 kDa and depends on calcium for functioning.[53]

Even though the exact neurotoxic route of PLA2 is not known, at least three mechanisms of action by which these enzymes exert their presynaptic toxic activity have been described: 1) the neurotoxin induces phospholipid hydrolysis of the presynaptic membrane and prevents it from interacting with the acetylcholine vesicles, 2) the damage described in the cell membranes favors the excessive influence of Ca^{++} inside the cells, which causes an exaggerated release of the acetylcholine neurotransmitter, as well as the alteration of its recycling process and its subsequent depletion, and 3) its enzymatic activity is left aside, so the protein complexes that favor the coupling of the vesicle and the presynaptic membrane are blocked.[16,23,47,54-56]

3FTx toxins

3FTx are non-enzymatic polypeptide structures made up of between 60 and 74 amino acid residues that are among the main components of elapid venoms.[57] Its structure has 3 loops of β-sheets that extend from a small globular hydrophobic core that is linked by 4 preserved disulfide bonds; this structure resembles that of a hand with 3 fingers, which is why they are known as three-finger toxins.[57,58]

Neurotoxicity (main effect of coral snake venom), cytotoxicity and cardiotoxicity are some of the pharmacological effects identified in 3FTx. However, it should be noted that its biological activity varies depending on the affinity it has with the receptors[59-61] (Table 3).

Table 3. Three-finger toxins found in elapid venoms.

3FTx type		Mechanism of action	Example
Neurotoxins	α	α1 and/or α7 nAChR antagonists.	α-bungarotoxins
	κ	They recognize different subtypes of neuronal α3β4 nAChR	κ-bungarotoxins
	MT	They selectively bind to mAChR.	*Dendroaspis angusticeps* MT1
Cardiotoxins		They form ionic pores in lipid membranes.	Cardiotoxin V4II from *Naja mossambica*
β-cardiotoxins and others alike		They bind to β1 and β2 adrenergic receptors.	CTX9, CTX14, CTX15, CTX21 and CTX23 from *Ophiophagus Hannah*
Non-conventional		Weak neurotoxins with nanomolar affinity to AChR α1 (reversible binding) and α7 (poorly reversible binding)	Candoxin
Acetylcholinesterase inhibitors		They bind to acetylcholinesterase	Fasciculins
L-type Ca^{2+} channel blockers		They block L-type Ca^{2+} channel in skeletal and heart muscles	Calciseptine
Platelet aggregation inhibitors		They interfere with the interaction between fibrinogen and the glycoprotein IIB-IIIa receptor ($\alpha_{IIB}\beta3$).	Dendroaspins

nAChR: nicotinic acetylcholine receptor; mAChR: muscarinic acetylcholine receptors; MT: muscarinic toxin; CTX: cardiotoxin
Source: Elaboration based on Utkin[59], Kini & Doley[60] and Nirthanan *et al.*[61]

Depending on the amino acid sequence, 3FTx neurotoxins can be classified into two types: short-chain neurotoxins (type I) and long-chain neurotoxins (type II), both with a molecular mass varying between 6 and 9 kDa.[58,61] Short-chain α-neurotoxins are the main responsible for the neurotoxic effect of elapid venoms due to their high- affinity binding for nicotinic acetylcholine receptor (nAChR) and the inhibitory control they exert on this receptor without affecting the release of neurotransmitters from the presynaptic terminal.[59,62,63]

Functional characterization of the neurotoxic activity of venoms of the *Elapidae* family and the *Micrurus* genus in particular

Recent studies on the classical biochemistry of elapid venom composition in Colombia have made major progress in the description of the ontogenetic characteristics and phylogenetic trees of these species.[16,19,21] In this regard, multiple research works assess the peripheral neurotoxic and myotoxic activity of the venom of *M. dissoleucus, M. mipartitus*,[19] *M. lemniscatus*, *Micrurus frontalis* and *M. surinamensis,*[13] or the PLA2 of the species *Micrurus nigrocinctus.*[64]

Specifically, for Australian elapids (*Pseudechis* spp.), Hart *et al.*[65] indicated that binding of neurotoxins to nAChR is more effective in preparation from chick muscle than in human and rat skeletal muscles. This difference is explained because the synaptic junctions are almost absent in birds, even though neuromuscular junctions in avian tissues are almost the same size as in rats; this makes nAChR more susceptible to post-synaptic neurotoxins and more sensitive to the neurotoxic action of elapid venoms.[65]

Although the neurotoxic activity of *Micrurus* snake venom has not been sufficiently studied, several methodologies have been used as complementary techniques besides organ baths, e.g. cell culture models, that can contribute to the understanding of the actions of the full venom and its main components (PLA2 and 3FTx). For example, hippocampal tissue has been used to prove viability and conservation of cellular function (integrity of mitochondria and intracellular calcium variability) after the administration of different doses of venom from these species,[66] to establish toxin binding to transmembrane receptors,[67] and determine electroencephalographic and neuropathological changes in behavioral studies of animal models.[68]

Conclusions

Advances in venomics, the global study of venom through omic techniques, allow for a better understanding of the protein constituents of snake venoms. In the specific case of the genus *Micrurus*, such advances are of vital importance due to the small amount of venom produced by these species and the limitations of having them in captivity. Furthermore, the identification of proteins with neurotoxic properties such as α-neurotoxins and β-neurotoxins, main components of *Micrurus* venom, and the understanding of their mechanism of action in *ex vivo* muscle tissue preparations, are fundamental tools for the development of toxinology and allows understanding the mechanism of action of these components and the great protein variability of each species and each individual.

Establishing the characteristics of snake venom proteomes has potential benefits for basic research on these substances, such as the identification of new molecules in the venom. This also contributes to a better understanding of the evolution and biological effects that these venoms can have. Likewise, this characterization is useful for the clinical diagnosis of snakebites and for the development of new research tools and drugs with clinical potential as specific antivenoms.

Studies with *ex vivo* muscle and nerve preparations to assess the effect of neurotoxins are a good model to characterize the pre-synaptic and post-synaptic effect of *Micrurus* snake venom. Moreover, these preparations serve as a support for muscle tissue histopathology to determine myotoxicity resulting from exposure to poison.

Conflicts of interest

None stated by the authors.

Funding

None stated by the authors.

Acknowledgements

None stated by the authors.

References

1. Gutiérrez JM, Williams D, Fan HW, Warrell DA. Snakebite envenoming from a global perspective: Towards an integrated approach. Toxicon. 2010;56(7):1223-35. http://doi.org/bqxp9z.
2. Rojas-Bárcenas AM. Informe de evento accidente ofídico, Colombia, 2017. Bogotá D.C.: Instituto Nacional de Salud; 2017.
3. Lynch J, Angarita-Sierra T, Ruiz-Gómez FJ. Programa nacional para la conservación de las serpientes presentes en Colombia. Bogotá D.C.: Instituto Nacional de Salud; 2016.
4. Feitosa DT, Da Silva NJ, Pires MG, Zaher H, Prudente AL da C. A new species of monadal coral snake of the genus *Micrurus* (Serpentes, *Elapidae*) from western Amazon. Zootaxa. 2015;3974(4):538-54. http://doi.org/d3pm.
5. Campbell JA, Lamar WW. The venomous reptiles of the western hemisphere. New York: Comstock Publishing Associates; 2004.
6. Uetz P, Hošek J. The Reptile Database. 2018 [cited 2020 Jul 13]. Available from: https://bit.ly/32g3yGa.
7. Roze JA. Coral Snakes of the Americas: Biology, Identification, and Venoms. Malabar, FL: Krieger Pub Co; 1996.
8. Meier J, Sotcker KF. Biology and distribution of venomous snakes of medical importance and the composition of snake venoms. In: Meier J, White J, editors. Handbook of clinical toxicology of animal venoms and poisons. New York: Informa Healthcare; 2008. p. 752.
9. Rosenberg HI. Histology, histochemistry, and emptying mechanism of the venom glands of some elapid snakes. J Morphol. 1967;123(2):133-55. http://doi.org/fp69vf.
10. Costa-Cardoso J, de Siqueira-Franca F, Hui-Wen F, Santána-Melaque C, Haddad V. Animais Peconhentos no Brasil. 2nd ed. Butantan: Sarvier; 2009.
11. Johnston P. Homology of the jaw muscles in lizards and snakes-a solution from a comparative gnathostome approach. Anat Rec (Hoboken). 2014;297(3):574-85. https://doi.org/fcq2.
12. Boldrini-França J, Cologna CT, Pucca MB, De Castro K, Bordon F, Amorim FG, *et al.* Minor snake venom proteins: structure, function and potential applications. Biochim Biophys Aacta Gen Subj. 2017;1861(4):824-38. http://doi.org/f93c28.
13. Cecchini AL, Marcussi S, Silveira LB, Borja-Oliveira CR, Rodrigues-Simioni L, Amara S, *et al.* Biological and enzymatic activities of *Micrurus sp*. (Coral) snake venoms. Comp Biochem Physiol A Mol Integr Physiol. 2005;140(1):125-34. http://doi.org/bx4jgn.
14. Henao-Duque AM, Núñez-Rangel V. Maintenance of red-tail coral snake (*Micrurus mipartitus*) in captivity and evaluation of individual venom variability. Acta Biol. Colomb. 2016;21(3):593-600. http://doi.org/d3pn.
15. Lomonte B, Rey-Suárez P, Fernández J, Sasa M, Pla D, Vargas N, *et al.* Venoms of *Micrurus* coral snakes: Evolutionary trends in compositional patterns emerging from proteomic analyses. Toxicon. 2016;122:7-25. http://doi.org/f9bbzd.
16. Rey-Suárez P, Núñez V, Saldarriaga-Córdoba M, Lomonte B. Primary structures and partial toxicological characterization of two phospholipases A2 from *Micrurus mipartitus* and *Micrurus dumerilii* coral snake venoms. Biochimie. 2017;137:88-98. http://doi.org/gbhbbg.
17. Olamendi-Portugal T, Batista CVF, Restano-Cassulini R, Pando V, Villa-Hernández O, Zavaleta-Martínez-Vargas A, *et al.* Proteomic analysis of the venom from the fish eating coral snake *Micrurus surinamensis*: novel toxins, their function and phylogeny. Proteomics. 2008;8(9):1919-32. http://doi.org/bx74br.
18. Bon C, Choumet V, Delot E, Faure G, Robbe-Vincent A, Saliou B. Different evolution of phospholipase A2 neurotoxins (beta-neurotoxins) from *Elapidae* and Viperidae snakes. Ann N Y Acad Sci. 1994;710:142-8. http://doi.org/dzsvbg.
19. Renjifo C, Smith EN, Hodgson WC, Renjifo JM, Sanchez A, Acosta R, *et al.* Neuromuscular activity of the venoms of the Colombian coral snakes *Micrurus dissoleucus* and *Micrurus mipartitus*: an evolutionary perspective. Toxicon. 2012;59(1):132-42. http://doi.org/d6wmnk.
20. Da Silva NJ, Aird SD. Prey specificity, comparative lethality and compositional differences of coral snake venoms. Comp Biochem Physiol C Toxicol Pharmacol. 2001;128(3):425-56. http://doi.org/cr82pn.
21. Rey-Suárez P, Núñez V, Fernández J, Lomonte B. Integrative characterization of the venom of the coral snake *Micrurus dumerilii (Elapidae)* from Colombia: Proteome, toxicity, and cross-neutralization by antivenom. J Proteomics. 2016;136:262-73. http://doi.org/f8fxzv.
22. Sanz L, Pla D, Pérez A, Rodríguez Y, Zavaleta A, Salas M, *et al.* Venomic analysis of the poorly studied desert coral snake, *Micrurus tschudii tschudii*, supports the 3FTx/PLA2 dichotomy across *Micrurus* venoms. Toxins (Basel). 2016;8(6):178. http://doi.org/d3pq.
23. Hodgson WC, Wickramaratna JC. *In vitro* neuromuscular activity of snake venoms. Clin Exp Pharmacol Physiol. 2002;29(9):807-14. http://doi.org/d7vqfp.
24. Su MJ, Coulter AR, Sutherland SK, Chang CC. The presynaptic neuromuscular blocking effect and phospholipase A2 activity of textilotoxin, a potent toxin isolated from the venom of the Australian brown snake, *Pseudonaja textilis*. Toxicon. 1983;21(1):143-51. http://doi.org/fbcv8z.
25. Crachi MT, Hammer LW, Hodgson WC. A pharmacological examination of venom from the Papuan taipan (Oxyuranus scutellatus canni). Toxicon. 1999;37(12):1721-34. http://doi.org/ckswr9.
26. Harvey AL, Barfaraz A, Thomson E, Faiz A, Preston S, Harris JB. Screening of snake venoms for neurotoxic and myotoxic effects using simple *in vitro* preparations from rodents and chicks. Toxicon. 1994;32(3):257-65. http://doi.org/brg4wt.
27. Wickramaratna JC, Hodgson WC. A pharmacological examination of venoms from three species of death adder (Acanthophis antarcticus, Acanthophis praelongus and Acanthophis pyrrhus). Toxicon. 2000;39(2-3):209-16. http://doi.org/cvm439.
28. Rowan EG, Pemberton KE, Harvey AL. On the blockade of acetylcholine release at mouse motor nerve terminals by beta-bungarotoxin and crotoxin. Br J Pharmacol. 1990;100(2):301-4. http://doi.org/d3pr.

29. Rey-Suárez P, Núñez V, Gutiérrez JM, Lomonte B. Proteomic and biological characterization of the venom of the redtail coral snake, *Micrurus mipartitus (Elapidae)*, from Colombia and Costa Rica. J Proteomics. 2011;75(2):655-67. http://doi.org/cjq7n7.
30. Chang CC, Lee JD, Eaker D, Fohlman J. Short Comunicatios The presynaptic neuromuscular blocking action of Taipoxin. A comparison with Beta-Bungarotoxin and Crotoxin. Toxicon. 1977;15(6):571-6. http://doi.org/cpn2cs.
31. Su MJ, Chang CC. Presynaptic effects of snake venom toxins which have phospholipase A2 activity (beta-bungarotoxin, taipoxin, crotoxin). Toxicon. 1984;22(4):631-40. http://doi.org/dqdsxh.
32. Wilson HI, Nicholson GM, Tyler MI, Howden MEH. Induction of giant miniature end-plate potentials during blockade of neuromuscular transmission by textilotoxin. Naunyn Schmeideberg's Arch Pharmacol. 1995;352(1):79-87. http://doi.org/cx4kqk.
33. Hodgson WC, Dal Belo CA, Rowan EG. The neuromuscular activity of paradoxin: a presynaptic neurotoxin from the venom of the inland taipan (Oxyuranus microlepidotus). Neuropharmacology. 2007;52(5):1229-36. http://doi.org/dd84dd.
34. da Silva Jr NJ, Griffin PR, Aird SD. Comparative chromatography of Brazilian coral snake *(Micrurus)* venoms. Comp Biochem Physiol B. 1991;100(1):117-26. http://doi.org/b3qdpf.
35. Moreira KG, Prates MV, Andrade FAC, Silva LP, Beirão PSL, Kushmerick C, *et al.* Frontoxins, three-finger toxins from *Micrurus* frontalis venom, decrease miniature endplate potential amplitude at frog neuromuscular junction. Toxicon. 2010;56(1):55-63. http://doi.org/cvdjvz.
36. Fernández J, Alape-Girón A, Angulo Y, Sanz L, Gutiérrez JM, Calvete JJ, *et al.* Venomic and Antivenomic Analyses of the Central American Coral Snake, *Micrurus nigrocinctus (Elapidae)*. J Proteome Res. 2011;10(4):1816-27. http://doi.org/cb766h.
37. Corrêa-Netto C, Junqueira-de-Azevedo I de LM, Silva DA, Ho PL, Leitão-de-Araújo M, Alves MLM, *et al.* Snake venomics and venom gland transcriptomic analysis of Brazilian coral snakes, *Micrurus altirostris* and *M. corallinus*. J Proteomics. 2011;74(9):1795-809. http://doi.org/bjvtgw.
38. Ciscotto PHC, Rates B, Silva DAF, Richardson M, Silva LP, Andrade H, *et al.* Venomic analysis and evaluation of antivenom cross-reactivity of South American *Micrurus* species. J Proteomics. 2011;74(9):1810-25. http://doi.org/d5zxjg.
39. Vergara I, Pedraza-Escalona M, Paniagua D, Restano-Cassulini R, Zamudio F, Batista CVF, *et al.* Eastern coral snake *Micrurus fulvius* venom toxicity in mice is mainly determined by neurotoxic phospholipases A2. J Proteomics. 2014;105:295-306. http://doi.org/d3pt.
40. Bénard-Valle M, Carbajal-Saucedo A, de Roodt A, López-Vera E, Alagón A. Biochemical characterization of the venom of the coral snake *Micrurus tener* and comparative biological activities in the mouse and a reptile model. Toxicon. 2014;77:6-15. http://doi.org/f5pt7k.
41. Fernández J, Vargas-Vargas N, Pla D, Sasa M, Rey-Suárez P, Sanz L, *et al.* Snake venomics of *Micrurus alleni* and *Micrurus mosquitensis* from the Caribbean region of Costa Rica reveals two divergent compositional patterns in New World elapids. Toxicon. 2015;107(Pt B):217-33. http://doi.org/d3pv.
42. Casais-E-Silva LL, Teixeira CFP, Lebrun I, Lomonte B, Alape-Girón A, Gutiérrez JM. Lemnitoxin, the major component of *Micrurus lemniscatus* coral snake venom, is a myotoxic and pro-inflammatory phospholipase A2. Toxicol Lett. 2016;257:60-71. http://doi.org/f8vdxx.
43. Lomonte B, Sasa M, Rey-Suárez P, Bryan W, Gutiérrez JM. Venom of the Coral Snake *Micrurus clarki*: Proteomic Profile, Toxicity, Immunological Cross-Neutralization, and Characterization of a Three-Finger Toxin. Toxins (Basel). 2016;8(5):138. http://doi.org/d3pw.
44. Liu S, Zhang C, Xu YF, Yang F, Sun MZ. Electrospray ionization mass spectrometry as a critical tool for revealing new properties of snake venom phospholipase A2. Rapid Commun Mass Spectrom. 2009;23(8):1158-66. http://doi.org/b4wv7n.
45. Favreau P, Menin L, Michalet S, Perret F, Cheneval O, Stöcklin M, *et al.* Mass spectrometry strategies for venom mapping and peptide sequencing from crude venoms: case applications with single arthropod specimen. Toxicon. 2006;47(6):676-87. http://doi.org/c48gh9.
46. Calvete JJ, Juárez P, Sanz L. Snake venomics. Strategy and applications. J Mass Spectrom. 2007;42(11):1405-14. http://doi.org/bx25tm.
47. Chang CC, Chen TF, Lee CY. Studies of the presynaptic effect of b-bungarotoxin on neuromuscular transmission. J Pharmacol Exp Ther. 1973;184(2):339-45.
48. Folhman J, Eaker D, Karlsson E, Thesleff S. Taipoxin, an Extremely Potent Presynaptic Neurotoxin from the Venom of the Australian Snake Taipan (Oxyuranus s. scutellatus). Isolation, Characterization, Quaternary Structure and Pharmacological Properties. Eur J Biochem. 1976;68(2):457-69. http://doi.org/d6qjc9.
49. Karlsson E, Eaker D, Rydén L. Purification of a presynaptic neurotoxin from the venom of the Australian tiger snake Notechis scutatus scutatus. Toxicon. 1972;10(4):405-13. http://doi.org/ccdh56.
50. Fohlman J. Comparison of two highly toxic Australian snake venoms: The taipan (Oxyuranus s. scutellatus) and the fierce snake (Parademansia microlepidotus). Toxicon. 1979;17(2):170-2. http://doi.org/dfzn4x.
51. Chang CC, Lee JD. Crotoxin, the neurotoxin of South American rattlesnake venom, is a presynaptic toxin acting like beta-bungarotoxin. Naunyn Schmiedebergs Arch Pharmacol. 1977;296(2):159-68. http://doi.org/cp8942.
52. Sampaio SC, Hyslop S, Fontes MRM, Prado-Franceschi J, Zambelli VO, Magro AJ, *et al.* Crotoxin: novel activities for a classic beta-neurotoxin. Toxicon. 2010;55(6):1045-60. http://doi.org/dn6b4b.
53. Harris JB, Scott-Davey T. Secreted phospholipases A2 of snake venoms: Effects on the peripheral neuromuscular system with comments on the role of phospholipases A2 in disorders of the CNS and their uses in industry. Toxins (Basel). 2013;5(12):2533-71. http://doi.org/f5nb6k.
54. Rigoni M, Paoli M, Milanesi E, Caccin P, Rasola A, Bernardi P, *et al.* Snake Phospholipase A2 Neurotoxins Enter Neurons, Bind Specifically to Mitochondria, and Open Their Transition Pores. J Biol Chem. 2008;283(49):34013-20. http://doi.org/dzg9xh.
55. Gutiérrez JM, Lomonte B. Phospholipases A2: Unveiling the secrets of a functionally versatile group of snake venom toxins. Toxicon. 2013;62:27-39. http://doi.org/f4nh46.
56. Ranawaka U, Lalloo DG, de Silva HJ. Neurotoxicity in Snakebite—The Limits of Our Knowledge. PLoS Negl Trop Dis. 2013;7(10):1-18. http://doi.org/gbfmcw.
57. Xiong S, Huang C. Synergistic strategies of predominant toxins in snake venoms. Toxicol Lett. 2018;287:142-54. http://doi.org/gc7t2h.
58. Barber CM, Isbister GK, Hodgson WC. Alpha neurotoxins. Toxicon. 2013;66:47-58. http://doi.org/d3p2.
59. Utkin YN. Three-finger toxins, a deadly weapon of elapid venom--milestones of discovery. Toxicon. 2013;62:50-5. http://doi.org/f4ntp2.
60. Kini RM, Doley R. Structure, function and evolution of three-finger toxins: mini proteins with multiple targets. Toxicon. 2010;56(6):855-67. http://doi.org/bq2s2g.
61. Nirthanan S, Gopalakrishnakone P, Gwee MCE, Khoo HE, Kini RM. Non-conventional toxins from Elapid venoms. Toxicon. 2003;41(4):397-407. http://doi.org/fbtc8v.

62. Kopper RA, Harper GR, Zimmerman S, Hook J. Comparison of total protein and phospholipase A(2) levels in individual coral-snake venoms. Toxicon. 2013;76:59-62. http://doi.org/f5mwks.
63. Rey-Suárez P, Floriano RS, Rostelato-Ferreira S, Saldarriaga-Córdoba M, Núñez V, Rodrigues-Simioni L, *et al.* Mipartoxin-I, a novel three-finger toxin, is the major neurotoxic component in the venom of the redtail coral snake *Micrurus mipartitus (Elapidae).* Toxicon. 2012;60(5):851-63. http://doi.org/f374z6.
64. Alape-Girón A, Stiles B, Schmidt J, Girón-Cortes M, Thelesman M, Jörnvall H, *et al.* Characterization of multiple nicotinic acetylcholine receptor-binding proteins and phospholipases A2 from the venom of the coral snake *Micrurus nigrocinctus.* FEBS Lett. 1996;380(1-2):29-32. http://doi.org/bccfq4.
65. Hart AJ, Isbister GK, Hodgson WC. *In vitro* neurotoxic effects of *Pseudechis spp*. venoms: A comparison of avian and murine skeletal muscle preparations. Toxicon. 2013;63(1):112-5. http://doi.org/f4pzwq.
66. de Carvalho ND, Garcia RC, Kleber-Ferreira A, Batista DR, Cassola AC, Maria D, *et al.* Neurotoxicity of coral snake phospholipases A2 in cultured rat hippocampal neurons. Brain Res. 2014;1552:1-16. http://doi.org/f5v8x2.
67. da Silva DC, de Medeiros WA, Batista Ide F, Pimenta DC, Lebrun I, Abdalla FMF, *et al.* Characterization of a new muscarinic toxin from the venom of the Brazilian coral snake *Micrurus lemniscatus* in rat hippocampus. Life Sci. 2011;89(25-26):931-8. http://doi.org/bjmd4x.
68. Oliveira DA, Harasawa C, Seibert CS, Silva LLC, Pimenta DC, Lebrun I, *et al.* Phospholipases A2 isolated from *Micrurus lemniscatus* coral snake venom: Behavioral, electroencephalographic, and neuropathological aspects. Brain Res Bull. 2008;75(5):629-39. http://doi.org/cddsgq.