Bothropic and crotalic venoms: main aspects and derivative products for therapeutic and diagnostic purposes - a brief approach

Venenos botrópicos e crotálicos: principais aspectos e produtos derivados para fins terapêuticos e diagnósticos - uma breve abordagem

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ABSTRACT

Ophidian accidents, mainly by snakes of the Bothrops and Crotalus genera, are an important public health problem, especially in tropical countries due to the high rate of occurrence and lethality, being considered as a neglected disease and with challenging treatment. Thus, more knowledge that can minimize the devastating effects of snakebites is required. This brief review addressed aspects related to snakebite accidents belonging to the Bothrops and Crotalus genera,

with regard to their epidemiology, some biochemical characteristics of the respective venoms and the development of pharmaceutical products from snake venoms for therapeutic and diagnostic purposes.

Keywords: ophidian accidents, bothrops spp, crotalus spp, snakebite, venoms.

RESUMO
Os acidentes ofídios, principalmente por cobras dos géneros Bothrops e Crotalus, são um importante problema de saúde pública, especialmente em países tropicais devido à elevada taxa de ocorrência e letalidade, sendo considerados como uma doença negligenciada e com tratamento desafiante. Assim, é necessário mais conhecimento que possa minimizar os efeitos devastadores das mordidas de cobra. Esta breve revisão abordou aspectos relacionados com acidentes com mordidas de cobra pertencentes aos géneros Bothrops e Crotalus, no que diz respeito à sua epidemiologia, algumas características bioquímicas dos respectivos venenos e o desenvolvimento de produtos farmacêuticos a partir de venenos de cobra para fins terapêuticos e de diagnóstico.

Palavras-chave: acidentes ophidian, Bothrops spp, Crotalus spp, mordida de cobra, venenos.

1 HISTORY

Snakes are members of a group of reptiles that first appeared as aquatic forms and later emerged onto the land [12]. There are fossil records suggesting that snakes appeared about 135 million years ago [54]. The great movement and human occupation in the different habitats have caused important changes in ecosystems resulting in an increase in the number of accidents caused by venomous snakes. Ophidian accidents have been shown to be an important public health problem, especially in tropical countries due to the high rate of occurrence and lethality [67].

Effective treatment for patients who have suffered snakebite is a challenge that the man has long been trying to solve. In 1894, Albert Calmette, Phisalix and Bertrand were able to demonstrate the possibility of transferring resistance from an immunized animal to another non-immunized one, thus providing passive protection against venoms [45].

The first epidemiological study of ophidian accidents in Brazil was carried out by Vital Brazil in 1901, when the number of deaths from this type of accident in the State of São Paulo was surveyed [20]. Later, in the same year, the first vials of antiophidic serum were delivered for consumption. These vials started to be distributed together with a type of bulletin investigating the data on the accident that demanded the administration of the antivenom serum [91]. Over the years, new data collection models have emerged for the notification and identification of these accidents, but these models still basically follow the same variables established by Vital Brazil [15].

Since 1986, the notification of such occurrences became mandatory in Brazil, which has contributed a lot to a better understanding of its epidemiology. In 2007, given its importance in assisting victims who suffered an snakebite accident, antiophidic serum was added to the list of essential drugs [65]. According to Rojas et al. (2007) [75], ophidian accidents do not occur in a specific location and, therefore, cases have been recorded in different geographic areas in our country. It should be noted that the epidemiology of snakebites accidents in Brazil shows a profile that has remained unchanged for the past 100 years. These accidents have a constant profile related to climatic conditions and increased human activity in the countryside. They occur more frequently in the period from November to April and most of them during the day affecting the lower limbs (about 75% of cases) of predominantly male individuals aged over 15 years. Such characteristics are explained by the type of work developed by the victims, since rural workers are the most affected. Snakes of *Bothrops* genus are responsible for most accidents followed by *Crotalus* genus, although other genera can also cause fewer accidents.

According to Gutierrez et al. (2013) [44], despite the global impact caused by snakebite accidents, they have not yet received the necessary attention from the community, the pharmaceutical industry, governments and public health advocacy groups. Thus, snakebite accident is still considered a neglected disease, although it was already included in the WHO list of Neglected Tropical Diseases (NTDs) in 2009. Still according to the same authors, snakebite affects mainly impoverished rural populations with no political voice; therefore, victims cannot influence regional and national political and administrative decisions. In this way, their needs remain virtually unknown and politically neglected.

Snake accidents are life threatening and bring immeasurable damage to victims, and may even lead to death. However, venoms extracted from different types of snakes have shown great utility as a source of raw material for an important range of pharmaceutical products. Such venoms are complex mixtures of small molecules and peptides / proteins, and most of them exhibit activities such as neurotoxic, cytotoxic, cardiotoxic and myotoxic, among others [25].

Given the importance and scarcity of information on the subject, this brief review sought to address the development of pharmaceutical products from snake venoms for therapeutic and diagnostic purposes relevant to human medicine. In our view, this review may be of some utility to professionals and academics seeking quick information about products derived from herpeto fauna, as well as serving as a stimulus to researchers to search for new products derived from Brazilian and world biodiversity. The need to seek improvements in the treatment of victims of snakebites and other illnesses, in addition to launching new diagnostic solutions on the market, corroborate the idea of a didactic, practical, and motivating review for those who work in

science, technology and innovation. Undoubtedly, substances derived from snake venom have great therapeutic and diagnostic potential, deserving further exploration of this still poorly investigated potential.

2 METHODOLOGY

The articles cited in this review were searched based on Medline, Embase, LILACS, Web of Science and Scielo, without date limit, in English, Portuguese and Spanish, using as keywords "snake venoms", "bothropic venom", "crotalic venom" associated with "pharmaceutical products", "diagnostic products", "therapeutic products" and "biochemical characteristics".

3 EPIDEMIOLOGY OF THE TWO MAIN SPECIES IN BRAZIL: *BOTHROPS* AND *CROTALUS*

According to Azevedo-Marques et al. (2003) [5], in Brazil there are four genera of venomous snakes, with dozens of recognized sub-species. The *Bothrops* genus (jararaca, jararacuçu and urutu, among others) and *Micrurus* (corals) can be found throughout the national territory, while the *Crotalus* genus (rattlesnakes) is preferentially distributed in the Southeast and South of the country, and the *Lachesis* genus (surucucus), in the Amazon Region.

Among the species present in the Brazilian fauna, snakes belonging to the *Bothrops* and *Crotalus* genera are responsible for the highest frequency of snakebites in our country. There is a predominance of bothropic accident, which corresponds to approximately 87 % of cases, followed by crotalic accident with approximately 9 % of cases, and the remaining 4 % occur by laquetic and elapitic accidents (caused by the genus *Micrurus*). Although crotalic accidents are in second place as to their occurrence, they occupy the first place in relation to the lethality index [18; 73; 19; 13]. In Brazil, in 2019, 30,482 cases of snakebites were reported. Of this total, 3,309 accidents occurred in the State of Minas Gerais, where the authors of this review live. Snakes of the *Bothrops* genus caused 2,080 accidents while snakes of the genus *Crotalus* caused 599 [17].

Bothrops genus (*e.g.* jararaca, jararacuçu, urutu) has more than 60 species found throughout Brazil and represents the most significant group of venomous snakes, due to the high occurrence of cases and lethality. They are widely distributed in the country, show aggressive behavior, and attack their prey silently. This genus of snake occurs, more frequently, in humid areas, such as rivers and ponds. *Bothrops jararaca* is the species that predominates in Brazil and is found mainly in the south of Bahia, and southeast and south regions of the country

(92; 19; 32).

In Brazil, there is a single species of snake of the *Crotalus* genus, *Crotalus durissus*, which has six subspecies. The *Crotalus durissus terrificus* subspecies is the most frequently found and is seen from Rio Grande do Sul (south region) to Minas Gerais (southeastern region), and is popularly known as rattlesnake, four-wind rattlesnake, boicininga, among other popular names (79). Snakes of the *Crotalus* genus are very rarely found in forests or coastal areas, as they prefer drier and stony places, and are commonly found in open fields, hillsides, savannahs and sandy areas in general (49; 47).

4 MAIN ASPECTS OF SNAKE VENOMS

Snake venoms are complex mixtures of many substances, including small molecules and peptides / proteins. Most of these substances exhibit bioactivity presenting an action that can be toxic, enzymatic, activating and inhibiting, with deleterious effects on cells (87; 25). Although several toxins extracted from snake venom can have a fatal effect on living beings, potential uses as diagnostic tools, therapeutic agents or drugs have been reported. The potential therapeutic properties, such as antitumor, antimicrobial, anticoagulant and analgesic activities were reviewed by Yau Sang Chan et al. (2016) (25).

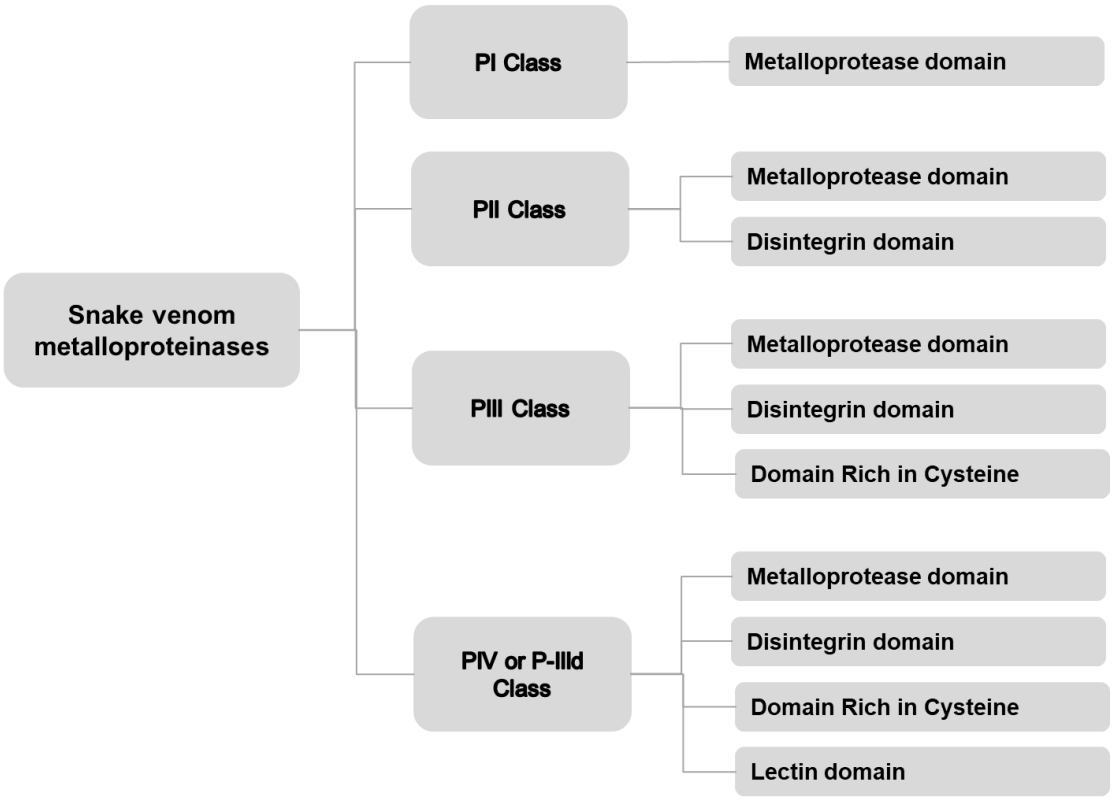
4.1 BOTHROPIC VENOM

The effect on the human body caused by inoculation of snake venom is closely related to its chemical composition. The venom of snakes of the *Bothrops* genus basically has proteolytic, anticoagulant and hemorrhagic actions. However, composition of the venom may vary between the same species in different geographic regions due to the possible difference in diet and even in the same animal depending on their age (35). Its venom has numerous enzymes with a wide spectrum of actions, such as phospholipase A2 and L-amino acid oxidase. Metalloproteinases, serinoproteases, phospholipases, disintegrins, myotoxins and neurotoxins stand out as the main components of this venom. They have an immense variety of biological effects, such as anticoagulant and myotoxic, which are related to their proteolytic, hemorrhagic and coagulant properties (23; 63).

The metalloproteinases found in snake venoms are proteolytic enzymes that play important biological functions in living beings and, in snakes, are synthesized in the venom gland (41). In *Bothrops jararaca* venom, metalloproteinases occupy the largest percentage of its composition (26), being one of the best known and most studied of this species, the metalloproteinase called jararagin (66). Snake venom metalloproteinases are classified based on

the organization of their domains, as described in Figure 1.

Figure 1. Classification of metalloproteinases present in *Bothrops* snake venom [14; 34]



The main effect of these metalloproteinases is the disturbance of the hemostatic system. From there, these substances can be classified into coagulating and anticoagulant enzymes. Therefore, the coagulating enzymes activate factors of the coagulation cascade (prothrombin or factor II, factor X) and those anticoagulants present fibrinolytic activities, which makes the fibrinogen protein non-coagulable [72]. The hemorrhagic, inflammatory and necrotic effects observed in cases of snakebites of this family are related to the proteolytic activity of these metalloproteinases found in venoms [43].

The serine proteases found in snake venoms are enzymes that have an effect on the hemostatic system, as they are able to act on the components of the coagulation cascade and, consequently, leads to an imbalance of this system [53].

Another component found in venoms is phospholipases, mainly phospholipase A2. These are enzymes that act as catalysts for the hydrolysis of ester bonds at the sn2 position of glycerophospholipids, which have fatty acids and lysophospholipid as products [28]. These phospholipases can be classified according to their regulation, mechanism, location, amino acid sequence and structure into 15 groups [82; 22]. Those found in snake venoms are part of the

category of secreted phospholipases A2 and, according to this classification, those of the *Viperidae* family, belong to group II [80], which has antiangiogenic properties and the ability to modulate cell adhesion and proliferation *in vitro* [10; 11; 50]. They are mainly responsible for the local damage observed in snake accidents of the *Bothrops* genus [93].

As mentioned, *Bothrops* venom has in its composition the enzyme L-amino acid oxidase, a flavoenzyme that belongs to the class of oxidoreductases that catalyze the deamination of L-amino acids, which leads to the formation of the corresponding alpha-keto acid, ammonia and peroxide of hydrogen [88].

The proteolytic properties of bothropic venom result from its cytotoxic action directly on tissues. Cytotoxins induce the release of histamine and phospholipase A2, which release arachidonic acid from membrane phospholipids, initiating the synthesis of prostaglandins (which increase capillary permeability). It is known that the venom contains proteases that can act in two ways that is, cleaving kininogen releasing kinins, and leading to the production of nitric oxide and prostaglandins and / or degrading the patient's own tissue proteins [53]. The hemorrhagic action of the venom is associated with the role of hemorrhagic toxins that act directly on the capillary and venule endothelium, increasing permeability and causing lesions in its basement membrane, in addition to being associated with thrombocytopenia and coagulation changes. Such hemorrhagic toxins have a vasculotoxic action causing damage to the blood vessel endothelium, which can lead to local and / or systemic bleeding. In this process factors V and VIII, and platelets are also consumed, in addition to the generation of fibrin degradation products. Clinically, blood incoagulability can be observed leading to hemorrhage, local tissue edema and myonecrosis [3; 18]. On the other hand, the coagulant action of bothropic venom is due to its ability to act directly on the victim's coagulation cascade presenting an action like thrombin, that is, capable of converting fibrinogen into fibrin. In addition, bothropic venom also activates factor X and prothrombin contributing to the coagulant effect [57; 86].

4.2 CROTALIC VENOM

The venom of the *Crotalus* genus presents greater toxicity in relation to the *Bothrops* genus, being composed of a variety of peptides and proteins with enzymatic activity, among which is mentioned the crotoxin, a neurotoxin of pre-synaptic action, the main substance responsible for its neurotoxic and lethal effect [73]. Crotoxin corresponds to 50 % of crotalic venom and is formed by two fractions. A fraction, called crotoxin B, has phospholipase A2 activity; and the other fraction, crotoxin A, has no enzymatic activity, the first being basic and the second acidic, respectively [69; 76]. This interaction between these two fractions is responsible

for the high toxicity of the *Crotalus* genus venom [6]. The phospholipases A2 present in the crotoxin B fraction of the venom, are already well described in the literature, as they are mainly responsible for the myotoxic, cytotoxic and neurotoxic actions of the venom. As already mentioned, they are enzymes that are part of type II secreted phospholipases A2 that, with their action, release fatty acids, mainly arachidonic acid and lysophospholipids. This free arachidonic acid, together with prostaglandins, are important mediators related to neuronal effects [81].

As previously mentioned, the neurotoxic effect of the venom of the *Crotalus* genus is due to the crotoxin that acts, basically, by inhibiting the release of acetylcholine in the nerve endings. This inhibition leads to a neuromuscular block responsible for the locomotion disorders observed in patients, and may lead to respiratory paralysis in more severe cases [7; 18]. In addition, other substances are also present in the venom, such as crotamine, gyroxin and convulxin, among other thrombin-like enzymes, which can cause respiratory paralysis, severe hypotension, coagulation disorders, myotoxicity and acute kidney failure [46]. Serinoproteases, hyaluronidases, L-amino acid oxidases, peptides, carbohydrates, serotonin, calcium, magnesium, copper, iron, as well as enzyme inhibitors are also found in this venom [72]. Thus, it is possible to infer that the venom of snakes of the *Crotalus* genus has mainly neurotoxic, myotoxic and coagulant actions [18].

The crotamine present in *Crotalus* venom is nothing more than a 42 amino acid polypeptide that can be considered a myotoxin [74]. Studies show that this toxin may be responsible for inducing the depolarization of the skeletal muscle membrane by increasing its permeability to Na^+, leading to the occurrence of short contractions in the muscles and their prolonged relaxation. These effects on muscle fibers can be explained by the action of crotamine on them through the sodium channels [62].

Gyroxin is a glycoprotein that can be considered similar to thrombin and has esterasic activities [1]. This similarity to thrombin makes it responsible for the coagulating action of *Crotalus* venom [51]. Studies in rats have shown a behavioral activity caused by this toxin, such as rotation of the body in the longitudinal axis, hypoactivity, opistotonia, immobility and a phenomenon called gyroxin syndrome, characterized by interspersed periods of prostration [77].

First, it was believed that convulxin was responsible for the seizures observed in the victims of snakebites caused by snakes of the genus *Crotalus*. However, studies have reported that this toxin does not have such convulsive activity [55]. It has also been described that convulxin is a potent blood platelet activator capable of leading to platelet aggregation [68].

The myotoxic effect of this venom occurs due to damage to skeletal muscle fibers (rhabdomyolysis). As a result of this generalized muscle destruction, myoglobin is released

and, consequently, there is myoglobinuria that gives urine a dark color. This condition can evolve to acute renal failure in more severe cases [18; 59]. The coagulant effect is due to the presence in the venom of enzymes like thrombin, which convert fibrinogen into fibrin. Excessive consumption of fibrinogen can lead to coagulation disorders, such as blood incoagulability, at the same time that changes in prothrombin time (PT) and activated partial thromboplastin time (aPTT) confirm the existence of disorders of coagulant activity [4; 18; 5].

5 DEVELOPMENT OF PHARMACEUTICAL PRODUCTS FROM SNAKE VENOMS FOR THERAPEUTIC AND DIAGNOSTIC PURPOSES

As already mentioned, thousands of cases of snakebites are reported annually in Brazil and worldwide. Therefore, several studies have been developed in order to understand the mechanism of action of venoms, as well as their therapeutic potential. It is encouraging that several studies have emerged, bringing innovations and improvements not only in the modality of treatment, but also an expansion in the range of diagnostic possibilities.

A very curious question would be how can the deadly power of snake venom be converted into medicine? Since the beginning, many healers, sorcerers, physicians, and scientists have sought in venoms the remedy to cure diseases. As an example, the physician Vital Brazil can be cited who developed the antiophidic serum from the snake venom to cure the evils caused by it [20]. The venoms are also a source of greed for their potential to generate wealth, as they serve as a model for new drugs and pesticides.

It has been reported for many years that venoms from snake families such as *Elapidae*, *Crotalidae* and *Viperidae* had satisfactory effects on Yoshida sarcoma cells [16], highlighting the *Crotalus durissus terrificus* venom for ~~through~~ its direct action on tumor cells [84]. In a broader context, the therapeutic effects of venoms have also been described in diseases such as arterial hypertension, melanoma, leishmaniasis, as well as antimicrobial, antiviral and antitumor activities. A few years after the discovery of the angiotensin-converting enzyme (ACE), responsible for the formation of angiotensin II which causes arterial hypertension, the Brazilian scientist Sergio Ferreira described a potentiating factor for bradykinin present in the *Bothrops jararaca* venom. This discovery led the scientists David Cushman, Miguel Ondetti and their collaborators to produce the first oral ACE-inhibiting drug, called captopril [33; 64]. In 2002, Correa [27] reported that a hemorrhagic metalloproteinase taken from *Bothrops jararaca* venom, jararagin, showed encouraging results in melanoma cell cultures by altering cell morphology and biological activity, which led to a decrease in the number of metastases.

Castilhos (2011) [24] developed a study focusing on the effects of bothropic venom on

promastigote forms of *Leishmania* sp, and reported a decrease in the infectivity of parasites by the action of the venom and its protein fractions.

Numerous studies using raw venoms of *C. durissus* subspecies, or their isolated fractions, have been reported with several pharmacological actions, such as antifungal, antileishmania, antiplasmodic, antiviral, antibacterial and antitumor activities [29; 85; 9; 56; 71; 90; 8; 58].

The first studies related to antibacterial activity in snake venoms were carried out in 1948 and 1968 [39; 2]. Since then, several other studies have been carried out along this same line. Diz Filho and collaborators (2009) [29] reported that the phospholipase A2 present in the venoms of *Crotalus durissus ruruima* had a great antibacterial (*Xanthomonas axonopodis pv passiflorae*) and antifungal (*Candida albicans*) activity.

Due to its structural similarity to β-defensins, crotamine present in crotalic venoms has also been identified in studies of antimicrobial activity. Oguiura et al. (2011) [61] observed that crotamine (*Crotalus durissus terrificus)* had an antibacterial activity (*Escherichia coli*), whose action occurs through permeabilization of the membrane. In addition, Maluf et al. (2016) [31] reported in their study a dose-dependent antiparasitic activity (*Plasmodium falciparum*) for crotamine.

Another study showed *that Crotalus durissus terrificus* crude venom was able to confer resistance to infections of Vero E6 cells by dengue or yellow fever viruses. It is noteworthy that the cells treated with crotoxin showed greater protection against viral infections [56].

According to Sajevic et al. (2011) [78] and El-Aziz et al. 2019 [30], as an example of approved drugs from snake venoms that target coagulation disorders, fibrinolysis and platelet function can be cited Tirofiban / Aggrastat®, Epitibatide / Integrilin® (acute coronary syndrome, prevention of thromboembolic complications after angioplasty, stenting); Batroxobin / Defibrase® (stroke, unstable angina), and Haemocoagulase / Pentapharm® (prevention and treatment of bleeding).

Snake venoms toxins can initiate or inhibit various stages of the coagulation, and fibrinolysis pathways or platelet aggregation. Thus, such components of venoms that influence hemostasis can also be used as tools in the diagnosis of coagulopathies. Some uses are exemplified below and include:

a) monitoring of anticoagulation during treatment with hirudin by adding Ecarin, which is derived from the venom of the snake *Echis carinatus* [60]; Ecarin is also used in combination with textarin, also a component of snake venom, as a highly sensitive test for lupus anticoagulants [89];

b) dosage of the levels of the natural anticoagulation proteins C and S, by adding to the plasma ACC-C / Protac® (Pentapharm), a serinoproteinase extracted from the venom of the snake *Agkistrodon contortrix* [38]; and

c) evaluation of fibrinogen levels in plasma containing heparin, fibrin degradation products and defects in fibrin polymerization, using Batroxobin / Reptilase®, an enzyme that is a component of *Bothrops jararaca* venom and acts in a similar way to thrombin [37].

Finally, it should be noted that the continuous study of toxins present in snake venoms has led to the discovery of new substances that, extracted from the venom, can be used as important tools for the development of new types of diagnostic procedures and kits for various laboratory tests. Some examples of kits already on the market in which compounds derived from snake venoms play an essential role are: Pefakit®, Reptilase® Time, PiCT® and Pefakit® APC-R Factor V Leiden. Such kits rely on one or more compounds extracted from snake venoms as primary reagents [21; 36].

Given the above, it is possible to understand the importance of continuous research development addressing the components of the Brazilian herpeto-fauna, an inexhaustible source of resources and inputs of scientific interest. Such pharmaceutical products, including those for therapeutic and diagnostic uses, developed from the knowledge about the properties of different types of snake venom, motivate the search for new discoveries improving the quality of life of human beings. The continuous evolution of Science, making use of different types of snake venoms and their fractions, will allow an expansion of the range of benefits to the medical clinic now offered. An important suggestion within the list of diagnostic application would be the development and/or improvement of existing kits for use in the quick and reliable identification of the species or genus of snake causing the ophidian accident. This identification will allow the immediate administration of monovalent antivenom specific for the venom ensuring greater efficacy of this therapy and minimization of adverse effects [70]. This is of great value, since an early and correct treatment is related to a better outcome of victims of snakebites [48; 70] . In addition, the use of this kind of diagnostic kits can contribute to the improvement of services provided in emergency care units with poor infrastructure, since their results can quickly guide the treatment decision by less experienced medical teams, favorably impacting treatment and outcome of victims of snakebites [52] . A study carried out in Sri Lanka has reported the importance of improving diagnostic kits, given that delays in the administration of antivenom are directly related to the absence of methods for the early diagnosis of envenoming [83].

In view of the above, the crude venom and the isolated fractions of snake venoms have a

high biotechnological potential. Thus, the clinical application of proteins extracted from snake venoms with an impact on hemostasis assumes enormous importance and has aroused the interest of many researchers.

6 CONCLUDING REMARKS

Considering the number of cases reported annually, snakebite can be considered a public health problem, often neglected. This fact can be confirmed by the scarce number of recent national studies on this topic. Therefore, there is a lack of information on the various clinical, laboratory and therapeutic aspects, a fact that may negatively impact the performance of health professionals, who are often poorly trained to provide proper clinical care to victims of snakebites. The realization of this gap should motivate strategies that promote greater knowledge on this subject, aiming at better patient care and, thus, minimize the chances of clinical complications resulting from snakebites.

Therefore, the need for and importance of national studies to reduce the impact of accidents by snakebites should be emphasized, as an initiative coordinated by the World Health Organization to ensure safe and effective treatments. In this context, new therapeutic options are being explored, including recombinant antibodies and natural and synthetic toxin inhibitors, in addition to the conventional treatment that consists of administering antivenoms of animal origin[42]. Another alternative that deserves greater effort, especially from Brazilian scientists, is the development of ethnobotanical studies, since these have been able to identify medicinal plants and active compounds that inhibit the action of snake venom [40]. The great biodiversity of Brazil undoubtedly deserves to be better explored in the search for biotechnological innovations that can contribute to the solution of a wide range of health problems. Due to the relevance of the theme, the need for revisions with a brief approach is highlighted dedicated to professionals and students working in this area. It is also emphasized the need for efforts in the development of diagnostic methods for the rapid identification of the snake responsible for the accident, as well as for the early detection of complications in patients who are victims of such accidents. Strategies that can optimize the management of patients, mainly impacting on reducing the time elapsed between the patient's entry into emergency service and the administration of treatment, especially in cases where it was not possible to capture the snake, are highly desirable. It is widely known that a long time between the accident and the administration of treatment can be responsible for a more severe clinical picture or even death. Finally, efforts aimed at the development of pharmaceutical products from snake venoms for therapeutic and diagnostic purposes should be strongly encouraged due to the potential for

clinical application of components from the rich Brazilian herpetofauna.

This study has some limitations, considering that it is a literature review based on few recent studies on the subject, due to its scarcity or inappropriateness for the topic addressed.

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COMPETING INTERESTS

The authors declare no competing interests.

REFENCES

Alexander G, Grothusen J, Zepeda H, Schwartzman RJ. Gyroxin, a toxin from the venom of Crotalus durissus terrificus, is a thrombin-like enzyme. Toxicon. 1988; 26(10):953-60.

Aloof-Hirsch S, de Vries A, Berger A. The direct lytic factor of cobra venom: purification and chemical characterization. Biochim Biophys Acta. 1968; 154(1):53-60.

Amaral CF, Rezende NA, Pedrosa TM, da Silva OA, Pedroso ER. [Afibrinogenemia secondary to crotalid snake bite (Crotalus durissus terrificus)]. Rev Inst Med Trop Sao Paulo. 1988; 30(4):288-92.

Amaral CFS, Rezende NA, Silva OA, Ribeiro MMF, Magalhães RA, Reis RJ, *et al.* Insuficiência renal aguda secundária a acidentes ofídicos botrópico e crotálico. Análise de 63 casos. . Revista Instituto de Medicina Tropical de São Paulo. 1986; 28(4):220-7.

Azevedo-Marques MM, Cupo P, Hering SE. Acidentes por animais peçonhentos:serpentes peçonhentas. Simpósio: urgências e emergências dermatológicas e toxicológicas. Ribeirão Preto: Medicina; 2003.

Bancher W, Rosa RR, Furlaneto RS. Estudos sobre a fixação eletiva e quantitativa do veneno de Crotalus durissus terrificus nos tecidos nervoso, renal, hepático e muscular de Mus musculus Linnaeus, 1758. Mem Inst Butantan. 1973; 37:139-48.

Barravieira B, Peraçoli MTS. Soroterapia heteróloga. In: B. B, editor. Venenos Animais: uma visão integrada. Rio de Janeiro: EPUC; 1994.

Barros GA, Pereira AV, Barros LC, Lourenco A, Jr., Calvi SA, Santos LD, *et al.* In vitro activity of phospholipase A2 and of peptides from Crotalus durissus terrificus venom against amastigote and promastigote forms of Leishmania (L.) infantum chagasi. J Venom Anim Toxins Incl Trop Dis. 2015; 21:48.

Barros LC, Soares AM, Costa FL, Rodrigues VM, Fuly AL, Giglio JR, *et al.* Biochemical and biological evaluation of gyroxin isolated from Crotalus durissus terrificus venom. J Venom Anim Toxins incl Trop Dis. 2011; 17(1).

Bazaa A, Luis J, Srairi-Abid N, Kallech-Ziri O, Kessentini-Zouari R, Defilles C, *et al.* MVL-PLA2, a phospholipase A2 from Macrovipera lebetina transmediterranea venom, inhibits tumor cells adhesion and migration. Matrix Biol. 2009; 28(4):188-93.

Bazaa A, Pasquier E, Defilles C, Limam I, Kessentini-Zouari R, Kallech-Ziri O, *et al.* MVL-PLA2, a snake venom phospholipase A2, inhibits angiogenesis through an increase in microtubule dynamics and disorganization of focal adhesions. PLoS One. 2010; 5(4):e10124.

Bellairs AD, Underwood G. The origin of snakes. Biol Rev Camb Philos Soc. 1951; 26(2):193-237.

Bernardes CP, Menaldo DL, Mamede CC, Zoccal KF, Cintra AC, Faccioli LH, *et al.* Evaluation of the local inflammatory events induced by BpirMP, a metalloproteinase from Bothrops pirajai venom. Mol Immunol. 2015; 68(2 Pt B):456-64.

Bjarnason JB, Fox JW. Hemorrhagic metalloproteinases from snake venoms. Pharmacol Ther. 1994; 62(3):325-72.

Bochner R, Struchiner CJ. [Snake bite epidemiology in the last 100 years in Brazil: a review]. Cad Saude Publica. 2003; 19(1):7-16.

Braganca BM, Patel NT, Badrinath PG. Isolation and properties of a cobra venom factor selectively cytotoxic to Yoshida sarcoma cells. Biochim Biophys Acta. 1967; 136(3):508-20.

Ministério da Saúde. [cited 03 April 2020]. Available from: http://tabnet.datasus.gov.br/cgi/tabcgi.exe?sinannet/cnv/animaisbr.def.

BRASIL. Manual de diagnóstico e tratamento de acidentes por animais peçonhentos. Brasília: Ministério da Saúde; 2001.

BRASIL. Fundação Nacional de Saúde. Manual de diagnósticos e tratamento de acidentes por animais peçonhentos. 3rd ed. Brasília: Ministério da Saúde; 2005.

Brazil V. Contribuição ao estudo do veneno ophidico. Rev med S Paulo. 1901; 4.

Britten AHF, Card RT, Mielke CH. Reptilasetime – Useful monitor of progress in disseminated intravascular coagulation. . Abstr XIII Int Congr Haematol; Munich 1970.

Burke JE, Dennis EA. Phospholipase A2 biochemistry. Cardiovasc Drugs Ther. 2009; 23(1):49-59.

Cardoso DF, Lopes-Ferreira M, Faquim-Mauro EL, Macedo MS, Farsky SH. Role of crotoxin, a phospholipase A2 isolated from Crotalus durissus terrificus snake venom, on inflammatory and immune reactions. Mediators Inflamm. 2001; 10(3):125-33.

Castilhos P, Pereira CG, Silva ALN, Napolitano DR, Oliveira F, Souza MA. Effects of Bothrops moojeni venom on Leishmania amazonensis promastigote forms. Journal of Venomous Animals and Toxins including Tropical Diseases [online]. 2011; 17(2):150-8.

Chan YS, Cheung RCF, Xia L, Wong JH, Ng TB, Chan WY. Snake venom toxins: toxicity and medicinal applications. Appl Microbiol Biotechnol. 2016; 100(14):6165-81.

Cidade DA, Simao TA, Davila AM, Wagner G, Junqueira-de-Azevedo IL, Ho PL, *et al.* Bothrops jararaca venom gland transcriptome: analysis of the gene expression pattern. Toxicon. 2006; 48(4):437-61.

Corrêa MC, Maria DA, Moura-da-Silva AM, Pizzocaro KF, Ruiz IRG. Inhibition of melanoma cells tumorigenicity by the snake venom toxin jararhagin. Toxicon. 2002; 40(6):739-48.

da Silva Cunha KC, Fuly AL, de Araujo EG. A phospholipase A(2) isolated from Lachesis muta snake venom increases the survival of retinal ganglion cells in vitro. Toxicon. 2011; 57(4):580-5.

Diz Filho EB, Marangoni S, Toyama DO, Fagundes FH, Oliveira SC, Fonseca FV, *et al.* Enzymatic and structural characterization of new PLA2 isoform isolated from white venom of Crotalus durissus ruruima. Toxicon. 2009; 53(1):104-14.

El-Aziz TMA, Soares AG, Stockand JD. Snake venoms in drug discovery: Valuable therapeutic tools for life saving. Toxins. 2019; 11(10).

El Chamy Maluf S, Dal Mas C, Oliveira EB, Melo PM, Carmona AK, Gazarini ML, *et al.* Inhibition of malaria parasite Plasmodium falciparum development by crotamine, a cell penetrating peptide from the snake venom. Peptides. 2016; 78:11-6.

Fenwick AM, Gutberlet Jr RL, Evans JA, Parkinson CL. Morphological and molecular evidence for phylogeny and classification of South American pitvipers, genera Bothrops, Bothriopsis, and Bothrocophias (Serpentes: Viperidae). Zoological Journal of the Linnean Society. 2009; 156(3):617-40.

Ferreira SH, Rocha e Silva M. Potentiation of bradykinin and eledoisin by BPF (bradykinin potentiating factor) from Bothrops jararaca venom. Experientia. 1965; 21(6):347-9.

Fox JW, Serrano SM. Insights into and speculations about snake venom metalloproteinase (SVMP) synthesis, folding and disulfide bond formation and their contribution to venom complexity. FEBS J. 2008; 275(12):3016-30.

França FOS, Malaque CMS. Acidente botrópico. In: Cardoso JLC, Francisco Franca F, Wen FH, Malaque CMS, Haddad Junior V, editors. Animais peçonhentos do Brasil: biologia, clínica e terapêutica dos acidentes. São Paulo: Sarvier; 2009.

Funk C, Gmur J, Herold R, Straub PW. Reptilase-R--a new reagent in blood coagulation. Br J Haematol. 1971; 21(1):43-52.

Funk C, Gmür J, Herold R, Straub PW. Reptilase®-R—A New Reagent in Blood Coagulation. British Journal of Haematology. 1971; 21(1):43-52.

Gempeler-Messina PM, Volz K, Bühler B, Müller C. Protein C Activators from Snake Venoms and Their Diagnostic Use. Pathophysiology of Haemostasis and Thrombosis. 2001; 31(3-6):266-72.

Glaser HSR. Bactericidal Activity of Crotalus Venom in Vitro. Copeia. 1948; 1948(4):245-7.

Gomez-Betancur I, Gogineni V, Salazar-Ospina A, Leon F. Perspective on the Therapeutics of Anti-Snake Venom. Molecules. 2019; 24(18).

Gutiérrez JM. Comprendiendo los venenos de serpientes: 50 años de investigaciones en América Latina. Revista de Biología Tropical. 2002; 50:377-94.

Gutierrez JM, Albulescu LO, Clare RH, Casewell NR, Abd El-Aziz TM, Escalante T, *et al.* The Search for Natural and Synthetic Inhibitors That Would Complement Antivenoms as Therapeutics for Snakebite Envenoming. Toxins (Basel). 2021; 13(7).

Gutierrez JM, Rucavado A. Snake venom metalloproteinases: their role in the pathogenesis of local tissue damage. Biochimie. 2000; 82(9-10):841-50.

Gutierrez JM, Warrell DA, Williams DJ, Jensen S, Brown N, Calvete JJ, *et al.* The need for full integration of snakebite envenoming within a global strategy to combat the neglected tropical diseases: the way forward. PLoS Negl Trop Dis. 2013; 7(6):e2162.

Hawgood BJ. Doctor Albert Calmette 1863-1933: founder of antivenomous serotherapy and of antituberculous BCG vaccination. Toxicon. 1999; 37(9):1241-58.

Hernandez Cruz A, Garcia-Jimenez S, Zucatelli Mendonca R, Petricevich VL. Pro- and anti-inflammatory cytokines release in mice injected with Crotalus durissus terrificus venom. Mediators Inflamm. 2008; 2008:874962.

Jim J, Sakate M. Biologia das serpentes. In: Barraviera B, editor. Venenos animais: uma visão integrada. São Paulo: Publicações Científicas; 1994.

Johnston CI, Brown SG, O'Leary MA, Currie BJ, Greenberg R, Taylor M, *et al.* Mulga snake (Pseudechis australis) envenoming: a spectrum of myotoxicity, anticoagulant coagulopathy, haemolysis and the role of early antivenom therapy - Australian Snakebite Project (ASP-19). Clin Toxicol (Phila). 2013; 51(5):417-24.

Jorge MT, Ribeiro LA. Acidentes por serpentes peçonhentas do Brasil. AMB rev Assoc Med Bras. 1990; 36(266-77).

Khunsap S, Pakmanee N, Khow O, Chanhome L, Sitprija V, Suntravat M, *et al.* Purification of a phospholipase A(2) from Daboia russelii siamensis venom with anticancer effects. J Venom Res. 2011; 2:42-51.

Kini RM. Anticoagulant proteins from snake venoms: structure, function and mechanism. Biochem J. 2006; 397(3):377-87.

Knudsen C, Jurgensen JA, Fons S, Haack AM, Friis RUW, Dam SH, *et al.* Snakebite Envenoming Diagnosis and Diagnostics. Front Immunol. 2021; 12:661457.

Matsui T, Fujimura Y, Titani K. Snake venom proteases affecting hemostasis and thrombosis. Biochim Biophys Acta. 2000; 1477(1-2):146-56.

Melgarejo AR. Serpentes peçonhentas do Brasil. In: Cardoso JLC, Francisco Franca F, Wen FH, Malaque CMS, Haddad Junior V, editors. Animais peçonhentos do Brasil: biologia, clínica e terapêutica dos acidentes. São Paulo: Sarvier; 2009.

Mello LE, Prado-Franceschi J, Giglio JR, Cavalheiro EA. Convulxin does not induce convulsions when injected into the rat dorsal hippocampus. Acta Physiol Pharmacol Latinoam. 1989; 39(4):353-8.

Muller VD, Russo RR, Cintra AC, Sartim MA, Alves-Paiva Rde M, Figueiredo LT, *et al.* Crotoxin and phospholipases A(2) from Crotalus durissus terrificus showed antiviral activity against dengue and yellow fever viruses. Toxicon. 2012; 59(4):507-15.

Nahas L, Kamiguti AS, Barros MA. Thrombin-like and factor X-activator components of Bothrops snake venoms. Thromb Haemost. 1979; 41(2):314-28.

Neves MS, Sousa DRT, Ferreira MPSBC, Frota MZM, Souza JVB, Lozano JLL. Evaluation of antifungal activity of snake venoms from the Amazon forest. Journal of Yeast and Fungal Research. 2015; 6(2):11-6.

Nogueira RMB, Sakate M, Sangiorgio F, Laposy CB, Melero M. Experimental envenomation with Crotalus durissus terrificus venom in dogs treated with antiophidic serum - part I: clinical

evaluation, hematology and myelogram. Journal of Venomous Animals and Toxins including Tropical Diseases. 2007; 13:800-10.

Nowak G. The Ecarin Clotting Time, a Universal Method to Quantify Direct Thrombin Inhibitors. Pathophysiology of haemostasis and thrombosis. 2003; 33:173-83.

Oguiura N, Boni-Mitake M, Affonso R, Zhang G. In vitro antibacterial and hemolytic activities of crotamine, a small basic myotoxin from rattlesnake Crotalus durissus. J Antibiot (Tokyo). 2011; 64(4):327-31.

Oguiura N, Boni-Mitake M, Radis-Baptista G. New view on crotamine, a small basic polypeptide myotoxin from South American rattlesnake venom. Toxicon. 2005; 46(4):363-70.

Oliveira RBd, Ribeiro LA, Jorge MT. Fatores associados à incoagulabilidade sangüínea no envenenamento por serpentes do gênero Bothrops. Revista da Sociedade Brasileira de Medicina Tropical. 2003; 36:657-63.

Ondetti MA, Cushman DW. Inhibitors of angiotensinconverting enzyme. . In: Soffer RL, editor. Biochemical

regulation of blood pressure New York: Wiley; 1981. p. 165-204.

Organization. WH. Guidelines for the management of snake-bites2010.

Paine MJ, Desmond HP, Theakston RD, Crampton JM. Purification, cloning, and molecular characterization of a high molecular weight hemorrhagic metalloprotease, jararhagin, from Bothrops jararaca venom. Insights into the disintegrin gene family. J Biol Chem. 1992; 267(32):22869-76.

Pinho FMO, Pereira ID. Ofidismo. AMB rev Assoc Med Bras. 2001; 47:24-9.

Prado-Franceschi J, Brazil OV. Convulxin, a new toxin from the venom of the South American rattlesnake Crotalus durissus terrificus. Toxicon. 1981; 19(6):875-87.

Puig J, Vilafranca M, Font A, Closa J, Pumarola M, Mascort J. Acute intrinsic renal failure and blood coagulation disorders after a snakebite in a dog. J Small Anim Pract. 1995; 36(7):333-6.

Puzari U, Mukherjee AK. Recent developments in diagnostic tools and bioanalytical methods for analysis of snake venom: A critical review. Analytica Chimica Acta. 2020; 1137:208-24.

Quintana JC, Chacón AM, Vargas L, Segura C, Gutiérrez JM, Alarcón JC. Antiplasmodial effect of the venom of Crotalus durissus cumanensis, crotoxin complex and Crotoxin B. Acta Tropica. 2012; 124(2):126-32.

Ramos OH, Selistre-de-Araujo HS. Snake venom metalloproteases--structure and function of catalytic and disintegrin domains. Comp Biochem Physiol C Toxicol Pharmacol. 2006; 142(3-4):328-46.

Rangel-Santos A, Lima C, Lopes-Ferreira M, Cardoso DF. Immunosuppresive role of principal toxin (crotoxin) of Crotalus durissus terrificus venom. Toxicon. 2004; 44(6):609-16.

Rizzi CT, Carvalho-de-Souza JL, Schiavon E, Cassola AC, Wanke E, Troncone LR. Crotamine inhibits preferentially fast-twitching muscles but is inactive on sodium channels. Toxicon. 2007; 50(4):553-62.

Rojas C, Gonçalves, Almeida-Santos S. Epidemiologia dos acidentes ofídicos na região noroeste do estado de São Paulo, Brasil. Revista Brasileira de Saúde e Produção Animal ISSN 1519 9940. 2007; 8:193-204.

Roodt AR, Dolab JA, Segre L. Fisiopatologia y diagnóstico del ataque por serpientes venenosas: una breve actualización. Rev Med Vet. 1996; 77:64-71.

Ruiz de Torrent RM, Bongiovanni B, Leiva LC, Evangelista de Duffard AM, Rodriguez JP, Acosta de Perez OC, *et al.* Neurotoxicological effects of a thrombin-like enzyme isolated from Crotalus durissus terrificus venom (preliminary study). Toxicon. 2007; 50(1):144-52.

Sajevic T, Leonardi A, Krizaj I. Haemostatically active proteins in snake venoms. Toxicon. 2011; 57(5):627-45.

Sakate M. Terapêutica das intoxicações. In: Andrade SF, editor. Manual de terapêutica veterinária. 2nd ed. São Paulo: Roca; 2002.

Samy RP, Gopalakrishnakone P, Stiles BG, Girish KS, Swamy SN, Hemshekhar M, *et al.* Snake venom phospholipases A(2): a novel tool against bacterial diseases. Curr Med Chem. 2012; 19(36):6150-62.

Schaeffer EL, Gattaz WF. Requirement of hippocampal phospholipase A2 activity for long-term memory retrieval in rats. J Neural Transm (Vienna). 2007; 114(3):379-85.

Schaloske RH, Dennis EA. The phospholipase A2 superfamily and its group numbering system. Biochim Biophys Acta. 2006; 1761(11):1246-59.

Silva A, Hlusicka J, Siribaddana N, Waiddyanatha S, Pilapitiya S, Weerawansa P, *et al.* Time delays in treatment of snakebite patients in rural Sri Lanka and the need for rapid diagnostic tests. PLoS Negl Trop Dis. 2020; 14(11):e0008914.

Silva RJ, Fecchio D, Barraviera B. Antitumor effect of snake venous. J Venom Anim Toxins incl Trop Dis. 1996; 2(2).

Soares MA, Pujatti PB, Fortes-Dias CL, Antonelli L, Santos RG. Crotalus durissus terrificus venom as a source of antitumoral agents. J Venom Anim Toxins incl Trop Dis. 2010; 16(3).

Spadacci-Morena DD, de Tomy SC, Sano-Martins IS, Katz SG. The effect of experimental Bothrops jararaca envenomation on pregnant mice. Toxicon. 2006; 47(2):196-207.

Sunitha K, Hemshekhar M, Thushara RM, Santhosh MS, Sundaram MS, Kemparaju K, *et al.* Inflammation and oxidative stress in viper bite: an insight within and beyond. Toxicon. 2015; 98:89-97.

Tan N, Fung S, editors. Snake Venom L-Amino Acid Oxidases and Their Potential Biomedical Applications. 2008.

Triplett DA, Stocker KF, Unger GA, Barna LK. The Textarin/Ecarin ratio: a confirmatory test for lupus anticoagulants. Thrombosis and haemostasis. 1993; 70(6):925-31.

Vargas LJ, Quintana JC, Pereañez JA, Núñez V, Sanz L, Calvete J. Cloning and characterization of an antibacterial l-amino acid oxidase from Crotalus durissus cumanensis venom. Toxicon. 2013; 64:1-11.

Vaz E. Vital Brazil. . Anais Paulistas de Medicina e Cirurgia. 1950; 60:347-66.

Wen FH, Cardoso JLC, Málaque CMSa, França FOS, Sant´anna SS, Fernandes W, *et al.* Influência das alterações ambientais na epidemiologia dos acidentes ofídicos e na distribuição geográfica das serpentes de importância médica nos Estados de São Paulo e Paraná, 1988-1997. Informe Epidemiológico do Sus. 2002; 11:45-7.

Zamuner SR, da Cruz-Hofling MA, Corrado AP, Hyslop S, Rodrigues-Simioni L. Comparison of the neurotoxic and myotoxic effects of Brazilian Bothrops venoms and their neutralization by commercial antivenom. Toxicon. 2004; 44(3):259-71.