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PATHOLOGY

Creolin® administered by different pathways in rats experimentally poisoned with *Bothrops jararaca* venom

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ABSTRACT: *This study aimed to evaluate the effects of Creolin® when administered by different pathways in rats experimentally poisoned with Bothrops jararaca venom. In female Wistar rats, the Bothropic venom was inoculated intramuscularly, and then the rats were either treated with Creolin® (administered orally, topically, or intramuscularly), or with amixture of venom + Creolin® intramuscularly. Animals that received Creolin®, apart from the venom, by oral, topical, or intramuscular routes developed local symptoms and showed laboratory findings similar to those animals that received only the venom. Conversely, animals inoculated with the venom incubated with Creolin® showed no signs of local venom toxicity (necrosis or hemorrhage) and displayed hematological parameters within the normal range for the species. These results suggest that Creolin® exhibited an antiophidian effect only when it is mixed with the venom and administered intramuscularly.*
Key words: *antivenom, folk medicine, bothropic venom.*

Creolina® administrada por diferentes vias em ratos envenenados experimentalmente com veneno de *Bothrops jararaca*

RESUMO: *Esse estudo objetivou avaliar os efeitos da Creolina® quando administrada por diferentes vias de acesso em ratos experimentalmente envenenados pela peçonha de Bothrops jararaca. Em ratas Wistar fêmeas foi inoculada a peçonha botrópica por via intramuscular, e em seguida as ratas foram tratadas com Creolina® (administrada oralmente, topicamente e intramuscularmente) ou a mistura de veneno + Creolina®. Os animais que receberam a Creolina®, além do veneno, por via oral, tópica e muscular desenvolveram a sintomatologia local e achados laboratoriais semelhantes ao grupo que recebeu apenas o veneno. De forma controversa, os animais inoculados com o veneno misturado a Creolina® não apresentaram sinais característicos da ação local do veneno (necrose, hemorragia) e apresentaram parâmetros hematológicos dentro da normalidade para espécie. Esses resultados sugerem que a Creolina® apresentou efeito antiofídico apenas quando misturada ao veneno e administrada intramuscularmente.*
Palavras-chave: *antiveneno, medicina popular, veneno botrópico*.

INTRODUCTION

Snakebite accidents are a serious public health problem worldwide, especially in tropical countries, due to their high morbidity and mortality rates. In Brazil, snakes of the genus *Bothrops*-more popularly known as "jararaca," "ouricana," and "caiçara," among other names-correspond to more than 90% of the poisonings (BRASIL, 2001).

Bothrops venom is a complex mixture composed of several proteins and non-protein compounds capable of triggering local effects (edema, hemorrhage, and necrosis) and systemic effects (hemostatic changes, hypovolemic shock, and renal damage) in the animal organism (SENISE et al., 2015).

The specific treatment recommended in cases of ophidic accidents involving *Bothrops* snakes is the use of antibothropic serum (pentavalent) in combination with symptomatic auxiliary therapy. The use of this serum has significant disadvantages, some of which require specific treatment; this serum product is not readily available in most remote regions of the country, presents a high cost of production, may not

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be effective on local symptomatology, and may cause immunological responses, such as development of early or hypersensitive reactions (serum sickness), as a result of its heterogeneity (BRASIL, 2001).

Therefore, several substances of animal, vegetal, or mineral origin are commonly used as antiophidics, in an attempt to deliver alternative treatments of low cost that can effectively reverse the symptomatology caused by the venom (GOMES et al., 2016; MOTTA et al., 2017).

Among the substances deemed as antiophidics by popular medicine, in the Brazilian semi-arid region, we have highlighted Creolin® as the more commonly used. This commercial product is used empirically in cattle and small ruminants to reverse the clinical condition caused by bites from snakes of the genera *Bothrops* and *Crotalus*. Creolin® in its pure form is administered in small amounts on the tongue of the animal, immediately after a snake accident is suspected. This practice is widespread among farmers of Paraíba, and it is currently the only resource for cases of snakebite accidents in domestic animals (unpublished data).

Creolin® is a commercial product used as a veterinary disinfectant. It has in its composition a mixture of hydrocarbons derived from coal tar, cresols, and phenols. It is used as a germicidal, antiseptic, and anthelmintic solution, and also for other non-specific purposes (STEFFEN et al., 2010; HAVERKOS et al., 2017).

According to the manufacturer, this product presents health hazards. The main symptoms of Creolin® poisoning are stomach pain, headache, pain and erythema on the skin, eye pain, narcotic effects, nausea, vomiting, diarrhea, and tremors. Therefore, this product is classified as: "Veterinary combustible disinfectant, dangerous for human health" (INTERTOX, 2011).

The efficacy and mechanism of action of Creolin® are not yet completely understood. It is believed that because it is a phenolic compound, it can inactivate enzymes, denature proteins, and alter the membrane permeability of microorganisms (JAIGOBIND et al., 2007).

Such suspicions associated with the known empirical use in domestic animals support the theory of a possible effect of this compound on bothropic venom. In light of this-and the need to develop alternative therapies that are easily accessible, the objective of the present study was to investigate the antiophidic potential of Creolin® against bothropic venom and evaluate the viability of the use of this compound to foment studies that develop low-cost and easily accessible alternative protocols for snakebite accidents.

MATERIALS AND METHODS

We used adult female animals of the species *Rattus norvegicus* and of Wistar line, with a mean age of 16 weeks, weighing between 170 and 220 g, obtained from the Biotério Prof. Thomas George, Campus I of the UFPB, João Pessoa-PB, which is accredited in the National Council of Control of Animal Experimentation (CONCEA). The animals were kept in plastic cages of 40x50x20 cm, under controlled temperature (22-24 °C) and natural light cycle (12/12 h). Water and dry commercial feed (Purina - Rodents) were supplied *ad libitum*.

Bothrops jararaca lyophilized venom samples (Bja - Lot 01/08-10) were supplied by Instituto Butantan, Brazil. The venom was maintained at -20 °C and at the time of use, weighed and dissolved in sterile saline at a concentration of 1 mg/mL. Inoculation of the venom was done in the middle third of the lateral side of the right hind limb of the animals, at a dose of 3.2 mg/kg intramuscularly, after trichotomy and antisepsis. The dose recommended in this study was determined in a pilot experiment, where increasing doses of the same venom sample were inoculated into Wistar rats intramuscularly aiming to determine the lowest dose capable of triggering local and systemic symptomatology consistent with natural inoculation without death after 36 hours.

Because there is no knowledge of the dose of Creolin® used empirically by the farmers, the standard dose assessment of 0.3 mL/kg was initially established in this study. The volume was adjusted proportionally based on the volumes traditionally used in folk medicine for cattle and small ruminants.

The animals were weighed, identified by cardinal numbers, and randomly distributed into seven groups composed of five animals. The first group received a sterile saline solution in order to compose the control group, identified as "Control." One group received only the bothropic venom intramuscularly, and another only Creolin® intramuscularly, composing groups that aimed to evaluate the isolated effects of the compounds; these were identified respectively as "Bj" and "imC". The next groups received the bothropic venom and immediately after the inoculation were treated with either oral (administration on the tongue), topical (application with sterile gauze on the region of inoculation of the venom), or intramuscular Creolin®; these were respectively identified as: "Bj+voC", "Bj

+ vtC", and "Bj+imC". The last group received a mixture of the doses of Creolin® and bothropic venom intramuscularly and was identified as imBj+C.

After inoculation (hour zero), the animals were evaluated clinically at each hour for eight hours (hour eight) by adapting the evaluation model used by MOTTA et al. (2017). After the end of the clinical evaluation, all animals were anesthetized with a mixture of xylazine (10 mg/kg) and ketamine (75 mg/kg) intraperitoneally and blood samples were collected by cardiocentesis, as advocated by CONCEA (2015). Euthanasia was then performed with the addition of a complementary methodology (exsanguination) followed by necropsy with tissue collection, including fragments of the heart, spleen, liver, kidneys, nervous tissue (cortex), and muscle tissue of each animal, using 10% formaldehyde as a fixative solution.

Blood samples were processed by the veterinary hematology analyzer pocH-100iV Diff (SYSMEX). Plasma fibrinogen dosage was determined by the heat precipitation technique, and the total plasma protein determined by refractometry, following the methodology of the Laboratory of Clinical Pathology of the Veterinary Hospital of the UFPB, where the tests were performed. The tissue fragments were subjected to the standard histological process according to the protocol used by the Laboratory of Animal Histology of the UFPB, and histopathological analysis was performed using light microscopy with the Zeiss Axio Scope A1 microscope. We adapted the methodology used by GOIS et al. (2016) for histopathological evaluation, with results being classified according to the following score: absent (-) when there were no alterations, (+) when it affected up to 30% of the evaluated area, moderate (++) when it affected from 30% to 70%, and intense (+++) when it affected more than 70% of the area.

The hematological values were subjected to analysis of variance (ANOVA) and compared using the Tukey test, considering a significance margin of 5%. The statistical evaluation was performed using the "R Studio" software, version 1.0.136.

RESULTS AND DISCUSSION

After administration of the compounds and initiation of the evaluation (hour zero), all animals that received the venom-irrespective of the route of administration of Creolin®-presented prostration, reluctance to move, and discomfort when moving the limb, most likely due to the local myotoxic effects, coagulants, and hemolytic effects of bothropic venom (YAMASHITA, 2013).

In the animals of the Control, imC, Bj+imC, and Bj groups, there were no motor alterations or states of consciousness during the evaluation. However, the animals in the groups that had contact with the venom and Creolin® orally, topically, and the mix intramuscularly, presented varying degrees of sedation and involuntary muscle spasms that persisted for two hours after inoculation. Two animals from the Bj+vtC group developed severe neurological signs followed by coma and death two hours after the topical application of Creolin® to the site of venom inoculation (Table 1). The changes in mental state, coma, and death, in addition to episodes of myoclonus, corroborated what is described in the material safety data sheet (INTERTOX, 2011). These characteristics supported the non-use of this substance as it can cause

Table 1 - Identified changes and their time intervals in a group of Wistar rats after experimental bothropic poisoning (*Bothrops jararaca*) and treatment with Creolin® by different access routes.

Changes	Control	imC	Bj	Bj+voC	Bj+vtC	Bj+imC	imBj+C
Claudication	Absent	0 h-3 h	0 h-8 h	0 h-8 h	0 h-8 h	0 h-8 h	0 h-8 h
Prostration	Absent	Absent	0 h-5 h	0 h-4 h	0 h-3 h	0 h-4 h	0 h-4 h
Myoclonus	Absent	Absent	Absent	0 h-2 h	0 h-2 h	Absent	0 h-2 h
Bleeding	Absent	Absent	1 h-8 h	2 h-8 h	2 h-8 h	01-8 h	Absent
Pain	0 h-1 h	0 h-2 h	0 h-8 h	0 h-8 h	0 h-8 h	0 h-8 h	0 h-8 h
Dark urine	Absent	Absent	1 h-8 h	2 h-5 h	2 h-5 h	2 h-5 h	2 h-3 h
Anorexia	0 h-1 h	0 h-3 h	0 h-5 h	0 h-5 h	0 h-3 h	0 h-4 h	0 h-5 h
Death	0/5	0/5	0/5	0/5	2/5	0/5	0/5

Control: sterile saline solution; Bj: only intramuscular bothropic venom; imC: only intramuscular Creolin®; Bj+voC: bothropic venom followed by oral Creolin® treatment; Bj+vtC: bothropic venom followed by topical Creolin® treatment; Bj+imC: bothropic venom and followed by intramuscular injection of Creolin®; imBj+C:intramuscular injection of a mixture of Creolin® and bothropic venom.

undesirable and harmful changes to animal health and lead to accidents involving cutaneous exposure in humans (VEARRIER et al., 2015).

All animals that had contact with bothropic venom—regardless of the administration of Creolin®-presented blood in the urine, most likely due to the hemolytic and myotoxic effects of venom (Table 1). Histopathological evaluation confirmed the harmful effect of venom on the victim's renal system (hydropic degeneration, acute tubular necrosis, and the presence of hyaline and granular cylinders), with little variation between groups after eight hours of observation (Table 2). The presence of hyaline and granular cylinders in the lumen of the renal tubules was evident in all animals except the Control group. Animals of the imBj+C group presented a slight decrease in the proportion of hyaline cylinders when compared to the others; this change was classified as moderate (++). The presence of tubular necrosis and hydropic degeneration in the Bj and Bj+voC groups suggested a glomerular lesion with loss of protein, corroborating the findings of MOTTA et al. (2017) and TAKAHIRA (1999), who reported similar alterations in studies with bothropic venom in rats and dogs, respectively.

After two hours of evaluation, the animals that received the venom and then Creolin® by different routes had blackened musculature, with a friable and hemorrhagic appearance, and progressive edema at the inoculation site (right thigh), where the venom seemed to have spread (Figure 1B, 1C). Histologically, the musculature presented severe hemorrhage and edema, with hyaline degeneration of muscle fibers (Table 2). The extremity of the limb was not affected. This change persisted until the end of the clinical evaluation (hour eight), corroborating the local effects reported in the natural and experimental cases of bothropic venom inoculation in animals (YAMASHITA, 2013; GOMES et al., 2016; MOTTA et al., 2017). The animals of the imC and imBj + C groups did not present hemorrhagic or necrotic changes, only progressive edema of the inoculated region until the end of the evaluation. However, the infiltration of neutrophils was evident in the region of inoculation of the compounds, possibly due to inoculation of a phenolic compound that is highly immuno reactive and not formulated for injectable administration (Figure 1A, 1D).

All animals were necropsied, with no macroscopic changes. Histopathological evaluation of the spleen, liver, heart, and nervous tissue samples did not show any noticeable changes, indicating that the venom and Creolin® did not cause acute lesions in these tissues (within eight hours), consistent with the pathophysiology of the bothropic poison reported in the literature (YAMASHITA, 2013; SENISE et al., 2015). Regarding the toxic effects of Creolin®, more specific studies are necessary due to its known toxic potential for some systems (INTERTOX, 2011); histopathological changes may occur with longer exposure time.

With regard to the hematological findings, there was a significant difference in the leukocyte values of the groups that received the bothropic venom and treatment when compared to those of Control and Bj groups (Table 3); these results corroborate those from the studies on the hematological effects of the bothropic venom, which also demonstrated leukocytosis (MOTTA et al., 2017).

Table 2 - Renal and muscular histopathological changes in Wistar rats after experimental bothropic poisoning (*Bothrops jararaca*) and treatment with Creolin® by different access routes after eight hours of inoculation.

Renal Changes	Control	imC	Bj	Bj+voC	Bj+vtC	Bj+imC	imBj+C
Granular cylinders	-	-	+++	+++	+++	+++	++
Hyaline cylinders	-	-	++	+++	+++	+++	+
Hydropic degeneration	-	-	+	+	+	+	-
Necrosis of tubules	-	-	++	+	-	+	-
Muscular Changes	Control	imC	Bj	Bj+voC	Bj+vtC	Bj+imC	imBj+C
Bleeding	-	-	+++	+++	+++	++	-
Edema	-	++	+++	+++	++	++	+
Neutrophilic infiltrate	-	+++	-	-	++	+	+++
Hyaline degeneration	-	-	+	+	-	+	-

Control: sterile saline solution; Bj: only intramuscular bothropic venom; imC: only intramuscular Creolin®; Bj+voC: bothropic venom followed by oral Creolin® treatment; Bj+vtC: bothropic venom followed by topical Creolin® treatment; Bj+imC: bothropic venom and followed by intramuscular injection of Creolin®; imBj+C:intramuscular injection ofa mixture of Creolin® and bothropic venom.

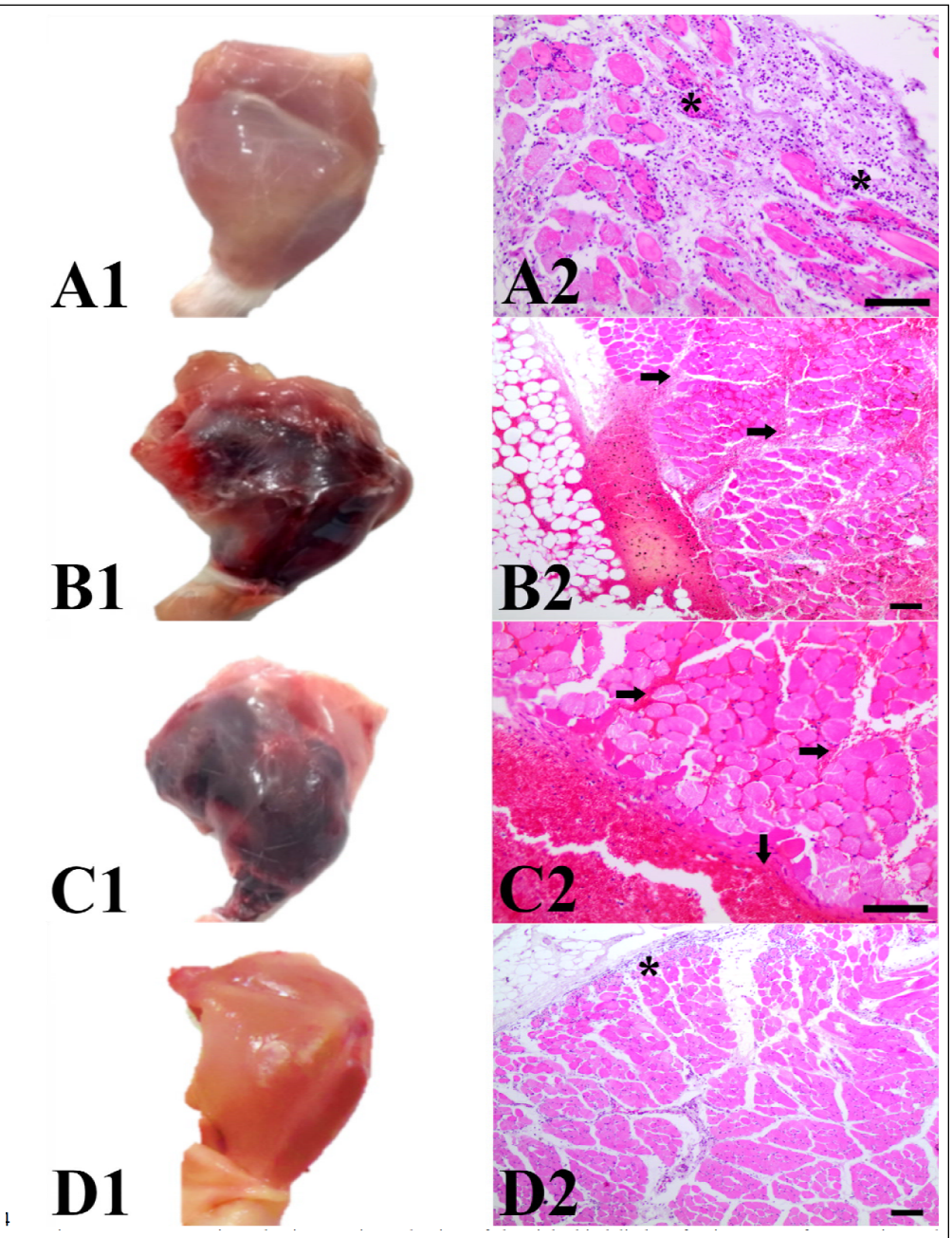

Figure 1 - Macroscopic and microscopic evaluation of the right hind limbs of Wistar rats after experimental bothropic poisoning (*Bothrops jararaca*) and treatment with Creolin®. A1) Photographic image of the right-side face of the limb inoculated only with Creolin® intramuscularly. A2) Photomicrography of the inoculation region of Creolin® with evident neutrophil infiltrate (*). Hematoxylin-Eosin staining. Bar: 200 micrometers. B1) Photographic image of the right-side face of the limb inoculated only with bothropic poison intramuscularly presenting friable appearance, edema, and hemorrhage. B2) Photomicrography of the venom inoculation region with diffuse hemorrhage (arrows). Hematoxylin-Eosin staining. Bar: 100 micrometers. C1) Photographic image of the right-side face of the limb inoculated with bothropic venom and then Creolin® intramuscularly, presenting friable appearance, edema, and hemorrhage similar to inoculation only with the venom. C2) Photomicrography of the venom inoculation region and the Creolin® injected intramuscularly with diffuse hemorrhage (arrows). Hematoxylin-Eosin staining. Bar: 200 micrometers. D1) Photographic image of the right-side face of the limb inoculated with the venom + Creolin® mixture intramuscularly. D2) Photomicrography of the inoculation region of the venom + Creolin® mixture injected intramuscularly with the presence of neutrophil infiltrate (*). Hematoxylin-eosin staining. Bar: 100 micrometers.

Table 3 - Hematological values of Wistar rats, eight hours after experimental bothropic poisoning (*Bothrops jararaca*) and treatment with Creolin® by different access routes (*). Values expressed as mean ± standard deviation.

Hemogram	Control (n=5)	imC (n=4)	Bj (n=5)	Bj+voC (n=4)	Bj+vtC (n=3)	Bj+imC (n=5)	imBj+C (n=4)
Leukocytes (x10³/µL)	5.2±0.9b	7.8±1.7ab	9.5±1.3a	6.9±0.7ab	5.2±0.9b	7.3±2.3ab	6.2±0.2b
Blood Cells (x10⁶/µL)	7.2±0.4ab	7.5±0.7a	6.6±0.5abc	4.6±0.4d	4.9±0.2d	6.0±0.3c	6.3±0.2bc
Hemoglobin (g/dL)	15.2±1.0a	15.8±0.5a	13.4±1.0b	9.5±0.9c	9.2±0.4c	12.2±0.6b	13.1±0.3b
Hematocrit (%)	42.0±2.6ab	45.2±1.2a	37.3±2.6c	26.7±3.0d	28.1±0.2d	34.1±1.8c	38.0±1.0bc
Plasma Protein (g/dL)	6.4±0.2a	6.7±0.3a	5.2±0.3c	5.6±0.1bc	5.3±0.3bc	5.2±0.3c	6.0±0.1ab
Fibrinogen (mg/dL)	225.0±125.8a	225.0±50.0a	100.0±89.4a	100.0±115.5a	66.6±115.5a	140.0±260.8a	160.0±167.3a
Platelets (10³/µL)	704.3±135.3a	840.5±82.1a	13.6±6.9b	77.2±61.7b	88.3±31.3b	11.0±4.0b	838.0±209.6a

*Mean in the same line, followed by distinct letters differed by Tukey test and $p<0.05$. Control: sterile saline solution; Bj: only intramuscular bothropic venom; imC: only intramuscular Creolin®; Bj+voC: bothropic venom followed by oral Creolin® treatment; Bj+vtC: bothropic venom followed by topical Creolin® treatment; Bj+imC: bothropic venom and followed by intramuscular injection of Creolin®; imBj+C: intramuscular injection of a mixture of Creolin® and bothropic venom.

Animals that had contact with the venom presented a reduction in the total number of red blood cells, hemoglobin and hematocrit, unlike the group that received only Creolin®, which did not develop such alterations. The hematological parameters are expressed in table 3. These findings may be associated with the presence of hemorrhage in the right hind limb (the site of the bothropic venom inoculation) and may also indicate an inability of Creolin® to reverse the hemorrhagic and myotoxic activity of the venom.

Platelets and coagulation factors are the main features related to the mechanism of action of bothropic venom. Several components contained in the venom cause disturbances in platelet function and thrombocytopenia. The thrombocytopenia caused by the venom is temporary, yet its action on the mechanisms that involve aggregation is lingering (SANTORO et al., 2004; YAMASHITA, 2013). In this experiment, the animals that had contact with the venom and were treated with Creolin® had a significantly lower platelet count when compared to the values of the Control, imC and imBj+C groups (Table 3), in addition to the presence of aggregation in all samples, similar to the effects of bothropic venom reported in the literature (MOTTA et al., 2017).

In the animals where the mixture of venom and Creolin® was administered, the symptomatology consistent with exposure to bothropic venom did not occur, suggesting a possible direct action of Creolin® on the venom, most likely due to the partial denaturation of its bioactive components. However, this compound is highly toxic to organic systems and has no antiophidic activity when administered separately, irrespective of the route of administration.

CONCLUSION

Creolin® has the ability to inhibit the development of local symptomatology when mixed with *Bothrops jararaca* venom. However, in isolation, it does not present properties useful for the treatment of bothropic poisoning and may present potentially toxic adverse effects to the animals due to the variable sensitivity among the species.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The study was approved by the Ethics Committee on the Use of Animals of the Universidade Federal da Paraíba (CEUA-UFPB), protocol n° 8447140518.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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