Four physiopathological effects of the venom of *Bothrocophias colombianus* (Rendahl & Vestergren, 1940) endemic to the colombian Pacific

Cuatro efectos fisiopatológicos del veneno de *Bothrocophias colombianus* (Rendahl & Vestergren, 1940) endémica del Pacífico colombiano

Running title: Toxinological profile of the venom of *Bothrocophias colombianus*
Título corto: Perfil toxicológico del veneno de *Bothrocophias colombianus*

Yesica Yulisa Moreno García1a
Karen Leonor Ángel-Camilo2a
Mary Luz Bueno-Ospina1b
Santiago Ayerbe-González2b
Jimmy Alexander Guerrero-Vargas 3

Grupo de Investigaciones Herpetológicas y Toxinológicas (GIHT), Centro de Investigaciones Biomédicas—Bioterio (CIBUC-Bioterio), Museo de Historia Natural, Programa de Maestría en Biología, Facultad de Ciencias Naturales, Exactas y de la Educación, Universidad del Cauca, Popayán 190003, Colombia.
a. E-mail: ysicamor@unicauca.edu.co
ORCID: https://orcid.org/0009-0006-4512-7859
b. E-mail: mlbueno@unicauca.edu.co
ORCID: https://orcid.org/0000-0003-2973-6399

Grupo de Investigaciones Herpetológicas y Toxinológicas (GIHT), Centro de Investigaciones Biomédicas—Bioterio (CIBUC-Bioterio), Departamento de Biología, Facultad de Ciencias Naturales, Exactas y de la Educación, Universidad del Cauca, Popayán 190003, Colombia.
a. E-mail: klangel@unicauca.edu.co
ORCID: https://orcid.org/0000-0001-5517-5674
b. E-mail: klangel@unicauca.edu.co
ORCID: https://orcid.org/0000-0001-8449-763X

Grupo de Investigaciones Herpetológicas y Toxinológicas (GIHT), Centro de Investigaciones Biomédicas—Bioterio (CIBUC-Bioterio), Museo de Historia Natural, Departamento de Biología, Facultad de Ciencias Naturales, Exactas y de la Educación, Universidad del Cauca, Popayán 190003, Colombia.
E-mail: guerrero@unicauca.edu.co
ORCID: https://orcid.org/0000-0002-0193-5508

* Correspondence to: Dr. Jimmy Alexander Guerrero Vargas, Grupo de Investigaciones Herpetológicas y Toxinológicas, Universidad del Cauca, Colombia. Popayán 190003, Colombia
guerrero@unicauca.edu.co

Historia del artículo

Fecha de recepción: 10-02-2024
Fecha de aceptación: 20-08-2024

DOI: 0.47374/novcol.2024.
v19.2471

Abstract

Belonging to the Viperidae family, Crotalinae subfamily, *Bothrocophias colombianus*, the Colombian toad-headed pitviper or equis colorada, is a species of venomous snake found in the humid rainforests of the Pacific coast of Colombia, where the lack of knowledge of the effects of its venom greatly hinders medical care. Therefore, in an effort to knowledge the effects of the venom *B. colombianus* venom, gaining an understanding of the local effects by calculating the minimum edematous (MED) and minimum hemorrhagic (MHD) doses, establishing the lethal dose 50 (LD50) in mice of the CFW strain, and examining any systemic effects from a determination of the minimum coagulant dose (MCD) in human blood plasma. The results revealed the LD50 of this venom as 199.5 µg of venom/mouse. There were hemorrhagic (1.06 ± 0.47 µg of) and edematizing effects (0.45 ± 0.15 µg), in addition to a slight coagulant effect (21.02 ± 0.82 µg). In conclusion, the clinical picture of envenomation caused by this species could be characterized by strong hemorrhagic and edematous alterations and slight impairments in the coagulation cascade.

Keywords: Bothrocophias colombianus, toxicity, coagulant, edematogenic, hemorrhagic, LD_{50}.

Resumen

La víbora pitviper o equis colorada, *Bothrocophias colombianus*, perteneciente a la familia Viperidae, subfamilia Crotalinae, es una especie de serpiente venenosa que se encuentra en las selvas húmedas de la costa Pacífica de colombiana, donde el desconocimiento de los efectos de su veneno dificulta enormemente la atención médica. Esta investigación es un esfuerzo por conocer los efectos del Veneno de *B. colombianus*, comprendiendo mejor los efectos locales, mediante el cálculo de las dosis mínima edematosa (MED) y mínima hemorrágica (MHD), estableciendo la dosis letal en 50 (DL50) en ratones de la cepa CFW, y evaluando cualquier efecto sistémico derivado de la dosis mínima coagulante (MCD) en plasma sanguíneo humano. Los resultados revelaron que la DL50 de este veneno es de 199,5 µg veneno/ratón. Se observaron efectos hemorrágicos (1,06 ± 0,47 µg de) y edematizantes (0,45 ± 0,15 µg), además de un ligero efecto coagulante (21,02 ± 0,82 µg). En conclusión, el cuadro clínico del envenenamiento causado por esta especie podría caracterizarse por fuertes alteraciones hemorrágicas y edematosas, y ligeras alteraciones en el proceso de coagulación.

Palabras clave: Bothrocophias colombianus, toxicidad, coagulante, edematógena, hemorrágica, LD_{50}.

Introduction

Currently, the World Health Organization (WHO) is the entity in charge of managing prevention, promotion, and intervention guidelines worldwide in health. Within its policies, snakebite envenomation is reported as a tropical disease that is neglected worldwide. Snake bites affect approximately 5.4 million people in the world, causing between 81,000 and 138,000 deaths per year. Most cases are reported in Africa, Asia, and Latin America; for the latter, around 150,000 cases and the death of at least 5,000 people are estimated (WHO, 2019).

In their natural environment, venomous snakes use their venom to immobilize or kill their prey while simultaneously initiating digestion. However, it can also be employed as a defense mechanism when the snake feels in danger. In many cases, encounters with humans end in an event (Lynch, 2012). The families of snakes causing this problem and of medical interest for Colombia are the Viperidae and Elapidae families since they have species capable of injecting venom with great precision into humans, generating serious clinical symptoms of envenomation (Campbell and Lamar, 1989; Gutiérrez, 2002).

Bothropic envenomation, including in events caused by snakes of the Viperidae family, comprises bites caused by species of the genera *Bothriechis*, *Bothrocophias*, *Bothrops and Porthidium*, generalizing the following symptoms for these species: alteration in the coagulation cascade, myotoxicity, local reactions, mixed shock and massive capillary, pulmonary and/or mesenteric microthrombosis (Ayerbe-González and Latorre-Ledezma, 2010; Ayerbe, 2021). However, the venom of each species can show intraspecific and interspecific variability due to climatic conditions, age of the individual, feeding habits, and seasonal changes (Chippaux et al., 1991).

The genus *Bothrocophias* is a group separate from the genus *Bothrops*. For *Bothrocophias*, there is only one citation of the effect caused by its venom (Ayerbe, 1998b). This means that clinically, whenever events caused by this species occur, the principle of cross-reactivity has resorted to polyvalent antibothropic antivenom serum, which is designed to neutralize the venom of 18 species of snakes, including *Porthidium nasutum*, *Porthidium lansbergii*, *Bothriechis schlegelii*, *Bothrops punctatus*, *Bothrops asper*, and *Bothrops atrox*, among others (Campbell & Lamar, 2004).

The species of the genus *Bothrocophias* previously belonged to the genus *Bothrops*. In 2001, the genus *Bothrocophias* was recognized as a separate group, explained by the division of a large group into three monophyletic groups: a predominantly North American clade (*Agkistrodon*, *Crotalus*, *Sistrurus)*, a Central American clade *(Atropoides*, *Bothriechis*, *Cerrophidion*, *Ophryacus*, *Porthidium)* and a South American clade *(Bothriopsis*, *Bothrocophias*, *Bothrops)* (Gutberlet and Campbell, 2001). The characteristics that allow us to differentiate the species belonging to the genus *Bothrocophias* include small, smooth intersupraocular scales; distinctive white spots on gular and infralabial scales; and tuberculate keels on scales on the caudal portion of the back (Gutberlet and Campbell, 2001).

Bothrocophias colombianus is the Colombian toad-headed pitviper (locally known as "equis colorada." It has an ovoviviparous embryonic development, nocturnal habits, an approximate size of 80-150 cm long, and feeds mainly on small mammals, lizards, and birds. It differs from *B. campbelli* in having a more reddish coloration on the body, most noticeable in juvenile snakes. Commonly, species can be found in plantations of lulo *(Solanum quitoense)*, corn *(Zea mays)*, banana, and yucca *(Manihot esculenta)* and pastures bordering the forest (Castro-Herrera et al, 2005).

The venom of *B. colombianus*, the species of interest for this research, causes severe toxicity. A young specimen of *B. colombianus* caused nonoliguric acute renal failure by acute tubular necrosis, pedial artery, and colliquative mesenteric thrombosis, which required ileocecal segment resection (Ayerbe, 1998a; Ayerbe-González and Latorre-Ledezma, 2010). Taking this into account, in the present work, the toxinological profile of the venom of B. colombianus was determined by calculating the LD50, myotoxic activity, and hemorrhagic, edematous, and coagulant effects.

Materials and methods

Venom

The venom was obtained from two specimens of *B. colombianus* (Figure 1) already in captivity at the Biomedical Research Center of the University of Cauca (CIBUC). The venom was extracted manually by inducing the animals to bite into a falcon tube. The venom was then lyophilized and stored at −20°C until use.



Figure 1. *B. colombianus.*

Biomodels

The experiments were conducted with male and female CFW strain *Mus musculus* mice weighing between 16 and 20 grams (average 18 grams), provided by the CIBUC. The animals were kept in appropriate conditions with controlled temperature, humidity, and feeding.

Ethical considerations

The research was carried out under the authorization of the ethics committee of the University of Cauca, established through endorsement No. 6.1 - 1.25/34 of March 27, 2020.

Median Lethal Dose (LD_{50})

The LD50 was performed by intraperitoneal *(i.p.)* injection, with the application of 0.5 mL of solutions with different concentrations of venom (from 3.075 to 225 µg/mouse) diluted in phosphate-buffered saline (PBS). These venom concentrations were injected into groups of five mice, each weighing between 16 and 20 grams. Observations were made after 48 hours, recording how many mice died and the signs and symptoms of toxicity observed throughout the experiment (Gutiérrez et al., 1990).

Coagulant activity

This experiment was carried out with human plasma extracted from healthy volunteer donors. The blood was centrifuged at 3000 rpm for 10 min in a centrifuge, maintaining a stable temperature (17°-19°C). Subsequently, the plasma was extracted with a micropipette. For the experiment, 100 µL of venom was mixed with 200 µL of human blood plasma, and the clotting time was recorded with a stopwatch (Gutiérrez et al., 2015).

Edematogenic activity

Venom (50 µL) in varying concentrations (from 0.25 µg/mouse to 4 µg/mouse) was injected into groups of five

mice (16 – 20 grams), using PBS as a diluent and 50 µL of PBS as a negative control. The doses of venom and control were administered subcutaneously *(s.c.)* into the footpad of the mouse's right and left hind paws, respectively. After 0.5, 1, 6, 9, 12, and 24 hours, the thickness of the two paws was measured with a caliper. The magnitude of the edema (percentage of increase in paw thickness) was estimated via the following formula: [(Thickness of the right paw/ thickness of the left paws) x 100] -100 (using the values at the time of 1 hour) (Winter et al., 1962). The minimum edematous dose (MED) was determined from a dose-response analysis, defined as the dose of venom that induces 30% edema (Lomonte et al., 1993).

Local hemorrhagic activity

For the hemorrhagic activity, a range of venom concentrations was prepared (3.18 to 50.8 µg/mouse) diluted in PBS. Doses of venom (0.1 mL) were injected intradermally *(i.d.)* into four groups of 4 mice weighing 18 and 20 grams, and the control group was injected with 0.1 mL of PBS. The biomodels were euthanized by cervical dislocation two hours after applying the injection. To determine the area and intensity of the hemorrhagic lesion, a hemorrhagic quantification method by Jenkins et al., 2017 was used using the Inkscape v. 0.92 program. The minimum hemorrhagic dose was defined by the authors as the minimum amount of venom that produces an area of 10 mm or 50 hemorrhagic units (Jenkins et al., 2017).

Statistical analysis

In each test, dose-response graphs were constructed to calculate the minimum dose of each biological activity. These are reported with their respective standard deviations. A regression analysis was performed to determine the statistical relevance between the groups. The statistical analyses and figures were obtained in R v. 4.2.1 based on a confidence index of 95% for each of the tests.

Results and discusión

In the present work, a number of different parameters of toxicity for the venom of *Bothrocophias colombianus*, a pitviper endemic to the Colombian Pacific, were determined for the first time, including the LD50 and the hemorrhagic, edematous, and coagulant activities in mice. In the event of such a pitviper envenomation, these data enable medical personnel to focus on the most relevant effects and provide more rapid care to those affected.

Lethal dose 50 of Bothrocophias colombianus

The LD_{50} of B. colombianus in mice was found to be 199.5 µg of venom/mouse. Figure 2 shows the graph obtained from the probit análisis (where LD50 is the amount of venom that causes the death of 50% of the experimental population). The dose-response curve constructed for the calculation approximates a linear trendline, represented by a coefficient of determination of 0.85.

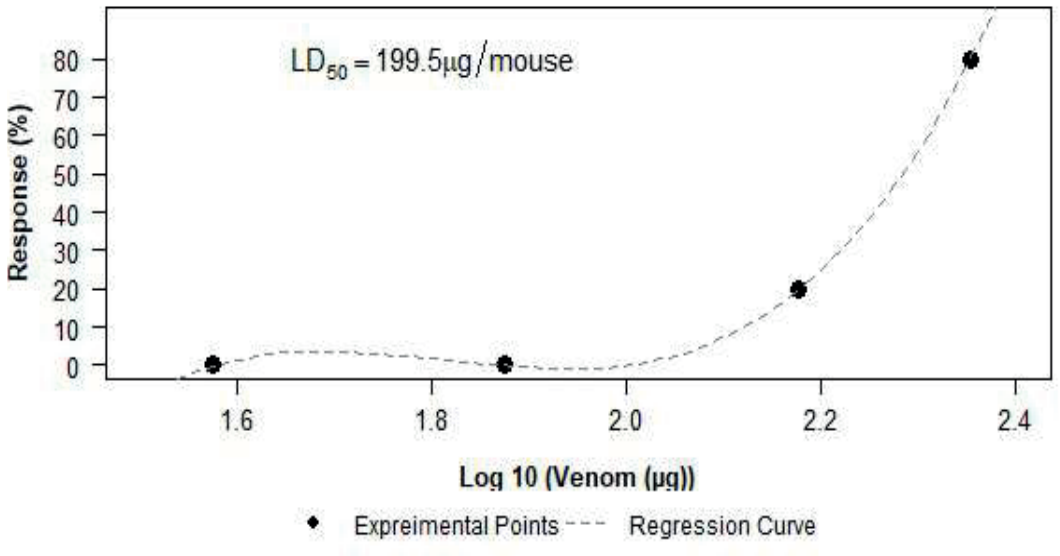


Figure 2. *LD_{50} of the venom of B. colombianus. The Log_{10} of the micrograms of venom is the abscissa, along the x-axis, and the percentage of responses is the ordinate, on the y-axis.*

The venom of *B. colombianus* cannot be classified solely by the most relevant effect produced after a bite. Its lethality and toxicity are based on its heterogeneous chemical composition and the multiple pathophysiological actions it exerts on the body's systems (Jiménez-Porras, 1970). This protein compound is designed to exert an effective action when capturing their prey, which is why the environmental, climatic, and geographical conditions of the place in which they are distributed are factors that influence their variation compared to other species, even species belonging to the same genus (Chippaux et al., 1991).

In this case, the lethality of the *B. colombianus* venom (199.5 µg/mouse) is similar to B. campbelli from Ecuador (Salazar-Valenzuela et al., 2014), *B. campbelli* from Nariño (Sevilla-Sánchez *et al*., 2024), *B. microphthalmus*, also from Ecuador (Terán and Lomonte, 2016), two phylogenetically close species. In contrast, it has a lower potency than the venom of *Bothrops asper* from El Tambo, Cauca (Mora-Obando et al., 2020) and *Bothrocophias myersi* from the Isthmus of Pichido (Pereañez *et al*., 2020), both locations in the Pacific region of Colombia (Sevilla-Sánchez *et al*., 2024).

Within the lethality test, symptoms of toxicity such as tachypnea, prostration, and hypoactivity were recorded, symptoms generally attributed to local pain, mainly in Bothropic type events. Respiratory difficulty was also observed in prolonged periods of tachypnea, which evolved into slow-labored breathing and periods of apnea. These symptoms were also reported for *B. campbelli* from Nariño (Sevilla-Sánchez *et al*., 2024), *Bothrops colombiensis*, and *Bothrops venezuelensis* and are signs attributed to a neurological condition (Méndez A. et al, 2009). In addition to these symptoms, opisthotonus, piloerection, flaccid paralysis of the forelimbs, and ataxia were detected.

Coagulant activity

This test found that the minimum coagulant dose of *B. colombianus* venom was 21.02 ± 0.82 µg of venom. The dose-response curve constructed to calculate this activity approximates an exponential trendline, represented by a coefficient of determination of 0.9534 (Figure 3).

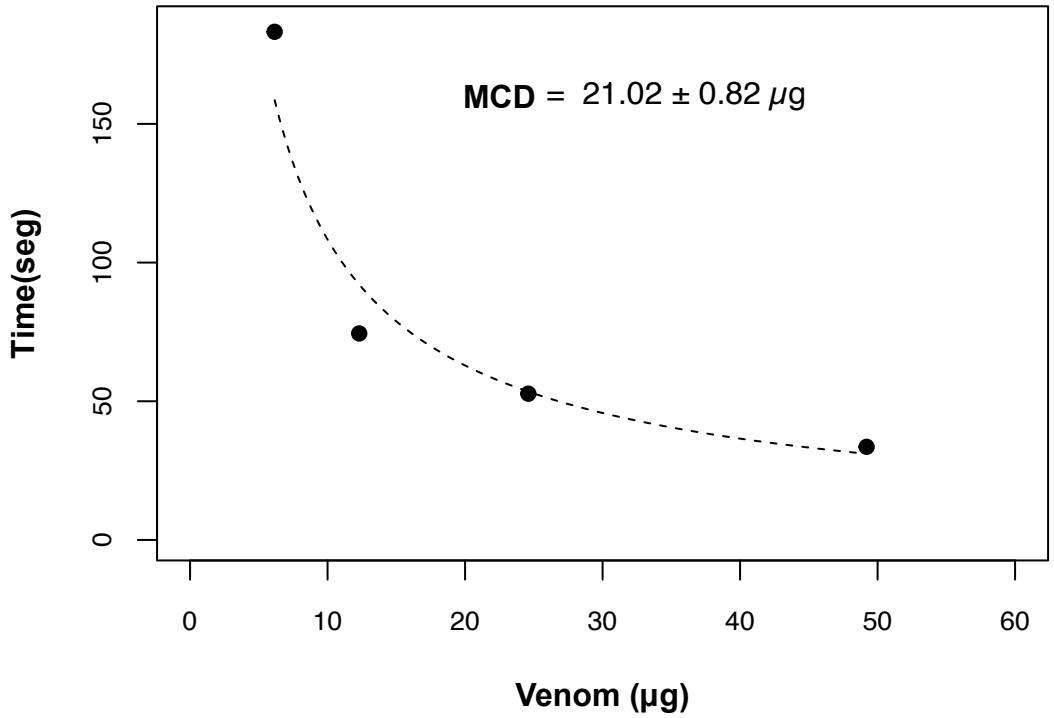


Figure 3. *Minimum coagulant dose of the venom of B. colombianus, a pitviper endemic to the Colombian Pacific. The quantity of venom is shown as the abscissa, with coagulation time as the ordinate.*

Serine proteases, another component present in snake venom, can affect many parts of hemostasis, from the activation of coagulation factors to the induction of platelet aggregation (Castro et al., 2004). In the case of *B. colombianus*, the venom showed a weak coagulant activity, which may be related to the presence of serine proteases. However, a proteomic profile is necessary to affirm this relationship. Taking the above into account, we can consider that this venom has only a mild coagulant activity, similar to that reported for *B. campbelli* from Ecuador (Salazar-Valenzuela et al., 2014), and a lower coagulant activity compared to *B. campbelli* from Nariño (Sevilla-Sánchez et al., 2024), *Bothrops asper* from El Tambo (Mora-Obando et al., 2020), Cauca, *B. myersi* from Valle del Cauca (Pereañez et al., 2020) and *Lachesis acrochorda* from Colombia southwestern (Ángel-Camilo et al., 2020).

Edematogenic activity

The MED of *Bothrocophias colombianus* was 0.45 ± 0.15 µg of venom. The dose-response curve constructed for the calculation approximates a logarithmic trendline, represented in a coefficient of determination of 0.8885 (Figure 4).

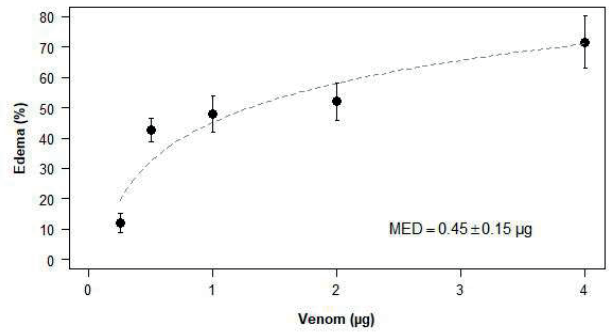


Figure 4. *The dose-response curve for determining edemantogenic activity at a time of 1 hour produced by varying doses of B. colombianus venom. Each point represents the average of 4 replicates. The abscissa is the micrograms of venom and the ordinate the percentage of edema.*

The maximum percentage of edema was recorded between 1 to 3 hours after inoculation with venom, with the maximum peak at 3 hours (Figure 5).

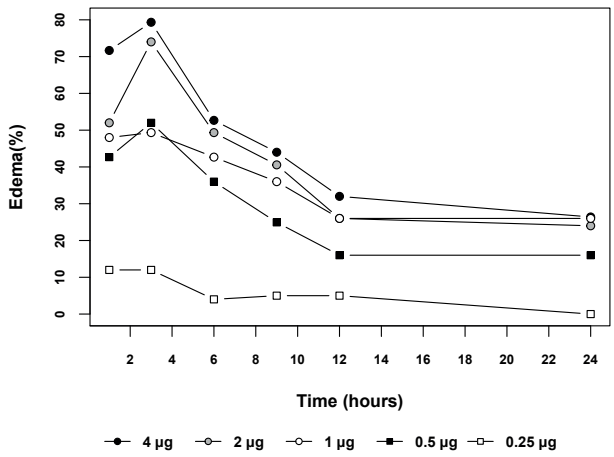


Figure 5. *Monitoring over 24 hours of the edematous activity produced by varying doses of B. colombianus venom. Each line represents a dose of venom, and each point represents the average of 4 replicates. T he abscissa shows the hours elapsed and the ordinate represent the percentage of edema produced.*

Edema is a characteristic of envenomation. It is defined as an effect that indicates an increase in interstitial fluid. This can be produced by deterioration in the cell membrane through the action of a peptide factor such as kinin or by the release of anaphylatoxins, histamine or other autacoids and also by the presence of phospholipase A_2 enzymes (Lloret & Moreno, 1993; Martínez-Vargas, 1990).

The presence of phospholipase A_2 is related to the production of edema. Phospholipase A_2 allows the triggering of processes that promote damage to the cell membrane, with loss of membrane phospholipids and/or the generation of hydrolysis products such as lysophosphatides and free fatty acids, including arachidonic acid (Martínez-Vargas, 1990). Other protein factors within a venom that can induce edema are serine proteases and metalloproteinases (Pereañez et al., 2020; Salazar-Valenzuela et al., 2014).

The MED of *B. colombianus* is similar to those reported for *Bothrops myersi* from Valle del Cauca (Pereañez et al., 2020). The edematizing effect produced by the venom of *B. colombianus* may be related to the possible presence of phospholipase A2, metalloproteinases and serine proteases reported for *B. myersi* and *B. campbelli* respectively, species for which a rapid and strong edematizing activity was also reported (Pereañez *et al*., 2020; Salazar-Valenzuela *et al*., 2014).

Hemorrhagic activity

The minimum hemorrhagic dose produced by *B. colombianus* venom was found to be 1.06 ± 0.47 µg of venom. The dose-response curve constructed for the calculation again approximates a linear trendline, represented by a coefficient of determination of 0.9327 (Figure 6). The hemorrhagic lesions produced by the varying doses of venom are shown in Annex 1, along with the values of the measured area and the hemorrhagic units obtained, representing the amount of hemoglobin extravasated or leaked.

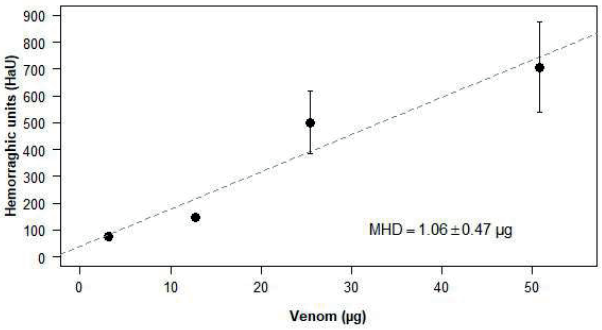


Figure 6. *Dose response curve of hemorrhagic activity produced by varying doses of venom of B. colombianus. Each point represents the measurement of four replicates. The abscissa corresponds to the micrograms of venom and the ordinate to the hemorrhagic units.*

Hemorrhage is considered a frequent manifestation in the description of the main clinical characteristics of snakebite envenomations, which is consistently associated with the action of snake venom metalloproteinases (SVMPs), an abundant component in venoms (Gutiérrez & Rucavado, 2000) and that have also been reported in species of the genus *Bothrocophias* (Pereañez et al., 2020; Salazar-Valenzuela et al., 2014).

The hemorrhagic effect produced by the venom of *B. colombianus* is similar to that reported for B. asper from El Tambo, Cauca (Mora-Obando *et al*., 2020) and presents a more toxic effect than that reported for *B. myersi* from Isthmus of Pichido (Pereañez et al., 2020), *B. campbelli* from Nariño (Sevilla-Sánchez *et al*., 2024) and *B. campbelli* from Ecuador (Salazar-Valenzuela *et al*., 2014).

Conclusions

The venom of the toad-headed pitviper, *Bothrocophias colombianus*, is hemorrhagic, edematous, and has a mild coagulant effect. The clinical picture of envenomation caused by this species could be characterized by strong hemorrhagic, edematous alterations and slight impairments in the coagulation cascade. The LD_{50} of the venom of B. colombianus is considered a mild lethality similar to that reported for other species of the genus Bothrocophias. The signs of toxicity produced by the venom of B. colombianus are similar to those reported for *B. campbelli* from Ecuador and *B. campbelli* from Nariño, ensuring that, in the event of envenomation by this species, health professionals are better prepared to act and thus avoid complications for the patient.

Acknowledgments

To the Universidad del Cauca and the Museo de Historia Natural de la Universidad del Cauca for their support with the specimens. To María José Sevilla Sanchez, Andres Felipe Olaya, David Pino Velasco, Angie Camila Caicedo, and Ana Maria Bravo Valencia, for their support in the development of the experimental phase. To Henry Daza Chaves for their support in carrying graphics.

Funding

Thank you at Universidad del Cauca (501100005682) by financial support with the project “Convocatoria 2 - Jóvenes investigadores e innovadores en el departamento del cauca”.

Authors contribution

YYMG, KAC, MLBO, SAC and JGV were responsible for the conception of the work, formal analysis, writing - original draft preparation, writing–reviewing, and editing. All authors have read and agreed to the final version of the manuscript.

Declaration of interests

The authors declare that there are no conflicts of interest.

References

Angel-Camilo, K.L., Guerrero-Vargas, J.A., de Carvalho, E.F., Lima-Silva, K., de Siqueira, R.J.B., Freitas, L.B.N., de Sousa, J.A.C., Mota, M.R.L., dos Santos, A.A., Neves-Ferreira, A.G.C., Havt, A., Leal, L.K.A.M. & Magalhaes, P.J.C. 2020. Disorders on cardiovascular parameters in rats and in human blood cells caused by Lachesis acrochorda snake venom. *Toxicon* 184, 180–191. https://doi.org/10.1016/j.toxicon.2020.06.009

Ayerbe, S. 1998. Aspectos clínicos y epidemiológicos del accidente ofídico en el departamento del Cauca, pp. 91-100. In Otero Patiño, R., Ángel Mejía, R. & García, M.E. (eds): Primer Simposio Colombiano de Toxinología. Memorias, 276 pp. Editorial Ecográfica Ltda, Medellín, Antioquia (Colombia).

Ayerbe-González, S. & Latorre-Ledezma, J. P. 2010. Manual para la prevención y mejoramiento en la atención del paciente con accidente ofídico (Tercera ed).

Ayerbe, S. 2021. Serpientes, Accidentes & Soluciones. https://ayerbedocserpientes.com, Popayán, Cauca, Colombia, pp. 1-116.

Campbell, J.A. & Lamar, W.W. 1989. The Venomous Reptiles of Latin America. Cornell University Press, Ithaca, New York.

Campbell, J.A. & Lamar, W.W. 2004. The Venomous Reptiles of the Western Hemisphere. Cornell University Press, Ithaca, New York.

Castro, F., Ayerbe, S., Calderón, J.J. & Cepeda, B. 2005. Nuevo registro para Colombia de Bothrocophias campbelli y notas sobre B. colombianus y B. myersi (serpentes: Viperidae). Noved. Colomb. 8, 1–9. Castro, Zingali, R.B., Albuquerque, M.G., Pujol-Luz, M. & Rodrigues, C.R. 2004.Snake venom thrombin-like enzymes: from reptilase to now. *Cell. Mol. Life Sci.* 61,843–856.
https://doi.org/10.1007/s00018-003-3325-z

Chippaux, J.-P., Williams, V. & White, J. 1991. Snake venom variability: methods of study, results and interpretation. *Toxicon* 29, 1279–1303. https://doi.org/10.1016/0041-0101(91)90116-9.

Gutberlet Jr., R.L. & Campbell, J.A. 2001. Generic recognition for a neglected lineage of South American pitvipers (Squamata: Viperidae: Crotalinae), with the description of a new species from the Colombian Chocó. *Am. Mus. Novit.* 3316, 1–15.

Gutiérrez, J.M., Rojas Céspedes, G., Lomonte, B., Gené, J.A., Chaves Mora, F., Alvarado, J., Rojas & Umaña, E., 1990. Standardization of assays for testing the neutralizing ability of antivenoms. *Toxicon* 28 (10), 1127–1129. https://doi.org/ 10.1016/0041-0101(90)90110-s

Gutiérrez, J.M. & Rucavado, A., 2000. Snake venom metalloproteinases: their role in the pathogenesis of local tissue damage. Biochimie 82, 841–850.

Gutiérrez, J., 2002. Comprendiendo los venenos de serpientes: 50 años de investigaciones en América Latina. *Revista de Biología Tropical*, *2*.

Gutiérrez, J.M., Rucavado, A., Solano, G., Lomonte, B., Herrera, M., Segura, Á., Villalta, M., Vargas, M., Escalante, T., León Vargas, G., Escalante, T., León, G., Pazos, F. & Álvarez, C. 2015. Métodos para el estudio de actividades tóxicas de venenos de serpientes. Estrategias y Avances en el Estudio de Toxinas de Interés Para la Biomedicina. Benemérita Universidad Autónoma de Puebla, México, pp. 19–45.

Jenkins, T.P., Sánchez, A., Segura, Á., Vargas, M., Herrera, M., Stewart, T.K., León, G. & Gutiérrez, J.M. 2017. An improved technique for the assessment of venom-induced haemorrhage in a murine model. *Toxicon* 139, 87–93. https://doi.org/10.1016/j.toxicon.2017.10.005

Jiménez-Porras, J.M. 1970. Bioquímica, farmacología y fisiopatología de los venenos de serpientes. Rev. Univ. Costa Rica 28: 43-55.

Lomonte, B, Tarkowski, A. & Hanson, L.A. 1993. Host response to Bothrops asper snake venom: analysis of edema formation, inflammatory cells, and cytokine release in a mouse model. Inflammation 17, 93–105.

Lloret, S., & Moreno, J. J. 1993. Oedema formation and degranulation of mast cells by phospholipase A2 purified from porcine pancreas and snake venoms. *Toxicon*, *31*(8), 949–956. https://doi.org/10.1016/0041-0101(93)90254-G

Lynch, J. D. 2012. El contexto de las serpientes de Colombia con un análisis de las amenazas en contra de su conservación. *Revista Académica Colombiana*, *XXXVI*(1), 15.

Martínez-Vargas, A. Z. 1990. Química y farmacología del veneno de serpientes (1 Parte). *Revista de Química*, *IV*(1), 25–46.

Méndez, A.R.M. & Moreno, R.M.F. 2009. Dosis letal 50 del veneno de Bothrops venezuelensis y Bothrops Colombiensis (Serpientes, Viperidae) en ratones NMRI y BALBC. Trabajo de Grado. Escuela de Ciencias de la Salud, Departamento de Medicina Interna, Universidad de Oriente.

Mora-Obando, D., Salazar-Valenzuela, D., Pla, D., Lomonte, B., Guerrero-Vargas, J.A., Ayerbe, S., Gibbs, H.L. & Calvete, J.J. 2020. Venom variation in Bothrops asper lineages from north-western South America. *J. Proteomics* 229, 103945. https://doi.org/10.1016/j.jprot.2020.103945

Pereañez,J.A., Preciado, L.M., Fernández, J., Camacho, E., Lomonte, B., Castro, F., Cañas, C.A., Galvis, C. & Castaño, S. 2020. Snake venomics, experimental toxic activities and clinical characteristics of human envenomation by Bothrocophias myersi (Serpentes: Viperidae) from Colombia. *J. Proteomics 220*, 103758. https:// doi.org/10.1016/j.jprot.2020.103758

Salazar-Valenzuela, D., Mora-Obando, D., Fernández, M.L., Loaiza-Lange, A., Gibbs, H.L. L., & Lomonte, B., 2014. Proteomic and toxicological profiling of the venom of Bothrocophias campbelli, a pitviper species from Ecuador and Colombia. Toxicon 90, 15–25. https://doi.org/10.1016/j.toxicon.2014.07.012

Sevilla-Sánchez, M.J., Guerrero-Vargas, J.A., Ayerbe-González, S., Calderón-Leytón, J.J., Lomonte, B. & Mora-Obando D. 2024. Toxinological profile and histopathological alterations induced by Bothrocophias campbelli venom from Colombia. *Acta Trop. 250:*107094. https://doi.org/10.1016/j.actatropica.2023.107094

Terán, M. & Lomonte, B., 2016. Actividad letal de seis venenos de serpientes de importancia médica en el Ecuador. Rev. Ecuat. Med. Cienc. Biol. 37, 25–30.

WHO. 2019. Snakebite Envenoming. https://www.who.int/news-room/fact-sheets/detail/snakebite-envenoming.

Winter, C. A., Risley, E. A. & Nuss, G. W. 1962. Carrageenin-Induced Edema in Hind Paw of the Rat as an Assay for Antiinflammatory Dkugp. *Proceedings of the Society for Experimental Biology and Medicine,11*(3):544-547. https://doi.org/10.3181/00379727-111-27849