# Homeopathy and biotherapy in *Bothrops asper* envenomation

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**Abstract**

Snake envenomation by *Bothrops asper* is a common problem affecting cattle raising and rural workers in farms across Latin America. Control of hemorrhage must often be ensured at the site of the accident, as medical care and antivenom therapy might not be available on the premises. The aim of the present study was to investigate the effects of homeopathic medicine *Phosphorus* 6cH, biotherapy *Bothrops asper* 6cH and a homeopathic formula on hemorrhage induced by *B. asper* envenomation. Groups of mice received the investigated treatments before and after envenomation (minimal hemorrhagic dose) and the diameter and intensity of hemorrhage were assessed. When administered before envenomation, all 3 treatments reduced the hemorrhage diameter; the best results were achieved with formula administered 14 days before envenomation and *Phos* 6cH 7 days before. Among the animals treated after envenomation, *Phos* 6cH in 4 doses/hour exhibited the best results in terms of hemorrhage diameter and intensity. We conclude that both homeopathy and biotherapy exhibit considerable potential as alternative treatment to reduce hemorrhage induced by *B. asper* venom.

**Keywords**

Snake envenomation; B*othrop*s asper; Homeopathy; Biotherapy

# Homeopatia e bioterápico em acidente botrópico

**Resumo**

Acidentes por *Bothrops asper* afetam frequentemente o gado e trabalhadores rurais em toda a América Latina. Dado que tanto atenção médica quanto tratamento antiofídico podem não estar disponíveis no local do acidente, é necessário controlar a hemorragia *in loco*. O objetivo do presente estudo foi avaliar os efeitos do medicamento homeopático *Phosphorus* 6cH, bioterápico *Bothrops asper* 6cH e um complexo homeopático na hemorragia induzida por *B. asper*. Diferentes grupos de camundongos receberam os medicamentos testados antes e depois do envenenamento (dose hemorrágica mínima) e foram medidos o diâmetro e a intensidade da hemorragia. Quando administrados antes do envenenamento, os 3 tratamentos reduziram o diâmetro da hemorragia, obtendo-se os melhores resultados com o complexo administrado 14 dias antes e *Phos* 6cH 7 dias antes. Entre os animais tratados depois do envenamento, os melhores resultados em termos de diâmetro e intensidade da hemorragia foram obtidos com *Phos* 6cH em 4 doses/hora. Conclui-se que tanto a homeopatia quanto o bioterápico apresentam grande potencial como tratamento alternativo para reduzir a hemorragia induzida por *B. asper*.

**Palavras-chave**

Acidente ofídico; *Bothrops asper*; Homeopatia; Bioterápico

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## Introduction

Snakebites are one of the most oblivious and neglected tropical diseases, although the mortality rates are comparable to the ones of other serious disorders, such malaria and AIDS [1-3]. While the outcomes of snake envenomation differ as a function of the involved snake species, injected venom amount, affected body part and time to receive appropriate treatment, in most cases patients lose their ability to live normal lives after the accident [4,5]. Patients bitten by venomous snakes exhibit variable signs and symptoms, but the vast majority develops shock, local necrosis, hemostatic problems including severe hemorrhage and acute kidney failure [6].

Accidents caused by snakes from the *Bothrops* genus are characterized by proteolysis, coagulation due to occurrence of thrombin-like activity, platelet aggregation, fibrinogenolytic activity and hemorrhage [7]. In Central America and some places in South America, *Bothrops asper* is responsible for severe cases of snakebite envenomation [8,9].

The local effects of *B. asper* venom on the site of the bite appear promptly after injection. Patients report excruciating pain and, in few hours, other effects, such as blistering, dermonecrosis and myonecrosis; edema and hemorrhage manifest immediately [10].

The pathophysiology of edema induced by *B. asper* envenomation is related to various factors, including capillary extravasation due to microvessel damage, release of inflammatory mediators and increase of the microvessel permeability [11].

Relative to hemorrhage caused by *Bothrops asper* envenomation, there is a group of hemorrhagic components isolated from the venom known as snake venom metalloproteinases (SVMP), which are an M12 reprolysin family of metalloproteinases [11,12]. SVMP are classified in 4 groups, being group III responsible for major hemorrhage for targeting the microvasculature and affecting coagulation [10-13].

These aspects of snake envenomation are a cause of concern not only for human beings, but also for domestic animals and livestock. In dogs, for instance, symptoms of snakebite envenomation include vomiting, dark red urine, breathing difficulty and reduced jaw tone [14]. Cats might become paralyzed, in addition to exhibiting salivation and impossibility to swallow [15]. Cattle might be envenomed during the working hours and also during their rest time (i.e. herding) or at night. The symptoms of snakebite in cattle encompass hemorrhage at the bite site, edema, bleeding from the nose or ears and respiratory tract edema [16].

While administration of veterinary antivenom is the acceptable treatment and efficiently prevents death [14-16] some of the effects of local envenomation might be reduced with alternative therapies. Thus, herbal medicines are extensively used in some places of South America, especially in the Amazonian forest, where extracts of some plants, like *Eclipta prostrata* L., *Brownea rosademonte* Bergius, *Phyllanthus klotzschianus* Müll. Arg., *Casearia sylvestris* Sw. and *Citrus limon* L. are used to relieve the symptoms of envenomation until the patient receives medical care. The extracts of all these plants neutralize the effects of *B. atrox* venom [17-19].

As is known, homeopathy is based on the principle of therapeutic similitude. In turn, biotherapics (nosodes) are chemically indefinite products obtained from different biological sources and prepared according to the homeopathic pharmacotechnics [20].

Biotherapics are advantageous because they allow diminishing the interference of ideological variations in prescription [21] since the very same pathogen that causes the disease is used to prepare the medicine. Lately, stimulated by advances in microbiology, histopathology and immunology, researchers are improving the preparation of nosodes with scientific validation and standardization [22]. There are several studies involving homeopathy, biotherapy and animal models [23,24].

Although use of snakebite antivenom is the therapy accepted by the medical establishment, in some places of Latin America it is difficult to rapidly achieve this type of treatment, and if it takes too long the patient might die [2,16]. Thus, animal models especially mice, are used to test the biological activities of venoms from snakes, as well as the efficacy of antivenom treatments used in snakebite accidents, because there are few models capable of equalizing animal models to *in vitro* models for this type of studies [25-29].

Several authors reported use of homeopathy not only with curative intention, but also to prevent disease [30,31]. For instance, Hahnemann used *Belladonna* to prevent infection with scarlet fever, and some contemporary clinicians stated this practice could protect as vaccination [32]. Recently, homeopathic medicines were used to prevent bacterial infections by *Leptospira* spp [33] and also hemorrhagic dengue fever in Brazil [34,35].

Another possible clinical approach in homeopathy is to use formulas combining several medicines (complexes) which might be prescribed when a combination of effects is necessary or for a specific conventional diagnosis [36-38]. For instance, one such complex was used for dengue fever in Brazil, including *Eupatorium perfoliatum*, *Crotalus horridus* and *Phosphorus* [34,35].

Homeopathy might be used in combination with other treatments, such as antivenom therapy, to minimize the effects of snakebite envenomation. We report the first use of homeopathy, biotherapy and a homeopathic formula for *B. asper* envenomation. The aim of the present study was to assess the effectiveness of a new biotherapic medicine, *Bothrops asper* 6cH, as possible alternative therapy for snakebite accidents, by establishing whether hemorrhagic effects decrease when tested in mice.

## Materials and methods

### *Ethical statement*

All procedures involving the animals used in this study were approved by the Institutional Committee for Care and Use of Laboratory Animals of Costa Rica University (approval: CICUA-37-11 and CICUA-82) and met the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, 1986). In addition, legislation for protection of animals used for scientific purposes (Directive 2010/63/EU, Commission Implementing Decision 2012/707/EU, Recommendation 2007/526/EC) and Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines were followed in the design and performance of all the experiments involving animals. All procedures were performed under the strict supervision of biologists and technicians.

***Preparation of homeopathic and biotherapic medicines***

The homeopathic and biotherapic medicines were prepared according to the Brazilian Homeopathic Pharmacopoeia [39]. Three medicines were used: *Phosphorus* 6cH, *Bothrops asper* 6cH and a homeopathic complex made of equal parts of *Phos* 6cH and *B. asper* 6cH.

*B. asper* 6cH was prepared from lyophilized crude venom provided by Clodomiro Picado Institute, Costa Rica. When preparing medicines that are not cataloged or were never been prepared before, the Brazilian Homeopathic Pharmacopeia [39] asserts that is necessary to follow all instructions starting by grinding solid raw materials to then dissolve them in lactose until potency 3cH, after which the following potencies can be dissolved in liquid vehicle. *B. asper* 6cH was prepared in lactose until potency 3cH; thereafter purified water was used until potency 6cH was reached. *Phos* 6cH was prepared by dilution and agitation from lower potencies according to the Brazilian Homeopathic Pharmacopoeia [39]. The homeopathic complex was prepared by mixing equal parts of *Phos* 6cH and *B. asper* 6cH, both in aqueous form [39]. Potency 6cH was chosen targeting to treat physical effects of snake envenomation. All remedies were prepared in purified water to avoid any interference of ethanol in the animal model.

***Animal model***

Mice were provided by the Animal Facility of Clodomiro Picado Institute. The species selected was *Mus musculus*, strain CD-1/ICR, from both sexes, juveniles, weighting 16-18 g, without genetic modifications and not blood-related. The animals were kept in polycarbonate transparent cages (Eurostandart 1291) measuring 42.5cm x 26.5cm x 18.0cm. Wood chippings were used for bedding. Continuous breeding program 2:1 (male: female) and 12-hour light/dark cycle were applied. Room temperature 24-26°C, relative humidity 85-90%, mice extruded food (Aguilar y Solís, S.A., Costa Rica) and water *ad libitum* were provided. The animals were maintained according to the Welfare Animal Law (n° 7451) of Costa Rica. The number of animals (n=6 per group) and administration of treatments were based on previous studies on snake envenomation [40,41] and comply with NC3Rs - National Centre for Replacement, Refinement, and Reduction of Animals in Research [42].

***1. Pre-envenomation treatment***

Prophylactic treatment was applied for 14 (4 groups, n=6) and 7 (4 groups, n=6) days before administration. The positive control group (n=6) was treated with 50 µL of purified water 3 times per day (9:00h, 12:00h and 15:00h) per oral route through feeding tube. Three groups of 6 mice each received treatments (respectively *B. asper* 6cH, n=6; *Phos* 6cH, n=6; and complex, n=6) 3 times per day (9:00h, 12:00h and 15:00h) also per oral route and with feeding tube.

After 14/7 days, all the mice received an intradermal injection of 50 µL of *B. asper* venom in PBS on the abdomen. Concentration 150 µg/ml of the venom was the minimal hemorrhagic dose (MHD) namely, the amount of venom that induces a hemorrhagic area of 10 mm diameter. MHD is an assay developed and standardized by the World Health Organization for assessing the biological activity of venoms and the neutralizing capacity of antivenoms [40,41,43].

Two hours after the injection of *B. asper* venom, all the mice were sacrificed in $CO_2$ chamber and the hemorrhage diameter [44,45] and intensity were measured. According to the American Veterinary Medical Association Guideline, $CO_2$ has rapid depressant,

analgesic and anesthetic effects, being the euthanasia method chosen in the present study [46].

***2. Post-envenomation (curative) treatment***

Following intradermal injection of 50 µL of *B. asper* venom (MHD 150 µg venom/ml PBS) on the abdomen, the positive control group (n=6) received purified water per oral route via feeding tube, every 15 minutes for 2 hours. Three groups of 6 mice each received the treatments (*B. asper* 6cH, *Phos* 6cH or complex) every 15 minutes for 2 hours. After 2 hours of treatment, all the mice were sacrificed in $CO_2$ chamber, and the hemorrhagic diameter and intensity were measured.

***Parameters***

*Diameter of hemorrhagic lesion*

The determination of hemorrhage in mice promoted by ventral injection of snake venom is already standardized at Clodomiro Picado Institute, University of Costa Rica [44] having been adapted from previous experiments for determination of hemorrhagic activity of snake venom [45] as described below.

Two hours after intradermal injection of 50 µL of *Bothrops asper* venom (150 µg/ml) on the ventral region, all the mice were sacrificed, and their skin was removed. The cross-diameter of hemorrhagic spots was measured through the glass plate, and the hemorrhage diameter was calculated with equation: diameter = 2 x (√ hemorrhagic area / π).

*Hemorrhage intensity*

The intensity of hemorrhage was quantified using software Inkscape 0.91 (r13725; www.inkscape.org, 1989, 1991, Free Software Foundation). This software allows estimating the intensity of the color red based on the number of pixels. This evaluation was performed on photographs of mice that received the pre- and post-envenomation treatments. For this purpose, a blank area was selected next to each hemorrhagic ring, and the pixel count [i.e. R (red), B (blue), G (green)] was defined as zero (blank). Then, an arbitrary area of the hemorrhagic lesions was selected and the RBG pixel counts were registered. Then the values of the blank area were subtracted from the values of the hemorrhagic area, obtaining a value on the RBG scale used as intensity of hemorrhage.

***Statistical analysis***

Hemorrhage diameter and intensity are expressed as mean ± standard deviation. The dataset was assessed for normality and homogeneity of variances by means of the Shapiro-Wilk and Levene tests. Difference in hemorrhagic diameter and hemorrhagic intensity was analyzed by one-way ANOVA. Specific comparisons were performed by means of the t-test. The combined hemorrhage diameter and intensity effect was assessed through a multivariate test. P-value $<0.05$ was considered as statistically significant in all the tests.

**Results**

***Prophylaxis 14 days before envenomation***

*Effects on hemorrhagic diameter*

Use of *B. asper* 6cH, *Phos* 6cH and complex for 14 days before envenomation promoted variation of the hemorrhagic diameter (F=41.648, df=4;25, p<0.001). The group treated

with *B. asper* 6cH exhibited reduction of the hemorrhagic diameter compared to the positive control (t=3.672, df=10, p=0.004). The group treated with *Phos* 6cH did not show difference in hemorrhagic diameter compared to the positive control (t=1.807, df=10, p=0.101) (Fig. 1A). In turn, the group treated with complex for 14 days exhibited reduction of the hemorrhagic diameter (t=3.280, df=10, p=0.008) compared to the positive control.

Medicines *B. asper* 6cH and *Phos* 6cH acted differently on the reduction of the hemorrhagic diameter (t=-2.895, df= 10, p=0.016) the former inducing greater reduction. No significant difference was found upon comparing *B. asper* 6cH and complex (t=0.107, df= 10, p=0.917). However, difference was found between *Phos* 6cH and complex (t=2.373, df= 10, p= 0.039). These findings show that the complex was the best treatment to achieve hemorrhagic diameter reduction (Fig. 1A).

*Effects on hemorrhagic intensity*

Effects on hemorrhagic intensity in the groups treated with medicines for 14 days before envenomation was observed (F=3.254, df= 3;20, p=0,043). Effect on hemorrhagic intensity was promoted by *B. asper* 6cH compared to the control group (t=-3.696 df=10, p=0.004). *Phos* 6cH (t=-1.671, df=10, p= 0.126) and complex (t=-0.484, df=10, p=0.639) showed no difference.

No differences were found in comparisons: *B. asper* 6cH vs. *Phos* 6cH (t=1.785 df= 10, p=0.105), *B. asper* 6cH vs. complex (t=2.186 df= 10, p=0.054) and *Phos* 6cH vs. complex (t=0.826 df= 10, p=0.428) (Fig. 1B). *B. asper* 6cH increased the hemorrhagic intensity and decreased the hemorrhagic diameter (Pillai's Trace=0.890, F=5.345, p<0.0001) (Fig. 1).

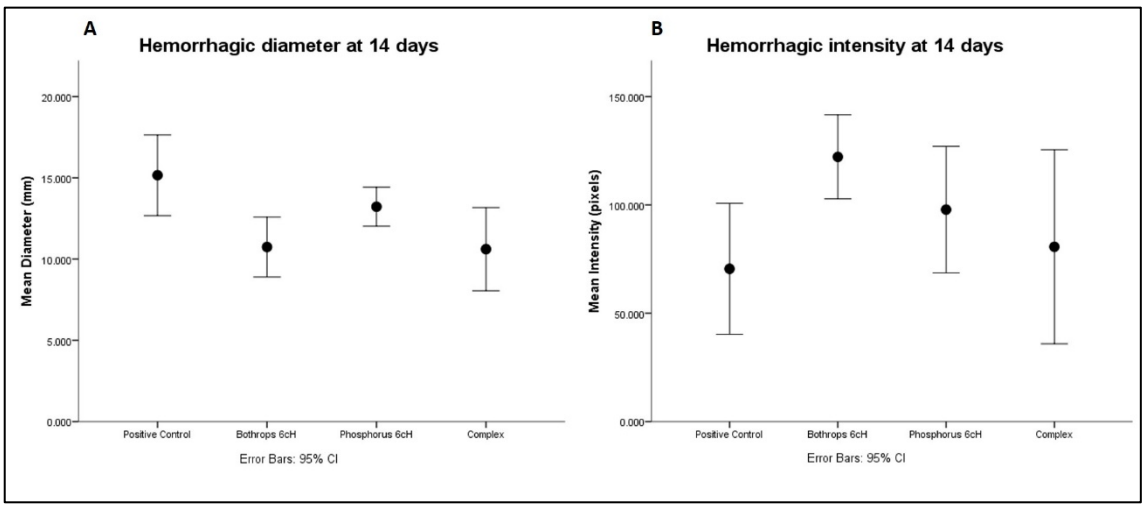


**Figure 1**. Effects of prophylactic treatment (*Bothrops asper* 6cH, *Phosphorus* 6cH and complex) administered for 14 days before *B. asper* envenomation in mice. **A**. Effects of prophylactic treatment on hemorrhagic diameter. **B.** Effects of prophylactic treatment on hemorrhagic intensity. P< 0.05 was considered to be statistically significant.

***Prophylaxis 7 days before envenomation***

*Effects on hemorrhage diameter*

Variation of the hemorrhagic diameter was found when *B. asper* 6cH, *Phos* 6cH and complex were administered 7 days before envenomation (F=69.756, df=4;25, p<0.001).

All 3 treatments reduced the hemorrhage diameter: *B. asper* 6cH (t=5.462, df=10, p<0.001), *Phos* 6cH (t=7.248, df=10, p<0.001) and complex (t=3.323, df=10, p=0.008) (Fig. 2A).

Significant difference was not found in comparisons: *B. asper* 6cH vs. *Phos* 6cH, or *B. asper* 6cH and complex (t =-0.110, df=10, p=0.914; t=-2.248, df=10, p=0.05, respectively). However, *Phos* 6cH was better to reduce the hemorrhage diameter compared to the complex (t =-2.908, df= 10, p=0.016) (Fig. 2A).

*Effects on hemorrhagic intensity*

Significant effects on hemorrhagic intensity were not found after administration of *B. asper* 6cH, *Phos* 6cH and complex 7 days before envenomation (F=1.175, df=3;20, p= 0.344) (Fig. 2B).

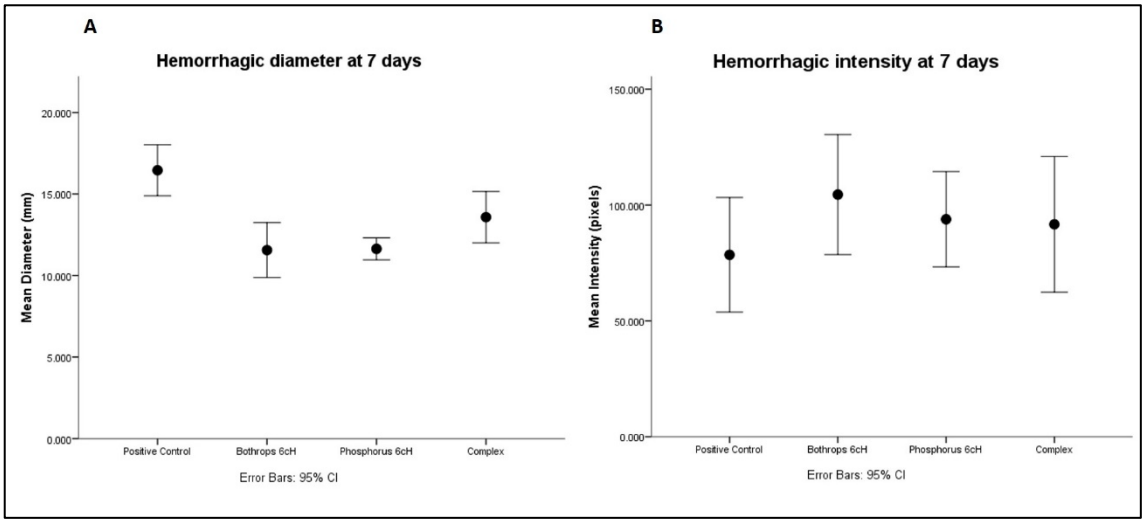


**Figure 2**. Effects of prophylactic treatment (*Bothrops asper* 6cH, *Phosphorus* 6cH and complex) administered 7 days before *B. asper* envenomation in mice. **A**. Effects of prophylactic treatment on hemorrhagic diameter. **B.** Effects of prophylactic treatment on hemorrhagic intensity. P<0.05 was considered to be statistically significant.

***Post-envenomation (curative) treatment***

*Effects on hemorrhage diameter*

Effect was observed when *B. asper* 6cH, *Phos* 6cH and complex were administered every 15 minutes after envenomation (F=4.294, df=4;25, p=0.009). The best treatment to reduce the hemorrhage diameter was *Phos* 6cH (t=2.676, df=10, p=0.023) by comparison to the control group. *B. asper* 6cH (t=0.549, df=10, p=0.595) and complex (t =1.863, df=10, p=0.092) showed no difference compared to the control group (Fig. 3A).

*Effects on hemorrhage intensity*

Difference in the red color intensity was found with the post-envenomation treatments *(*F=2.951, df=4;25, p=0.04*)*. Reduction of hemorrhage intensity was observed when groups *B. asper* 6cH (t=2.499, df=10, p=0.031), *Phos* 6cH (t=3.591, df=10, p=0.005) and complex (*t=3.398, df=10, P=0.007)* were compared to the control group (Fig. 1B).

Considering the hemorrhage diameter and intensity both were reduced when the mice received the treatments every 15 minutes for 2 hours (Pillai's Trace=0.989, F=3.418,

p=0.003). Differences in diameter reduction (F=4.294, df=4;25, p=0.04) and intensity (F=2.951, df = 4;25, p= 0.04) were found.

Also differences in time and frequency of treatment (i.e. 7 and 14 days pre-envenomation and post-envenomation) were detected (F=4.844, df=11;60, p=0.001) relative to the hemorrhagic diameter. Differences in hemorrhagic intensity were found for *B. asper* 6cH (F= 14.838, df=2;15, p<0.001) and *Phos* 6cH (F=8.640, df=2;15, p=0.003); the complex, in turn, did not show significant difference (F = 2.814, df = 2;15, p=0.092) compared to the control group.

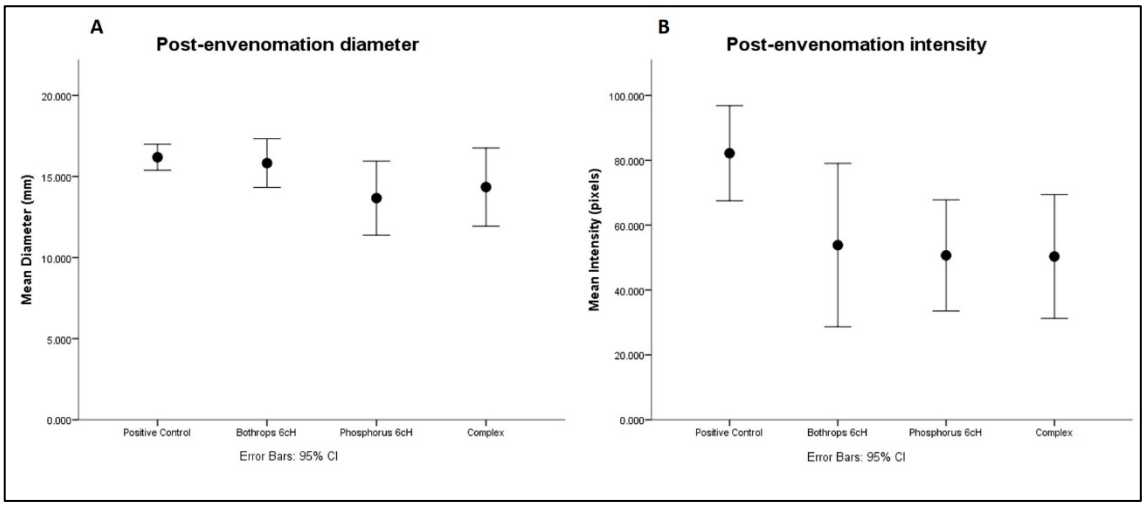


**Figure 3.** Effects of curative treatments (*Bothrops asper* 6cH, *Phosphorus* 6cH and complex) administered every 15 minutes for 2 hours after *B. asper* envenomation in mice. **A**. Effects of prophylactic treatment on hemorrhagic diameter **B.** Effects of prophylactic treatment on hemorrhagic intensity. P<0.05 was considered to be statistically significant.

## Discussion

Although prophylaxis by means with biotherapics is not common among classical homeopathy practitioners, it can be advantageous as a function of its specificity against diseases [21]. Different venom snakes contain particular sets of enzymes, proteins carbohydrates, lipids and minerals which characterize the profile of envenomation. *B. asper* bites promote severe bleeding due to presence of metalloproteinases, traditionally expressed as hemorrhage diameter. While histopathological assessment is commonly performed to evidence the tissue damage induced by enzymes, specifically metalloproteinases, it is not suitable to evidence the hemorrhagic damage caused by the whole venom [10,41,44,45,47,48].

Even though there are some homeopathic remedies made of snake venoms, such as *Lachesis muta* [49,50] and *Bothrops lanceolatus* [51], included in the homeopathic materia medica, in the present study we used a medicine made from *B. asper* venom, acting as isopathy. The venom of *Lachesis* spp. provokes severe hypotension, bradycardia and respiratory depression [52], a profile slightly different from the one of *B. asper* envenomation. The venom of *B. lanceolatus* lacks coagulant components [53,54], but the one of *B. asper* venom contains several coagulant and procoagulant toxins, such as thrombin-like serine proteinases [55,56], SVMP [13] and phospholipase $A_2$ [58].

Therefore, the profile of envenomation is also different, which justifies the preparation of *B. asper* 6cH in the present study.

To help patients who suffered snakebite accidents, maintaining hemostasis by reducing venom-induced systemic effects, such as hemorrhage and coagulopathy, is highly relevant [58]. *B. asper* 6cH and the complex were the best medicines for diminishing the hemorrhagic diameter when administered 14 days before the envenomation.

Although *B. asper* 6cH decreased the hemorrhagic diameter, unfortunately it was associated with increase of the hemorrhage intensity. In this case, it would be interesting to test a milder potency, such as 30cH, the LM scale or, as Hahnemann suggests in the Organon of Medicine § 159 [59], reduce the dose.

It seems that presence of *Phos* 6cH in the complex composition boosted synergetic effects with *B. asper* 6cH, while it did not reduce the intensity of hemorrhage. In turn, the tested complex showed to be more effective, because it diminished the hemorrhage diameter without increasing the hemorrhage intensity.

*Phos* 6cH for prophylactic use was influenced by the demonstration of its effects on prevention of hemorrhagic dengue [34,36]. However, it did not show effects on reduction of the hemorrhage diameter or intensity when administered 14 days before envenomation. This action was different when *Phos* 6cH was administered with curative intention.

While *B. asper* 6cH and the complex showed effects on hemorrhage when they were administered 14 days before envenomation, and *Phos* 6cH did not, paired comparisons did not detect difference between the action of all 3, due to variations in the animal model developed for studying hemorrhage induced by snake venoms. Such variation was previously studied by various authors [41].

When *B. asper* 6cH, *Phos* 6cH and the complex were used for 7 days before envenomation, all of them reduced the hemorrhage diameter, but had no influence on the intensity of hemorrhage. These findings suggest homeopathy or biotherapy might be used to achieve similar protection against snakebite envenomation. In Organon of Medicine § 158, Hahnemann asserts that a slight homeopathic aggravation indicates that the acute disease will succumb to the first dose [47,60]. However, considering the severity of the clinical symptoms of snakebite envenomation [10,16,61] it would be desirable to reduce hemorrhage without previous aggravation.

Relative to the curative homeopathic treatment, although *B. asper* 6cH, complex and *Phos* 6cH could decrease the hemorrhage diameter, the best medicine in this regard was *Phos*. Conceptually, we used *Phos* 6cH as homeopathic medicine, because it is described in various works of homeopathic materia medica as a medicine that induces, and thus can treat, a set of signs and symptoms similar to the ones of snake envenomation. *Phos* has broad action on the hemorrhagic diathesis and can be homeopathically used to treat from tuberculosis to frequent and copious bleeding from the nose, stomach, anus, urethra and metrorrhagia [62,63]. Despite its possible use in cases of hemorrhage, it is reported it cannot be used for long periods and in low potencies [62-65]. Therefore, *Phos* might serve as adjuvant for snake envenomation when it is used early in the accident.

Dinges made an extensive study showing that Hahnemann communicated in letters to associates about using double medication in homeopathic treatment, even though he did not like the idea of implanting double medication [66]. The use of complexes is somewhat common when clinicians need a combination of medical effects [37,38]. However, the complex tested in the present study did not show any outstandingly different results when compared to *B. asper* 6cH and *Phosphorus* 6cH alone 7 days before or after envenomation. Moreover, for prevention 7 days before or healing bleeding induced by *B. asper* venom in murine models, it is convenient to pursue the teachings of Hahnemann, who in Organon of Medicine § 273 claims: "In no case under treatment is it necessary and therefore not permissible to administer to a patient more than one single, simple medicinal substance at one time" [59].

Despite the low dilution and frequent repetition of the medicines, no changes in the animals' behavior occurred with either the prophylactic or the curative treatment. Nevertheless, the results of the present study need to be complemented in future trials by collecting the symptoms induced by *B. asper* 6cH on healthy animals.

In the case of snakebite envenomation, parenteral administration of antivenoms is the only therapy accepted because it can neutralize the deleterious effects of snake venom [67]. Unfortunately, in Latin America medical assistance is often 2 or 3 hours away, especially in farms and agriculture plantations, and this delay worsens the symptoms of snake envenomation [68,69]. Considering the results shown, more studies using homeopathy or biotherapy will produce more information on how to provide some comfort to patients until antivenom administration.

## Conclusion

Homeopathy is considered a low-cost therapy and is easily available. In addition, homeopathy or biotherapy (isopathy) as alternative therapies have a great potential for therapeutic and research purposes. Such is the case of the medicines *Bothrops asper* 6cH, which used for prophylactic treatment in a murine model reduced the diameter of hemorrhage, and *Phosphorus* 6cH as curative therapy for diminishing hemorrhage (diameter and intensity) induced by *B. asper* venom. In future studies, we will investigate the histological outcomes of homeopathy on the pathological effects of purified toxins, especially metalloproteases. *Bothrops asper* 6cH might help lessening the severity of venomous snakebites when administered in combination with antivenom therapy. This especially in the case of veterinary patients, which might take advantage of homeopathic therapies since snakebites are common occurrences in cattle [16]. The effectiveness exhibited by the medicines analyzed in the present study might stimulate other researchers to seek to understand how alternative therapies might help patients until they can receive antivenoms.

## Acknowledgements

Experimental work was supported by Clodomiro Picado Institute, University of Costa Rica, and all authors are very grateful for it. Additionally, the authors thank all the comments made by the editorial reviewers of Revista de Homeopatia, which improved the manuscript.

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