

Murine model evaluation of biochemical and tissue-damage induced by *Bothrops asper* (Nauyaca) snake venom

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Alteraciones bioquímicas y daño tisular inducido por el veneno de la serpiente *Bothrops asper* (Nauyaca) en ratón

Recibido: 3 de julio de 2024

Aceptado: 9 de octubre de 2024

Abstract

Introduction: Venomous snakes Bites are a serious public health issue, and in Mexico, *Bothrops asper* is responsible for the majority of viper bites.

Objective: To evaluate, in a mouse model, the effect of *Bothrops asper* venom snake on liver enzymes LDH, AST, and ALT, as well as to assess its effect on renal and hepatic histology.

Methods: The LD50 was determined using a dose-response curve after intramuscular administration of increasing doses of the venom to mice (0.5, 1, 2.5, 5, and 10 mg of protein/kg). Subsequently, the mice were administered the LD25 value of the venom (or saline solution for control), and 24 hours after the injection, LDH, AST, and ALT enzymes were measured in the blood, and the liver and kidneys were collected from each animal.

Results: Animals treated with poison (LD25 of 1 mg of protein/kg) had greater activity of AST, ALT and LDH enzymes, structural damage in the cortex and renal medulla, as well as in the liver after 24 hours of administration in the experimental group.

Conclusion: *B. asper* venom causes an increase in LDH and transaminase enzymes, as well as renal and hepatic histopathological alterations.

Resumen

Introducción: Las mordeduras de serpientes venenosas son un grave problema de salud pública, y en México, *Bothrops asper* es responsable de la mayor proporción de mordeduras de víbora.

Objetivo: Evaluar en un modelo de ratón, el efecto del veneno de serpiente *Bothrops asper* sobre las enzimas hepáticas LDH, AST y ALT, así como evaluar su efecto sobre la histología renal y hepática.

Métodos: La LD50 se determinó con una curva dosis-muerte después de administrar a ratones por vía intramuscular con dosis crecientes del veneno (0.5, 1, 2.5, 5 y 10 mg de proteína / kg). Posteriormente, se administró a los ratones, el valor de la LD25 de veneno (o solución salina para el control) y después de 24 horas de la inyección, se determinaron las enzimas LDH, AST y ALT en sangre y se obtuvieron hígado y riñón de cada animal.

Resultados: Los animales tratados con veneno (LD25 de 1mg de proteína/kg) tuvieron una mayor actividad de enzimas AST, ALT y LDH, daño estructural en la corteza y médula renal, así como en hígado después de 24 hrs de administración en el grupo experimental.

Conclusión: El veneno de *B. asper*. provoca aumento de las enzimas LDH y transaminasas, así como alteraciones histopatológicas renales y hepáticas.

PALABRAS CLAVE

Poison, Damage, Medicinal plant, Public health.

KEY WORDS

Veneno, Daño, Planta medicinal, Salud Pública

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Introducción

Snakebite is a great public health problem affecting almost every part of the world. Every year, it is estimated that this problem causes about 50,000 deaths and 22,000 permanent disabilities, such as amputations, and loss of normal function.¹ An epidemiological study carried out from 2003-2007 in Mexico, it was found a total of 18,848 snakebite accidents. The states with the highest incidence about this kind of injury were Oaxaca (2266 cases), Veracruz (2231 cases), San Luis Potosí, Hidalgo and Puebla, because their geographical and climatic characteristics favor a greater diversity of snakes.²

The species responsible for the greatest number of accidents is *Bothrops asper* (48% of all cases),³ which is known in Mexico as “the Nauyaca”.³ The toxicity mechanisms of the snake, well described in the literature, are mainly related to myotoxic, neurotoxic and hemotoxic effects.⁴ Moreover, it is known that the crotoxins and phospholipases of this snake are responsible for causing renal and hepatic dysfunction in some patients.⁵ In Mexico, there is only one study on this subject, done with pediatric patients, where it was found that less than 5 % of the cases presented kidney or liver damage.⁶ In general, clinical profiles related to alterations in the tissues of these organs, patients had been bitten by rattlesnakes, are from the same family (Viperidae) as *Bothrops asper*.⁷

The objective of the present study was to evaluate in a murine model the effect of *Bothrops asper* venom on lactate dehydrogenase enzyme (LDH), aspartate transaminase enzyme (ALT), and alanine transaminase enzyme (AST), as well as its effect on kidney, and liver histology.

Material and methods

Venom

The venom of *Bothrops asper* was donated by Edgar Reina Ponce and Orlando Reina Ponce, director of the Living Museum “Wild Veracruz”. It was obtained by hand milking two healthy adult male snakes. In the laboratory, the poison was stored at -20°C and the proteins were quantified by the Bradford method.⁸

Five groups were formed (n=3) to test the poison with doses: 0.5, 1, 2.5, 5 and 10 mg protein/Kg. The venom was administered by intramuscular (IM) injection, and the LD₅₀ was obtained after constructing the dose-death curve, as reported previously.⁸

Treatment of Animals

Sixteen adult male NIH mice (20 and 25 g) were kept in 40X40 acrylic boxes under controlled temperature, and humidity, with free access to food and water. All experimental procedures were performed according to the Mexican law for the production, care and treatment of laboratory animals.⁹

Mice were randomly divided into two groups: 1) the control animals (n = 8) received 0.9% saline solution, and 2) the experimental group (n=8) was administered the LD₂₅ of *Bothrops asper* venom. In all cases, the treatment was applied by intramuscular injection. 24 h after administration of the poison or saline solution, the animals were recorded on camera. Then the body weight was taken, and then the mice were sacrificed, and the blood, livers and kidneys from each animal were immediately obtained. From blood samples, LDH, ALT, AST were determined by using commercial kits. The organs were weighed and the relative weight calculated. The organs were then used for histological analysis.

Histological Study

Livers and kidneys were fixed for 72 hours, and then embedded in paraffin. Eight coronal section cuts of 7 μm of each organ were obtained and were subsequently stained with hematoxylin-eosin technique. The microscopic study was conducted in a double-blind manner, analyzing 20 tissue sections for each evaluated organ.¹⁰

Statistical analysis

Values are expressed as the mean $\pm$ standard error (SE). Differences between groups were analyzed using the Students' *t* test. Statistical significance was considered with $P < 0.05$.

Results

The LD₂₅ for intramuscular administration was 1 mg/kg. Although the relative liver weight was not changed by the administration of the snake venom (Figure 1A), the relative kidney weight presented a significant decrease (experimental versus control group; Figure 1B).

For histological evaluation, kidney and liver tissue of the experimental animals were analyzed. In the kidney, glomerular cortical area of control, and experimental animals were observed (Figure 2). In the animals that received the venom, there was a marked atrophy of glomerulus, and convoluted tubules, with distortion of cellular continuity, cell nuclei loss, and it was observed glomerular discontinuity in the medulla. On the other hand, in the liver, it was found hemorrhage, necrosis, and hepatocytes atrophy.

Comparing the serum enzymes evaluated, it was found that poison-treated animals had greater levels of ALT enzyme (A), AST enzyme (B), and LDH enzyme (C) (Figure 3).

Discussion

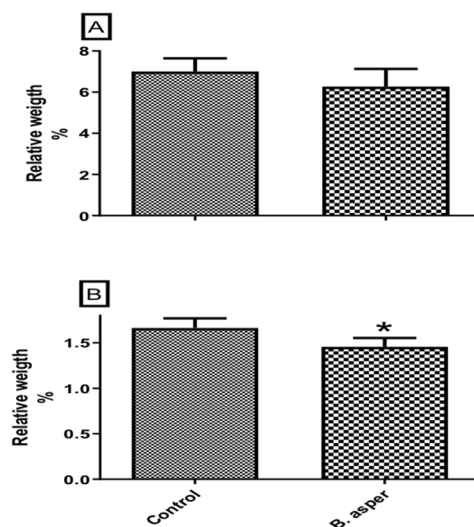
Snakebites are considered as a public health problem in Mexico. The main species responsible for snakebites in this country is *Bothrops asper*.¹¹ The most studied effects of the venom of this snake are hemotoxicity and myotoxicity.¹² Only a few studies have evaluated venom-related kidney and liver toxicity or alterations in enzymatic activities in Mexico.¹

Regarding the Viperidae genus, there have been reports of renal and hepatic cytotoxic activity corresponding to the activity of enzymes such as phospholipases, phosphodiesterases, phosphomonoesterases, alpha-aminoacidooxidases, acetylcholinesterase, proteolytic enzymes from the serine proteinase, several kinds of metalloproteinases, arginine esterase, 5'-nucleotidase, hyaluronidase, and NAD nucleosidases. This cytotoxic effect, here in reflected by the systemic organ damage found, is related to hemorrhagic, and proteolytic activity, furthermore, exist almost exclusively with bothropic poisons.¹³

In other countries, there are various reports of hepatic, and renal,¹⁴ impairment caused by batroxobin from snakes of the *Crotalus* genus and cobra (*Naja* sp). In another hand, it has been found that poisoning with crude cobra venom caused histological alterations such as compression, and degeneration of glomerular epithelial cytoplasm, leading to death by necrosis.¹⁵

Figure 1

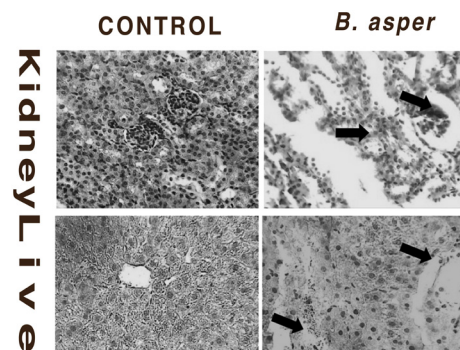
RELATIVE WEIGHTS OF A) LIVER AND B) KIDNEYS OF THE GROUPS STUDIED. THE RESULTS SHOW THE MEAN $\pm$ STANDARD ERROR. T STUDENT TEST. * $P < 0.05$ VS CONTROL GROUP



Source: own elaboration.

Figure 2

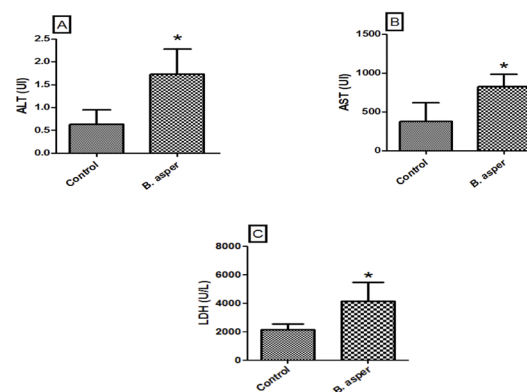
PHOTOMICROGRAPHS OF RENAL AND LIVER TISSUE, HEMATOXYLINE-EOSINE TECHNIQUE



The arrows indicate the histological alterations submitted for the administration of *B. asper* poison. It was observed kidney necrosis with cellular continuity loss, and glomerulosclerosis. Liver hemorrhage, necrosis, and hepatocytes atrophy were observed. Source: own elaboration.

Figure 3

EVALUATION OF ALT (A), AST (B) AND LDH (C) ENZYMES ACTIVITY IN THE PLASMA OF MICE TREATED WITH *B. asper* VENOM



Bar represents the mean $\pm$ SEM. t student test. * $P < 0.05$ vs control group. Source: own elaboration.

AST, ALT and LDH are present as cytosolic, and mitochondrial isoenzymes in many tissues, and they can be used to determine damage to the kidney, and liver tissue. They are also used to measure heart damage, which may increase in response to severe muscle damage, and it was reported in relation to the same snake in 2012.¹⁶ Studies on mice inoculated with viperin snake venom show that serum levels of AST rise from the first to the 24 hours of exposure.¹⁷ In humans the level of this enzyme increases from the third to the 24 hours after a snake bite.¹⁸

In the case of the *Bothrops colombiensis* snake (distribution in Venezuela), it has been identified that an injection of 40 µg of venom significantly increased the markers of creatine phosphokinase (CK), AST, ALT, and urea; where it was observed that CK increased significantly between 3 and 15 hours, with a maximum value at 6 hours (4001 ± 196.58 U/L), attributing a myotoxic effect. The elevation of the AST enzyme had an increase between 1-15 hours and at 144 hours and the ALT only increased in a period of 1-24 hours, which can compromise liver mitochondria and create damage in this organ.¹⁹

At the renal level, urea increased significantly in the period from 1 to 144 hours, up to 59 % in the first 6 hours of venom exposure, while creatinine remained unchanged; explaining this alteration by a damage in the renal microcirculation and its subsequent hypoxia and oxidative stress; activating endothelial cells with new expression of cell surface markers, which could promote an increase in MCP-1 secreted by mononuclear leukocytes, cortical tubular epithelial cells, and podocytes; being associated with renal inflammation, glomerular damage and tubular atrophy, mainly in proximal and distal convoluted tubules.²⁰

The venom of snakes of the *Bothrops* genus has been extensively studied in Latin America, since it has been observed that this substance causes local damage such as flushing, myonecrosis and edema, as well as systemic effects related to coagulation disorders, cardiovascular shock and acute kidney injury²¹ (which reports a prevalence of 1.4-38 % depending on the species involved and reaches a mortality of 13-19 %); and that are induced by components such as phospholipases A2 (44.3 %), thrombin, metalloproteins (30.8 %), either in animal models or in humans.^{19,22}

Conclusion

B. asper venom elicited LDH enzyme, and transaminases enzymes, and induced histopathological changes in kidney and liver tissue in murine model. the present study demonstrates that *Bothrops asper* venom (LD₂₅ with 1mg/kg weight in mice weighing 20 to 25g) caused renal and hepatic toxicity by increasing the levels of transaminase and LDH enzymes and inducing necrosis in the liver. Parenchyma of both organs in mouse models in the first 24 hours after venom administration.

For this reason, this study identified the in-mouse models once the was administered; being an important advance for research in animal models in Mexico, to identify the possible effects that are triggered by a possible snakebite accident in this country.

Financiamiento

Los autores declaran que no existió financiamiento alguno.

Conflictos de intereses

Los autores declaran que no existe conflicto de interés.

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