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Short communication

Crotalus durissus sp. rattlesnake venom induces toxic injury in mouse sperm

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ABSTRACT

Accidents involving snakebites constitute a serious public health problem in many regions around the world. However, there are no study about a possible action of snake venom on the reproductive system. Herein we show that *Crotalus durissus* sp. (Linnaeus) rattlesnake venom (25 μg/kg of body weight) affected chromatin condensation, and increased the number of sperm with abnormal morphology and the sperm count. In conclusion, besides the known hazards of the *C. durissus* sp. venom to animal health, this study was the first to show its effect also on male germ cells.

Accidents involving snakebites constitute a serious public health problem in many regions around the world (Kasturiratne et al., 2008). According to the World Health Organization (WHO), around 81,000 to 138,000 people die each year as a result of snake bites, and around three times as many amputations and other permanent disabilities are caused by snakebites annually (WHO, 2018). In Brazil, among registered snake bites, those by Crotalus are responsible for the highest mortality rate (Frezzatti and Silveira, 2011). Several studies have demonstrated the hazards of the snakebites on human health (Gutiérrez et al., 2017). Damage to the nervous system (Del Brutto and Del Brutto, 2012), muscles (Mebs and Ownby, 1990), and acute renal failure (Pinho et al., 2005) have been reported. However, as far as we kwon, there are no study about a possible action of snake venom on the reproductive system of the people who were bitten. Thus, the effect of *Crotalus durissus* sp. (Linnaeus) venous on mouse spermatozoa was investigated. The protocol (Protocol 034) used in this study was approved by the Bioethics Committee of the University for the Development of the State and the Pantanal Region (UNIDERP).

Crotalus durissus sp. venom was obtained from the serpentarium of the UNIDERP (Campo Grande, Mato Grosso do Sul State, Brazil). A dehydrated venom sample was weighed and dissolved in saline solution (0.9% NaCl), immediately before use in the experiments. Three-month-old and sexually mature male CF-1 mice (38–41 g) were obtained from the animal facilities of the University of Chile and maintained in a room under controlled conditions of temperature (22 ± 2 °C) and a 12 h light/dark cycle, with *ad libitum* access to commercial diet and tap water. Then, the animals were distributed into two groups (n = 6/each): 1- Negative control – which was treated only with the saline solution; 2 - Venom group, that received the *Crotalus durissus* sp. venom (25 μg/kg body weight – bw). Both saline and venom were injected via gastrocnemius muscle, in order to simulate the snakebite. Venom dose used corresponded to the intramuscular lethal dose 50 (LD50) of the *Crotalus durissus terrificus* venom in mice (0.56 mg/kg), as cited by Ribeiro et al. (1993). Four days after treatments, animals were killed (intraperitoneal injection of ketamine - 44 mg/kg/bw ip.), and sperm cells collected from the cauda epididymis (Fornés and Bustos-Obregón, 1994). Sperm morphology and chromatin condensation were analyzed using the protocol described by Vigil and Bustos-Obregon (1985) and by Fornés and Bustos-Obregón (1994), respectively. DNA integrity was evaluated using the fluorescence acridine orange test (Tejada et al., 1984). Statistical analysis was performed using the Mann-Whitney test.

The data showed that *Crotalus durrisus* sp. venom affected chromatin condensation (decreased the ability of condensation), and increased the number of sperm with abnormal morphology (Fig. 1) and the sperm count (Table 1). No effect was detected on DNA integrity (Table 1).

According to Tasoulis and Isbister (2017), the main component in

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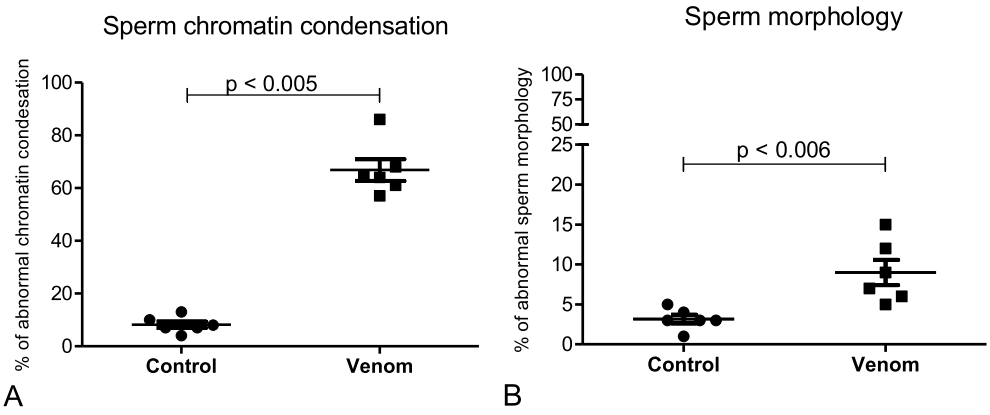

Fig. 1. Percentage of sperm with abnormal condensed chromatin (A) and morphology (B) in mice treated with *Crotalus durissus* sp. venom (25 μg/kg body weight). Mean ± SEM.

Table 1
Sperm count and DNA integrity in mice treated with a single dose of *Crotalus durissus* sp. venom (25 μg/kg body weight).

Endpoints/Groups	Control[a]	Venom
Sperm count (10^6 sperm/g)	47.3 ± 13.9	102.6 ± 56.1*
Normal sperm DNA integrity (%)	98.5 ± 1.0	97.1 ± 2.8

[a] 0.9% NaCl; mean ± SD;*p < 0.05.

the venom of *Crotalus durissus* sp. is the phospholipase A_2 (PLA_2). Studies have shown that this ophidic enzyme induces damage in membrane of goat (Rahmy and Ayoub, 2002) and mouse spermatozoa (Escoffier et al., 2010). Therefore, it is plausible to suggest the increase of sperm with abnormal morphology could have been mediated by the PLA_2. Similarly, the modification of the sperm chromatin condensation could be due to phosphodiesterases, since these enzymes are able to degrade the H2B histones (Gronow and Chapleo, 1979). Data obtained by Georgieva et al. (2010) confirm the presence of this class of enzymes in the venom of the South American rattlesnakes (*Crotalus durissus terrificus)*.

Regarding the increased number of sperm in the cauda epididymis of those mice treated with the venom, it is difficult to explain. Perhaps, some venom toxin could have stimulated spermiation or sperm disengagement (instantaneous event when sperm are rapidly swept away from the spermiation site to make their way to epididymis), favoring the sperm released by contractions of the peritubular myoid cells surrounding the seminiferous tubules (O'Donnell, 2014; O'Donnell et al., 2011).

In conclusion, besides the known hazards of the *Crotalus durissus* sp. venom to animal health, this study was the first to show its effect also on male reproductive cells.

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