



General biochemical and immunological characterization of the venom from the scorpion *Tityus trivittatus* of Argentina

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ABSTRACT

Tityus trivittatus is the Argentinean scorpion reported to cause the majority of human fatalities in the country, however no systematic studies have been conducted with the venom of this species. This communication describes a general biochemical and immunological characterization of the venom obtained from *T. trivittatus* scorpions collected in the city of Buenos Aires and various provinces of Argentina: Catamarca, Cordoba, Entre Rios, La Rioja, Santa Fe and Santiago del Estero. These are places where human accidents were reported to occur due to this scorpion. For comparative purposes two types of samples were assayed: whole soluble venom obtained by electrical stimulation and supernatant from homogenized venomous glands. Two strains of mice (NIH and CF-1) were used for LD₅₀ determinations by two distinct routes of administration (intravenously and intraperitoneally). Important variations were found that goes from 0.5 to 12 mg/kg mouse body weight. Samples of soluble venom were always more potent than Telson homogenates. More complex pattern was observed in homogenates compared to soluble venom, as expected. This was supported by gel electrophoretic analysis and high performance liquid chromatographic (HPLC) separations. Additionally, the HPLC profile was enriched in proteins resolved at similar elution times as other known toxins from scorpion venoms studied. Immune enzymatic assays were also conducted comparatively, using four different anti-venoms commercially available for treatment of scorpion stings (Argentinean antidote from INPB, two anti-venoms from Butantan Institute of Brazil and Alacramyn from the Mexican Bioclon Institute). Cross-reactivities were observed and are reported among the various venoms and anti-venoms used. Lung, heart, liver and pancreas pathological modifications were observed on tissues of intoxicated mice. It seems that

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there are important variations on the venom compositions of the various samples studied and reported here, depending on the geographical area where the scorpions were captured. The results reported here are important for the clinical outcome of human accidents.

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1. Introduction

Envenomation due to scorpion stings is a problem of growing medical importance in Argentina. In the period from 1993 to 1999 three deadly accidents in humans were registered (de Roodt et al., 2003), and in the period from 2000 to 2006, this number increased to twelve children (Saracco et al., 2006; de Roodt 2007).

The scorpion species responsible for the majority of these deadly accidents is *Tityus trivittatus*, here abbreviated *T. trivittatus*. Its geographical distribution goes from the North of the Patagonian region to the Northern provinces (Southern latitudes 40°–26°) (de Roodt et al., 2003). This scorpion belongs to the Buthidae family and is one of the six species of *Tityus* found in Argentina: *T. trivittatus*, *T. bahiensis*, *T. confluens*, *T. uruguayensis*, *T. paraguayensis* and *T. argentinus* (Ojanguren-Affilastro, 2005). *T. trivittatus* is a well adapted species to cohabit with humans dwellings, which increases the probability of accidents (Salomón and de Roodt, 2001; de Roodt et al., 2003; de Roodt 2007).

During the last years the Health Ministry of Argentina (Ministerio de Salud, 2004, 2005, 2008) reported a considerable increase on the total number of accidents and a fourth fold increment of deadly cases, due to stings by scorpions (de Roodt, 2007). Additionally, in recent years fatal events were registered in provinces where this scorpion, although known to occur, did not have record tracks of severe human envenomations. Examples of these geographical localizations are the provinces of Entre Ríos, Santa Fe, Catamarca, La Rioja, San Juan and Tucumán. In these provinces, despite the potential risk associated with possible stings by *T. trivittatus* species, it was not considered a medical problem until recently, when deadly cases were registered to occur in children. Other provinces such as Buenos Aires, including the federal city of Buenos Aires, although having the presence of *T. trivittatus* species, have not reported severe human accidents, yet (de Roodt et al., 2003).

The treatment of scorpion envenomation consists in the application of the specific anti-venom, which is produced and freely distributed by the National Institute for Production of Biologicals of the Ministry of Health (INPB). Production of this anti-venom is difficult, since it depends on the capture of this species of scorpion for obtaining venom or venom glands for horse immunization procedures. This is a synanthropic species showing in some regions problems for correct taxonomic identification, apart from the rather low number of specimens that can be collected in the endemic areas. These facts not only limited the production of anti-venoms but hampered the conduction of systematic biochemical studies of its venom components, as well as the estimation of its toxicity and neutralization potency by the existent anti-venoms.

The toxicity symptoms caused by *T. trivittatus* venom vary according to distinct geographical regions and the clinical manifestations due to their stings seems to change abruptly in some provinces. It is thought that these changes might reflect changes in venom compositions, among other possible unknown factors. The human deaths in provinces where the stings were frequent but severe envenomation absent, strengthens this observation.

In view of the above mentioned difficulties and due to the lack of information on the regional characteristics of this scorpion venom, the present communication reports toxicological and biochemical data from different samples of telson homogenates and/or soluble venom obtained by electrical stimulation of *T. trivittatus* scorpions from different provinces of Argentina. Among the data here considered are: the chromatographic and electrophoretic profiles, immunochemical reactivity and lethal potency of venom samples in mice. We observed very different lethal potencies and chromatographic profiles in the different samples even in those from the same province. The chromatographic pattern of the whole soluble venom showed clear differences regarding those observed from telson gland homogenates. The lethal potencies observed were in the range of potency described for Brazilian species of *Tityus*.

2. Material and methods

2.1. Venom

Venom was obtained from telson homogenates of scorpion of the provinces of Santiago del Estero (SdeE), Catamarca (Cat), La Rioja (LaR), Córdoba (Cba), Entre Ríos (ER), Santa Fe (SFe) and from the city of Buenos Aires (BsAs). Telsons were obtained from scorpions captured in different provinces (Cba, SdeE, SFe), dried *in situ*, and sent to the Institute in Buenos Aires for processing. In other cases (ER, Cat and LaR) the scorpions were obtained in the provinces but the telson extraction was done in our laboratory in Buenos Aires, using always similar methodologies. Telsons from BsAs were obtained in our laboratory from scorpions captured in the city. In all the cases the methodology to obtain the telsons was the same. Briefly, the scorpions were killed by freezing and immediately the telsons were cut and vacuum dried for two to six hours, depending on the number of telsons been processed. In our laboratory the telsons were dried again in the same way, prior making the homogenates. The venom from homogenates was obtained from the supernatant of centrifuged macerated telsons made by conventional methods (de Roodt et al., 2001; Ozkan et al., 2006). The pool of telsons used to prepare the homogenates from the different samples in all the cases ranged from 20 (minimum) to 160 telsons (maximum). Venom was diluted in NaCl 0.15 M,

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