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Peripheral and central effects of intracerebroventricular microinjection of *Hottentotta gentili* (Pallary, 1924) (Scorpiones, Buthidae) venom

Moulay Abdelmonaim El Hidan [a, *], Oulaid Touloun [a, b], Omar El Hiba [c], Jawad Laadraoui [d], Hind Ferehan [d], Ali Boumezzough [a, **]

[a] Laboratory of Ecology and Environment L2E, (URAC 32, CNRST, ERACNERS 06) Faculty of Sciences Semlalia, Cadi Ayyad University, BP 2390-40080, Marrakesh, Morocco
[b] Polyvalent Laboratory of Research & Development LPVRD, Department of Biology, Polydisciplinary Faculty, University Sultan Moulay Slimane, Beni Mellal, Morocco
[c] Neurosciences, Pharmacology and Environment Unit, Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakesh, Morocco
[d] Laboratory of Pharmacology, Neurobiology and Behavior, Department of Biology, Faculty of Sciences Semlalia Cadi Ayyad University, Marrakesh, Morocco

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ABSTRACT

Central effects of scorpion venom toxins have been neglected, due both to the common belief that scorpion venoms act by targeting peripheral organs and also to the misunderstanding that these peptides do not cross the brain-blood barrier (BBB). Determining whether scorpion neurotoxicity is restricted to peripheral actions or whether a central mechanism may be partly responsible for systemic manifestations could be crucial in clinical therapy trends. The present study therefore aims to assess histopathological damages in some organs (heart, kidney, liver, and lungs) and the related biochemical impairments, together with a neurobehavioral investigation following an intracerebroventricular (i.c.v) micro-injection of *Hottentotta gentili* (Scorpiones, Buthidae) venom (0.47 μg/kg). I.c.v. injection of venom produced focal fragmentation of myocardial fibers, while lungs showed rupture of the alveolar structure. Concurrently, there was a significant rise in the serum enzymes levels of ASAT, ALAT, CPK and LDH. Meanwhile, we observed behavioral alterations such as a hypoactivity, and in addition the venom seems to have a marked anxiogenic-like effect. The present investigation has brought new experimental evidence of a peripheral impact of central administration of *H. gentili* venom, such impact was manifested by physiological and behavioral disturbances, the last of these appearing to reflect profound neuromodulatory action of *H. gentili* venom.

1. Introduction

Scorpion envenomation constitutes one of the most important health problems in many countries, including North-Africa, the Middle East and South America (Al-Sadoon and Jarrar, 2003; De Roodt et al., 2003; Patil, 2009).

Depending on the species and the venom dose injected, the body region of venom inoculation, and on the patient's age, the clinical symptoms of scorpion venom can vary from mild to moderate local pain and paresthesia (90% of poisoning accidents) to serious systemic dysfunction such as cardio-vascular alterations, gastrointestinal dysfunction, lung edema, pancreatitis, convulsions, neurological lesions, coma, and death (Amaral et al., 1993; Osnaya-Romero et al., 2001). The most serious poisoning cases are observed in children and in senior citizens (Guidine et al., 2008). Currently, 25,000 scorpion stings are recorded per year in Morocco and 90% of fatal cases are younger than 10 years old (Abourazzak et al., 2009).

Among all components of scorpion venoms, toxins that affect ion-channels are the most important venom components responsible for human intoxication (Quintero-Hernández et al., 2013). The scorpion α-toxins are the most important neurotoxins, consisting of 61–76 polypeptides that act on a specific site on the voltage-gated sodium channel. These toxins inhibit the inactivation of the

* Corresponding author.
** Corresponding author.
E-mail addresses: Elhidan@gmail.com (M.A. El Hidan), aboumezzough@gmail.com, boumezzough@uca.ac.ma (A. Boumezzough).

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channel, inducing a prolonged depolarization and neuronal excitation. There are other toxins with less important effects on human. These toxins bind on potassium and calcium channels (Quintero-Hernández et al., 2013).

Scorpion venom action on the central nervous system (CNS) was previously neglected due to the common perception that peptides constituting scorpion venom didn't cross the brain-blood barrier (BBB). However recent studies have emphasized that scorpion venom could act on CNS and even cross the BBB. In fact, Clot-Faybesse et al. (2000) and Nunan et al. (2003) report finding scorpion toxins in the CNS after systemic injection in newborn mice without a fully developed BBB. Additionally, Nunan et al. (2003) had observed the presence of tityostoxin (TsTX) toxin in the brain of young rats after subcutaneous injection. These authors moreover reported that the distribution of TsTX in the brain of young rats was threefold higher than that of adult rats, indicating a higher BBB permeability. Moreover, Mesquita et al. (2002), had reported that intra-muscular (i.m.) injections of Phenobarbital, a GABAergic agonist, were able to block the lung edema induced by intracerebroventricular (i.c.v.) injections of TsTX, suggesting an important role of the central nervous system (CNS) in the mechanism of action for this fraction of the *Tityus serrulatus* scorpion venom. Nencioni et al. (2009) also demonstrate central changes in levels of homovanillic acid, a metabolite of the dopamine, after intraperitoneal injection of venom of both *T. serrulatus* and *Tityus bahiensis*.

Hottentotta gentili is one of the most dangerous scorpions in Morocco, causing severe envenoming and even death. It was previously observed in our laboratory that subcutaneous injection of this scorpion species induced behavioral alterations characterized by hypersalvation, respiratory difficulty, squeaking, mouth rubbing, mastication, wild-running, jumping, trembling, humpback and wet dog shakes. Also when neurobehavioral disturbances were evaluated based on the locomotor activity and other cognitive approaches such as depression and anxiety; we found that *H. gentili* venom causes time dependant neurobehavioral changes. In fact, *H. gentili* venom induces a hypoactivity state, and also elicits depression and anxiogenic effects mainly after the three first hours post envenomation (El Hidan et al., 2015a).

Based on the above data and in view of the very small number of investigations on venom-elicited effects following either central or peripheral injection of scorpion venoms, especially with venom from Old World Scorpions common in North-Africa and the Middle East, the present study was designed to determine whether venom from the Old World *H. gentili* scorpion centrally microinjected could reproduce the same effects observed after subcutaneous injection.

We performed a study to investigate, in experimental Sprague–Dawley rats, the possible histopathological damages in some organs (heart, kidney, liver, and lungs) and the subsequent biochemical impairments, together with a neurobehavioral investigation following a i.c.v micro-injection of a sublethal dose (0.47 μg/kg) of *H. gentili* venom.

2. Material and methods

2.1. Animals

Twelve Sprague–Dawley Male rats (200–250 g) were used for determination of histopathological, biochemical and behavioral changes after intracerebroventricular (i.c.v) injection of *H. gentili* venom. Animals were kept at a constant room temperature (25 °C), with a 12 h dark–light cycle and free access to food. All animals were treated according to the European decree relating to the ethical evaluation and authorization of projects using animals for experimental procedures, 1st February 2013, NOR: AGRG1238767A. Thus, all efforts were made to minimize the number and suffering of animals used.

2.2. Scorpions

Scorpions were collected from Zagora province in the South-Eastern region of Morocco. They were housed in well ventilated wooden cages with free access to food and water. The species was determined according to the identification key as described by Kovarík (2007).

2.3. Methods

2.3.1. Venom extraction

Venom was obtained from mature *H. gentili* (Fig. 1) scorpions by electrical stimulation of the telson as described by Ozkan et al. (2007). The venom was dissolved in double distilled water and centrifuged at 15,000 g for 15 min. The protein content of supernatant was determined according to the method of Bradford (1976). Until use, the sample was stored at −20 °C.

2.3.2. Surgery

The animals were anesthetized with chloral hydrate (6% ip) then they were submitted to stereotaxic surgery for guide-canula implantation in the right lateral ventricle (AP −0.8; LL −1.5; DV −2.5), coordinates derived from the Paxinos and Watson (1998) stereotaxic atlas. A stainless steel guide cannula, fastened to the skull bone with dental acrylate cement was used. After surgery, animals were housed individually and allowed to recover for 5–7 days.

Animals were divided into two groups control groups (6 animals) injected with sterilized saline solution (0.9%) and treated groups (6 animals) injected with 0.47 μg/kg of *H. gentili* venom.

The i.c.v microinjections were made as described by Mesquita et al. (2003), briefly a 5.0-μl Hamilton syringe was connected to the injector cannula through a polyethylene tube filled with distilled water, used to drive toxin into ventricular space. A small 1.0-μl bubble was inserted just before loading the injection needle with 4.0-μl *H. gentili* venom or Nacl (0.9%) solution, fourfold the amount actually injected. The injection setup, therefore, was designed to have no dead volume space, in order to confirm *H. gentili* venom injection, and to separate toxin from the distilled water. The i.c.v injections had always a volume of 1.0-μl.

2.3.3. Histological study

Rats were sacrificed by decapitation 6 h post-injection and their

Fig. 1. *Hottentotta gentili* from Zagora.