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In vivo EFFECTS OF *Buthus occitanus tunetanus* AND *Androctonus australis garzoni* SCORPION VENOMS ON PREGNANT AND NON-PREGNANT RATS

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ABSTRACT: Scorpion envenomation in pregnant victims has been scarcely studied. Accidents with venomous animals can induce serious injuries for both mothers and embryos. In the current work, the lethality of *Buthus occitanus tunetanus* (*Bot*) and *Androctonus australis garzoni* (*Aag*) venoms was assessed in pregnant and non-pregnant murine rat models. The median lethal dose (LD_{50}) was determined following the Spearman-Karber method. Our results showed great similarities of envenomation symptoms between term-pregnant and nonmated rats. An unusual vaginal bleeding was also seen in pregnant rats envenomed with *Bot* and *Aag* venoms. Our findings suggest that gestation may increase the venoms toxicity in rats.

KEY WORDS: scorpion venom, *Buthus occitanus tunetanus, Androctonus australis garzoni*, *in vivo* effects, gravid rats.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Scorpion envenomation is widely spread in many regions of the world (5). In Tunisia, approximately 30,000 to 45,000 cases of scorpion-stung humans are reported every year (8). The morbidity and lethality of scorpion venoms are due to the action of different neurotoxins affecting the activity of a large variety of membrane ion-channels components (17, 18). They vary with many factors related both to the victim's state (age, body weight, strain and sex) and to the involved venomous animal (genus, geographical and seasonal distribution, and individual intraspecificity) (1, 3, 6–8, 13, 15, 16). Manifested symptoms vary from local burning to cardiorespiratory arrest, which is usually responsible for the victim's death.

Although there are numerous data on scorpion venoms and their adverse effects, scarce information has been reported about their toxicity in pregnant victims (9).

In the present paper, the toxicity of venoms from *Buthus occitanus tunetanus* (*Bot*) and *Androctonus australis garzoni* (*Aag*), two scorpion species frequently involved in human envenomation in Tunisia, were assessed in late primigravida Wistar rats by determining the LD_{50} and recording the envenomation symptoms.

White Wistar virgin female rats were used. Each six rats were bred in a separate cage at the animal house of the Laboratory of Pharmacology, University of Sfax, Tunisia. The conditions at the animal house were kept constant with temperature of 23–25°C and light-dark cycle of 14 light : 10 dark cycles. Drinking water and standard food were provided *ad libitum*. After one week of acclimatization, females were allowed to mate with mature males in order to obtain timed-pregnant rats. The day in which spermatozoa appeared in the vaginal smear was considered the first day of pregnancy. In such conditions, fetal delivery may occur 21±1 days post coitus. Non-pregnant rats were kept under the same conditions.

Buthus occitanus tunetanus and *Androctonus australis garzoni* crude venoms were obtained from the Pasteur Institute of Tunis, Tunisia. They were collected by electrical milking, then water-extracted, freeze-dried, and finally rehydrated, according to the method of Miranda *et al*. (12). On the experiment day, venoms were adequately diluted with physiologic saline solution in order to obtain four different doses (0.5, 1, 2, or 4mg/ml of total proteins for *Bot* venom and 0.19, 0.37, 0.75, or 1.5mg/ml for *Aag* venom).

The median lethal dose (LD_{50}) was determined according to the method of Spearman-Karber (4) for both pregnant and non-pregnant rats. Briefly, a group of six mated or nonmated rats received one of the prepared crude venom doses in order to obtain four dose points in the response curve (animal mortality) within the linear portion, covering the full response range from 0% to 100% during 24h after the venom intraperitoneal injection. To prevent body weight-induced variability of the venoms effects, the administered volume was kept constant at 1ml/kg of body weight (BW). Crude venom doses were intraperitoneally injected. Almost all tests on pregnant and non-pregnant rats were carried out at the same day (corresponding to the 22^{nd} day of gestation). The BW on the experiment day was 370±15g for pregnant rats and 273±14g for non-pregnant rats. Envenomation symptoms were recorded for each group during the experimental period.

The LD_{50} was lower in pregnant (472µg/kg for *Aag* venom and 1.587µg/kg for *Bot* venom) than in non-pregnant (595µg/kg for *Aag* venom and 1.781µg/kg for *Bot* venom) rats (Table 1).

Similar envenomation symptoms (squeaking, mouth rubbing, salivation, chewing, agitation, respiratory dysfunction, loss of equilibrium, and paralysis) were observed in both pregnant and non-pregnant rats (Table 2). An unusual vaginal bleeding manifested in pregnant rats for both *Aag* and *Bot* envenomation. At high venom doses (2mg/kg and 0.75mg/kg for *Bot* and *Aag*, respectively), fetal delivery was observed before the animal death.

To our knowledge, few studies have focused on the toxicity of scorpion venoms in gravid females. Elsewhere, a teratogenic chronic effect of scorpion venom was observed in pregnant rats (6). In this work, the toxicity of two scorpion species frequently involved in human envenomation in Tunisia was investigated in murine gravid model. Our results showed great similarities of envenomation symptoms between pregnant and nonmated rats. Such signs are mainly observed in scorpion envenomation victims (5). However, an unusual vaginal bleeding, a sign of ongoing labor, was seen when high venom doses were used in term-pregnant rats. Uterine contractility is a key process of parturition. Thus, such effects may be a result of the direct venom-induced hypercontractility of the uterine myometrium (10, 11, 14). The LD_{50} of *Bot* venom in term- and non-pregnant rats was higher than that obtained by Devaux and Rochat (2) in non-pregnant mice (1,428µg/kg). However, the LD_{50} of *Aag*

venom was similar to that of its congener *Androctonus australis*, whose lethality ranged from 0.32 to 0.7mg/kg. The scorpion venom lethality (LD_{50}) increases with the increase in body weight (7, 8, 13, 14), but herein a controversial result was observed. Pregnant rats weighing more than non-pregnant rats (370±15g and 273±14g, respectively) presented a lower LD_{50}. Thus, we can suggest that gestation may increase female sensitivity to scorpion envenomation.

In conclusion, females may be more vulnerable to scorpion envenomation during gestation. In the present study, envenomation may have disturbed the pregnancy process, corroborating the data on other venomous animal bites and stings.

Table 1. Particular toxicity symptoms and median lethal dose (LD_{50}) of *Buthus occitanus tunetanus* (*Bot*) and *Androctonus australis garzoni* (*Aag*) venoms in pregnant and non-pregnant rats.

Particular symptoms	*Aag* venom [0.75mg/kg (BW)]		*Bot* venom [1mg/kg (BW)]	
	Pregnant rats	Nonmated rats	Pregnant rats	Nonmated rats
Vaginal bleeding (n)	2	0	2	0
Delivering rats (n)	2	---	1	---
Delivered fetuses (n)	2	---	1	---
LD_{50} [µg(protein)/kg (BW)]	472	595	1,587	1,781

BW: body weight

Table 2. Envenomation symptoms developed after intraperitoneal injection of crude scorpion venoms.

Scorpion venoms		***Androctonus australis garzonï***								***Buthus occitanus tunetanus***							
		Pregnant rats				**Non-pregnant rats**				**Pregnant rats**				**Non-pregnant rats**			
Venom doses (mg/ml)		**0.19**	**0.37**	**0.7**	**1.5**	**0.19**	**0.37**	**0.7**	**1.5**	**0.5**	**1**	**2**	**4**	**0.5**	**1**	**2**	**4**
Envenomation symptoms	Squeaking	+	++	+++	+++	+	++	++	+++	+	+	+++	+++	+	++	+++	+++
	Mouth rubbing	++	++	+++	+++	+	++	+++	+++	+	++	+++	+++	+	+++	+++	+++
	Chewing	+	+++	+++	+++	+	++	+++	+++	+	+	+++	+++	+	++	++	+++
	Salivation	++	+++	+++	+++	+	++	++	+++	+	++	+++	+++	+	+++	+++	+++
	Agitation	+	+	++	+++	+	+++	+++	+++	+	++	+++	+++	+	++	+++	+++
	Respiratory dysfunction	++	++	+++	+++	+	++	+++	+++	++	+++	+++	+++	++	+++	+++	+++
	Loss of equilibrium	+	++	+++	+++	--	++	+++	+++	--	++	+++	+++	--	++	+++	+++
	Paralysis	--	++	+++	+++	--	+	+++	+++	--	+	++	+++	--	++	++	+++

(--) symptoms were less frequently observed; (+): symptoms were very frequently observed.

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