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# A newly described scorpion species, *Leiurus abdullahbayrami* (Scorpion: Buthidae), and the lethal potency and *in vivo* effects of its venom

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**Abstract:** Currently, medically significant scorpion species belong to the *Buthidae* family and are represented by the genera *Androctonus, Buthus, Mesobuthus, Hottentotta, Parabuthus, Tityus, Centruroides, Leiurus*. Although *Leiurus* was originally considered a monotypic genus, four additional species have since been described. *Leiurus abdullahbayrami* (previously identified as *L. quinquestriatus* in Turkey) was classified as a new *Leiurus* species. This is the first report conducted on the lethality and biologic effects of *L. abdullahbayrami* scorpion venom in mice. In this study, the electrophoretic protein pattern of its venom was also determined. Two protein bands with molecular masses of 4 and 6 kDa were more strongly detected than other protein bands in the venom sample. Electrophoresis showed that *L. abdullahbayrami* scorpion venom possesses both short- and long-chain neurotoxins. The median lethal dose of this venom was found to be 0.19 mg/kg by subcutaneous (SC) injection in mice. Animals experimentally envenomed with *L. abdullahbayrami* venom exhibited hyperexcitability, agitation, aggressive behavior, squeaking and fighting, tachypnea, weakness, convulsions, and death due to cardiac and respiratory failure. In further studies, the potency of antivenom should be investigated in relation to the scorpion venom. Molecular and pharmacological studies are also required to identify and characterize *L. abdullahbayrami* scorpion venom.

**Key words:** scorpions, *Leiurus* spp., scorpion venom, lethality, *in vivo* effects.

## INTRODUCTION

Scorpions are venomous arthropods, members of the Arachnida class and order Scorpiones (1). The scorpion species that are of current medical importance belong to the Buthidae family, and are known to cause considerable public health problems in North Africa, Asia, the Middle East, India, Mexico, and Central and South America (2, 3).

The genus *Leiurus* was considered to be monotypic for many years, comprising the one species *Leiurus quinquestriatus* containing subspecies *L. quinquestriatus quinquestriatus* and *L. quinquestriatus hebraeus* (4-6). As of 2009, four species were added: *L. jordanensis* from Jordan, *L. savanicola* from Cameroon, *L. nasheri* from Yemen, and *L. abdullahbayrami* from Turkey (6-9).

In Turkey, Tulga (10) was the first to record *L. quinquestriatus* from Adıyaman Province in the southeastern region, which was identified by Prof. A. Shulov. It was later reported in Gaziantep, Hatay, Kilis, Sanlıurfa, and Mardin provinces in Turkey (6, 11). Recently, Yagmur *et al.* (6) described *Leiurus abdullahbayrami*, which had previously been identified as *L. quinquestriatus* in the Turkish scorpiofauna and has also been reported in Syria (12).

Five decades ago, Tulga (10) obtained venom from the telson of *L. quinquestriatus* using the maceration technique. The author observed that toxicity was directly related to the telson portion. In addition, Ozkan *et al.* (13) evaluated the

neutralizing capacity of *Androctonus crassicauda* antivenom against *L. quinquestriatus* from Iraq. Apart from these studies, no information has been found regarding the toxicity, *in vivo* effects, and venom proteins of Turkish *Leiurus* specimens (14).

Since there are no data on lethality and the effects of *Leiurus abdullahbayrami* scorpion venom, research must be done to find out its effects on population health. Therefore, the main objective of this study was to determine the protein profiles (molecular weight), lethality, and *in vivo* effects of venom from a new *Leiurus* scorpion species.

## MATERIALS AND METHODS

### Scorpion Origin

During summer 2009, scorpions (n = 27) were collected using an ultraviolet (UV) lamp at night in the Southeastern region of the Gaziantep and Sanlıurfa provinces in Turkey (Figure 1). The animals were housed in individual plastic boxes at the Department of Entomology, Faculty of Veterinary Medicine, Ankara University, Turkey. The scorpions were fed crickets or cockroaches and received water daily. Taxonometric identification followed Yagmur *et al.* (6).

### Experimental Animals

Swiss mice of both genders (20 ± 2 g) – bred in the animal facilities of Refik Saydam Public Health Agency (RSPHA) – were employed to determine the median lethal dose ($LD_{50}$) via SC administration. The animals were housed under controlled temperature (20 ± 2°C), in 12-hour light/dark cycle and fed commercial rodent pellets and water *ad libitum* throughout the experiment.

### Venoms

The venom was obtained from mature *L. abdullahbayrami* scorpions by electrical

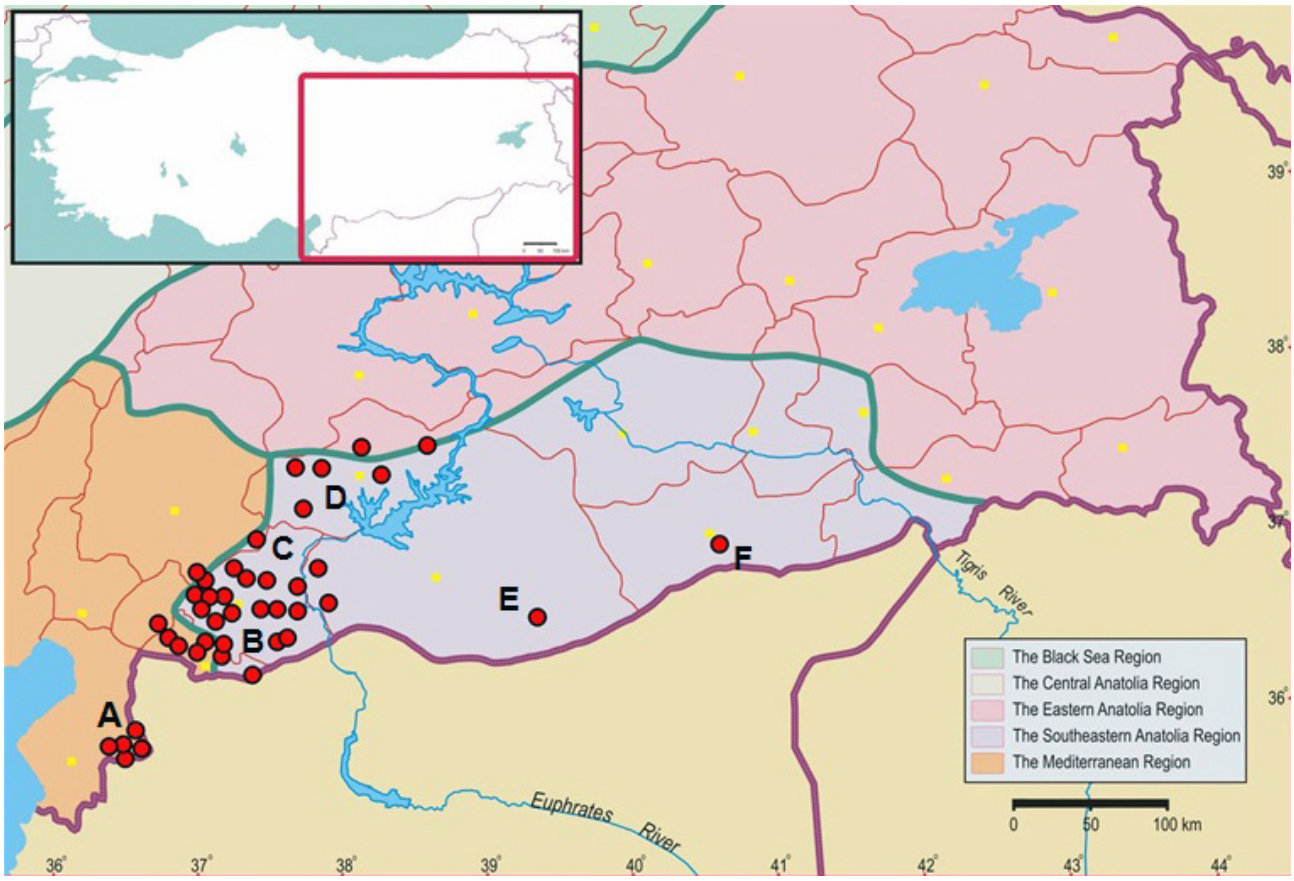


**Figure 1.** Map of the Southeastern Anatolia region with its provinces – (**A**) Hatay, (**B**) Kilis, (**C**) Gaziantep, (**D**) Adiyaman, (**E**) Sanliurfa, (**F**) Mardin – and the distribution (dots) of *Leiurus abdullahbayrami* according to Yagmur *et al.* (6). The scorpions were captured in Gaziantep (**C**) and Sanliurfa (**E**) provinces.

stimulation of the telson. It was dissolved with sterile double-distilled water and centrifuged at 14,000 rpm for 15 minutes at 4°C. The supernatant was stored at –20°C until use. Protein concentration was determined using a BCA kit (Pierce, USA) according to the manufacturer's instructions. Bovine serum albumin was employed as standard.

### Lethality and *In Vivo* Venom Effects

Experimental protocols for the animal experiments were approved by RSPHA Ethics Committee. Venom $LD_{50}$ was assessed by SC injections in mice (20 ± 2 g) whereas lethality was determined as described by Behrens and Karber (15). For both lethality and *in vivo* effects, five mice per dose group were SC injected with various doses (3.41, 3.63, 3.85, 4.05, and 4.27 µg/mouse) of *L. abdullahbayrami* venom, diluted in a 0.5-mL physiologic saline solution (0.85% NaCl). An equivalent volume of saline was injected into five mice as negative control group (Table 1). The animals were observed for 24 hours after venom injection to determine $LD_{50}$. Deaths occurring after 24 hours were recorded. In terms of *in vivo* effects, the mice injected with venom for the lethality assay were monitored and the evolution of their signs was recorded for up to 24 hours.

### Gel Electrophoresis of Venom

The venoms were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (16). Venom of the *L. abdullahbayrami* scorpion was run on NuPAGE® Novex Bis-Tris 4-12% gel in MES SDS running buffer (Invitrogen, USA, NP0002; 50 mM MES, 50 mM Tris-HCl, 1% SDS, 1.025 Mm EDTA) using Xcell SureLock® Mini-cell (Invitrogen, USA) following standard manufacturer protocol. SeeBlue® Plus2 Pre-Stained Standard (Invitrogen, USA) was run in parallel in order to calculate molecular weights of the proteins. The gel was stained with Coomassie blue, then scanned and molecular weights of the proteins were calculated with Molecular Imaging Software® (Kodak MIS, USA).

## RESULTS

The new species was distinguished from other members of the genus according to size of pedipalp, chela, metasomal V segment, and location of trichobothrium db (dorsal basal) on the fixed finger of the pedipalp. The scorpions were then identified as *L. abdullahbayrami* (Figure 2) under stereomicroscope (Stemi 2000-C®, Zeiss, Germany).

### Lethality Assay and *In Vivo* Effects on Mice

A reduced number of mice were used in order to avoid causing unnecessary pain and suffering to the animals. Therefore, both lethality and *in vivo* venom effects on the same Swiss mouse were determined. The lethal toxicity of *L. abdullahbayrami* venom was determined after SC injections of different venom doses and the death/survival ratio (Table 1) was collected over a 24-hour period. The $LD_{50}$ of *L. abdullahbayrami*

**Table 1.** Median lethal dose for *L. abdullahbayrami* venom in mice

| Concentration of venom solution | | Venom (µg/mouse) | Death/total |
|---|---|---|---|
| Venom (µL) | PSS (µL) | | |
| 40.0 | 3000 | 3.41 | 0/5 |
| 42.5 | 3000 | 3.63 | 1/5 |
| 45.0 | 3000 | 3.85 | 2/5 |
| 47.5 | 3000 | 4.05 | 3/5 |
| 50.0 | 3000 | 4.27 | 5/5 |
| Control | 3000 | – | 0/5 |
| $LD_{50}$ (µg/mouse) | 3.9 | | |

PSS: physiologic saline solution.

**Figure 2.** A newly described scorpion species, *Leiurus abdullahbayrami*.

scorpion venom was found to be 0.19 mg/kg by SC injection (Table 1).

Experimental mice showed the following intoxication symptoms after injection: hyperexcitability, agitation, aggressive behavior, squeaking and fighting, tachypnea, convulsions, weakness, paralysis, coma, and death. However, not all animals exhibited hypersalivation or lacrymation.

### SDS-PAGE Analysis of Venom

Venom protein content was found to be 0.513 mg protein/mL. The protein profile of *L. abdullahbayrami* venom was analyzed using NuPAGE® Novex 4-12% Bis-Tris gradient gel (Invitrogen, USA) followed by Coomassie blue staining. Proteins in the venom (10 μg) were found to be between 3 and 188 kDa by gradient gel electrophoresis (Figure 3). Four different protein bands with molecular masses of 4, 6, 31, and 46 kDa were detected in the venom sample by Kodak MIS.

## DISCUSSION

About 1700 different species of scorpion distributed over fourteen families exist in the world (17). Up until now, in Turkey, Buthidae is the family of medical significance, comprising four genera (*Androctonus*, *Leiurus*, *Mesobuthus*, and *Hottentotta*) with eight different species.

The *L. quinquestriatus* scorpion is considered one of the most dangerous, and is blamed for many scorpion envenomation cases in countries from North Africa and the Middle East. The species is therefore considered most significant to humans and an influential cause of morbidity, especially in children (18-22).

To the best of our knowledge, there have been no reports on the toxicity and biological effects of *Leiurus* species from Turkey since 1960. This work is the first to determine the electrophoretic protein pattern, lethality, and effects of the venom of the new scorpion species, *L. abdullahbayrami*.

In a study, Tulga (10) declared that the

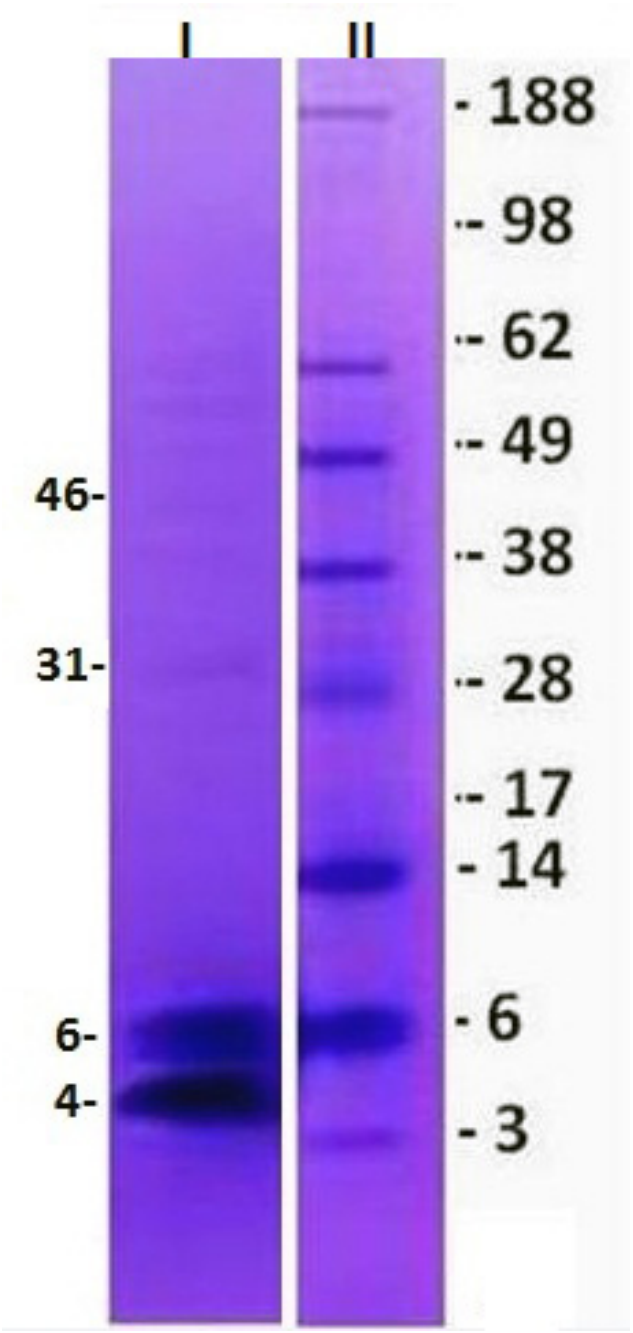


**Figure 3.** (**I**) Proteins from the venom of *Leiurus abdullahbayrami* from Sanliurfa were separated by NuPAGE® Novex 4-12% Bis-Tris gradient gel (Invitrogen, USA). Venom secretion proteins of 4 and 6 kDa were more strongly detected than other bands after staining. (**II**) Molecular weight: 188 kDa – myosin, 98 kDa – phosphorylase, 62 kDa – BSA, 49 kDa – glutamate dehydrogenase, 38 – kDa alcohol dehydrogenase, 28 kDa – carbonic anhydrase, 17 kDa – myoglobin-red, 14 kDa – lysozyme, 6 kDa – aprotinin, 3 kDa – insulin, B Chain (SeeBlue® Plus2 Pre-Stain Standard, Invitrogen, USA).

scorpion venom toxicity was related to the telson portion per mouse. The author emphasized that *L. abdullahbayrami* venom is four to five times more toxic than *A. crassicauda* venom. However, Ozkan *et al.* (13) stated that venom from the Iraqi *L. abdullahbayrami* is double the toxicity of *A. crassicauda* scorpion venom. Ismail (18) also reported that smaller quantities of *L. quinquestriatus* venom are 26 to 28 times more lethal than the largest quantity of *L. quinquestriatus* scorpion venom. Conversely, even if the venom was extracted using a single method, lethal dose depends on many factors such as genus, species, gender, geographic origin of the scorpion, and structure and quantity of the venom (23). The median lethal dose of *L. quinquestriatus* venom was previously reported as 0.25 mg/kg, which makes it one of the most toxic species of scorpion in the world (22, 24-26). In the current work, the $LD_{50}$ of *L. abdullahbayrami* venom was calculated to be 0.19 mg/kg in mice.

The venom of scorpions contains neurotoxic polypeptides that consist of low molecular weight peptides, which can cause a variety of symptoms, ranging from pain at the sting site to death (27, 28). The neurotoxic peptides responsible for the envenomation symptoms interact with ion channels and have the potential to cause massive damage to the nervous system of both vertebrates and invertebrates (29, 30). The short-chain neurotoxins with 3 to 4.4 kDa act on potassium or chloride channels. Long-chain neurotoxins, which have 6.5 to 8.5 kDa, mostly act on sodium channels (29, 31-33). Fatani *et al.* (22) stated that sodium and calcium channel blockers ameliorated the effects of *L. quinquestriatus quinquestriatus* scorpion venom. Hence, they said that these ion channel blockers could be useful in scorpion sting cases if antivenoms were not readily available. In our study, we showed the electrophoretic protein pattern of the scorpion venom to be between 3 and 188 kDa by gradient gel. Two protein bands with molecular masses between 4 and 6 kDa were more strongly detected than other protein bands in our venom samples. As a result, electrophoresis analysis indicates that *L. abdullahbayrami* scorpion venom possesses both short and long-chain neurotoxins according to electrophoretic protein patterns.

In earlier epidemiological and clinical studies, *L. quinquestriatus* venom was capable of provoking cardiac complications such as pulmonary edema, myocarditis, changes in heart

rate and rhythm, and cardiac failure (19, 34-39). In Turkey, Söker and Haspolat (40) reported 64 cases of scorpion stings in children from the provinces of Mardin, Sirnak, Batman, and Siirt in the southeastern Anatolia region, who were admitted to the Pediatric Emergency Department in Dicle University Hospital at Diyarbakir between 1995 and 1999 (Figure 1). Those authors also recorded a 12.5% lethality rate, which was due to cardiac and respiratory failure during the first 24 hours of hospitalization. Recently, Bosnak *et al.* (41) showed that 2.2% of all cases of scorpion stings admitted to a referral hospital in Turkey were caused by *L.quinquetriatus*.

In addition, Bosnak *et al.* (41) stated that pulmonary edema developed in 9.6% and dyspnea in 23.0% of victims; the predominant signs of cardiovascular system compromise were tachycardia (36.5%), dyspnea (23.0%), paleness (15.3%), hypertension (7.6%), and hypotension (3.8%), and manifestations of cholinergic stimulation, including excessive sweating (32.6%) and vomiting (3.8%), were seen after scorpion stings in children in southeast Turkey. However Al *et al.* (42) reported 120 cases of scorpion envenomation in the Batman province, but cardiac dysfunction, myocardial damage and death were not recorded as secondary symptoms to the major systemic envenomations. Scorpion toxins cause the direct release of neurotransmitters, such as acetylcholine and catecholamines, which produce signs of autonomic system over-activity or autonomic storms (43, 44).

In our study, mice experimentally envenomed with *L. abdullahbayrami* venom displayed hyperexcitability, agitation, aggressive behavior, squeaking and fighting, tachypnea, convulsions, weakness, paralysis, and death due to cardiac and respiratory failure. Fatani *et al.* (22) reported that animal symptoms resulting from *L. quinquestriatus* scorpion envenomation included agitation, aggressive behavior, hyperventilation, hypersalivation, lachrymation, micturition, defecation, shivering, periodic spastic contractions, gasping, convulsions, and in fatal cases, cardiac and respiratory arrest. Although we also observed these sympathetic signs such as agitation, excitability, aggressive behavior, tachypnea, convulsions and death due to cardiac and respiratory failure, we did not observe any hypersalivation or lachrymation (parasympathetic signs) in envenomed mice. Results of the present study showed that *L. abdullahbayrami* venom manifested sympathetic effects that were more marked than the parasympathetic effects according to the signs of intoxication.

As far as we know, the *Leiurus* species was only found in Adıyaman province, but has now been recorded in Hatay, Kilis, Gaziantep, and Mardin provinces in the southeastern region of Turkey, where scorpionism is a known significant medical problem (Figure 1). On the other hand, monovalent antivenom (*A. crassicauda*) has been administered in the treatment of all scorpion species sting cases in Turkey (45). Therefore, further studies should investigate the potency of antivenom against the scorpion venom. Molecular and pharmacological studies are also required to identify and characterize *L. abdullahbayrami* scorpion venom.

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## CONFLICTS OF INTEREST

There is no conflict.

## FINANCIAL SOURCE

The present study was supported by Refik Saydam Public Health Agency, Ankara, Turkey.

## ETHICS COMMITTEE APPROVAL

The present study was approved by the Ethics Committee of Refik Saydam Public Health Agency (under protocol number 2009/3).

## CORRESPONDENCE TO

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