

# A Study on Venom Proteins of *Iurus dufourei asiaticus* Birula, 1903 (Scorpiones: Iuridae)

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**SUMMARY:** The scorpion *Iurus dufourei asiaticus* (Birula 1903) which is the largest scorpion in Europe and Turkey belongs to the family Iuridae and is endemic in Turkey. No data has been found about the venom components of *I. d. asiaticus*. In this study, the venom extract obtained from *I. d. asiaticus* specimens collected from Aydın were analyzed using the Tris tricine SDS-PAGE method. A total of 28 protein fractions or fraction groups were detected in the range of 6.5-205 kDa.

**Key Words:** *Iurus dufourei asiaticus*, Iuridae, venom, protein, TSDS polyacrylamide gel electrophoresis

## *Iurus dufourei asiaticus* Birula, 1903 (Scorpiones: Iuridae)'un Zehir Proteinleri Üzerine Bir Çalışma

**ÖZET:** Avrupa ve Türkiye'nin en büyük akrep türü olan *Iurus dufourei asiaticus* Birula, 1903 Iuridae familyasına dahildir ve Türkiye için endemiktir. *I. d. asiaticus*'un zehir bileşenleri hakkında herhangi bir veri bulunmamaktadır. Bu çalışmada, Aydın'dan toplanan *I. d. asiaticus* örneklerinin zehir ekstraktları Tris-tricine SDS-PAGE metoduyla analiz edilmiştir. 6.5 – 205 kDa aralığında toplam 28 protein fraksiyon yada fraksiyon grubu belirlenmiştir.

**Anahtar Sözcükler:** *Iurus dufourei asiaticus*, Iuridae, Zehir, Protein, TSDS Poliakrilamid Jel Elektroforezi

## INTRODUCTION

Scorpion venoms are complex mixtures containing neurotoxic polypeptides, proteolytic enzymes, mucoproteins, nucleotides, lipids, and also protease inhibitors and biological amines (2, 5, 11, 13, 15, 16, 17, 24, 26). Because of their evolutionary time, their medical importance and the presence in their venomous glands of a variety of biologically active component, scorpions are used in an enormous variety of approaches and interdisciplinary studies (14).

It has been estimated that 100.000 distinct peptides exist in scorpion venoms, but only limited number of these peptides have been described (16, 20). Most of the biochemical study performed with scorpion venom has been reported for family Buthidae, probably because they are dangerous to human (25).

The scorpion *Iurus dufourei asiaticus* Birula, 1903 (Iuridae) which is the largest scorpion in Europe and Turkey is distributed in southern part of Anatolia and is endemic for Turkey (9). No data has been found about the venom components of *I. d. asiaticus*.

A few numbers of the studies about Anatolian scorpion venoms are on *Mesobuthus gibbosus* and *Androctonus crassicauda* which belongs to the family Buthidae (6, 21, 24).

The present study aimed to investigate the proteins and peptides in the range of 205-6.5 kDa of the *I. d. asiaticus* venom.

## MATERIAL AND METHODS

*Iurus dufourei asiaticus* specimens used in this study was collected from Aydın (Turkey). Their venom was obtained by electrical stimulation, dissolved in ultrapure water, centrifuged at 14.000 RPM for 20 min and supernatants was applied to Tris-Tricine SDS gel electrophoresis.

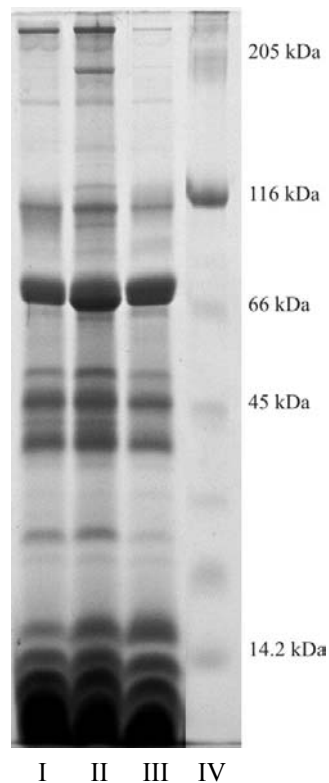
Tris-tricine-sodium dodecyl sulphate (TSDS) polyacrylamide gel electrophoresis was conducted according to procedures given by Schägger and von Jagow (18). This method is more sensitive for separating small protein component up to 2 kDa (19). Electrophoretic separations were carried out in discontinuous buffer system (cathode buffer: 0.1 M Tris, 0.1 M Tricine, 1 % SDS at pH 8.25 and anode buffer: 0.2 M Tris-HCL at pH. 8.9), using a 10 % separation gel and 4 % stacking gel. Equal amount of 10 µl venom extracts was denatured into the buffer solution of 100 % glycerol, 2-mercaptoethanol, 20 % SDS and 1 M Tris at pH 6.8 for 5 minutes at 95 °C, and

**Table 1.** The numbers and densities of electrophoretic fractions of the *Iurus dufourei* asiaticus venom (\* individual bands are dense as not distinguishable) NB: Number of bands.

| kDa | > 205 | 205–116 | 116–66 | 66–45 | 45–29 | 29–20 | 20–14.2 | 14.2–6.5 | < 6.5 | Total |
|-----|-------|---------|--------|-------|-------|-------|---------|----------|-------|-------|
| NB  | 4     | 5       | 5      | 4     | 4     | 3     | 1       | 2*       | *     | 28    |

then loaded to gels. Electrophoretic separations were maintained for 14 hours with 25 mA/gel stable current.

Separation gels were stained with 0.1% Coomassie Blue R-250 (Sigma) for 3 hours. Wide range standards (6.5 – 205 kDa) (Sigma) were used in order to calculate the molecular weights of proteins. Electrophoretic separation was possessed using a SE Ruby 600 (Amersham Bioscience) apparatus with gels having 18 X 16 X 0.15 dimension. Then, the photographs of the gels were taken and molecular weights of the proteins were calculated.



**Figure 1.** Tris-tricine SDS gel electrophoretic pattern of the venom extract of *Iurus dufourei* asiaticus Line I, II, III venom extracts, Line IV molecular weight markers, 205 kDa–Myosin, 116 kDa– $\beta$ -Galactosidase, 66 kDa–Albumin, 45 kDa–Ovalbumin, 14.2 kDa– $\alpha$ -Lactalbumin.

## RESULTS

The venom secretion of *Iurus dufourei* asiaticus, is colorless and has a higher viscosity than that of the water. The proteins and peptides of the venom secretion of *I. d. asiaticus* between

6.5 kDa–205 kDa were determined according to Tris-tricine-sodium dodecyl sulfate (TSDS) polyacrylamide gel electrophoresis (Fig.-1, Table 1). A Total of 28 protein fractions or fraction groups were detected. Most of the proteins (17 protein fractions) into the venom secretion were intensively found between 14 kDa – 205 kDa. Moreover, four clear fractions above 205 kDa, and at least four dense fractions having a molecular weights lower than 6.5 kDa were also observed. There were also individual variations in the number of bands between 66 – 205 kDa (Fig. 1).

## DISCUSSION

Determination of biochemical properties of scorpion venom secretion and their mode of actions has been of significant interest in recent years (1, 3, 4, 14, 23, 24). A large number of toxins have been isolated, purified and characterized from various scorpion species (16, 27). The toxic action of the scorpion venom is probably due to a small amount of low molecular weight peptide toxins basic in nature (27).

Although, scorpions are represented by 15 distinct species in Turkey (22), there have been only few records for venoms of the *Mesobuthus gibbosus* and *Androctonus crassicauda*. All of the studies about the venoms of these species are on the characterization and isolation of the neurotoxic peptides and their biological activities (6, 21, 23, 24).

*Iurus dufourei* asiaticus (Iuridae) is distributed in southern Anatolia (7, 8, 9, 12). No data has been found about the minimal lethal dose (MLD<sub>50</sub>) and the biochemical properties of the *I. d. asiaticus* venom.

In the present study, we have determined the electrophoretic protein pattern of *I. d. asiaticus* venom. TSDS-PAGE pattern gave a total 28 bands in the range of 205 – 6.5 kDa and above 205 kDa. Additional four bands are also observed. This dense fraction group is about >6.5 kDa. It has been previously reported that scorpion venoms contain short chain toxins with molecular weights ranging between 3.5 and 7.8 kDa which act by blocking ion-channels (1, 3, 10).

In further studies, at first it is necessary to identify minimal lethal dose (MLD<sub>50</sub>) and total protein content of *I. d. asiaticus*. Furthermore, a detailed biochemical genetic and analysis of the venom components should also be investigated.

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