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Age-Dependent Variations in the Venom Proteins of *Vipera xanthina* (Gray, 1849) (Ophidia: Viperidae)

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SUMMARY: In this study, polyacrylamide disc gel electrophoresis and densitometry analysis methods were used to analyze venom extracts of *Vipera xanthina* specimens of different lengths (35, 47 and 88 cm) collected from the same locality. The electropherograms of the venom protein samples showed age-dependent qualitative and quantitative variations.

Key Words: *Vipera xanthina,* Venom proteins, Polyacrylamide gel electrophoresis, Densitometry

Vipera xanthina (Gray, 1849) (Ophidia: Viperidae) Türünün Venom Proteinlerinde Yaşa Bağlı Varyasyonlar

ÖZET: Mevcut çalışmada aynı lokaliteden (Gümüldür-İzmir) toplanan üç farklı boyda (35 cm, 47 cm and 88 cm) *Vipera xanthina*'nın venom ekstraktları poliakrilamid jel elektroforezi ve densitometri yöntemleri ile analiz edilmiştir. İncelenen venom protein örneklerinin elektroferogramları arasında yaşa bağlı kalitatif ve kantitatif variasyonlar tespit edilmiştir.

Anahtar Sözcükler: *Vipera xanthina*, Zehir proteinleri, Poliakrilamid jel elektroforezi, Densitometri

INTRODUCTION

The Ottoman Viper, *Vipera xanthina* (Gray, 1849), an endemic species in Turkey, is known from Middle, South and West Turkey with vertical distribution to 2000 m (4). Age-dependent variations in biological features of venom extracts have been frequently reported by various researchers (10, 14, 15, 19, 20).

Studies related to snakes in Turkey have tended to deal with the taxonomy and distribution of species (3, 4, 5, 7, 21). Recently, studies were carried out on the pattern of the venom proteins of different colubrid and viperid species inhabiting Turkey (1, 2).

This study examines venom proteins of *Vipera xanthina* specimens collected from the same locality. All venom extracts, obtained from three different specimens having different lengths, were analyzed with polyacrylamide disc gel electrophoresis, and their venom electrophoretic patterns were compared.

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MATERIAL AND METHODS

Specimens used in this study were collected in the same locality (Gümüldür, İzmir 42º14'N - 27º02'E). The specimens were taken to the laboratory alive and total lengths were measured using a dial caliper with accuracy ±0.02 mm. Thereafter, venom was extracted without applying any pressure on the venom glands, as described by Tare et al. (18). As venom extracts also contain dead cells, they were centrifuged for 5 minutes at 600 g and stored in equal amounts (4 µl venom per sample) for each separation until they were analyzed. The venom proteins were separated according to methods given by Davis (8), slightly modified by Özeti and Atatür (17). Accordingly, a pH 6.7 stacking gel was layered above the pH 9 separation gels of 7.5% polyacrylamide, together with pH 8.3 tris-glycine buffer system. Electrophoretic separations were carried out at room temperature (approx. 20 – 25 °C), utilizing a Canalco Model 1200 electrophoresis apparatus. Separation gels were stained with 0.5 % Amido Black (Naphthol Blue Black 10-B), later destained passively with repeated 7 % acetic acid baths. Then, the stained gels were photographed. Qualitative evaluation of the gels was done directly from the electropherograms, and the densitometric curves of the separations were obtained by means of a Gelman ACD-15 Model 39430 densitometer at 500 nm.

RESULTS

The examined venom secretions of *Vipera xanthina* had a higher viscosity than that of the water. Although venom secretions of two specimens (47 and 88 cm total lengths) were light yellow, they were colorless in the 35-cm-long specimen.

The gel photographs of the venom protein samples are given in Fig. 1. According to the electrophoretic analyses, the proteins could be separated into 13 fractions or fraction groups in the two shorter specimens (35 and 47 cm), while 14 fractions were recorded in the longest individual (88 cm). Comparing the electropherograms of the three specimens revealed considerable quantitative differences in terms of the venom protein fractions of the two shorter specimens, but they were similar in the number of fractions and electrophoretic mobilities. The 88 cm individual, however, differed strongly from the other two in the number of fractions, electrophoretic mobilities and densities of the venom proteins (Figs. 1-4).

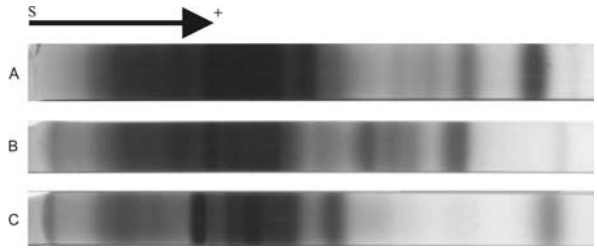

Figure 1. Polyacrylamid gel electrophoresis of venoms of snakes of different lenghts. **A.** 35 cm, **B.** 47 cm, **C.** 88 cm
(**S**: Start, junction between the stacking and separation gels).

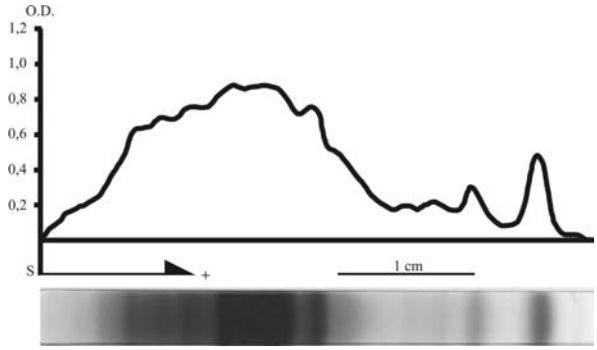

Figure 2. Gel photograph showing the electrophoretic separation of the venom protein sample obtained from the 35 cm length of *V. xanthina* specimen, together with its densitometric tracing curve (**O.D.:** Optical density, **S:** Start, junction between the stacking and separation gels).

DISCUSSION

Various studies on venom proteins of different snakes show that younger individuals have a higher potential than older ones with regard to certain activities such as lethality, coagulation and defibrination (6, 9, 10, 11, 14, 16, 19, 20).

Tun-Pe et al. (20) studied Russell's viper *Daboia russelli siamensis)* venoms using SDS-PAGE electrophoresis in different-sized

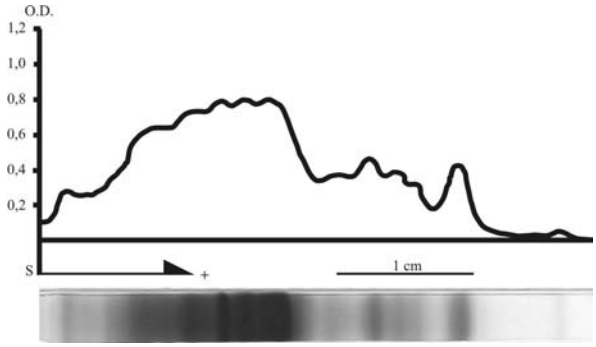

Figure 3. Gel photograph showing the electrophoretic separation of the venom protein sample obtained from the 47 cm length of *V. xanthina* specimen, together with its densitometric tracing curve. For further explanation, see legend to fig. 2.

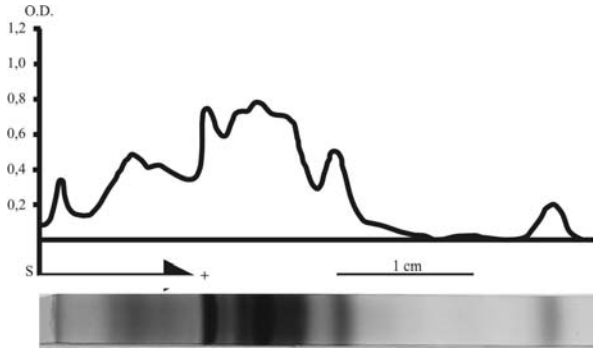

Figure 4. Gel photograph showing the electrophoretic separation of the venom protein sample obtained from the 88 cm length of *V. xanthina* specimen, together with its densitometric tracing curve. For further explanation, see legend to fig. 2.

specimens. The venom extracts of that species are colorless and have a lower activation of L-amino acid oxides. The authors also recorded the maximum activities in the venom of 40-60 cm long snakes and reported that these activities decreased at older snakes depending on length and age. Similar results were found in the early studies on venom proteins of various other snake species (6, 10, 11, 12, 19).

A study on the venom proteins of the Common Lancehead (*Bothrops atrox* Linnaeus, 1758) showed that youngest snakes have fewer protein bands; the number of bands increased as the snakes aged (13), corroborating the results of Tun-Pe et al (20).

Comparing our results on the color of the venom proteins with literature data, *Vipera xanthina* is similar to other viperid species (1, 2, 20).

Among the *Vipera xanthina* specimens we examined, the most aggressive behaviour was observed in the 47-cm-long snake, while the most submissive individual was the 88-cm-long one. This study indicated that the fractions and concentration of the venom proteins are higher in the older than in the youngest specimen. Our results support those of Meier (13) and Tun-Pe et al. (20) on venom proteins of different snake species.

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