

## Electrophoretic Patterns of Some Viper Venoms From Turkey

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**Abstract:** In this study, the venom extract of five viper species (*Vipera xanthina*, *Vipera ammodytes*, *Vipera kaznakovi*, *Vipera wagneri* and *Vipera lebetina*) collected from various regions of Turkey was subjected to polyacrylamide disc electrophoresis. No pressure was applied to the venom glands to provide venom extract. Important differences were detected among the electrophoretic patterns of venom proteins belonging to the five viper species.

**Key Words:** Vipera, snake venom, polyacrylamide disc electrophoresis

### Türkiye'den Bazı Engerek Yılan Zehirlerinin Elektroforetik Şekilleri

**Özet:** Bu çalışmada, Türkiye'nin çeşitli bölgelerinden toplanan 5 viperid türü (*Vipera xanthina*, *Vipera ammodytes*, *Vipera kaznakovi*, *Vipera wagneri* ve *Vipera lebetina*)'nın zehir ekstraktı poliakrilamid disk elektroforez yöntemine uygulanmıştır. Zehir ekstraktı zehir bezlerine hiçbir basınç uygulanmadan elde edilmiştir. Beş viperid türüne ait zehir proteinlerinin elektroforetik şekilleri arasında önemli farklılıklar saptanmıştır.

**Anahtar Sözcükler:** Vipera, Yılan zehiri, Poliakrilamid disk elektroforezi

### Introduction

The initial studies concerning snake venoms were on the secretion of the Duvernoy's gland of colubrid species (Hageman, 1961; Mebs, 1968; Robertson and Delpierre, 1969). Later on, based on technological developments, biological and clinical studies on the venoms of various colubrid species increased (Levinson et al., 1976; Kornalik et al., 1978; Hiestand and Hiestand, 1979; Vest, 1981; Ferlin et al., 1983; Rosenberg et al., 1985). In addition, many pharmacological, biochemical, immunological and toxicologic studies were carried out concerning the venoms of various viper, colubrid and elapid species (Mebs 1968; Kornalik et al., 1978; Boquet and Girons, 1972; Elliot, 1978; Kochva, 1978; Minton and Weinstein, 1987; Edstrom, 1992; Girons and Detrait 1992; Tun-Pe et al., 1995; Faiz et al., 1996; Gasmi et

al., 2001). Young and Miller (1974) compared the electrophoretic patterns of venom belonging to three colubrid species with those of vipers and elapids. Minton and Weinstein (1987), by using polyacrylamide gel electrophoresis, demonstrated the electrophoretic pattern of protein composition in colubrid venom.

Studies related to the snakes of Turkey are mostly taxonomic investigations. According to present references (Başoğlu and Baran, 1980; Baran and Atatür, 1998), most of the poisonous snakes living in Turkey belong to the family *Viperidae* and are represented by nine species. In addition, two poisonous snake species from the family *Colubridae* live in Turkey as well.

In this study, after analysing the venom of five viper species (*V. xanthina*, *V. ammodytes*, *V. wagneri*, *V.*

*lebetina* and *V. kaznakovi*) by means of polyacrylamide gel electrophoresis, the electrophoretic patterns of the venom proteins were compared.

### Materials and Methods

The material of this survey was obtained from different regions of Turkey (*V. xanthina* from Gümüldür, İzmir Vilayet; *V. ammodytes* from Perşembe, Ordu Vilayet; *V. kaznakovi* from Hopa, Rize Vilayet, *V. wagneri* from Aras Valley and *V. lebetina* from Ceylanpınar, Urfa Vilayet) on different dates. Specimens were brought to the laboratory alive and their venom was extracted according to Tare et al. (1986) without applying any external pressure to the venom glands. Since the venom extract includes dead cell pieces, it was centrifuged for 5 min in 600 g and stored at  $-20^{\circ}\text{C}$  until the electrophoretic separation was carried out.

Venom proteins were separated according to the polyacrylamide gel disc electrophoresis method of Davis (1964), slightly modified by Arıkan (1983). Tris-glycine buffer solution of pH 8.3 and 7.5% of separation gel at pH 9 were used at the bottom and 2.5% of stacking gel of pH 6.7 at the top. The electrophoretic separations were carried out at room temperature (approximately  $20-25^{\circ}\text{C}$ ) by using a Canalco Model 1200 electrophoresis apparatus. The separation gel was stained with 0.5% Amido Black (Naphthol Blue Black 10-B), and then the extra stain was removed from the gel by the help of 7% acetic acid baths passively; afterwards pictures of the gel were taken. The qualitative evaluation of the separations was performed on densitometric tracing curves obtained from a Gelman ACD-15 model 39,430 densitometer scanning at 500 nm. For analysis, 5  $\mu\text{l}$  of venom extract was used for each separation.

### Results and Conclusion

The venom extract of the five viper species examined is a light yellowish liquid. The vipers are solenoglyph snakes (fangs resemble a closed groove in shape) similar to crotalids. When the venom apparatus is examined, a large venom gland is present on each side and the lower part of the eyes. The front part of the venom gland is extended like a venom channel and opened to the base part of the fangs. When the snake bites, the muscles surrounding the venom glands contract and transmit the venom to the fangs by the channel.

All the specimens used in this study were sexually mature. The gel photograph of the venom proteins of five vipers (*V. xanthina*, *V. ammodytes*, *V. kaznakovi*, *V. wagneri* and *V. lebetina*) is given in Figure 1, and gel photographs showing the electrophoretic separations of each species, together with their densitometric tracing curves are given in Figures 2-6. As can be seen in Figure 1, the venom proteins of the five viper species can be separated into 10-12 fraction or fraction groups. Significant differences were established among the species in terms of the fraction number, speed and density of venom proteins. Among the five vipers, the total protein fraction number was highest in *V. lebetina*, *V. ammodytes* and *V. xanthina*. The protein fraction number was 12 in these vipers while it was 11 in *V. kaznakovi* and 10 in *V. wagneri*.



Figure 1. Venom protein electropherograms of five viper species A: *Vipera ammodytes* B: *Vipera xanthina* C: *Vipera kaznakovi* D: *Vipera wagneri* E: *Vipera lebetina*; S: Start (Junction between spacer and separation gels).

Minton and Weinstein (1987) stated that the colubrids' venom contains 7-10 protein fractions and the total number of proteins extracted by electrophoresis is lower than that of the viperids. However, the Duvernoy's secretion of the colubrid snakes was found to be as

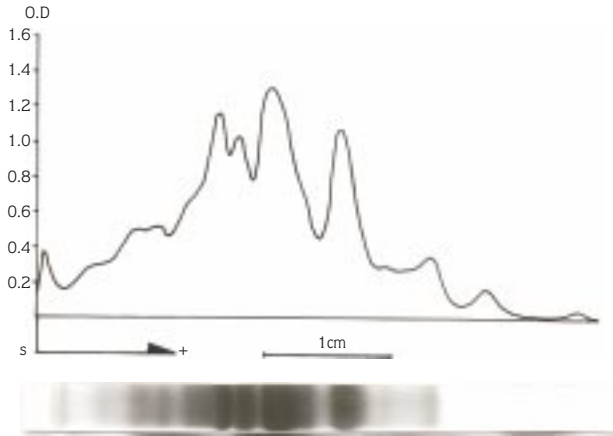


Figure 2. Gel photograph showing the electrophoretic separation of the specimen of *Vipera ammodytes*, together with its densitometric tracing curve. O.D.: Optical Density, S: Start (Junction between spacer and separation gels).

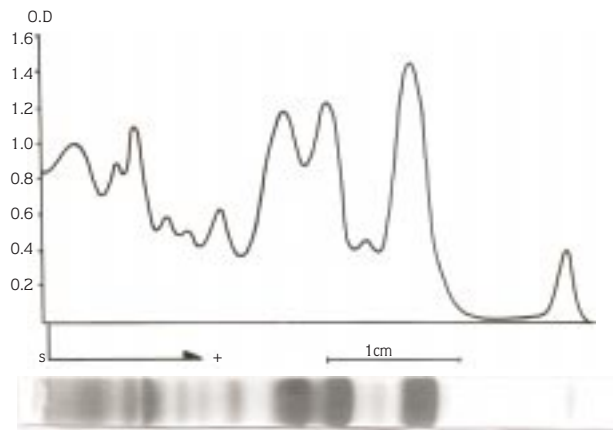


Figure 3. Gel photograph showing the electrophoretic separation of the specimen of *Vipera xanthina*, together with its densitometric tracing curve (For abbreviations, see caption of Fig. 2).

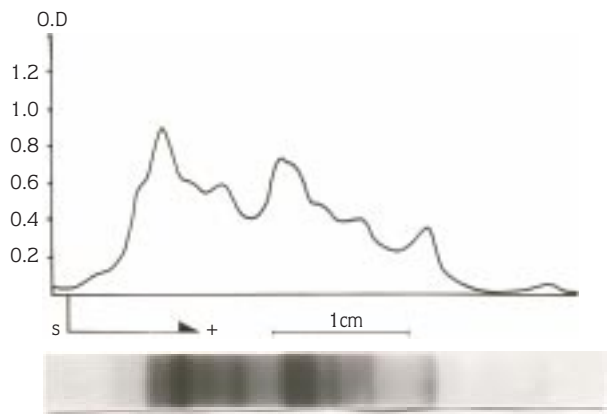


Figure 4. Gel photograph showing the electrophoretic separation of the specimen of *Vipera kaznakovi*, together with its densitometric tracing curve (For abbreviations, see caption of Fig. 2).

complex as the venom of proteroglyph snakes. The total number of protein fractions is higher in vipers than in colubrids. Accordingly, it can be concluded that the venom of vipers is more complex than that of colubrids.

According to various references (Elliot, 1978; Edstrom, 1992), all snake venom is rather complex and consists of a number of components. Venom contains a number of proteins, important for the digestion of food and inorganic ions such as calcium, magnesium, cobalt, nickel, zinc and manganese. In addition, a total of 25 different enzymes variable in number in different species have been described.

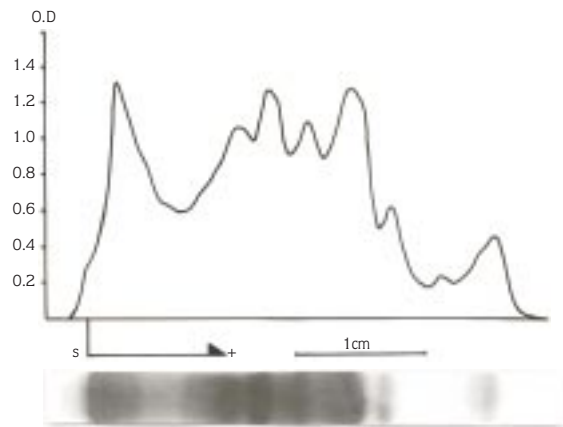


Figure 5. Gel photograph showing the electrophoretic separation of the specimen of *Vipera vagneri*, together with its densitometric tracing curve (For abbreviations, see caption of Fig. 2).

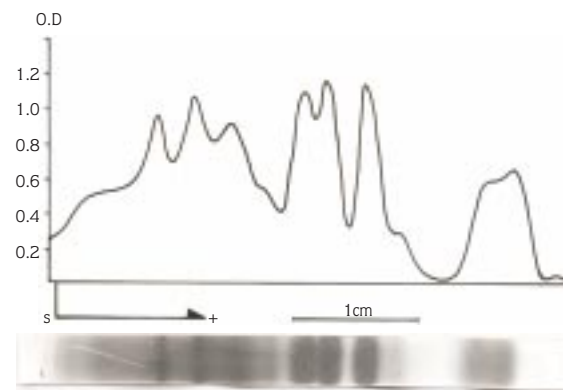


Figure 6. Gel photograph showing the electrophoretic separation of the specimen of *Vipera lebetina*, together with its densitometric tracing curve (For abbreviations, see caption of Fig. 2).

It was determined that the venom of vipers is rich in terms of proteolytic enzymes which facilitate the fragmentation of tissue proteins and, furthermore, that

venom includes trombhin-like components that can affect the blood coagulation mechanism of prey (Edstrom, 1992; Huang and Perez, 1980).

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