Advanced Studies in Biology, Vol. 12, 2020, no. 1, 29 – 36
HIKARI Ltd, www.m-hikari.com
https://doi.org/10.12988/asb.2020.91224

Study of the Effect of Some Physical and Chemical Factors on the Biological Properties and Chemıcal Composition of Viper Venom

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Abstract

In this article, the microelement composition of the venom of Macrovipera lebetina obtusa viper inhabiting different ecosystems of Azerbaijan, the effect of electromagnetic radiation on the spectral properties and molecular structure of viper venom, as well as the absorption spectra of standard zootoxins exposed to γ - radiation at 1.35, 2.7, 4.05 və 5.4 кGy doses and protein content of snake venom were studied. It was found that the chemical composition and toxic properties of viper venom depend to some extent on the characteristics of the ecosystem and the physicochemical factors of the environment. The obtained results can be used in radiation sterilization of snake venom and preparations based on it, and in the preparation of drugs in the pharmaceutical industry.

Keywords: Macrovipera lebetina obtusa, venom, abiotic factors, side effects

Introduction

Abiotic stress factors such as high and low temperatures, different salts, high light intensity, radioactivity, and γ-radiation cause various changes in the course of biological processes in living organisms [1, 2, 3]. There are changes in the amount, composition, toxicity, as well as physicochemical properties of the venom synthesized in the venom glands of snakes distributed in different ecosystems [4, 5].

Despite some results in the diagnosis and treatment of snake venom poisoning [6, 7, 8, 9], the general principles of the influence of environmental factors on the quantitative and qualitative composition, pharmacokinetics, metabolism [4, 5], chemical composition, physicochemical properties and toxicity of snake venom [10, 11, 12, 13] have not yet been clearly defined. It has not yet been determined how heavy and radioactive metals that contaminate snake habitats can affect viper venom. Therefore, the study of the effect of electromagnetic and ionizing radiation on the properties of viper venom can be considered an actual problem. Although Azerbaijan is a small country (the territory of the republic is 86,000 km^2), it is home to a large number of snakes (about 42 species), the reason is the most likely due to the country's climate, topography, and landscape. From this point of view, in the past, Azerbaijan was one of the main producers of Transcaucasian viper venom on the world market [5].

Today's reality requires that the venom from the Transcaucasian viper, which is widespread in the fauna of Azerbaijan, be competitive in the world market. All this requires a thorough study of the venom of the Transcaucasian viper.

Because of the above, the purpose of this article is to study the venom of the Transcaucasian viper, the study of the effect of some abiotic stress factors on the biophysical and toxicological properties of the venom.

Material and methods

It has been used as the object of the study the native venom of Transcaucasian viper (*Macrovipera lebetina obtusa*) dried in $CaCl_2$ vapor in the eccicator and samples of venom treated with γ-rays in different doses.

The chemical composition of the venom was determined by atomic absorption spectrophotometry (AAS) (AAS-300, Perkin-Elmer, USA, 2000) [14]. Statistical analysis of the results obtained by atomic absorption spectrophotometry was carried out based on the software of this device. The microelement composition of soil and plant samples was studied not only in the venom but also in the areas inhabited by snakes. It has been determined of trace elements such as Cd, Cr, Ni, Zn, Ca, Pb, Mn, Fe, Cu, Mg, etc. in the studied samples.

The venom quality indicators are studied by electron-paramagnetic resonance (EPR) [15], infrared (IR) (Specord-71 IR) and ultraviolet (UV) spectroscopy (Specord UV-VIS) [16], laser spectroscopy [17] methods. The changes in the molecular mobility of the venom after treatment with γ-rays were performed by radiothermoluminescence, dispersion analysis methods [18]. The radioecological condition of the areas (radiation background) was determined by a dosimeter-radiometer (SRP-88, Russia), and the total amount of proteins was determined by the Loury method [19].

A static analysis of the obtained results was performed according to Lakin [20].

Results and discussion

The results on the quantitative and qualitative composition of chemical elements taken from vipers hunted around the cities of Gobustan, Sabirabad, Kurdamir, Agsu, Baku and Sumgayit in Azerbaijan and in soil and plant samples taken from their settlements are given in the table.

Table.

The results on the amount of the heavy metals in samples taken from different parts of Azerbaijan

Nümunələr	Concentration of heavy metals, mg/kg				
	Cr	Pb	Cd	Ni	Zn
Gobustan district (Childag village)					
Plant	131.0 ± 1.30	23.0 ± 4.48	2.1 ± 0.06	40.0 ± 0.16	200.4 ± 0.09
Soil	89.9 ± 0.44	5.5 ± 0.08	0.7 ± 0.001	35.2 ± 0.54	52.7 ± 0.05
Venom	-	13.4 ± 0.03	1.9 ± 0.20	-	266.9 ± 0.03
Shamakhi region (Maraza village)					
Plant	130.0 ± 1.20	20.9 ± 3.48	2.0 ± 0.04	39.4 ± 0.50	200.3 ± 0.09
Soil	80.2 ± 0.36	4.9 ± 0.03	0.5 ± 0.03	35.5 ± 0.68	52.20 ± 0.07
Venom	-	13.4 ± 0.03	1.6 ± 0.18	-	26.37 ± 0.03
Sabirabad district (Shikhsalahli village)					
Plant	87.0 ± 0.99	4.9 ± 0.49	0.5 ± 0.59	10.7 ± 0.13	66.05 ± 0.44
Soil	90.6 ± 0.67	10.0 ± 0.57	0.5 ± 0.13	43.9 ± 0.23	67.09 ± 0.34
Venom	-	5.0 ± 0.28	-	-	260.9 ± 0.13
Agsu district (Garagoyunlu village)					
Plant	153.0 ± 1.32	8.5 ± 4.69	5.8 ± 0.06	33.7 ± 0.17	69.02±0.05
Soil	56.6 ± 0.46	9.5 ± 0.73	1.8 ± 0.004	28.0 ± 0.66	71.08±0.02
Venom	103.1 ± 2.79	2.1 ± 6.56	2.4 ± 0.99	-	250.0±3.06
Around Baku city (Buzovna settlement)					
Plant	90.8 ± 3.08	8.2 ± 0.15	0.42 ± 0.01	45.8 ± 0.36	100.7 ± 2.14
Soil	58.3 ± 0.60	52.1 ± 2.09	1.05 ± 0.04	20.1 ± 0.76	142.2 ± 6.42
Venom	-	18.5 ± 1.76	-	-	300.9 ± 4.23
Around Sumgayit city					
Plant	29.0 ± 7.71	26.9 ± 0.23	1.2 ± 0.04	19.0 ± 0.34	214.0 ± 0.03
Soil	29.0 ± 1.89	26.8 ± 0.05	1.1 ± 0.06	23.0 ± 0.15	65.0 ± 0.41
Venom	-	19.0 ± 1.32	-	-	377.6 ± 8.40

As shown the table, the microelement content of the venom obtained from *Macrovipera lebetina obtusa* viper, which inhabits different ecosystems of Azerbaijan, varies in a wide range (Cr – 87.0 - 103.1; Pb – 7.0 - 19.0; Cd – 1.6 –

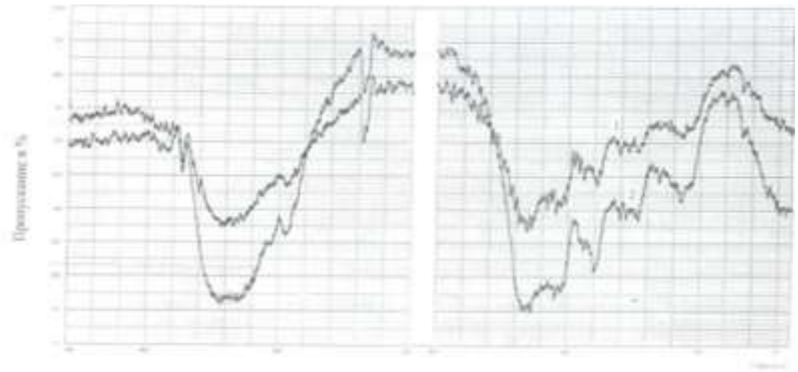
2.4; Zn – 300.9 - 377.6 mg/kg). It was determined that the concentration of Zn and Pb ions in the venom of snakes caught from nature is ~ 2 times higher than in the control samples taken from the Herpetological Combine of Baku.

Interestingly, despite the presence of Ni and Cr ions in the soil and plant samples of the study areas, these elements were not found in the venom samples.

Another interesting result is that although the highly toxic Cd element was present in venom samples taken from Baku, Sumgayit, and Sabirabad districts, the amount of this element in venom samples taken from vipers in other areas was quite small.

Based on our results, we can say that the quantitative diversity of heavy metal ions in snake venom taken from different parts of the country is due to the soil and plants, relief, and the level of biotic factors affecting the ecosystem of these areas.

To determine the effect of electromagnetic radiation on the spectral properties of the viper venom and structural changes in the molecule, the absorption spectrum of snake venom was studied at low – 1000 -7000 Wt / m^2 and high – 14000 - 20000 Wt / m^2 radiation levels and the results are given in Figures 1 and 2.



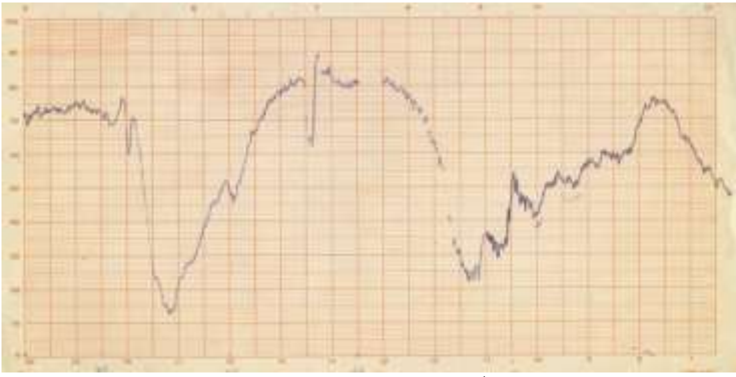
Wavenumber (in cm^{-1})

Fig. 1. IR – spectrum of venom samples of standard (1) and under the influence of low-intensity EMR (2).

Comparison of IR and UV spectra of snake venom with the results obtained on the orientation of N–H-groups in venom proteins suggests the following: 1) no structural changes occur in snake venom samples exposed to ER at low intensities; 2) in the venom samples exposed to EMR at high intensities, the changes related to structural changes in the protein molecule of zootoxin are observed (Fig. 1,2).

We believe that the obtained data can be used to identify viper venom and venom-based drugs. In this case, the structural analysis of the individual groups of venom allows to identify the venom and determine the presence of characteristic functional groups in the composition of venom-protein biomacromolecules, in the visible and UV regions, all samples of viper venom have characteristic absorption spectra, and the absorption maxima of snake venom proteins vary in the wavelength range of 220 to 800 nm.

As can be seen from the figure, the venom samples exposed to standard and low-intensity EMR have characteristic absorption maxima at wavelengths of 260 and 280 nm. In viper venom samples exposed to high-intensity EMR equal to 10000, 14000, 16000, 20000 W/m^2, the absorption bands of proteins change slightly and create maximum absorption at wavelengths of 262 and 285 nm (Fig. 1.2).



Wavenumber (in cm^{-1})

Fig. 2. IR – spectrum of venom samples under the influence of high-intensity EMR (2).

We believe that the change in the electronic state of the molecule is due to their absorption of light in the near IR, visible, and UV regions. Certain atomic groups are responsible for these absorptions, and the absorption spectra corresponding to these groups behave as structures with multiple bonds. In this case, the electrons that carry out the chemical bond are not localized but are common to the entire structure of snake venom. It should be noted that the obtained data from the study of the spectral properties of venom samples standard and treated with both low and high-intensity EMR can be used not only to determine the quality of zootoxins but also as a criterion for its environmental evaluation as a raw material.

The results obtained concerning the total amount of protein in the venom samples exposed to EMR showed that the amount of protein in the studied venom samples varied in the range of 96.5 ± 0.48 – 95.0 ± 1.90 mg/g, and in all cases the increase in radiation intensity indicates a slight decrease of the total amount of the protein (no results are given). In this case, the increase in the duration of radiation exposure in the case of low-intensity EMR (0.5, 1, and 2 hours) also reduces the total amount of proteins from 96.6 mg/g (control) to 95.3 mg/g. The results show that low-intensity EMR did not significantly change the amount of snake venom proteins. In contrast, high-intensity EMR can produce significant changes in the total amount of venom protein over 0.5, 1, and 2 hours compared to low-intensity EMR exposure samples. In this case, the amount of protein in the venom decreases from 96.6 mg/g to 90.0 mg/g, depending on the duration of exposure to

EMR. Given all this, we can say that high-intensity EMR, as an environmental factor, can significantly affect the chemical structure of the venom.

Also, the absorption spectra of standard zootoxin exposed to doses of 1.35, 2.7, 4.05 and 5.4 kGy of γ-rays were studied and the absorption spectra of various venom samples in the IR, visible and UV regions were obtained. It was found that at low doses of γ-rays (1.35 and 2.7 kGy) there are no significant differences in the structure of the standard venom, but at higher doses (4.05 and 5.4 kGy) there are small structural changes in the IR spectrum of irradiated venom samples. We believe that this fact can be used in radiation sterilization of snake venom and snake venom-based preparations.

An increase in the radiation dose from 2.7 kGy to 4.05 kGy, and then to 5.4 kGy leads to a decrease in the intensity of absorption of the venom during exposure to 0.5 hours, which results in a decrease of the toxicity and pharmacological activity of the venom. It is believed that γ-radiation at intensities higher than 14000 W/m^2 and at doses higher than 1.35 kGy are factors that reduce the toxicity of snake venom, and these facts can be used in the preparation of snake venom-based preparations in the pharmaceutical industry.

The obtained results can be used in the identification of zootoxins and their metabolic products. These results can also be a theoretical basis for the development of effective methods for the diagnosis of zootoxin poisoning.

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Received: July 4, 2020; Published: July 24, 2020