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Research Article

Comparative Data on the Biochemical and Physicochemical Characteristics of the Venom of Viper (Macrovipera Lebetina Obtusa Linnaeus, 1758) with different Shelf Life

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Abstract

The article presents data from experimental studies to identify the biochemical and physicochemical characteristics of the venom of the viper (Macrovipera lebetina obtusa Linnaeus, 1758) with different shelf life. The purpose of the presented work is to study the biochemical and physicochemical parameters of viper venom (Macrovipera lebetina obtusa Linnaeus, 1758) with different shelf life. To achieve this goal, we conducted experimental studies with samples of viper venom collected over the period of time 1989, 1991, 1993, 2010 and 2015. The activity of enzymes in samples of venom from the Transcaucasian viper was determined by the titrometric method. The resistance was measured with an E6-13A teraohmmeter. The photosensitivity of snake venom was studied based on photoconductivity measurements in the wavelength region of 0.2-2 μm. It has been established that viper venom crystals are not photosensitive in the wavelength range of 0.2-2 μm, but viper venom has photoconductivity in the wavelength range 2-4 μm. It has been experimentally established that in samples of viper venom collected in 2015, the content of proteolytic activity is 2.73, 2.0, 1.46, 1.71 times higher than in venom samples collected in 1989, 1991, 1993, 2010, respectively. The level of L-amino acid oxidase activity in venom samples collected in 2015 was detected, the level of which corresponded to 0.30 IU/mg. In samples of viper venom collected in 2015, the content of L-amino acid oxidase is 3.33, 2.73, 2.0, 1.15 times higher than in samples collected in 1989, 1991, 1993, 2010, respectively.

Keywords: Comparative Data; Biochemical; Physicochemical; Venom; Viper

Introduction

An important component of the poison, responsible for its toxic properties, are bioactive protein components and enzymes. Small doses of poison do not cause any clinical manifestations of poisoning and have long been used in the treatment of many serious diseases [1-8].

Freshly extracted snake venom is a slightly opalescent, viscous, clear liquid ranging from light to lemon yellow in color. The study of the physicochemical properties of solutions of venom from Central Asian snakes: surface tension coefficient, viscosity coefficient and density was carried out in the temperature range 298-318 K using densitometric and viscometric methods of physicochemical

analysis. When studying isotherms in the temperature range, deviations of these parameters were noted on the viscosity and density isotherms. The observed effect is associated with specific intermolecular interactions in them [9].

It should be noted that a wide range of peptides and proteins with different biological functions makes snake venoms a valuable source of new biologically active compounds, both for use in basic research and for the development of new drugs. The development and improvement of biochemical and physicochemical methods for identifying and standardizing snake venom and their components will provide the pharmaceutical industry with high-quality and environmentally friendly, natural products with specified pharmacological activity.

The purpose of the presented work is a biochemical and physicochemical analysis of the venom of vipers (Macrovipera lebetina obtusa Linnaeus, 1758) with different shelf life. We conducted experimental studies to determine the biochemical and physicochemical characteristics of viper venom collected over the period of time 1989, 1991, 1993, 2010 and 2015.

The research material was the whole venom of the viper, dried in a desiccator over calcium chloride vapor and with different shelf life. The activity of enzymes in samples of venom from the Transcaucasian viper was determined by the titrometric method. The activity of enzymes in samples of venom from the Transcaucasian viper was determined by the titrometric method. Statistical processing of experimental data was carried out using Student's test. Statistical processing of experimental data was carried out using Student's test.

To study the electrical parameters and photoconductivity of viper venom samples, the temperature dependence of the resistivity ρ for crystalline viper venom samples was determined. The resistance was measured with an E6-13A teraohmmeter. Determination of the chemical composition of snake venom was carried out by atomic absorption spectrophotometry using an AAS-300 device, Perkin-Elmer (USA).

Research Results and Discussion

In each venom sample, enzyme activity was determined using standard methods: proteolytic activity (PA) was determined by hydrolysis of sodium caseinate (22); -L-amino acid (AO) oxidase activity - using L-phenylalanyl as a substrate (23). The sex and age of the snakes were not taken into account when analyzing the biochemical data. Possible seasonal changes in proteolytic activity were not taken into account.

Research results In each individual sample, standard methods for determining enzyme activity were used: proteolytic activity (PA) was determined by hydrolysis of sodium caseinate [10]; -L-amino acid (AO) oxidase activity - using L-phenylalanyl as a substrate [11]. The sex and age of the snakes were not taken into account when analyzing the biochemical data. Possible seasonal changes in proteolytic activity were not taken into account.

In the venom of viper collected in 2015, the level of L-amino acid oxidase activity is 0.30 IU/mg. In samples of viper venom collected in 2015, the content of AO is higher by 3.33, 2.73, 2.0, 1.15 times than in samples collected in 1989, 1991, 1993 and 2010, respectively.

It was revealed that in the venom of viper collected in 2015, the PA content is higher by 2.73, 2.0, 1.46, 1.71 times than in venom samples collected in 1989, 1991, 1993, 2010, respectively.

When studying the electrical parameters and photoconductivity of samples of viper venom, the temperature dependence of the resistivity ρ for crystalline samples of the venom was determined. The snake venom sample was heated in a measuring cell at a constant rate of 9.1°C/sec. The resistance was measured with an E6-13A teraohmmeter. The photosensitivity of snake venom was studied based on photoconductivity measurements in the wavelength range 0.2 - 2 μm.

İn this case, a crystal of poison was glued to a metal substrate with silver paste. A second electrode was glued to the other surface of the metal substrate with silver paste. Thus, a ("sandwich") structure was created for further study of the electrophysical parameters of the viper's venom.

The snake venom sample was heated in a measuring cell at a constant rate of 9.1°C/sec. Next, the sample was heated to 1700C and the change in resistivity was observed, followed by its cooling and the process was repeated again. The heating process was repeated three times. The sample was heated again.

Figure 1 shows the dependence curves of the resistivity ρ on the heating temperature of the sample: ρ = f (T).

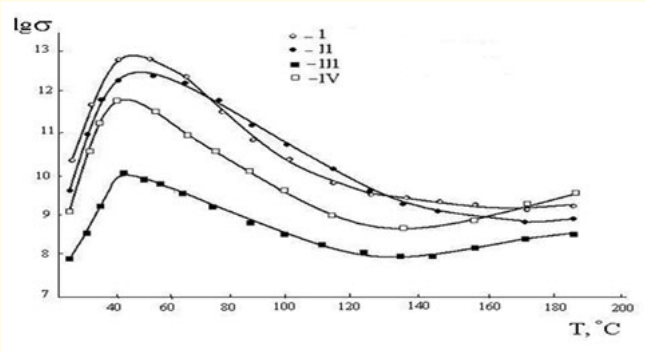

Figure 1: Curve of resistivity ρ versus temperature.

It can be seen from the figure that the resistivity increased each time. The experiments were repeated every other day. At the same time, the resistivity decreased. It was noted that the peaks on the resistivity curve shifted. When heated to a temperature of 1700C with subsequent reheating of the poison samples, a slight change in the resistivity of the viper venom samples was observed.

We assume that after each subsequent heating, structural changes occur in the poison samples, which, in turn, causes a change in both the pharmacological activity and toxicity of zootoxin enzymes.

ρ = ρoeb/T (5.1).

s = soe-b/T (5.2).

With subsequent heating of the snake venom with a 24-hour interval, which corresponds to curve 4, which resembles curve 1, the reverse process is most likely observed, that is, the destroyed structures are restored, which indicates the thermal stability of the venom. Based on the obtained resistivity values, we can say that snake venom crystals behave like semiconductors at temperatures up to 50°C. For semiconductors, the nature of the temperature dependence of resistivity and conductivity for a certain temperature range is determined by dependencies of the form:

ρ = ρoeb/T (5.1).

s = soe-b/T (5.2).

Where, ρo, so, b are some constants for a given temperature range, characteristic of a given crystal. Based on the results of the studies, it can be seen that under the influence of heat there is a change in the resistivity of snake venom crystals.

When heating of the venom is stopped, enzymatic activity is restored, as well as the physicochemical properties of the snake venom. Thus, as a result of the research, the electrophysical properties of viper venom were revealed. It has been established that the venom of the viper does not have photoconductivity. It has been established that viper venom crystals are not photosensitive in the wavelength range of 0.2-2 μm, but viper venom has photoconductivity in the wavelength range 2-4 μm.

District (Garagoyunlu village)					
Samples	**Concentration of metal ions, mg/kg (M±m)**				
	Cr	**Pb**	**Cd**	**Ni**	**Zn**
A plant	153.0 ± 1.316	8.5 ± 4.695	5.8 ± 0.063	33.7 ± 0.1685	69.02±0.050
Soil	56.6 ± 0.459	9.5 ± 0.073	1.8 ± 0.004	28.0 ± 0.658	71.08±0.020
Venom	103.1 ± 2.793	8.13 ± 6.560	2.42 ± 0.985		250.0±3.063

Table 1: Data on the content of metal ions in the studied poison samples, taken from the territory of Azerbaijan.

From the data presented in the table it can be seen that the content of metal ions was found in the venom of the viper: Cr, Pb, Cd and Zn. All poison samples are characterized by a higher content of lead and zinc ions. Another interesting fact is that the Ni present in the soil and plant samples is almost not present in the poison samples.

It should be noted that in samples of the standard viper venom taken from the Herpetological Plant of the city of Baku, we also identified trace concentrations of metal ions Cr and Ni, which were not present in many experimental samples of the poison. At the same time, in standard samples of poison, the concentrations of Pb and Zn were 1.92 ± 0.01, 0.23 ± 0.001 and 180.0 ± 0.05 mg/kg, respectively.

Thus, the main metal ions present in the venom of viper are the metal ions Cr, Pb, Cd and Zn, the concentrations of which correlate with their content in soils and are in the range: Cr - 103.1 ± 2.793, Pb - 8.13 ± 6.560, Cd - 2.42 ± 0.985 Zn - 250.0 ± 3 mg/kg. The study of the microelement composition of viper venom showed great variability in its elemental composition.

From the above it follows that the average values of the enzymatic activity of the venom collected in 1989 turned out to be significantly lower than the enzyme activity in the samples of viper venom collected in 2015.

It is known that the color of the poisonous secretion is determined by the presence in it of the coenzyme oxidase -L-amino acids - flavin adenine dinucleotide. Accordingly, in colorless samples of the venom, AO is close to zero. It is logical to assume that the activity of proteases in venom may depend on both diet, conditions and storage period of venom samples.

As a result of the research, the electrophysical properties of viper venom were revealed. It has been established that the venom of the viper does not have photoconductivity. The data we obtained can be used when storing preparations based on snake venom.

It must be noted that even well-known biologically active components of snakes can have unexpected or undesirable effects. This issue requires careful study on a case-by-case basis before a compound is considered as a drug source. Improving methods for assessing the authenticity of snake venoms in medicinal products continues to be an urgent task in the development of regulatory documentation for medicinal products and in the identification of venoms with different shelf life.

In conclusion, it must be stated that the results obtained were recommended and taken into account when storing, identifying and standardizing samples of viper venom.

Conclusions

- It has been established that in samples of viper venom collected in 2015, the PA content is higher by 2.73, 2.0, 1.46, 1.71 times than in venom samples collected in 1989, 1991, 1993, 2010, respectively.
- The level of activity of L-amino acid oxidase was experimentally determined. In venom samples collected in 2015, the level of L-amino acid oxidase activity is 0.30 IU/mg. In samples of viper venom collected in 2015, the content of AO is higher by 3.33, 2.73, 2.0, 1.15 times than in samples collected in 1989, 1991, 1993, 2010, respectively.
- It was revealed that under the influence of external factors, that is, temperature, there is a change in the electrophysical parameters of snake venom.
- It was revealed that the main metal ions present in the venom of the viper are the metal ions Cr, Pb, Cd and Zn, the concentrations of which correlate with their content in soils and correspond to: Cr - 103.1 ± 2.793, Pb - 8.13 ± 6.560, Cd - 2.42 ± 0.985, Zn - 250.0 ± 3 mg/kg.

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