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# The Study of the Physical Properties of Macrovipera Lebetina Obtusa Venom

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**ABSTRACT**

Photoconductivity of crystals of snake venom was measured depending on wave length. At the result it was established that crystals of Macrovipera Lebetina Obtusa venom doesn't have photoconductivity.
Thus, electrophysical properties of snake venom were experimentally revealed. It was established that at heating up to the temperature of $120^0$C, crystals of Macrovipera Lebetina Obtusa venom have maximum resistance, and after cooling its property restores. The obtained data can be applied at storage of preparations on the basis of snake venom.

The effect of gamma radiation on the spectral characteristics of the venom of Macrovipera lebetina obtusa was studied. Thus, the impact of venom γ--radiation doses in 1.35 kGy stabilizes both toxicity and pharmacological activity, respectively while increasing the shelf life of snake venom.

**KEYWORDS**: snake, venom, Macrovipera lebetina obtusa, photoconductivity, γ--radiation.

## 1. INTRODUCTION

Research of toxins of various kinds of snakes, and also huge attention to zootoxins is defined not only by inquiries of medical practice, but their use and studying represents the big interest for various branches of biology, physiology, bioorganic chemistry, biophysics, toxicology and other areas of sciences. These problems still are not perfect, demand deep theoretical and practical analyses and are still actual.

In references physical and chemical properties of solutions of venom of the Central Asian snakes have been studied . Factor of a superficial tension, viscosity and density factor was is spent in the range of temperatures 298-318K with use densitometries and viscosimetrics methods of the physical and chemical analysis. At the analysis at the temperature range of 303-308K deviations of these parameters on viscosity and density isotherms were noted. [6]. Some chemical agents, such as permanganate kalium, chloroform, ethanol, methyl dark blue operates as destroying on venoms. Snake venoms deactivate under the influence of physical factors, for example, at influence of an ultra-violet irradiation and X-rays [8]. On literary data, snake venom in the dried up kind keeps pharmacological and toxic properties for 22 years and even more. Various biological, ecological factors, and also chemicals agents and some physical factors have considerable influence on toxicity of venom [9]. Small doses of venom do not cause to any clinical displays of a venom and long ago were used at treatment of many heavy illnesses [5]. Influence of ecological factors on a chemical compound of venom Macrovipera lebetina obtusa was revealed [11, 13]. Researches on revealing of influence of γ -radiation on toxicity and on pharmacological properties of venom of Macrovipera lebetina obtusa were carried out [2,3].

Thus, the considerable number of works have been devoted to all-round studying of venom of venomous snakes. The picture of toxic and pharmacological action of snake venoms on an organism was in detail tracked. Inconsistent and many-sided characteristics of snake venoms were resulted. Influence of snake venom on various functional systems of an organism has been revealed. Toxicity of snake venom were studied. The comparative pharmacological and biochemical characteristic of venom of snakes was given.There are literary data on research of the snake venom, however many questions still remain not mentioned and demand the deep analysis and studying [12].

In the literature data on research of the snake venom were cited [1,4, 7,10], however many questions still remain not mentioned and demand the deep analysis and studying. Influence of temperature on electrophysical parametres of venom and photoconductivity of zootoxins was not studied. Proceeding from the above-stated, research of effect of gamma radiation on the spectral characteristics and electrophysical parametres of snake venom with a research objective of a photo-and thermal stability of crystals of snake venom is very actual.

## 2.The purpose of research.

The purpose of research is to study the influence of temperature on electrophysical parameters of venom and photoconductivity of zootoxins and to explore the effect of gamma radiation on the spectral characteristics of snake venom.

## 3.Material and research metod

Material of researches was venom of snake Macrovipera lebetina obtusa. In order to study electrophysical parametres and photoconductivity of snake venom, researches in temperature dependence of specific resistance of Macrovipera lebetina obtusa venom's crystal were carried out. Thus investigated crystal of snake venom was pasted on a metal substrate

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by silver paste. On other surface of a metal substrate silver paste the second electrode was pasted. Thus, the structure for the further research of electrophysical parametres of venom Caucasian Macrovipera lebetina obtusa was defined. Heating of the sample of snake venom has been spent in a measuring cell with constant speed of 2K/minute resistance. Measurements were provided by the thearometer E6-13A.

In comparative aspect, γ- radiation action on spectral characteristics of venom of Macrovpera lebetina obtusa was studied. Irradiation of vipera venom with small doses of γ- radiation (γ-radiation Д=0.75Gry/sec) was spent on K-25 isotope installation with application of $^{60}$Co. The sample was heated up and change of specific resistance was observed, further it was cooled and again process repeated. Heating of the sample was spent repeatedly (heating process repeated three times). In drawing curve, dependences of specific resistance ρ on temperature of heating of the sample ρ~f(T) are shown. Apparently from drawing and from the given tables, each time specific resistance increased. Experiments were repeated in a day. Thus as showed, in the received experimental data, specific resistance decreased (fig.1).

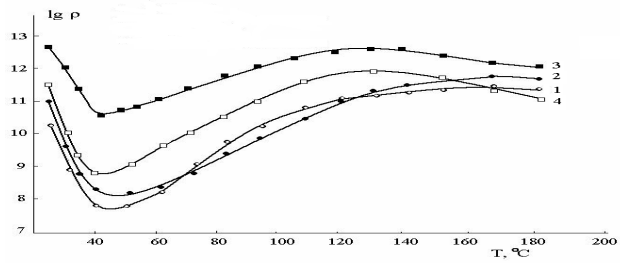


Fig.1 temperature dependences of snake venom specific resistance of crystals lgρ at various time of day

There was also a moving of maxima on a curve of specific resistance. At heating to temperature 170°C, with the subsequent repeated heating of samples of venom, minor alteration of specific resistance of zootoxin was marked. We assume, that after each heating in the sample there are structural changes and, that is in turn possibly causes to change of both pharmacological activity, and toxicity of snake venom. However, at the subsequent heating of Macrovipera lebetina obtusa venom with 24 hour interval corresponding to a curve 4 which reminds a curve 1, return process is most likely marked, it means that the destroyed structures are restored, which testifies to thermostability of snake venom. It is necessary to notice, that at the termination of heating of poison restoration of fermentative activity, and also physical and chemical properties of snake poison was marked.

Reduction of specific resistance at temperatures to 50$^0$C shows, that crystals of snake venom in this range of temperature behave as semiconductors. At semiconductors character of temperature dependence of specific resistance and conductivity for some interval of temperatures are defined by dependences:

$$\rho = \rho_o e^{\beta/T}$$
$$\sigma = \sigma_o e^{-\beta/T}$$

$\rho_o$, $\sigma_o$, β - constants for the given interval of temperatures characterizing the given crystal.

Proceeding from results of the spent researches we assume, that under the influence of external factors (temperature) change of electrophysical parameters of venom was marked.

Measurements of photoconductivity of Macrovipera lebetina obtusa venom were carried out. Typical spectral dependence of photoconductivity of the received structures at temperature 300K was investigated. During illuminating of crystals of venom, different values of the forward and reverse voltage photosensitivity was not observed.

Conductivity of snake venom crystals depending on temperature of heating of crystals, (fig. 2) was investigated. Curve dependences of conductivity σ on temperature of heating of the sample σ~f(T) on time were drove.

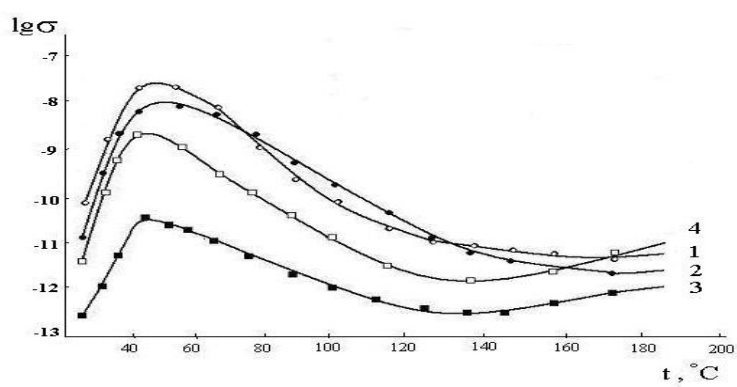


Fig. 2. temperature dependences of snake venom crystals conductivity lgσ at various time of days.

The sample was heated up and conductivity change was observed , and then it was cooled and process was repeated again. Experiments were repeated three times. Conductivity of venom increases to 43°C. At the further heating there was a conductivity reduction. Above 140°C, conductivity raises again. Experiments were repeated in a day. Thus the increase of conductivity and also moving of maxima at a curve of specific resistance was marked. At heating of the sample in temperature 170°C, with the subsequent repeated heating of samples of venom, minor alteration of conductivity of snake poison was observed. We assume, that after each subsequent heating, in the investigated sample of venom there are probably, structural changes and, in turn, changes of pharmacological activity of enzymes. However, at the subsequent heating of snake venom with 24 hour interval restoration of physical and chemical properties of snake poison that testifies to thermostability of zootoxin was noticed.

At the termination of heating of poison, restoration of fermentative activity, and also physical and chemical properties of snake poison was marked. Proceeding from results of the spent researches we assume, that under the influence of external factors (temperature) change of electrophysical parameters of Macrovipera lebetina obtusa venom was marked. Spectral dependence of photoconductivity of venom was experimentally investigated, it wasrevealed, that appeared Macrovipera lebetina obtusa venom crystals are not sensitive to light. In order to reveal the influence of radiation on spectral characteristics of vipera venom and structural changes in a molecule of biopolymer, we conducted researches of spectra of absorption of both standard venom, and the zootoxin irradiated with various doses of gamma radiation. We drew absorption of spectra of standard venom and the samples of snake venom subjected to irradiation with gamma radiation $^{60}$Co at doses of g-irradiation D=1.35, 2.7, 4.05, 5.4 kGy in infra-red, visible and ultra-violet areas. Infra-red spectra of absorption of samples of snake venom were drawn on spectrophotometer Specord-71 IR in tablets of potassium of bromide in the field of frequencies $\nu \approx$ 700-4000 sm$^{-1}$. For this purpose, standard vipera venom and samples of the venom, irradiated with gamma radiation, were mixed separately carefully in a porcelain mortar with chemically pure powder KBr. All samples of vipera venom in number of 1 mg (on 200 mg of potassium of bromide), after careful hashing with powder KBr were pressed in tablets under pressure 8×107 kg/m$^2$. We received the tablets pressed from potassium of bromide which were transparent enough and poorly disseminate infra-red beams. Considering the circumstance that the pressed tablets from a mix of poison with KBr at storage on air quickly grow turbid, they were pressed directly before record of IR spectra.

As a result, intensive strips of absorption in area 700-4000sm$^{-1}$ were received that has given the chance to carry out the effective identification of samples of snake venom, both standard, and irradiated by gamma radiation. For the structurally-group analysis and at interpretation of infra-red spectra of venom, references of strips of absorption of separate functional groups of biomolecules of zootoxin were spent. At comparing IR- spectrum of standard venom and irradiated at gamma- radiation $^{60}$Co at a dose of g-irradiation D=1.35 and 2.7 kGy, essential differences in structure of snake venom (fig. 3,4) was not revealed. However, insignificant structural changes was revealed in IR spectra of samples of the venom, subjected to irradiations with gamma radiation $^{60}$Co at doses D =, 4.05, 5.4 kGy (fig. 5).

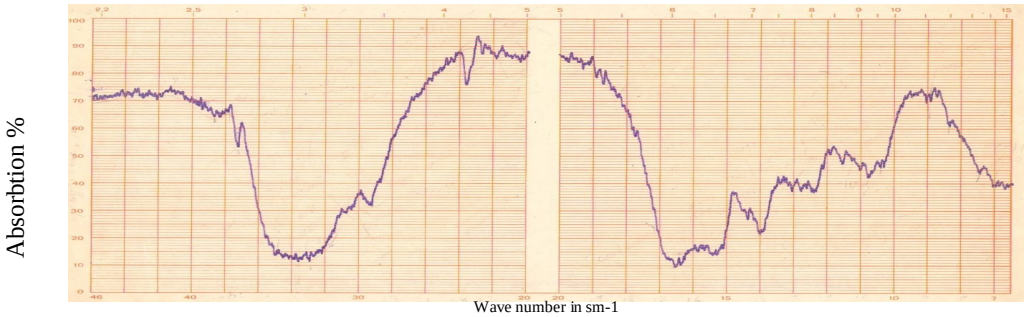


Fig. 3. Spectra of absorption of standart samples of snake vipera venom

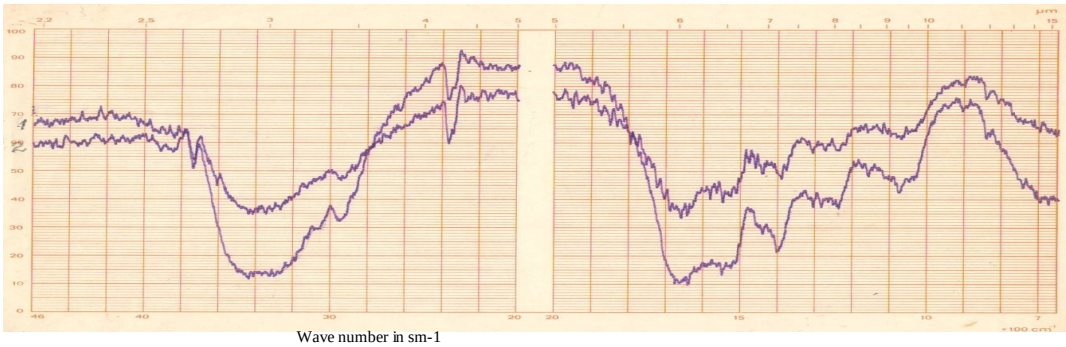


Fig. 4. Spectra of absorption of samples of vipera venom, irradiated with γradiation (1- D=1.35, 2- D=2.7 kGy):

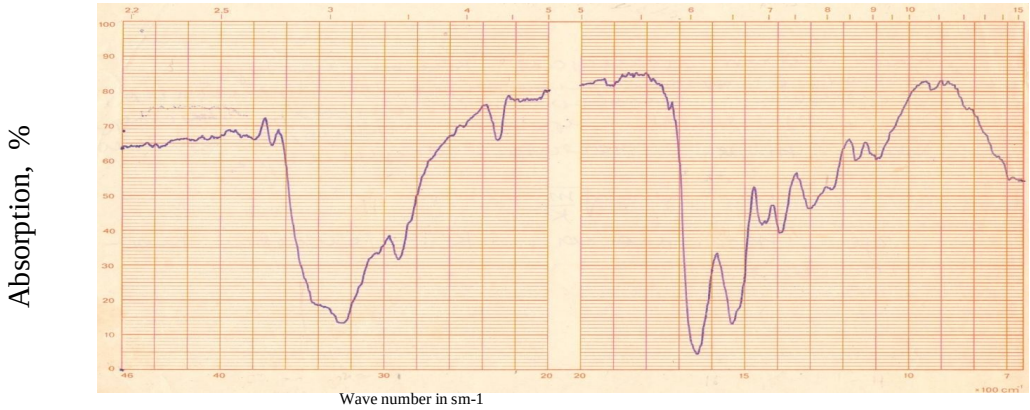


Fig. 5. Spectra of absorption of samples of vipera venom, irradiated with γ-radiation $^{60}$Co, D=5.4kGy

Thus, IR-spectra of absorption of snake venom were received and systematized, the structurally-group analysis which allows to reveal structural changes in venom, under influence of gamma radiation was established. From the above-stated it is possible to ascertain that small doses of radiation do not cause to structural changes in samples of vipera venom that can be considered at radiating sterilisation of snake venom and preparations on its basis.

By means of measurement of a spectrum of absorption of vipera venom, after an irradiation, it is possible to define places of attack and to establish chemical changes of zootoxin, both in the irradiated solutions, and in a firm phase.

It is necessary to notice that at comparing intensity of absorption of control samples (not irradiated) venom with samples of zootoxin irradiated to doses 1.35 кGy, essential changes was not revealed.

## 5. RESULTS AND CONCLUSIONS

Electrophysical properties and photoconductivity of Macrovipera lebetina obtusa venom were studied. The temperature dependence of specific resistance ρ~f(T) on time, and also temperature dependence of conductivity σ~f(T) on time were investigated. Electrophysical properties of snake venom were experimentally revealed and it was established, that Macrovipera lebetina obtusa venom does not possess photoconductivity.

Based on the results of the research, we assume that external factors (temperature) change in the electrical parameters of viper venom was marked.

On the basis of experimental data changes in electrical properties of snake venom was revealed. It was established that viper venom has no photoconductivity. There were changes in the conductivity of the snake venom under the influence of the temperature factor.

From the above it can be said that the conductivity of the poison increases with heating samples of venom to temperature $43^0$ C. However, as the subsequent heating temperature of venom increases, the opposite effect- the decline of conductivity are observed. In the future, when the temperature of sample heating snake venom is over $140^0$C, conductivity rises again.

As a result, we measured the photoconductivity of viper venom. In this case, we measured the photoconductivity of crystalline snake venom at different temperatures and wavelengths. Values of photoconductivity venom, depending on the wavelength was established. It was revealed that the crystals viper venom were not sensitive to light.

It is necessary to notice that at comparing intensity of absorption of control samples (not irradiated) venom with samples of zootoxin irradiated to doses 1.35 кGy, essential changes was not revealed.

Similar researches on influence of identification of gamma radiation on venom of snakes (Cerastes cerastes, by Bothrops jararacussu and others) are given in researches of some authors. After irridation of poison of snakes to gamma radiation $^{60}$ Co to doses 1 and 2 kGy, decrease in toxicity of venom was noted. However decrease in immunogene properties of toxins of snake poison was not noted. Authors revealed changes of spectral characteristics of the irradiated samples of toxins [1,4,6].

Macrovipera lebetina obtusa. Research of influence of gamma radiation on venom of Macrovipera lebetina obtusa in references are absent. We picked up for the first time radiation doses for stabilization of pharmacological and toxicological properties of venom of Macrovipera lebetina obtusa

Thus, the impact of venom γ-radiation doses to 1.35 kGy stabilizes both toxicity and pharmacological activity, respectively while increasing the shelf life of snake venom and in turn is important for a farmaceutical industry by manufacture of preparations on a basis zootoxins.

Thus, we can say that viper venom has no photoconductivity. The data obtained can be used for storage of drugs based on snake venom, as well as the identified physical properties of snake venom, can be applied as a criterion for establishing the authenticity, as a whole venom and its toxins and drugs based on zootoxins.

The experimental results can be applied in clinical practice in forensic science, the analysis of cadaveric material for authentication and identification, as snake venom toxins and products of its metabolism.

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The authors declare that they have no conflicts of interest in this research.

## REFERENCES

1. Boni-Mitake M., Costa H., P.J. Spencer, Vassilieff V.S., Rogero J.R.. Effects of $^{60}$Co gamma radiation on crotamine. Braz J Med Biol Res, December 2001, Volume 34(12), p.1531-1538
2. Caraballo, A., Navarro, J., Sánchez, E.E., Pérez, J. C., Rodriguez-Acosta, A., 2004, Epidemiological and clinical aspects of snakebites in Bolivar State, Venezuela. Revista de Medicina, (27): 25-28.
3. Galán, J.A., Sánchez, E.E., Bashir, S., Pérez, J.C., 2005.Characterization and identification of disintegrins in Crotalus horridus venom by liquid chromatography and tandem matrix-assisted laser desorption ionization quadrupole ion trap time-of-fligh (MALDI-QIT-TOF) mass spectrometry. Canadian J. Chem. (83): 1124-1131.
4. .Mirco Jessica, Baptista Janaina A, Caproni Priscila, Yoshito Daniele, Nascimento do Nanci. Evaluation of miotoxic activity of bothropstoxin-1 irradiated with $^{60}$Co gamma rays. International Nuclear Atlantic Conference - INAC 2009 Rio de Janeiro,RJ, Brazil, September27 to October 2, 2009: 978-85
5. Orlov B.N., Gelashvili D.B., Ibrahimov A.K, 1990, Venomous animals and plants of the USSR, The Higher school, Moscow, 271p.
6. Oussedik-Oumehdi Habiba , Laraba-Djebari Fatima. Irradiated *Cerastes cerastes* Venom as a Novel Tool for Immunotherapy. 2008, Vol. 30, No. 1 , p. 37-52.
7. Samel M., Subbi J., Siigur J., Siigur E., 2002, Biochemical characterization of fibrinogenolytic serine proteinases from Vipera lebetina snake venom. J. Toxicon, 40, (1): 51-54.
8. Sánchez, E.E., Ramírez, M.S., Galán, J.A. et all., 200, Cross reactivity of three antivenoms against North American snake venoms, J.Toxicon, (241): 315-320.
9. Sánchez, E.E., Galán, J.A., Powell R.L. et all, 2005. Disintegrins, hemorrhagic, and proteolytic activities of Mohave rattlesnake, Crotalus scutulatus scutulatus, venoms lacking Mojave toxin. J. Comp. Biochem. Physiol., (Part C): 124-132.
10. Topchiyeva Sh.A., 1998, On the dinamics of the changes of commen protein in the organs and tissues of Experimental animals under the transcancasian viper., Athens-Greece, 9th Panhellenic Pharmaceutical Congress.(7): 513-516.
11. Topciyeva Sh.A., Yolciyev Y.Y., Tagiev B.G., Jabbarov H.B., Tagiev O.B., Mamedov O.G., 2001, The influence of the environmental factors on chemical composition of Vipera's venom. 7th International Interdisciplinary Conference on the Environment, San Francisco/California, USA: 25.
12. Topchiyeva Sh.A., 2002, Chromatographic and spectral characteristics of snake venom, proteins and products of metabolism of ones. J. Farmakom, Kharkov. (4). 75-81.
13. Topchiyeva Sh.A, 2002, Fluorescent probes in snake venom investigation. J. Farmakom, Kharkov, (3): 174.-179.
14. Topchiyeva Sh.A., Iskenderov T.M., Jabbarov R.B., Musayeva N.N., 2003, Influence of the ecological factors to number Vipera lebetina obtusa and chemical composition of venom. Proceedings of the First International Conference on Environmental Research and Assessment, Bucharest, Romania, p: 34-37.
15. Topchiyeva Sh.A., Abiyev H.A.,2004. Ecological factor's and chemical structure of venom of Vipera lebetina obtusa J. Ekoenergetika, (1): 21-23.
16. Trummal K., Vija H., Subbi J., Siigur J., 2000, MADL-TOF mass spectrometry analysis of substrate specifity of lebetase, a direct-acting fibrinolytic metalloproteinase from Vipera lebetina snake venom. J.Biochim. Biophys. Acta, 1476, (2: 331-336.