Radiobiological and Biophysical Showing of Venom of Macrovipera Lebetina Obtusa

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Abstract— *Research objective was studying of influence of ecological factors on radiobiology-cal and biophysical properties of venom of Transcaucasian vipera Macrovipera lebetina obtusa. By method of atomic absorption spectrometry, metal ions Cr, Pb, Cd and Zn was revealed in samples of vipera venom. γ – radiospectrometric studies have shown that the venom samples also contain radionuclides as Ra228, Ra226, K40 and 137 Cs. Analysis of the data shows that the venom of Viperas caught from different regions of the Republic, are almost indistinguishable for the content of radionuclides. All samples of the venom have a lower content of Ra228.Generalizing results on research of infra-red, visible and ultra-violet spectra of absorption of vipera venom, it is possible to ascertain that infra-red, visible and ultra-violet spectra of standard samples of vipera venom and samples irradiated by □-radiation are studied and systematized. The received experimental data testify to perspectivity of spectral methods at biophysical and biochemical researches of both snake venom , and preparations on its basis, showing informative at studying of structural changes under the influence of ecological factors.It was established that the radiation dose (up to 1.35 kGy dose) for 3 minutes did not cause structural changes in the samples venom of vipera, but rather contribute to the stabilization of both toxicity and pharmacological activity while increasing the shelf life of aqueous solutions of vipera venom. As a result of experimental studies, a negative effect of metal ions and radiation on the biochemical and biophysical indices of snake venom*

Keywords— snake venom, Macrovipera lebetina obtusa, metal ions, radiation.

I. INTRODUCTION

In connection with intensive studying of snake Venoms the variability of pharmacological activity, physicochemical and biophysical parameters of Transcaucasian viper venom (Vipera lebetina obtusa) under the influence of ecological factors represents considerable interest from ekologo-physiological positions [11,12].

In references the review on studying of physical and chemical properties of solutions of venom of the Central Asian snakes is resulted. Factor of a superficial tension, viscosity and density factor is spent in the range of temperatures 298-318K with use densitometries and viscos metrics methods of the physical and chemical analysis. At the analysis at the temperature range 303-308K deviations of these parameters on viscosity and density isotherms are noted. Studying of isotherms has given the chance to ascertain, that the observable effect is connected with own intermolecular interactions in them [4]. The fraction which entirely reproduces all displays of an intoxication is identified and allocated In venom of Vipera lebetina. Venom of Vipera lebetina is a part of medical products. It is applied as a source of reception of commercial preparations FGN, phosphorus distresses and oxygen dases L-amino acids, and also as a diagnostic preparation at illnesses of curtailing system of blood [6, 7]. On literary data snake venom in the dried up kind keeps pharmacological and toxic properties till 22 years and even more. At cultivation of venom by water by physiological solution or glycerin toxic properties of snake venoms are not lost. Short-term influence of heats also does not render essential influence on toxicity of venom. Considerable influence on toxicity and quantity of allocated venom is rendered various biological, ecological factors, and also chemical agents and some physical factors [13,14,15]. Snake venoms are a great value for medicine and biology. Venoms of snakes are used at preparation of mono-and polyvalent whey. Venom of Vipera libetina is applied as blood the stopping means in otoloringology at operations during removing of glands, at nasal bleedings etc. [1, 5]. Influence of ecological factors on a chemical compound of venom Caucasian Vipera lebetina obtusa is revealed. Elementary structure of venom of Vipera lebetina obtusa caught of the various areas of Azerbaijan polluted by techno genic emissions of the industrial enterprises is studied by the atom -

absorption spectrophotometer method. In venom of Vipera lebetina obtusa are defined the maintenance of heavy metals: Cr, Pb, Cd, Ni and Zn [8, 9, 10]. Researches on revealing of influence of γ -radiation on toxicity and on pharmacological properties of venom of Vipera lebetina obtusa are carried outγ. [2, 3]. Thus, the considerable number of works have been devoted all-round studying of venom of venomous snakes. The picture of toxic and pharmacological action of snake venoms on an organism is in detail tracked. Inconsistent and many-sided characteristics of snake venoms have been resulted.Influence of snake venom on various functional systems of an organism has been revealed. Toxicity, pharmacological activity and physical and chemical properties both integral venom, and separate components of snake venom have been studied. The comparative pharmacological and biochemical characteristic of venom of snakes has been given.Studying of influence of ecological factors (abiotic and biotic factors and including heavy metals, radiation) on venom of Vipera lebetina obtusa, definition of concentration of heavy metals in venom of Vipera lebetina obtusa, studying of influence of heavy metals and radiation on biochemical indicators and venom spectral characteristics, on toxicity of venom Vipera lebetina obtusa, and also selection of conditions of radiating sterilisation of snake venom - an actual problem of biochemistry, toxicology, medicine and biology.The resulted review of references shows, that in experimental studying of snake venoms are available inconsistent both difficult enough and the unresolved questions demanding further deep theoretical and the experimental researches. Progressing environmental pollution by these products leads in turn to gradual pauperisation of fauna and flora of the given areas of Azerbaijan. Besides Direct impact makes on qualitative structure, biochemical, bio-physical and pharmacological properties of products of biosynthesis of animals In territory of Azerbaijan live 23 kinds of snakes, 4 kinds of them are venomous. Research of toxins of various kinds of snakes, and also huge attention to zootoxins is defined not only inquiries of medical practice, but their studying and use represents the big interest for various branches of biology, physiology, bioorganic chemistry, biophysics, toxicology and other areas of sciences. Studying of venomous products of biosynthesis of the snakes, what are unique on a chemical compound and physiological action group of biologically active substances, influence revealing biotics and abiotics environment factors on biochemical, biophysical and pharmacological properties of the venom, developed venomous gland Caucasian Vipera lebetina obtusa is an actual problem of a medical and biologic science. These problems still are not perfect, demand deep theoretical and practical analyses and are still actual. There are literary datas on research of the snake,venoms , however many questions still remain not mentioned and demand the deep analysis and studying.

Research objective was studying of influence of ecological factors, on biochemical and biophysical properties of venom of Transcaucasian vipera (Macrovipera lebetina obtusa).

II. MATERIALS AND METHODS

Research objective - studying of influence of ecological factors, including heavy metals and ionizing radiation on biochemical and biophysical characteristics of venom of Macrovipera lebetina obtusa. It has been defined the maintenance of heavy metals-polluters in venom of Macrovipera lebetina obtusa, caught from ecologically polluted sites of Absheron penensula by the atom-absorbtion spectrophotometer method (AAS-300, Perkin-Elmer). Measurements by definition of the maintenance of radionuclides in samples of venom of vipera, caught in districts of Azerbaijan with various degrees of impurity, and also in samples of soils, vegetation and water was spent on spectrometer Canberra. Experiment time-24 hour. The spectrum defining activity of radionuclides in snake venom has been removed on a spectrometer "CANBERRA"

Ultra-violet spectra passing of venom have been removed on device Specord UV-VIS.

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 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.

III. RESULTS

In various on impurity degree of Azerbaijans biosfere will be spent catching of snakes for definition of influence of polluters on biochemical characteristics and toxicity of venom (fig.1). Simultaneously researches will be carried out in laboratory conditions on obtaining of influences of ionizing radiation and electromagnetic radiation on biochemical parameters, on fermentative activity and toxicity of venom of Vipera lebetina obtusa. In laboratory conditions these samples will be analysed on the maintenance of toxic substances, such as heavy metals,

radionuklide. As a result of researches chemical compound changes, and also influence of heavy metals, ionizing radiation, electromagnetic radiation on albuminous structure, fermentative.activity and other biochemical indicators of venom of Vipera lebetina obtusa will be established. Conditions of radiation sterilization of snake venom will be picked up. Detection of heavy metals in snake Venom was carried out at direct influence on Venom a solution trichloroacetic acid. After precipitation of fibers in snake Venom by trichloroacetic acid the content of heavy metals in samples of snake Venom was performed. It was necessary to consider the fact of overestimate of the given experimental measurements at direct detection of the content of the above-stated metals in samples of snake Venom.The technique of study of viper Venom by atomic absorption spectrometry consists in the following. An exact amount of snake Venom in quantity of 20 mg was placed in centrifuge tube, 10 ml of solution HCl (1:1) was added and further a solution left in the thermostat at 40^0 C at 1 hour. After that 2 ml of 20 % solutions CCl_3COOH was added, with the subsequent keeping during of 1st hour at room temperature and centrifuged during 10 minutes at 1500 rpm. Fe, Cr, Cu, Cd was detected in the filtrate. It is necessary to consider that fact that standard solutions should contain 5 % trichloroacetic acid. Thus, we pick up optimum conditions for detecting Fe, Cr, Cu, Cd, Zn from trichloroacetic acid filtrate. For qualitative determination of concentration of investigated metals in bioobjects we constructed the graduated diagrams of determination of standard metals in coordinate's A-C. Under the graduated diagrams in coordinate's A-C concentration of detected elements was determined. For construction of graduated diagrams working standard solutions were entered serially into an air-acetylene flame of a burner, beginning from a solution with the minimum content of a detected element not less than four concentration, including the concentration close, to that which is expected in an analyzed solution. Each measurement repeated twice (not less than 2 times), at diagram construction average value was taken. We had conducted summer field researches in vicinities of Baku, and also areas of Azerbaijan: Gobustan, Shamakhi, Kurdamir and Sabirabad. During the expedition catching of viperas has been spent with a capture of venom, soil and vegetation tests. Snakes after milking have been released in the nature, venom is placed in exicators, dried up for analysis carrying out on the maintenance of heavy metals by a method of atom-absorbtion spectrometry. A part of venom of snake has been subjected to the analysis on the maintenance of radionuclides on installation Camberra.

Data by definition of the maintenance of heavy metals in investigated samples is presented in tables 1.

We had spent experimental measurements of the maintenance of radio nuclides in samples of venom of viperas, caught with various degree of impurity districts of Azerbaijan (fig.1).

The spectrum defining activity of radionuclides in venom of snakes has been removed on spectrometer Camberra. The radiating background of radionuclides (uranium, caesium), defined in venom of snakes is presented in the table 2,3,4. The radiating background of radionuclides in venom of snakes from various zones was identical (fig.2-6).

In the table 2,3,4 the radioactive elements which are available as a part of snake venom are presented. Natural radionuclides of low activity again have been defined in samples of snake venom. The revealed activity of an element of uranium in a spectrum of venom grows out of a radiating background which is formed by influence of environment.

Therefore protection of environment from technogenic pollutants on value ranks now with such problems as protection of soils.

It is necessary to note that an increase of a radiating background of environment which throughout last several millenia remained rather stable. The spectrum of activity of radionuclides in soil is presented in the drawing.

On the basis of the received data it is possible to ascertain that depending on degree of impurity of district where catching, milking venom, sampling of vegetation and soils fluctuations in the maintenance of heavy metals has been marked. However radiating activity of elements in snake venom and soils did not change almost. Natural radio nuclides of low activity again have been defined in samples of snake venom. The revealed activity of an element of uranium in a spectrum of Venom grows out of a radiating background which is formed by environment influence. Therefore protection of environment from technogenic pollutants on value ranks now with such problems as protection of soils. Studying of influence of small doses γ-radiations on spectral characteristics of venom Transcaucasian vipera. For revealing of influence of radiation on spectral characteristics of vipera venom and structural changes in a molecule of biopolymer, we have conducted researches of spectra of absorption of both standard venom, and the zootoxin irradiated with various doses of gamma radiation (table 39-40). We had removed absorption spectra of standard venom and the samples of snake venom subjected to an 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 removed 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/m2. We had received the tablets pressed from potassium of bromide which were transparent enough and poorly disseminate infra-red beams. Considering that circumstance that the pressed tablets from a mix of Venom 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 have been received that has given the chance carrying out of 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 have been spent. At comparing IR- spectrum of standard venom and irradiated at scale by radiation ^{60}Co at a dose of g-irradiation D=1.35 and 2.7 κGy, essential differences in structure of snake venom has not been revealed. However, insignificant structural changes have been revealed in IR spectra of samples of the venom, subjected irradiations with gamma radiation ^{60}Co at doses D =2.7, 4.05, 5.4 κGy. From investigated spectra standard and the irradiated samples of venom it is visible that at √<4000 sm-1 the strips of absorption concerning to OH group and molecules of water are observed, and intensive strips at 1350 sm-1 can be carried to valency fluctuations > CH2. Apparently from drawings equally intensive strips of absorption in an interval 3020 sm-1 allow to come out with the assumption that there is available grouping ≥C-H in a molecule of both standard snake venom, and irradiated with gamma radiation.

Absorption strips in all investigated samples of venom in the range of lengths of waves of 3000-3100 sm-1 have different intensity that testifies to possible presence in a grouping molecule =CH_2, -HC=CH-. Intensive strips of absorption in samples of both standard, and the irradiated venom at 1350, 1460, 1480 and 1100 sm-1 confirm the given assumption.

Apparently from spectra of absorption of investigated samples of vipera venom appears that valency fluctuations of C≡ shown by a strip in the field of 2100-2260 sm^{-1}, and valency fluctuations H-C≡ come to light in a strip of absorption of 3300 sm^{-1}. In the range of lengths of waves 2570, 3100-3700 sm^{-1} in venom spectra, strips of absorption which acknowledgement on presence of atom of sulfur give the chance are visible. The basic group of characteristic structures, contains atom of hydrogen. A distinctive feature of these spectra are strips of valency fluctuations N-H- of groups at 3300 and 3100 sm^{-1}.

Thus, IR-spectra of absorption of snake venom are received and systematised, the structurally-group analysis which allows to reveal structural changes in venom, under influence of gamma radiation is established. From the above-stated it is possible to ascertain that small doses of radiation do not cause structural changes in samples of vipera venom that can be considered at radiating sterilisation of snake venom and preparations on its basis. The structurally-group analysis of snake venom allows to spend venom identification, presence of the characteristic functional groups which are a part of a biomolecule, and also to establish presence of multiple communications in them and also to define a relative positioning of nuclear groups and communications in a zootoxin molecule. As absorption in infra-red area cannot give the high-grade characteristic about a biomolecule structure, therefore we had conducted comparative researches of ultra-violet and visible spectra of absorption of standard samples of venom and the samples of zootoxin subjected to influence of gamma radiation ^{60}Co at doses D=1.35, 2.7, 4.05, 5.4 kGy. Research material was vipera venom, dried up in dessicator over steams of chloride calcium. For studying of influence of small doses of gamma radiation on spectral characteristics of vipera venom, a number of experimental researches have been spent.

Samples of dry venom, and also their water solutions have been taken for experiment. Water solutions of venom have been subjected to γ- radiation to doses 1.35, 2.7, 4.05, 5.4 κGy throughout 0.5, 1.0, 1.5, 2.0 hours. Simultaneously dry vipera venom was exposed to gamma radiations of dose 5.4 κGy throughout 2 hours. In comparative aspect, γ- radiation action on spectral characteristics of venom of Vipera lebetina obtusa was studied. Irradiation of vipera venom with small doses of γ-radiation (γ-radiation D=0.75 Gy/sec) was spent on K-25 isotope installation with application of ^{60}Co. Ultra-violet spectra transmission of venom have been removed on device Specord UV-VIS.

On fig. 7. UV-VIS spectrum of absorption of a water solution of vipera venom was resulted.

Apparently from drawing, characteristic maxima of absorption in the field of 285nm and 800nm are peculiar to vipera venom. Group absorption in these molecules represents the big resounding structures with a great number of the interfaced communications. Electrons, carrying out a chemical bond, are not localised, but are common for entire structure. Therefore it is possible to speak about characteristic for zootoxin absorption spectra. 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. On fig. 8 spectra of absorption of samples of vipera venom, irradiated with γ-radiation (water solutions and dry venom) are resulted. At increase in a dose of an irradiation with γ-radiation of solutions of vipera venom, characteristic changes of optical density of investigated samples of snake venom are observed. The revealed reduction of intensity of absorption at 260 and 300 nanometers testifies to course of biochemical reactions in a firm phase of separate enzymes of zootoxin. As a result of the spent experimental researches it is established that under the influence of small doses of g-radiation at doses 2.7, 4.05, 5.4 κGy within 1.0, 1.5 and 2.0 hours leads to gradual decrease in toxicity and probably pharmacological activity of enzymes of snake venom (fig.8). 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 κGy, essential changes has not been revealed. From the above-stated follows that influence of small doses of γ-radiation to doses 1.35 κGy on vipera venom within 0.5 hours does not lead to reduction of intensity of absorption and accordingly to toxicity, and including pharmacological activity. The subsequent increase in a dose of γ-radiation to 2.7, 4.05, 5.4 κGy within 1.0, 1.5, 2.0 hours promotes proportional reduction of intensity of absorption of snake venom and accordingly reduction of both toxicity, and pharmacological activity. Thus, as a result of the spent researches it is revealed that under the influence of small doses of γ-radiation to doses 2.7, 4.05, 5.4 kGy throughout 1.0, 1.5 and 2.0 hours leads to reduction of intensity of absorption at 260 and 300 nm that testifies to course of biochemical reactions in a firm phase of separate enzymes of zootoxin, and in turn leads to decrease in toxicity and probably in pharmacological activity of enzymes of snake venom. However, influence on a solution of snake venom of γ-radiation to doses 1.35 κGy within 3 minutes promotes stabilization of toxicity, and also pharmacological activity with simultaneous increase accordingly a period of storage of water solutions of snake venom that can be used at sterilisation of water solutions of snake venom, and in turn is important for pharmaceutical industry by manufacture of injections on the basis of zootoxins.

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 κGy, 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 [16].

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

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.

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,inthe received experimental data, specific resistance decreased (fig.9). 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 50^0C 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:

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

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

ρ_o, σ_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. 10) was investigated. Curve dependences of conductivity σ on temperature of heating of the sample σ~f(T) on time were drove. 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 thermo stability 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 was revealed, 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. 11,12) 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. 13).

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 κGy, essential changes was not revealed. Generalizing results on research of infra-red, visible and ultra-violet spectra of absorption of vipera venom, it is possible to ascertain that infra-red, visible and ultra-violet spectra of standard samples of vipera venom and samples irradiated by γ-radiation are studied and systematized. Characteristic strips of absorption of snake venom are defined. The received experimental data testify to perspectivity of spectral methods at biophysical and biochemical researches of both snake venom, and preparations on its basis, showing informative at studying of structural changes under the influence of ecological factors.

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 140^0C, 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.

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.

IV. FIGURES AND TABLES

Fig. 1: The basic routes of catching of snakes and gathering of materials for researches in Absheron

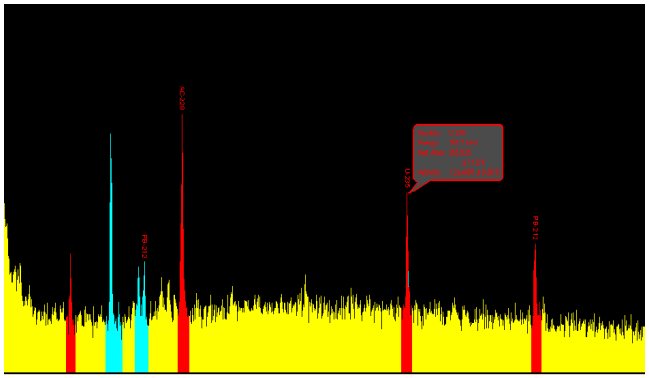


Fig. 2: A spectrum of activity of radionuclides in venom of snakes, caught in territory of Gobustan and Absheron peninsula of Azerbaijan

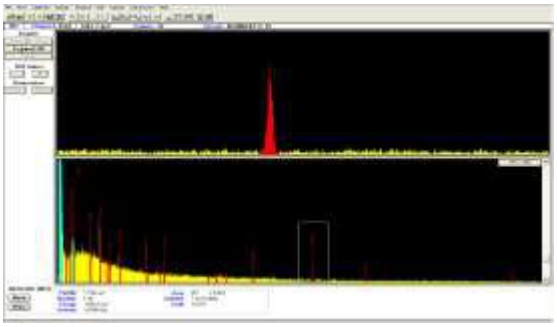


Fig. 3: A spectrum of activity of radionuclides in the snake venom, caught in territory of Shamakhi of Azerbaijan

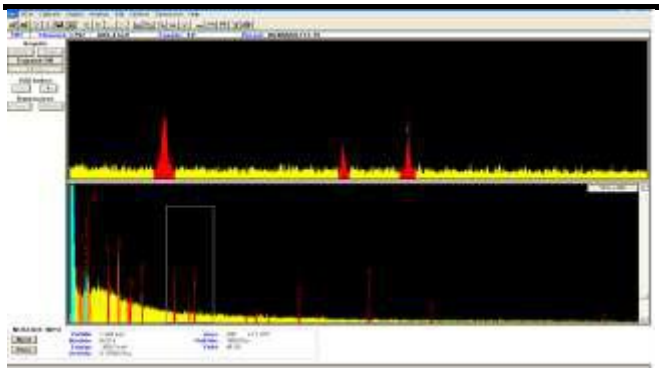


Fig. 4: A spectrum of activity of radionuclides in the snake venom, caught in territory of Sabirabad and Kurdamir.

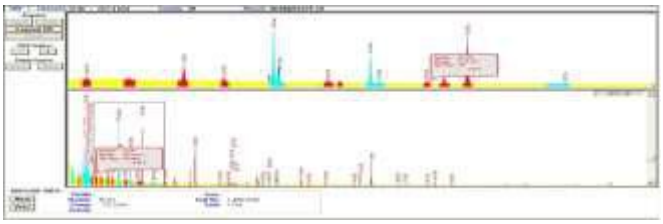


Fig. 5: A spectrum of activity of radionuclides in soils of Sabirabad and Kurdamir

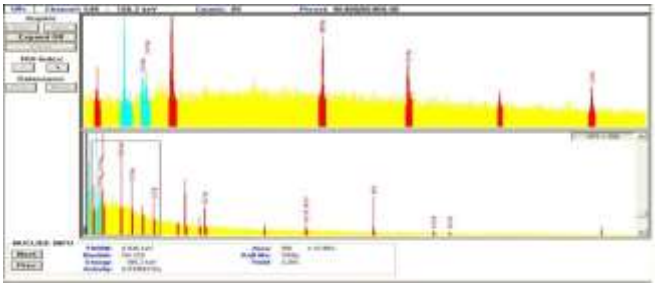


Fig. 5: A spectrum of activity of radionuclides in soils from vicinities of Bina

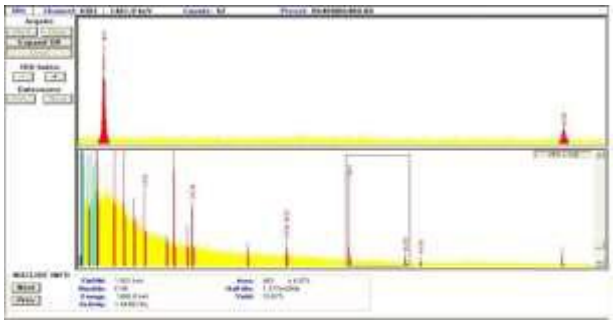
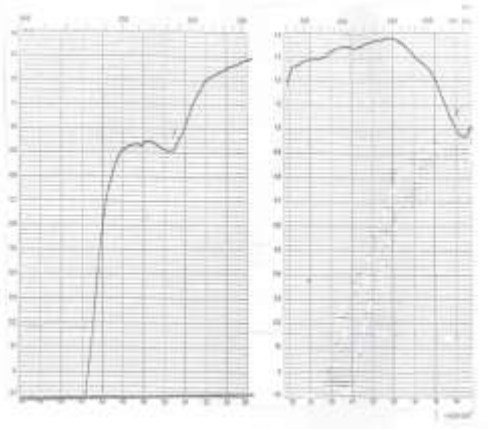
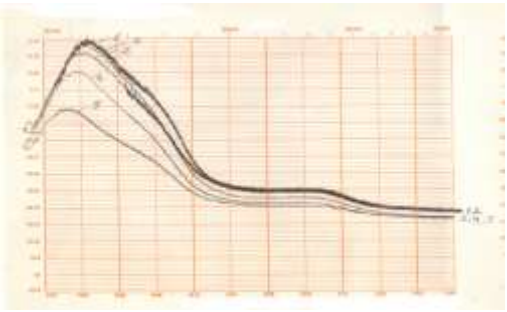


Fig. 6: A spectrum of activity of radio nuclides in soils from vicinities of Baku - the Airport



Wave number in sm-1

Fig.7: UV-VIS spectrum of absorption of vipera venom



Wave number in sm-1

Fig.8: Spectra of absorption of samples of vipera venom, irradiated with γ-radiation (1,2,3,4 water solutions, 5-dry venom):
1 control; 2,3,4 irradiated (D =1.35, 2.7, 4.05 kGy), 5 (D=5.4 kGy)

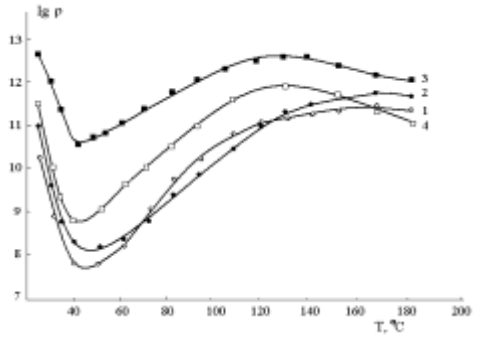


Fig.9: temperature dependences of snake venom specific resistance of crystals lgρ at various time of day

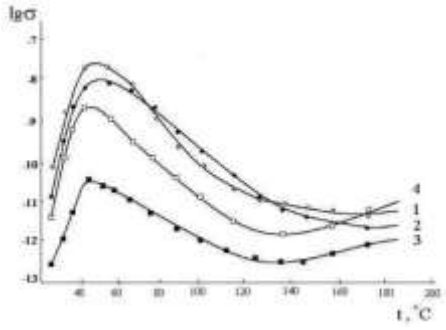


Fig. 10: Temperature dependences of snake venom crystals conductivity lgσ at various time of days

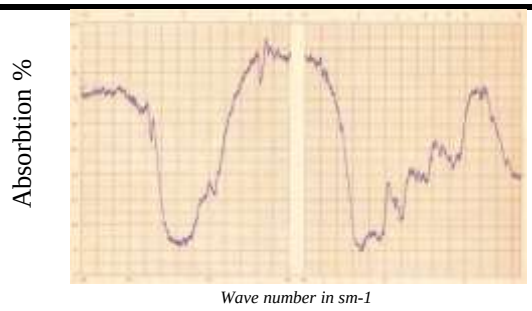


Fig. 11: Spectra of absorption of standart samples of snake vipera venom

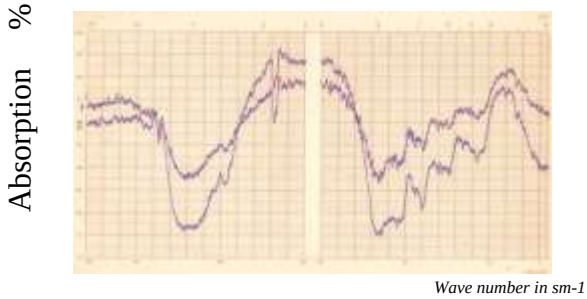


Fig. 12: Spectra of absorption of samples of vipera venom, irradiated with γradiation (1- D=1.35, 2- D=2.7 kGy):

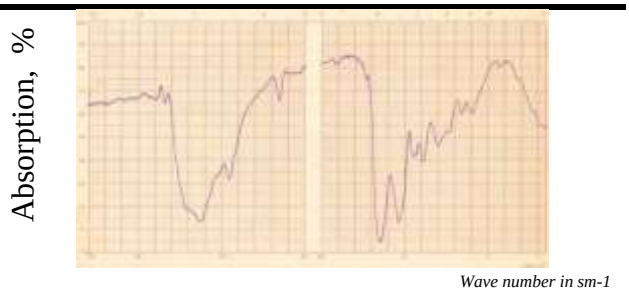


Fig.13: Spectra of absorption of samples of vipera venom, irradiated with γ-radiation ^{60}Co, D=5.4kGy

Table 1. The data content of heavy metals in investigated samples

Territory	*Baku - Airport*				
	Concentration, mg/kg (M±m)				
Samples	*Cr*	*Pb*	*Cd*	*Ni*	*Zn*
Plant	*130,4±2.528*	*23,0±1.379*	*2,06±0.064*	*40,30±0.307*	*701,39±0.303*
Soil	*80,7±4.842*	*5,9±0.154*	*0,59±0.007*	*36,11±0.275*	*53,00±0.098*
Venom		*133,9±14.464*	*1,9±0.949*		*665,0±3.989*
District Shamakhi					
Plant	*137.0±1.316*	*22,7±4.695*	*2,05±0.063*	*40,33±0.1685*	*701,4±0.077*
Soil	*83,0±0.459*	*5,3±0.073*	*0,62±0.004*	*28.0±0.658*	*53,0±0.055*
Venom		*134.9±0.056*	*1,76±0.245*		*644±0.099*
District Gobustan - Childag					
Plant	*131.0±1.300*	*23,0±4.480*	*2,05±0.058*	*39,99±0.1600*	*700,4±0.091*
Soil	*89,9±0.438*	*5,5±0.079*	*0,70±0.001*	*35,15±0.542*	*52,7±0.049*
Venom		*133.9±0.033*	*1,9±0.200*		*666,9±0.0034*
District Gobustan Maraza					
Plant	*130.0±1.200*	*20,9±3.480*	*1,99±0.038*	*39,36±0.500*	*700,3±0.090*
Soil	*80,2±0.356*	*4,9±0.030*	*0,53±0.025*	*35,15±0.678*	*52,20±0.071*
Venom		*133.7±0.029*	*1.6±0.177*		*663,7±0.027*
District Sabirabad Village Karatugay					
Plant	*66,5±1.290*	*4,9±0.090*	*1,0±3.480*	*40,4±0.670*	*280,1±0.040*
Soil	*100,4±0.556*	*7,3±0.027*	*0,6±0.030*	*45,6±0.798*	*98,0±0.088*
Venom	*87,0±0.049*	*87,0±0.030*			*669,0±0.076*
District Sabirabad Village Shihsalahli					
Plant	*87,0±0,990*	*4,9±0.487*	*0,5±0,589*	*10,7±0,133*	*66,05±0,440*
Soil	*90,6±0.670*	*10,0±0.567*	*0,5±0,131*	*43,9±0,228*	*67,09±0,344*
Venom		*50,1±0,285*			*860,9±0,129*
District Sabirabad Village Ahtachi					

Plant	*65,0±0,560*	*4,2±0,074*	*1,0±0,898*	*39,2±0,879*	*282,0±0,235*
Soil	*96,0±0,076*	*7,1±0,333*	*0,61±3,670*	*43.3±2,30*	*99,5±0,004*
Venom		*87,0±4,35*			*362,0±0,800*
District Sabirabad Village Uladjali					
Plant	*64,0±0,009*	*4,0±0,789*	*1,3±0,956*	*38,9±0,451*	*282,0±0,760*
Soil	*95,0±0,435*	*6,9±1,345*	*0,60±1,68*	*42.4±0,289*	*99,9±0,390*
Venom		*89,0±0,300*			*360,8±0,190*
District Kurdamir Village Atakishili					
Plant	*67,6±0,010*	*4,8±0,700*	*1,1±0,8906*	*41,5±0,400*	*284,3±0,799*
Soil	*102,3±0,336*	*7,6±1,355*	*0,7±0,489*	*46,7±0,8,90*	*99,0±0,480*
Venom		*89,1±0,280*			*371,1±0,230*
District Kurdamir Village Hirdapay					
Plant	*88,4±0,045*	*4,6±0,680*	*0,6±0,542*	*11,9±0,484*	*67,05±0,6500*
Soil	*91,4±0,501*	*9,3±1,500*	*0,6±0,980*	*44,9±0,389*	*68,05±0,200*
Venom		*49,13±0,478*			*863,6±0,315*

Table.2: Radiating activity of elements in snake venom

Elements	Radiating activity, Bk/g
Ra 228	0.174 +- 0.090
Ra-226	2.48 +- 0.05
Cs 137	MDA=0.315

Table.3: Radiating activity of elements in investigated samples

The crystals were Venom	Sample 1	Sample 2	Sample 3
The name radionuclide	Ra^{226}	K^{40}	Ra^{226} Pb^{212} U^{235} U^{238}
Energy radionuclide	186.2 keV	1460.8 keV	185.97 keV
Radiating activity	0.539663 Bk/g	1.44382 Bk/g	
Radiatsionno-chemical exit	3.28%	10.67%	5.26%
The peak area with a margin error	356; 10.98%	483;4. .897%	77, 770
Mass	56 mg	60mg	55 mg
Specific activity	-	-	0.427, 0.26, 12.64 Bk/kg
Width of semiheight	0.936 keV	1.501 keV	

Table.4: Radiating activity of elements in investigated samples

Soil	Sample 1	Sample 2	Sample 3
The name radionuclide	B^{212}	K^{41}	B^{214}
Energy radionuclide	727.2 keV	1460.4 keV	628.3 keV
Radiating	-	1.47844	0.176622

activity		Bk/g	Bk/g
Radiatsionno-chemical exit	11.8%	10.57%	46.7%
The peak area with a margin error	-	451,5.04%	208,11.37%
Mass	170 mg	110mg	155 mg
Specific activity	-	-	-
Width of semiheight	-	1.726 keV	1.205 keV

V. CONCLUSIONS

1.By the method of atomic absorption spectrometry, metal ions Cr, Pb, Cd and Zn was revealed in samples of vipera venom. It is shown that the metal concentrations in the venom of snakes fluctuate within: Cr – 87.0 ± 0.049; Pb – 7.01 ± 1.321 – 19.0 ± 1.321; Cd –1.6 ± 0.177 – 1,9±0.200; Zn – 360,8±0,190 – 863,6±0,315mg/kg, and the content of metal of Vipera venom of different regions differ significantly. In this case, all the samples of the venom of Viperas caught from different regions of the Republic, have a certain amount of lead and zinc ions; Cr is present in the venom of samples of snakes of Sabirabad and Agsu and, and Cd was detected in samples of the venom of Vipera of Gobustan and Shamakhi regions. It was revealed that the concentrations of metals in the venom correlate with their content in soils.

2. γ – radiospectrometric studies have shown that the venom samples also contain radionuclides as Ra^{228}, Ra226, K40 and 137 Cs, which are the specific activities of 228 Ra (0.08-0.174 Bq /kg), Ra^{226} (0.35-2.48, Bq /kg) K^{40} (1.35-23.4 Bq/kg), Cs^{137} (MDA = 0.315, respectively. Analysis of the data shows that the venom of Viperas caught from different regions of the Republic, are almost indistinguishable for the content of radionuclides. Thus K^{40} is present in larger quantities in all samples of venom. All samples of the venom have a lower content of Ra^{228}.

3.Thus, as a result of the spent researches it is revealed that under the influence of small doses of γ-radiation to doses 2.7, 4.05, 5.4 kGy throughout 1.0, 1.5 and 2.0 hours leads to reduction of intensity of absorption at 260 and 300 nm that testifies to course of biochemical reactions in a firm phase of separate enzymes of zootoxin, and in turn leads to decrease in toxicity and probably in pharmacological activity of enzymes of snake venom. However, influence on a solution of snake venom of γ-radiation to doses 1.35 кGy within 3 minutes promotes stabilization of toxicity, and also pharmacological activity with simultaneous increase accordingly a period of storage of water solutions of snake venom that can be used at sterilisation of water solutions of snake venom, and in turn is important for pharmaceutical industry by manufacture of injections on the basis of zootoxins. 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.

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