J. Basic. Appl. Sci. Res., 3(10)265-269, 2013
© 2013, TextRoad Publication
ISSN 2090-4304
Journal of Basic and Applied Scientific Research
www.textroad.com

Influence of Ionizing Radiation on the Physical and Chemical Properties of Venom of Snake Macrovipera Lebetina Obtusa

H.A. Abiyev[1], Sh.A.Topchiyeva[2]

[1]Azerbaijan Medical University, Baku, AZ 1070, Bakichanov str.23
[2]Institute of Zoology of Azerbaijan National Academy of Sciences,
Pass.1128, block 504, Baku, AZ 1073, Azerbaijan

Received: August 17 2013
Accepted: September 12 2013

ABSTRACT

As result of chemical analyses, significant concentration of heavy metals (Cd, Ni, Zn, Cr, Pb) was found out in structure of venom . As a result of cooling samples up to nitric temperature (77K), new narrow maxima appeared at 440 and 470 nm which is connected by metals taking place in structure of snake venom .

As a result of the lead experimental researches it was established, that under influence of small dozes γ-radiations at dozes 2.7, 4.05, 5.4 kGy within 1.0, 1.5 and 2.0 hours results in gradual decrease of toxicity and pharmacological activity of enzymes of snake venom.

KEYWORDS: Macrovipera lebetina obtusa, venom, environment, heavy metals,γ-radiations.

1.INTRODUCTION

The problem of pollution of biosphere of Apsheron of Azerbaijan by toxic, heavy metals like Pb, Hg, As, Cd, Ni, Cu, Va, Zn, Co, Mo, Sr and other metals, has arisen with technogen emissions of the industrial enterprises in an atmosphere. They are the most dangerous toxic elements for fauna and animals and the people by entering in the basic biological circulation of substances. Studying of the given problem is the actual problem which has important value for medicine, biology and ecology.

In territory of Azerbaijan 4 kinds from 23 kinds of snakes are venomous.

Insignificant degree of a level of scrutiny of venom Transcaucasian viper Macrovipera lebetina obtusa as representative of fauna of Azerbaijan being the large supplier of this invaluable medicinal raw material for a pharmaceutical industry has served as the precondition of its research. In the literature there are not numerous and rather inconsistent data on dependence of properties zootoxins from various ecological factors (biotics and abiotics). On the basis of literary given and our experimental researches it was revealed, that snake venom contains ions of metals of Cd, Cu, Ni, Hg, Zn and Cr quantities which varies depending on places of dwelling of snakes [1,2,3].

By the method of atom-absorption spectrophotometry (AAS-300, Perkin-Elmer) the content of heavy metals has been defined in the venom of vipers that live in serpentarium in ecological conditions and in the venom of snakes caught from polluted areas of Absheron Peninsula of Azerbaijan [4,5]. In the polluted zones fluctuation of maintenances of heavy metals in structure of biologically active polymer limits for Cr, Pb, Cd, Zn, Ni 29-101.1, 7.0-134.8, 1.8-24.2, 23.0, 600.89-863.6 mg / kg accordingly.

For venom of the snakes in a serpentarium, quantity indicators for Pb, Ni, Cd, Zn- is 15.12 0.28, 0.31, 180.0 mg / kg, accordingly.

Thus, essential changes of physical and chemical, pharmacological and toxicological properties of biologically active biopolymer have been revealed depending on conditions of their dwelling, biotics and abiotics factors.

It is known, that water solutions of venoms are unstable and they lose toxicity in some day. Snake venoms in the physiological solution containing 50 % of glycerin, on given to Poguda A.A. [6], at storage in a refrigerator within 6 months have not lowered toxicity. The venoms after drying (above chloride calcium) or liophylisation are more resistant to influence of factors of an environment.

Venom of a cobra at storage more than 20 years on a cold in the sealed - in ampoule does not change its toxicity. Snake venoms are thermostable and they don't lose activity in the sour environment and in the heat up to 120^0C. Destroying chemical agents: permanganate kalium, chloroform, ethanol, methyl dark blue operate on venoms [7,8,9,10,11]. Snake venoms are inactivated also under action of some physical factors: a ultra-violet irradiation, X-rays. According to data of Salafranca [12], in 7 days after an irradiation of Philippian cobras venom by Co^{60} in dozes 0.25, 0.5, 1 mrad, its toxicity is 83, 66 and 43 % accordingly in comparision to irradiated venom. Proceeding from above stated, studying of influence of small dozes of gamma radiation on spectral characteristics of venom and accordingly on toxicity and pharmacological activity of zootoxin is very actual.

***Corresponding Author:** Sh.A. Topchiyeva, Institute of Zoology of Azerbaijan National Academy of Sciences, Pass.1128, block 504, Baku, AZ 1073, Azerbaijan. shafiga.topchiyeva@mail.ru

2.The purpose of research.

The purpose of research – studying of influence of small dozes of gamma radiation on spectral characteristics and stability of venom vipera.

3.MATERIALS AND METHODS

The material of research is venom vipera, dried up in exicator above pairs chloride calcium and the venom irradiated with small dozes γ-radiations. For experiment tests of dry venom, and also their water solutions have been taken. Water solutions of venom have been subjected to γ-radiation up to dozes 1.35, 2.7, 4.05, 5.4 kGy during 0.5, 1.0, 1.5, 2.0 hours. Simultaneously dry venom vipera has been exposed to γ-radiations in continuation of 2.0 hours with doze 5.4 kGy.

In comparative aspect action of γ-radiation on spectral characteristics of venom Caucasian viper Macrovipera lebetina obtusa has been studied.Irradiation of venom vipera with small dozes of γ- radiation (γ-radiation D=0.75kG/sec) was carried out on K-25 to isotope installation with application ^{60}Co.

All experiment were carried in the Institute of Zoology of Azerbaijan National Academy of Sciences and in the Institute of Radiation Problems of Azerbaijan National Academy of Sciences.

4.RESULT AND DISCUSSION

A series of experimental studies were carried out to study the effect of low doses of gamma radiation on the spectral characteristics of the viper venom.

For the experiment, aqueous solutions of venom were taken. The solutions of venom were subjected to γ-rays to doses of 0.5, 1.0, 1.25, 1.35, 2.7, 4.05, 5.4 kGy in the course of 0.1, 0.3, 0.5, 1.0, 1.5, 2.0 hours.

At the irradiation of solutions viper venom with γ-radiation to doses of 0.5, 1.0, 1.25, 1.35kGr in continuation of 0.1, 0.3, 0.5, 1.0, 1.5, 2.0 hours, changes in toxicity, as well as the absorption spectra of snake venom were not observed. However, with increasing doses of gamma radiation and irradiation time, there are significant changes in both the toxicity of venom, and their absorption spectra.

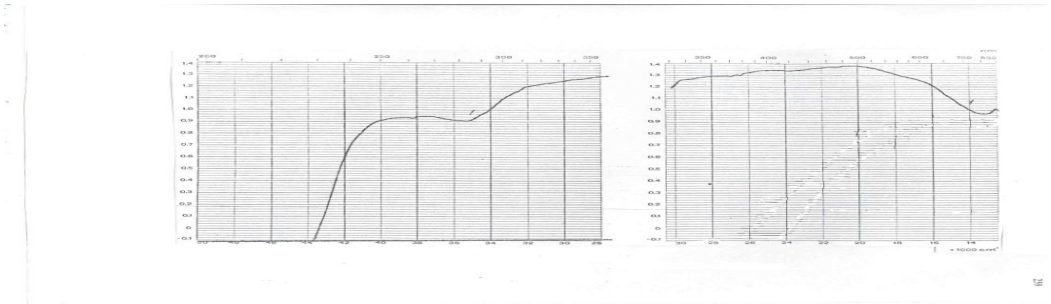
As a result of the lead experimental researches it is established, that under influence of small dozes g-radiations at dozes 2.7, 4.05, 5.4 kGy within 1.0, 1.5 and 2.0 hours results in gradual decrease of toxicity and it is possible pharmacological activity of enzymes of snake venom.

To prove this hypothesis, we studied the UV-VIS absorption spectra of not γ-irradiated and irradiated samples of viper venom, taken with a spectrometer Specord UV-VIS.

It should be noted that in comparison of the absorption intensity of control samples to samples of viper venom irradiated to a dose of 1.35 Gy ,there were no significant changes in the absorption of venom in the ultraviolet and visible regions.. This fact confirms the stability of the absorption spectra of viper venom. Thus, there is no shift of the absorption maxima in the ultraviolet and visible spectra of the samples poison.

On fig. 1 UV-VIS a spectrum of absorption of a water solution of venom vipera has been shown. Apparently from figure 1 venom vipera maxima of absorption are peculiar in the field of 285 nm and 800 nm. Absorption of group in these molecules represents the big resounding structures with the big number of the connected communications. Electrons, carrying out chemical communication are not located, but are the general for all structure. Therefore it is possible to speak about characteristic spectra of absorption for zootoxins.

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



Wave number in sm - 1

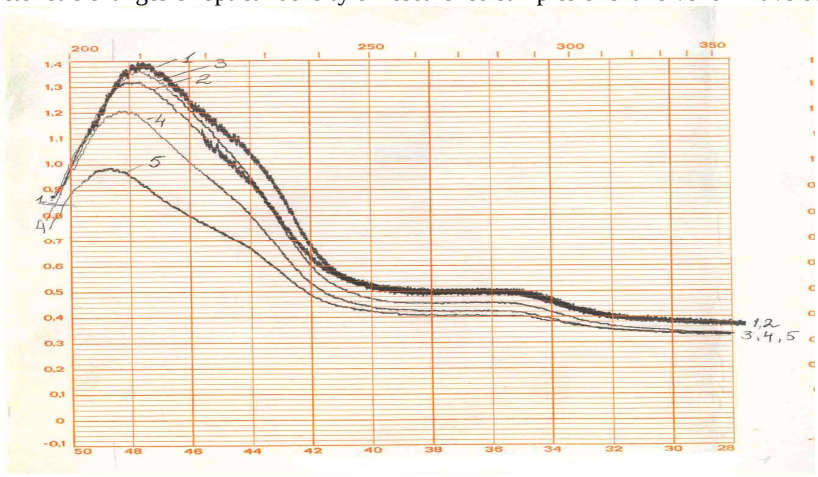
Fig. 1. UV-VIS a spectrum of absorption of venom Macrovipera lebetina obtusa

The revealed reduction of intensity of absorption at 260 and 300 nm testifies to course of biochemical reactions in a firm phase of separate enzymes zootoxins.

The absorption spectra of the venom in the infrared were studied on spectrometer Specord IR 75.

On fig. 2 spectra of absorption of samples of irradiated by γ-radiation venom vipera (water solutions and dry venom) have been shown.

Apparently from figure 2 at increase in a doze of an irradiation of solutions of venom vipera by γ-radiation characteristic changes of optical density of researched samples of snake venom have observed.



Wave number in sm – 1

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

It is necessary to note, that at comparison intensity of absorption of control samples (not irradiated) venom with samples zootoxin irradiated up to dozes 1.35 kGy it has not been revealed essential changes. From above-stated follows, that influence on venom vipers small dozes gamma-radiation up to dozes 1.35 kGy within 0.5 hours does not result in reduction of intensity of absorption and according to toxicity, and including pharmacological activity. The subsequent increase in a doze scale of radiation up to 2.7, 4.05, 5.4 kGy within 1.0, 1.5, 2.0 hours promotes proportional reduction intensity of absorption of snake venom and according to reduction, both toxicity, and pharmacological activity.

Thus, as a result of studies it was revealed that under the influence of low doses of γ-radiation to dose 2.7, 4.05, 5.4 kGy during 1.0, 1.5 and 2.0 hours leads to a decrease in the intensity of absorbance at 260 and 300 nm that indicates the occurrence of biochemical reactions in solid phase zootoxins individual enzymes, and in turn reduces possibly the toxicity and pharmacological activity of venom enzymes. However, exposure to a solution of venom to γ-radiation in doses to 1.35 kGy over 30 minutes stabilizes both toxicity and pharmacological activity, respectively while increasing the shelf life of aqueous solutions of snake venoms that can be used for sterilization of aqueous solutions of snake venom, and in turn, is important for the pharmaceutical industry in the production of injection-based zootoxins.

Summarizing the results on the infrared, visible and ultraviolet absorption spectra of the viper venom, it can be stated that the infrared, visible and ultraviolet spectra of standard and irradiated with gamma radiation patterns of viper venom was studied and systematized. The characteristic absorption band of snake venom were defined. The experimental data which are informative in the study of structural changes under the influence of environmental factors indicates the prospects of spectral methods for biophysical and biochemical studies as snake venom and also drugs on its basis.

The spectra were analyzed with a Jobin-Yvon HR 360 monochromator coupled with cooled CCD detector. The visible emitted light was collected from the same side. Luminescent properties at liquid N2 temperature was measured using a cold finger cryostat under vacuum.Fig.3. Photoluminescence spectra of venom Macrovipera lebetina obtusa at 300 K

Results of chemical analyses show that in structure of venom (Macrovipera lebetina obtusa) significant concentration of heavy metals (Cd, Ni, Zn, Cr, Pb) has been found out. In fig. 3 and fig. 4 emission spectra of Macrovipera lebetina obtusa under λ_{exc}= =337.1nm at room and 77 K temperature have been presented. It is visible, that PL specter of venom covers the area 350-800 nm and consists of the wide maximum at 520 nm and poorly appreciable maxima covering wavelengths of 400-500 nm at 300K. As a result of cooling samples up to nitric temperature (77K) new narrow appears maxima at 440 and 470 nm which is connected by metals taking place in structure of venom of Macrovipera lebetina obtuse.

Samples of venom were irradiated in special a ditch at 77K up to dozes of % 5 κGr. Before an irradiation samples were cleared from traces of oxygen. The irradiation was spent on air and in vacuum. Curves lighting were registered with a speed ~ 5^0/l.min. It is shown, that in an interval of temperatures 77-330K the curve lighting radiotermoluminence venom of viperas irradiated at 77K up to dozes 3 κGr is characterized not by a symmetric maximum at temperature 172K.

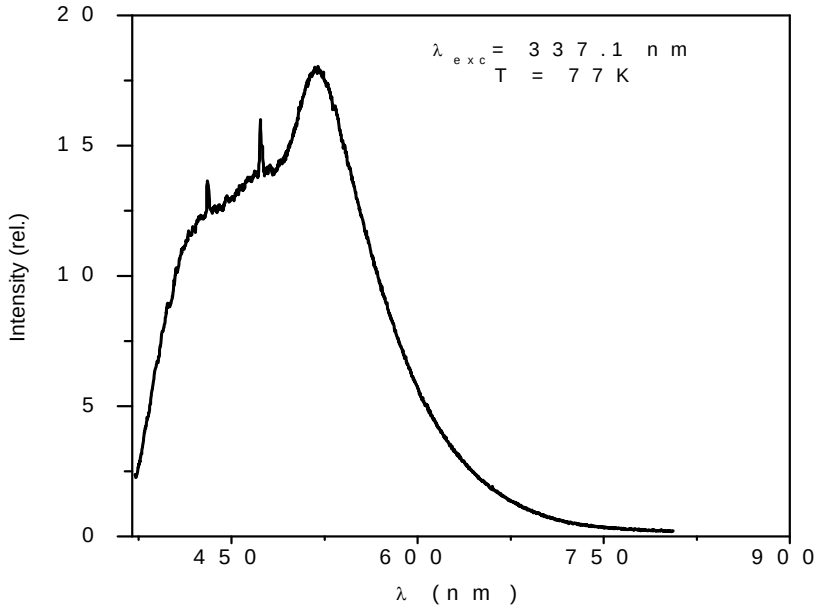


Fig.4. Photoluminescence spectra of venom Macrovipera lebetina obtusa at 77 K

Warming up to temperatures 320K results in monotonous decrease of intensity of a luminescence. It is established, that intensity of peak at 172K increases depending on doze D=0-15 kGr gamma irradiation under the linear law. The irradiation in air results in additional weak peak at 185K and to downturn of intensity at 172K. It is shown, that the peak of a luminescence at 172K can be dosimetric for an estimation of the absorbed doze.

The opportunity of change of medicinal properties of venom of Viperas is discussed at influence of small dozes of γ- radiation.

5. **CONCLUSION**

Thus, as a result of the lead researches it is revealed that under influence of small dozes γ-radiations up to dozes 2.7, 4.05, 5.4 kGy during 1.0, 1.5 and 2.0 hours results in reduction of intensity of absorption at 260 and 300 nm that testifies to course of biochemical reactions in a firm phase of separate enzymes zootoxin, and in turn results in decrease of toxicity and pharmacological activity of enzymes of snake venom.

However influence of γ-radiations on a solution of snake venom up to dozes 1.35 kGy within 30 minutes promotes stabilization as to toxicity, and pharmacological activity with simultaneous increase accordingly to the period of storage of water solutions of snake venom that can be used at sterilization of water solutions of snakevenom, and in turn is important for a farmaceutical industry by manufacture of injections on a basis zootoxins.

Acknowledgment

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

REFERENCES

1. Topchiyeva Sh.A., 2000. To the physics-chemical properties, application and some ecological aspects of changability of venom properties Journal Knowledge, (2): 48-52.
2. Topchiyeva Sh.A.et all.,2003. Influence of the ecological factors to number Vipera lebetina obtuse and chemical composition of venom Proceedings of the First International Conference on Enviromental Research and Assessment, pp: 34-37.

3. Topchiyeva Sh.A., Abiyev H.A., 2004. Comparative influence of impurity soils Apsheron of Azerbaijan on a chemical compound of venom Transcaucasian viper Vipera lebetina obtusa. J.Knowledge, (4): 74-78.
4. Iskenderov T.M., Topchiyeva Sh.A. ,2003. The influence of ecologicfl factors on chemical composition of venom and adaptation of Caucasian viper to the captivity conditions. J.Knowledge , (4) pp: 62-66.
5. Topchiyeva Sh.A., Isgenderov T.M., Mamedov O.G., 2003. The Influence of biospher pollution on chemical content of the Vipera venom in Azerbaijan. J. The contemporary successes of medicine and practical health of Azerbaijan, (1): 219-223.
6. Pavlovskiy E.N. ,1990. Poisonous the animal USSR, Moscow, pp: 242-271.
7. Pokuda A.A., 1972. Stability of solutions of venoms of snakes – vipera, halis, cobras at storage. Scientific reports of the higher school, biological sciences, (3) pp: 52-55.
8. Topchiyeva Sh.A., Magerramov A.M.,Musayeva N.N., et all. , 2004.Influence of small dozes γ-radiations on molecular mobility and pharmacological properties of venom of Vipera Lebetina Obtusa, 40th IUPOC International Symposium on Macromol, WORLD POLYMER CONGRESS MACRO, pp:119120.
9. Topchiyeva Sh.A., Mehrabova M.A.,Abiyev H.A., 2009. Investigationof electrophisical parameters of snake venom.The International conference on structural analysis of advanced materials,pp: 57-58.
10. Topchiyeva Sh.A., Mehrabova M.A., Abiyev H.A. , 2010.Investigation of electrophysical parameters of snake venom.WSEAS International Conference Advances in Biology, Bioengineering and Environment., pp: 85-89.
11. Topchiyeva Sh.A., Abiyev H.A., Mehrabova M.A., 2013. Investigation of Snake Venom of Vipera Lebetina Obtusa.5th WSEAS International Conference on Nanotechnology (NANOTECHNOLOGY '13), pp: 29-34.
12. Salafranca E.S., 1973. Irradiated cobra (Naja naja philippinensis) venom. Int. J. Appl. Radiatand isotop, 24(1): 59-60.