

Application of Ionising Radiation for Radiation Sterilization of Honey Bee Venom and Pollen (*Apis mellifera* L. Caucasica) and Preparations based on them

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ABSTRACT

The presented work presents data on study of radiation effects on bee venom using of methods radiothermoluminescence. During analysis, the samples were irradiated at 77 K and a dose of $0.2 \cdot 10^4$ Gy, while the heating rate when receiving the emission spectrum was 2.5 and 5.8 deg/min. Experimentally it was found that the radio-luminescence curve with a maximum at 200 K is typical for bee venom, and for pollen there is a maximum emission at 190 K, 240 K and a weak peak at 337 K. As a result of the studies conducted, γ -radiation doses ($D=0.5, 1.0, 1.5$ and 2.5 kGy) were selected for radiation sterilization of bee venom and preparations based on them. The obtained data can be recommended to the pharmaceutical industry for the radiation sterilization of poison and preparations based on bee venom in order to increase their shelf life.

Keywords: Honey bee, Venom, Pollen, Radiothermoluminescence, Radiation sterilization

INTRODUCTION

Among a huge number of biologically active substances of natural origin, one of the central places is occupied by animal poisons - a group of compounds unique in chemical nature and physiological action. Toxic and medicinal properties of them are known to man since ancient times. It should be noted that while some insects have more than 1.000.000 species, there are over 300.000 plant species in the world at present, Exceeding the plants in the number of species, the animals are incomparably poorer in their number of individuals, which probably explains the small study of the pharmacological and biochemical properties of biologically active substances of animal origin. From ancient times human beings, coming into contact with wildlife, came across various poisonous animals. Often, such a collision with poisonous insects, amphibians and reptiles led to the death of a person. At present, many biologically active substances of animal origin have been identified and their biochemical, physicochemical and pharmacological properties have been studied.

The Manual on International Statistical Classification of Diseases, Trauma and Causes of Death (WHO, Geneva) includes poisoning and toxic reactions due to exposure to poisonous plants and animals, including code E905.3 Bites

of hornets, wasps, bees. According to scientific data, bees existed for 56 million years before the appearance of primitive man [1].

The honey bee (*Apis mellifera* L.) is a part of the superfamily of Apoidea, which forms a large group (about 20% of the species composition) in the progressing order of Hymenoptera [2].

Animal venoms and toxins consist of proteins and peptides with potential medical and biotechnological use. Apitoxins are produced and secreted through a gland (acid gland) located in the posterior region of the *Apis mellifera* bee's abdomen, containing a complex mixture of substances with

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biological activity composed of enzymes, peptides, amino acids and other compounds. Apitoxin constituents have been used as analgesic, anticoagulant and anti-inflammatory agents for treating chronic diseases such as arthritis, rheumatism, tendinitis, bursitis, fibrosis, multiple sclerosis, and antineoplastic and have also been recently studied for its anti-acne and anti-fungal actions. Melittin is the main constituent of apitoxin. It has a low molecular weight (2.85 kDa) representing about 40-50% of apitoxin dry weight, where it synergistically acts with phospholipase A2 [3-10].

The venom proteome of the honeybee *A. mellifera* was recently investigated by integrating a combinatorial peptide ligand library approach with nanoLC FT-ICR MS/MS [11], resulting in 10^2 venom proteins and peptides, of which 33 were categorized as putative venom toxins.

The quantity and quality of bee venom depends on the age of the bees, forage quality and season. Bee venom contains trace elements phosphorus, copper, calcium, magnesium and other elements. Bee venom is composed of 18 amino acids. Enzyme action of bee venom is 30 times more potent than the snake. Its activity maintains for 7-8 years. No visible effect withstands freezing and heating to 110-115°C. Bee venom is resistant to the effects of acids and alkalis. Lead peptide in bee venom melittin is composed of 26 amino acids (50-55% dry matter venom) [12-15].

The study of the influence of environmental factors on living organisms, including on the products of their vital activity, the study of biochemical, physicochemical, pharmacological and toxicological properties of them is of great importance in the development of conditions for radiation sterilization of both venom and preparations based on them, which is the basis of the concept and the strategy of rational use of bioresources, one of the components of which in Azerbaijan is the honey bee.

Analyzing literary data, it is necessary to note that despite the thorough research of the products of vital activity of honey bees, there is a need for more in depth and comprehensive study of the products of their vital activity. This problem still does not lose its relevance.

Equally important is the increase in the shelf life of poison and other life products of the honey bee.

Based on the above, the purpose of the research was to identify doses of gamma radiation for the radiation sterilization of the products of life products of the honey bee.

MATERIALS AND METHODS

Experiments on the study of the effect of γ -radiation on the chemical composition of the products under study were carried out by the method of radio thermal luminescence. Irradiation and the effect of radiation on poison and pollen were performed on a K-25 isotope plant using ^{60}Co .

We have carried out experimental studies on the study and identification of the effects of low doses of gamma radiation on molecular mobility and changes in the characteristic temperatures of the poison and pollen.

As a result of experimental work, comparative studies were carried out on the effect of low doses of γ -radiation up to 10^4 Gy of exposure in air on molecular mobility, changes in characteristic temperatures, spectral characteristics and pharmacological properties of *Apis mellifera* L. honey bee products (poison, pollen), using radiothermoluminescence.

Gamma irradiation of the samples of poison and pollen in the form of tablets pressed at room temperature was carried out both in vacuum and in air from a source of ^{60}Co . The test samples in an amount of 0.2 mg were placed in metal cuvettes for irradiation with a gamma-ray source. Samples were irradiated with small doses of γ -radiation. On the basis of 5-fold measurements, their radio-thermoluminescence spectra were constructed.

THE RESULTS OF THE STUDY AND DISCUSSION

In radio thermoluminescent analysis, the samples were irradiated at 77 K and a dose of $0.2 \cdot 10^4$ Gy, while the heating rate upon receipt of the emission spectrum was 2.5 and 5.8 deg/min.

It is shown that for samples of bee venom, a characteristic of radio thermoluminescence with a maximum at 200 K is characteristic. The intensity of the glow from 200 K exponentially decreases to 300 K.

In contrast to the honeybee venom, for pollen, luminescence peaks are observed at 190 K, 240 K and a weak peak at 337 K. The nature of these peaks is currently being clarified. It is only established that the intensity and temperature position of the first peak do not depend on the heating rate. Irradiation in air leads to an increase in the intensity of luminescence of low-temperature peaks, which can be associated with the influence of dissolved oxygen O_2 in the samples under study.

Observing the change in the peaks of radio thermoluminescence, we can state the role of absorbed oxygen in changing the structure, physicochemical and pharmacological properties of the products of the life of honey bees (poison and pollen) *Apis mellifera* L. (**Figure 1**).

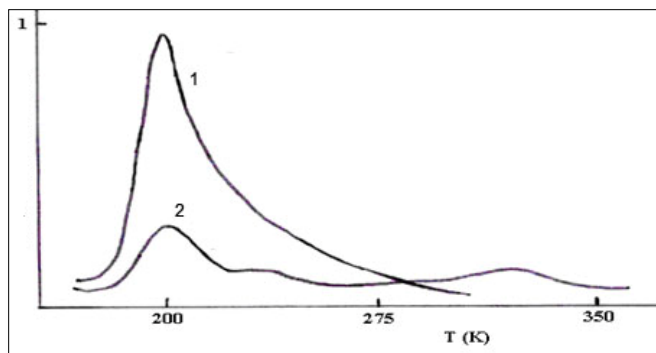


Figure 1. The spectrum of radio thermoluminescence of the products of the vital activity of honeybees *Apis mellifera* L. Caucasia: 1-poison, 2-pollen.

When choosing the conditions of radiation sterilization of bee venom, it was taken into account that not only quantum radiation, and also, γ -radiation is manifested as a result of processes that occur inside the particles themselves, including as a result of their transformation. It also appears when braking fast charged particles. From the literature it is known that the low radiation resistance of drugs, which are diluted aqueous solutions, eliminates the possibility of using ionizing radiation for their sterilization. In order for the radiation method to become suitable for these preparations, it is necessary to increase their resistance in the radiation-chemical relation. This can be achieved in two main ways. The first of these is to add to the solution of substances that protect the drug from the effects of radiolysis products of water. The second method consists in the irradiation of aqueous drugs in a frozen polycrystalline state. In this state, the phases of ice and solute are separated; therefore, the indirect effect of the products of ice radiolysis on the solute is practically impossible. The direct action at concentrations can be neglected. As a consequence, the radiation resistance of the drug increases significantly. Such an effect was found in the case of many aqueous drugs.

Many questions about the effect of low doses of γ -radiation, and other types of ionizing radiation on a living organism remain open. These questions are important for the technology of radiation sterilization of drugs.

The literature provides data on the study of bee venom, but many questions still remain unanswered and require in-depth analysis and study.

We used the method of radio-thermoluminescence to study the effects of radiation on bee venom, which allowed us to obtain information about the structural properties of the system, about the centers of charge stabilization of the primary products of the radiolysis of the poison, about the migration pathways absorbed during irradiation and so on.

Poison samples were irradiated in a special cuvette with gamma rays at 77 K to doses of 5 kGy. Before irradiation, the samples were cleaned from traces of oxygen. Irradiation

was carried out in air and in vacuum. The luminescence curves were recorded at a rate of $\sim 5^\circ/\text{min}$.

Studies conducted by us have shown that with an increase in the dose of radiation in solutions of a poison, there is a significant change in the optical density of the samples. In this case, there is a decrease in the intensity of absorption at 200 nm, which is a result of the occurrence of biochemical reactions in the solid phase of individual zootoxin enzymes.

Comparison of the intensity of absorption of non-irradiated samples of poison with samples of zootoxin irradiated to doses of 2.5 kGy did not reveal significant changes.

However, it was found that exposure to γ -radiation at doses of D=3.5, 4.0, 4.5 and 5.5 kGy for 3 min showed a decrease in toxicity of the poison. We assume that this is the result of a change in the pharmacological activity of the enzymes of the poison.

Thus, γ -radiation doses (D=0.5, 1.0, 1.5 and 2.5 kGy) are recommended for radiation sterilization of poison and preparations based on them.

Summarizing the experimental results obtained, it can be concluded that, under the influence of γ -radiation (up to doses of 2.5 kGy) on the poison per 3 min, there is no reduction in the intensity of absorption and accordingly, toxicity, including pharmacological activity.

A further increase in the dose of γ -radiation in the range from 3.5 to 5.5 kGy leads to a gradual decrease in both toxicity and pharmacological activity of the poison.

We assume that the effect of γ -radiation (up to doses of 2.5 kGy) on a solution of bee venom over a period of 3 min helps stabilize the toxicity and pharmacological activity of the poison. Undoubtedly, the stabilization of the pharmacological activity of the poison will lead to an increase in the shelf life of aqueous solutions of the poison and preparations based on the poison of the bees.

Therefore, experimentally proven figures (up to doses of 2.5 kGy and irradiation time of 3 min) can be used to sterilize aqueous solutions of bee venom.

CONCLUSION

It was revealed that radio-thermoluminescence with a maximum at 200 K is typical for poison and for pollen - maximum of luminescence at 190 K, 240 K and a weak peak at 337 K.

Recommended doses of-radiation (D=0.5, 1.0, 1.5 and 2.5 kGy) for radiation sterilization of poison and preparations based on them.

The obtained data can be recommended to the pharmaceutical industry for radiation sterilization of poison and their preparations in order to increase the shelf life.

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