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Prevention of Radiation Damage with the use of Biologically Active Components of the Honey Bee Apis Mellifera L. Caucasica

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ABSTRACT

The article deals with the problem of preventing radiation damage caused by external irradiation. As a radio protector, the use of a biologically active substance of animal origin, especially the venom of the honey bee Apis mellifera L. Caucasica and mellitine, is shown.

It was found that the lifespan of mice, injected with bee venom or mellitine 1 hour before irradiation 20 days after a single gamma irradiation of 60Co at a dose of D = 1.5G and 7 Gy at an irradiation dose rate of 1 Gy/min, increased from 10 % to 33.3% and from 25% to 71.1%, respectively.

Keywords: *Apis mellifera, venom, honey bee, mellitin;*

INTRODUCTION

The problem of preventing radiation damage caused by external irradiation, as well as increasing the resistance of the organism to the action of ionizing radiation, is a global issue and becomes more and more actual in recent times.An analysis of domestic and foreign literature has shown that scientists have studied the radioprotective properties of many drugs from various pharmacological groups, but the search is still ongoing.

The use of honey bee venom and its biologically active component -mellitine as a radioprotector has advantages over chemotherapy. The use of bee venom and mellitine as radioprotectors is based on its pharmacological properties, such as the general resistance of the organism to pathological processes.Contradictory data on the anti-radiation properties of bee venom are given in the literature.

Thus, the authors found that the at the injection of bee venom at a dose of 6 mg / kg w 24 hours before irradiation at a dose of 825 P, the survival rate of animals increased to 80% at 100% death of the control group mice. However, other authors in approximately the same methodological conditions failed to detect the anti-radiation effect of the venom. It was noted that the effect is absent when small doses of venom are injected, but with an increase in the doses of the venom, a tendency to detect the radiation effect appears [1, 2, 3, 4].

The lethal dose of bee venom for humans is about 0.2 g. For mice, the bee sting is toxic; they perish after the bite of one bee. On average, at the stinging, 0.3-0.8 mg of venom is injected to the bee. LD50, a bee venom for white laboratory mice, is 4 mg/kg of body weight [5].Later, the revealed dependence was confirmed, and it was found out that the effectiveness of the radio protection depends both on the effective dose of the venom and on the power of the radiation exposure.

It was established that bee venom is ineffective when irradiating animals with lethal doses (above 800 P). At the same time, when the dose of irradiation was reduced to 500-600 R, the survival of animals increased substantially. Thus, the survival rate of mice injected with bee venom 24 hours before irradiation 30 days after injury was 1.5 to 2 times higher than the survival of control animals [6].The authors also noted that with subcutaneous administration of melittin, the survival rate of mice 30 days after irradiation was 90 - 100%, while 60% of mice died in control. At the intraperitoneal injection of melittin 30 minutes prior to irradiation in a

lethal dose (1000 R), 42% of animals survived, while in control all mice died.

Koryagin (2006) studied the ability of bee venom at the course injection of in a dose of 0.1 mg / kg to increase the body's resistance to fractional gamma irradiation. The animals of the experimental group were injected intraperitoneally with venom for 7 days at a frequency of once a day.

The control group of animals was injected with physiological solution. One day after ending injections, the animals of the control and experimental groups were subjected to fractional total gamma irradiation for 5 days (0.6 Gy/day) at a dose rate of 1 Gy / min. The total dose was 3 Gy. Intact animals were not subjected to any effects [7].

A single total gamma irradiation (^{60}Co) at a dose of 3 Gy (dose rate of 1 Gy/min) was performed 7 days after the end of injections in the first series of experiments, after 14 days in the second series, 21 days later in the third series and 28 Days in the fourth series of experiments. The radioprotective effects of the bee venom were most pronounced in the first 3 weeks after the end of the injections of zootoxin. During this period, bee venom significantly increased the total number of surviving bone marrow cells. On the 28th day after the injection of venom, there was a significant decrease in its anti-radiation activity, although it did not disappear completely. The author believes that the radioprotective effect of bee venom is associated with the formation of a nonspecific adaptation reaction.

The results of the study showed that the bee venom had a clearly expressed radioprotective effect. The amount of megalocytes in the red bone marrow was 55% higher in the animals of the experimental group than in the control. In addition, in the animals of the experimental group, the content of the main germs of the hematopoiesis in the bone marrow of cells of the main hematopoiesis was marked in comparison with the control: myeloid (by 40%), lymphoid (by 70%), erythroid (3.3 times). The total number of leukocytes in the peripheral blood in the experiment was 63% higher than in the control group. The used dose of radiation had practically no effect on the total number of erythrocytes and hemoglobin content [8].

Bites of bees can cause life-threatening, and sometimes lethal, antigen-dependent anaphylactic reactions in the human body [9].

Melitin is the main component of bee venom. It has a molecular weight of 2840 D and is formed by 26 amino acid residues, 6 of which are charged. In aqueous media, melitin forms a tetramer consisting of two dimers with a molecular weight of 11.200 D. The N-terminal sequence of amino acid residues with 1-20 causes hydrophobicity of melitin, and the hydrophilic properties are due to residues 21-26 in the C-terminal region. Such amphiphilicity gives melitin the properties of a cationic detergent with a high surface activity [10]. Melitin has a hemolytic, antiviral, antifungal and cytolytic effect, causing the death of cells and their organelles. In view of the high cytotoxic and hemolytic activity of melitin, works are conducted for its practical use in oncology. Various mechanisms of melitin delivery to target cells, which can be cancer cells of the prostate, liver, and mammary glands, which's developement has inhibitory effect on the development, are proposed. Many foreign studies have reported that melitin can induce apoptosis and exhibit antiproliferative properties. Melitin could be an ideal remedy against cancer, but its use is limited by its lytic properties [11,12,13].

Also radio protective effect of zootoxins and medicines of animal origin isn't less actively studied. In particular, it has been established that the course injection of salamander venom inhibits the proliferation rate of bone marrow cells, which may be one of the mechanisms leading to an increase in the radio-resistance of hemopoietic cells [19]. The radioprotective properties of the salamander's venom have been revealed, as evidenced by the significantly higher content of irradiated animals in the blood of the formed elements, normalization of hemopoiesis processes, a decrease in the activity of free radical processes in comparison with the animals of the control groups. It was shown that the state of radioresistance, which arises from repeated injection of the venom in small doses, is preserved for a long time (up to 1 month). The authors conclude that the salamander venom injected into the body before irradiation causes the development of a general adaptation syndrome, a decrease in the proliferative activity of bone marrow cells, a decrease in the number of chromosomal aberrations, which determines

its ability to stimulate the radio-resistance of the organism [14].

In the studies of A.S. Koryagin (2006) the radioprotective effect of honey bee venom was studied in detail. For this purpose, rats were injected intraperitoneally with bee venom at a dose of 0.1 kg/kg for 7 days at a frequency of once a day. A single total gamma irradiation (^{60}Co) at a dose of 3 Gy (dose rate of 1 Gy/min) was performed 7 days after the end of injections in the first series of experiments, after 14 days in the second series, after 21 days in the third series and after 28 day - in the fourth series of experiments. The radioprotective effects of the bee venom were most pronounced in the first 3 weeks after the end of injections of zootoxin. During this period, bee venom significantly increased the total number of surviving bone marrow cells, effectively protected lymphoid and erythroid germs of hematopoiesis, and also had a definite radioprotective effect against the myeloid pool of cells. On the 28th day after the injection of the venom, there was a significant decrease in its anti-radiation activity, although it did not disappear completely. The author believes that the radioprotective effect of bee venom is associated with the formation of a nonspecific adaptation reaction [15].

At present, radioprotectors are the most studied and highly effective medical means of radiation protection [16]. However, their use is limited to the periods of use (exclusively till radiation exposure), often by a small therapeutic breadth and, as a consequence, enough high toxicity in optimal radioprotective doses [17, 18].

Thus, it was noted that bee venom at the course injection in a non-toxic dose is a long-acting radioprotector capable of effectively protecting the body from fractional gamma irradiation. It must be assumed that the basis for its anti-radial effect is the non-specific mechanisms of the activation reaction.

Proceeding from the foregoing, the purpose of this work was a comparative study of the radioprotective properties of native venom and mellitine.

Material and Methods

The material of the research was ecologically pure venom, collected from apiaries bees, located in the ecologically clean zone of Azerbaijan and mellitine, isolated from the venom of honey bees. The venom and mellitine were stored in a desiccator over the vapors of calcium chloride. Water solutions of venom and mellitine were prepared immediately before the experiment.

In order to determine the radioprotective effect of the native venom and mellitine, the experiments were performed on 3-month-old white mice weighing 18-22 grams. Animals were divided into two groups: control and experimental.

In order to study the radioprotective effect of the bee and mellitin venom, the mice of the control groups were injected with test samples dissolved in physiological solution at a dose of 0.1 mg/kg and 0.5 mg/kg. A single total gamma irradiation of ^{60}Co mice was carried out in doses of D = 1.5G and 7 Gy at irradiation dose rates of 1 Gy/min. The experiments were carried out in 12 series of experiments in vitro.

Results and Discussion

Preliminary experimental animals were injected intraperitoneally with solutions of bee venom and mellitine 30 minutes prior to irradiation.

It should be noted that the life expectancy of mice with intraperitoneal injection of bee venom at a dose of 0.1 and 0.5 mg / kg corresponds to 30 and 20 days, respectively.

The life span of control mice with intraperitoneal injection of mellitine at a dose of 0.1 and 0.5 mg/kg corresponds to 20 and 10 days, respectively.

The survival of mice with intraperitoneal injection of bee venom at a dose of 0.1 and 0.5 mg/kg of body weight followed by a single γ-irradiation of 60Co in a dose of D = 1, 5 and 7 Gy at a dose rate of 1 Gy/min is given in Table 1 and in Fig. 1.

From the data in Table 1 it follows that the lifespan of the first group of mice with the preliminary injection of the bee venom at a dose of 0.1 mg / kg and subsequent irradiation with doses of gamma radiation up to doses of D = 1 Gy increased to 35 days (in the control group, the life span was 30 days) that is increased by 14.3% and is 85.7%.

The lifespan of the second group of mice with the preliminary introduction of the bee venom at a dose of 0.1 mg/kg and subsequent irradiation with doses of gamma radiation up to doses of D = 5 Gy increased to 22 days (in the control group, the life span was 18 days), that is, increased by 22.2% and is 77.8%.

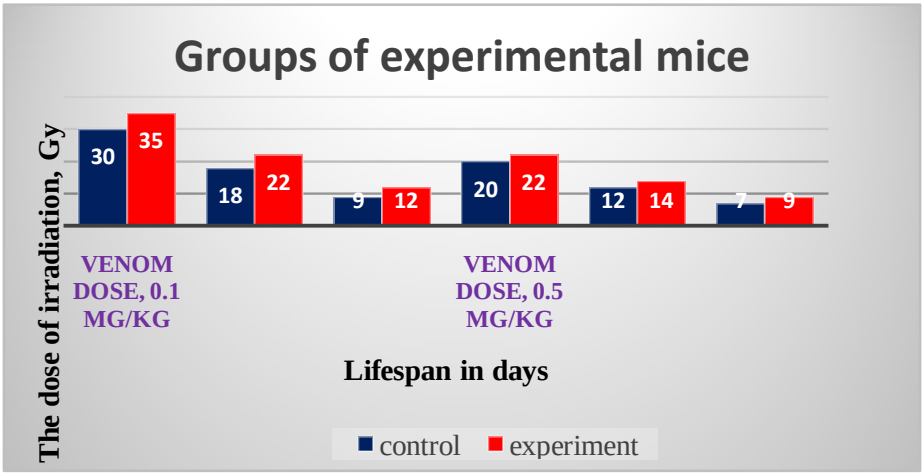

Figure1. *The lifespan of mice with intraperitoneal injection of bee venom at a dose of 0.1and 0.5 mg/kg of body weight followed by a single γ-irradiation of ^{60}Co at a dose of D = 1, 5 and 7 Gy at a dose rate of 1 Gy/min.*

Table1. *The survival of mice with intraperitoneal injection of bee venom followed by a single γ-irradiation of ^{60}Co*

The dose of irradiation, Gy	Groups of experimental mice			
	venom dose, 0.1 mg/kg		venom dose, 0.5mg/kg	
	control	experimental	control	experimental
	Lifespan in days			
1	30.0±0.5	35.0±0.7	20.0±1.2	22.0±0.3
5	18.0±0.9	22.0±0.4	12.0± 0.9	14.0±0.2
7	9.0±0.6	12.0±0.3	7.0±0.6	9.0±0.2

The life expectancy of the 3th group of mice with the preliminary injection of the bee venom at a dose of 0.1 mg / kg and subsequent irradiation with doses of gamma radiation up to doses of D = 7 Gy increased to 12 days (in the control group, the life span was 9 days), that is, increased by 33.3% and is 66.7%.

From the data in Table 1 it can be seen that the lifespan of the 4th group of mice with the preliminary injection of the bee venom at a dose of 0.5 mg / kg and subsequent irradiation with doses of gamma radiation up to doses of A = 1 Gy, increased the lifespan of the animals increased to 22 days (in the control group, 20 days), that is, increased by 10% and is 90%.

The lifespan of the 5th group of mice with preliminary injection of the bee venom at a dose of 0.5 mg/kg and subsequent irradiation with doses of gamma radiation up to doses of D = 5 Gy increased to 14 days (in the control group, the life span was 12 days), that is, increased by 16.7% and is 83.3%.

The lifespan of the 6th group of mice with preliminary injection of the bee venom at a dose of 0.5 mg/kg and subsequent irradiation with doses of gamma radiation up to doses of D = 7 Gy increased to 9 days (in the control group, the life span was 7 days), that is, increased by 28.6% and is 71.4%.

In order to study the radioprotective effect of the mellitin, a control group of mice was irradiated at D = 1, 5 and 7 Gy. Experimental groups of 2-3-month-old white mongrel mice with a total body weight of 18-22 grams were first intraperitoneally injected with bee venom at a dose of 0.1 (7,8,9 groups) and 0.5 mg/kg (10,11,12 groups) of body weight, 3 days at a frequency of once a day.

Then, the 7th experimental group of mice was subjected to a single gamma irradiation of ^{60}Co in a dose of D = 1 Gy at a dose rate of 1 Gy/min after 3 days, the 8th experimental group of mice after 3 days subjected to a single gamma irradiation of ^{60}Co in a dose of D = 5 Gy at a radiation dose rate of 1 Gy/min. The 9th experimental group of mice was subjected to a single γ-irradiation of ^{60}Co in a dose of D = 7 Gy at a dose rate of 1 Gy/min 3 days after irradiation.

Further, the 10th experimental group of mice after 3 days was subjected to a single gamma irradiation of 60Co in a dose of D = 1 Gy at an irradiation dose rate of 1 Gy/min, the 11th experimental group of mice after 3 days was

subjected to a single gamma irradiation of ^{60}Co in a dose of D = 5 Gy at an irradiation dose rate of 1 Gy/min, the 12th experimental group of mice was subjected to a single γ-irradiation of ^{60}Co in a dose of D = 7 Gy at a dose of 1 Gy/min 3 days after irradiation. The survival of mice with intraperitoneal administration of mellitine at a dose of 0.1 and 0.5 mg/kg of body weight followed by a single γ-irradiation of 60Co at a dose of D = 1, 5 and 7 Gy at a dose rate of 1 Gy/min is given in Table 2 and in Figure 2 . In experiments on mice with intraperitoneal fractional injection of venom or mellitin followed by a single γ-irradiation of ^{60}Co at a dose of A = 1, 3, 5 and 7 Gy, an increase in the lifespan of experimental groups of mice was noted at an irradiation dose rate of 1 Gy / min.

Table2. *The survival of mice with intraperitoneal injection of mellitin followed by a single γ-irradiation of ^{60}Co*

The dose of irradiation, Gy	Groups of experimental mice			
	Mellitin dose, 0.1 in mg/kg		Mellitin dose, 0.5mg/kg	
	control	experimental	control	experimental
	Lifespan in days			
1	20.0±0.5	**25.0**±0.6	8.0±0.3	8.0±0.3
5	11.0±0.3	16.0 ±0.4	6.0±0.2	4.0 ±0.2
7	4.0±0.2	5.0 ±0.2	3.0±0.1	1.0± 0.1

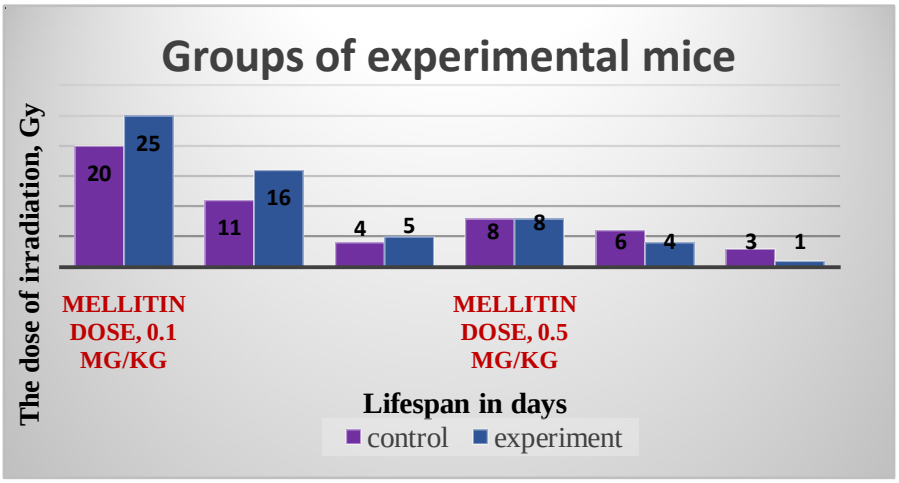

Figure2. *The lifespan of mice with intraperitoneal injection of mellitin at a dose of 0.1and 0.5 mg/kg of body weight followed by a single γ-irradiation of ^{60}Co at a dose of D = 1, 5 and 7 Gy at a dose rate of 1 Gy/min*

The survival rate of the experimental groups of mice (at the fractional injection of the bee venom and mellitine)), in comparision to the control group, ranged from 10% to 33.3% and from 25% to 71.1%, respectively.

We believe that the radioprotective effect of bee venom and mellitin is associated with the formation of a nonspecific adaptation reaction.

Thus, we detected a radioprotective effect of the honey bee venom and mellitin, which manifests itself in an increase in the lifespan of experimental animals subjected to γ-irradiation of ^{60}Co.

For the first time it was revealed that the injection of venom or mellitin is accompanied by a prolonged radioresistance, reducing the effect of ionizing radiation on the life span of mice under conditions of a single gamma irradiation.

Consecutive introduction of bee venom or mellitin to radiation exposure and in the early periods after irradiation allows to increase the survival time of experimental animals subjected to irradiation.

Investigation of the radioprotective effect of the course injection of small doses of bee venom under conditions of a single fractionated gamma irradiation makes it possible to broaden the notion of nonspecific radioresistance and suggests the possibility of creating new preparations on the basis of biologically active substances of animal origin that enhance the radioresistance of the organism.

Radioresistance, which develops in the body in response to multiple injections of bee venom, can successfully protect the body from fractionated gamma irradiation.

CONCLUSION

- At the intraperitoneal or intramuscular fractional injection of venom followed by a single gamma irradiation of ^{60}Co at a dose of D = 1, 5 and 7 Gy at an irradiation dose rate of 1 Gy/min, the life span of the experimental groups of mice increased in the range from 10% to 33.3 % .
- At the intraperitoneal or intramuscular fractional injection of mellitin followed by a single gamma irradiation of 60Co at a dose of D = 1, 5 and 7 Gy at an irradiation dose rate of 1 Gy/min, the life span of the experimental groups of mice increased in the range from 25% to 71.1%.

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