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# IDENTIGICATION OF THE INFLUENCE OF THE SHELF LIFE OF THE VENOM OF THE BLUNT-NOSED VIPER (MACROVIPERA LEBETINA OBTUSA) ON ITS TOXICITY

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# ბლაგვცხვირა გველგესლას (Macrovipera lebetina obtusa) შხამის მოქმედების ვადის განსაზღვრა მის ტოქსიკურობაზე

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## რეზიუმე

კვლევის მიზანი იყო ბლაგვცხვირა გველგესლას „Macrovipera lebetina obtus“ შხამის შენახვის ვადის გავლენის შეწავლა ლეტალური LD100 დოზის ტოქსიკურობაზე. ექსპერიმენტებისთვის გამოყენებული იქნა 1989, 1993, 2010 და 2015 წლების სხვადასხვა ვარგისიანობის შენახვის ვადის მქონე შხამის ნიმუშები. ექსპერიმენტები ჩატარდა 60 თეთრ ლაბორატორიულ თაგვზე (მამრებზე), რომელთა წონა იყო 18.0-22.0გ. ექსპერიმენტული კვლევების შედეგად დადგინდა, რომ თაგვებში სხვადასხვა ვარგისიანობის ვადის მქონე შხამის შეყვანა იწვევს ექსპერიმენტული ცხოველების სიცოცხლის ხანგრძლივობის ცვლილებებს საკონტროლო ცხოველებთან შედარებით, რაც გამოიხატება მათი სიცოცხლის ხანგრძლივობის მნიშვნელოვან ზრდაში 28-დან 90 წუთამდე. დადგინდა, რომ შხამის შენახვის ვადის ზრდასთან ერთად აღინიშნება შხამის ტოქსიკურობის შემცირება, რაც იწვევს ექსპერიმენტული თაგვების სიცოცხლის ხანგრძლივობის ზრდას. ასევე ექსპერიმენტის შედეგად, განისაზღვრა ბლაგვცხვირა გველგესლას შხამის LD50 ლეტალური დოზა თეთრ თაგვებზე ინტრაპერიტონეალური 3.0 მგ/კგ სხეულის მასაზე შეყვანით. დადგინდა ბლაგვცხვირა გველგესლას შხამის ლეტალური დოზა არის LD100, რომელიც „Macrovipera lebetina obtusa“ შეესაბება სხეულის წონის 6.0 მგ/კგ-ს.

**Introduction** Snake venoms are a complex of biologically active substances with extremely diverse properties and the ability to affect the main integrating systems of the body: blood and nervous system. Their toxic and medicinal properties have been known to man since ancient times. However, only in the 20th century did their scientifically substantiated use for therapeutic and diagnostic purposes begin. Snake venoms, including the venom of the blunt-nosed viper (Macrovipera lebetina obtusa), which exhibit pronounced biological effects, are complex protein mixtures. Proteins and peptides of snake venoms can influence all key processes of cell life. Proteases of viper and rattlesnake venoms cause local tissue damage, hemorrhagic edema, myonecrosis, and also have fibrinogenolytic, fibrinolytic, coagulating, and bradykinin-potentiating effects. Viper and rattlesnake venom's hemotoxins are represented by serine proteases and metalloproteases. Serine proteases are heat-labile endopeptidases; in terms of their action, they are similar to thrombin-like enzymes and kininogenases. The latter are heat-labile proteins that catalyze the hydrolysis of casein, hemoglobin, insulin, etc. Venom proteases can cause blood clotting and fibrinolysis disorders, leading to thromboembolism or hemorrhage. By acting on different links in the hemocoagulation cascade, proteases of most venoms have a dual effect; At first, intravascular blood

coagulation is observed, then the blood may lose its ability to coagulate for an extended period. PLA2 in abundance are present in the poisons of the snakes Elapidae and Viperidae. They share many crucial features with mammalian PLA2, such as the catalytic mechanism, the requirement for Ca2+, and a highly conserved primary and tertiary structure. In addition to a likely role in prey digestion, snake phospholipases typically exhibit a variety of pharmacological properties, including neuro-, myo-, and cardiotoxicity, as well as anticoagulant and proinflammatory effects [2]. They affect smooth muscle contraction, as well as induce muscle fiber depolarization [3], and can polarize the membrane of nerve endings [4]. In addition to myo- and neurotoxicity, some PLA2s exhibit antitumor activity and could be used as a new class of anticancer drugs. Some authors suggest that the anticarcinogenic effect depends not only on their enzymatic activity [6-8]. PLA2, acting as apoptosis inducers, exhibit antiproliferative properties on tumors such as Ehrlich ascites tumor, leukemia (Jurkat), and breast adenocarcinoma [9]. In addition to the anticarcinogenic effect, various PLA2s from snake venoms exhibit antiangiogenic properties, inhibiting cell adhesion and migration in vitro and in vivo [10]. Some PLA2s exhibit antiintegrin activity. For example, $PLA_2$ from the venom of the viper Macrovipera lebetina inhibits cell adhesion and migration of human microvascular endothelial cells (HMEC-1) and causes disturbances in the actin cytoskeleton and distribution of αvβ3 integrins [11]. $PLA_2$ from the venom of the viper Daboia russelii siamensis exhibits indirect hemolytic anticoagulant and cytotoxic activities. $PLA_2$ inhibits migration and reduces the survival of melanoma cells (SK-MEL-28), and also exhibits an anti-metastatic effect in vivo, reducing the colonization of melanoma cells (B16F10) in the lungs of mice (BALB/c) [12]. Currently, the main elements of snake venom are well studied. It was found that, like all other products of animal origin, the complex compounds contained in them consist mainly of four elements of the periodic table: carbon, hydrogen, nitrogen, and oxygen. The dry residue of the venom contains 43-49% carbon, 11-17% nitrogen, 6-9% hydrogen, and 15-22% oxygen. All other elements make up 5-8% of the weight of the dried venom. The largest part of this weight falls on sulfur (1.62-4.27% of the dry residue of the venom). The biological activity of snake phospholipases is extremely diverse and depends on both the structure of the enzyme and the type of cells on which they act [13,14,15]. According to the literature, the venom of the black-nosed viper at a dose of 2.8±0.7 mg/kg caused the death of 50% of white mice. Was to determine the effect of the venom of the blunt-nosed viper, Macrovipera lebetina obtusa, on its toxicity. The experiments used leopard viper venom samples obtained from several snakes caught simultaneously (2 to 5 specimens caught in 1989, 1993, 2010, and 2015). Aqueous solutions of leopard viper venom were prepared at concentrations of 3.0 and 6.0 mg/kg body weight. To determine the median lethal dose, increasing doses of aqueous venom solutions were injected into groups of experimental animals of the same weight. Mice were divided into experimental (20 heads) and control (20 heads) groups. Five mice were used in the experiment - four groups of five mice; animals in the groups were injected with 5, 10, 15, 20, 25, 30 μl of venom solution. Injections of leopard viper venom solutions were performed intraperitoneally into the right lower quadrant of the abdominal surface of mice using a 10-100 μl microsyringe (Brand, Germany). A group of mice (5 pieces) was used as a control group, which were injected intraperitoneally with leopard viper venom collected in 2016. Toxicometric experiments were performed at room temperature (25–27°C), and observations were terminated 24 hours after venom administration by counting the number of dead and surviving animals.

**Research results:** After poisoning mice with leopard viper venom, all experimental animals developed depression and died. However, life expectancy differed depending on the degree of toxicity of the venom. Throughout the experiment, mice were monitored every hour for survival, general condition, seizures, and activity, taking into account behavioral reactions. All experimental animals were kept in the

same conditions and on a common diet, with free access to water and food. The results of dead and surviving mice were recorded within 24 hours after injection in each test group. Visual observation of the condition of experimental animals showed that after the introduction of venom, both in the control and experimental groups, with the same concentration of leptodon venom, but with different retention times, in all groups of mice, after 5-10 minutes, a deterioration in the general condition was noted. Death in experimental mice occurred in the same way as in the control group after 25-36 minutes of intoxication. After the administration of Leptodon venom, in the first minutes after the administration of the zootoxin, all mice showed an increase in respiratory rate and impaired motor coordination. After 20-30 minutes, the condition of the experimental groups of mice worsened. The mice developed shortness of breath, lethargy, and edema. Then the condition of the mice gradually worsened, and the mice died. The life span of the experimental groups of mice, which were injected with venom for different storage periods, corresponded to 28-90 minutes. Thus, the analysis of the LD50 of the median lethal dose of the venomous secretion samples of the black-nosed viper revealed differences in their toxicity depending on the storage period. The results of the dead and surviving mice were recorded within 24 hours after the introduction of the poison in each test group. Toxicity is the inverse of the median lethal dose (concentration), that is, the higher the median lethal dose (concentration), the lower the toxicity of the substance. The toxicity indicator is the median lethal dose. The median lethal dose (LD50) is the dose of a substance that causes the death of 50% of animals with a single injection, expressed in milligrams of the substance per 1 kilogram of animal weight. Data on the identification of the effect of the storage period of the poison on the median lethal dose, including the lifespan of experimental mice, are presented in the table.

**Table 1. Effect of blunt-nosed viper venom with different shelf life on the lifespan of white mice (dose of i/p administered venom 3 mg/kg)**

| № | Venue shelf life of venom in years | Number of dead mice | Lethality, % | Lifespan of mice in minutes | |
|---|---|---|---|---|---|
| | | | | Control | Experiment |
| 1 | 1989 | 2 | 40 | 40-60 | 90-110 |
| 2 | 1993 | 2 | 40 | 40-60 | 80-120 |
| 3 | 2010 | 3 | 60 | 40-60 | 65-150 |
| 4 | 2015 | 3 | 60 | 40-60 | 40-60 |

Based on the results of the conducted experimental studies, the lethal dose LD50 of the studied samples of lebetina viper venom when administered intraperitoneally to white mice was 3.0 mg/kg of body weight. Based on the results of the conducted study, the lethal dose LD100 of the studied samples of dry lebetina viper venom (Macrovipera lebetina obtusa) was 6.0 mg/kg of body weight when administered intraperitoneally to white mice with a venom solution (Table 2).

**Table 2. Results of dead and surviving mice within 24 hours after injection of poison at a dose of 6.0 mg/kg body weight**

| № | Venue shelf life of venom in years | Number of dead mice | Lethality, % | Lifespan of mice in minutes | |
|---|---|---|---|---|---|
| | | | | Control | Experiment |
| 1 | 1989 | 3 | 60 | 28-36 | 70-90 |
| 2 | 1993 | 4 | 80 | 28-36 | 60-70 |
| 3 | 2010 | 5 | 100 | 28-36 | 45-60 |
| 4 | 2015 | 5 | 100 | 28-36 | 28-36 |

Thus, comparing the obtained data with the results of literary sources, it can be stated that with an increase in the shelf life, a noticeable decrease in the toxicity of the poison samples was noted. With an increase in the shelf life, in all likelihood, a change is noted in both the physicochemical properties of the leopard viper venom and its toxicity and pharmacological activity. From the above, it can be stated that the effect of the shelf life on the toxicity and pharmacological properties of the leopard viper venom has been revealed. In addition, a pattern has been revealed in the decrease in the toxicity of the poison depending on the shelf life. It has been experimentally established that with an increase in the shelf life, a corresponding decrease in the toxicity of the poison is observed. Experimentally, on 40 white outbred mice, changes in the toxicity of the leopard viper venom were established depending on the shelf life of the samples (Figs 1 and 2). Fig. 1. Diagram of the dynamics of survival of mice intoxicated with leopard viper venom at 3mg/kg

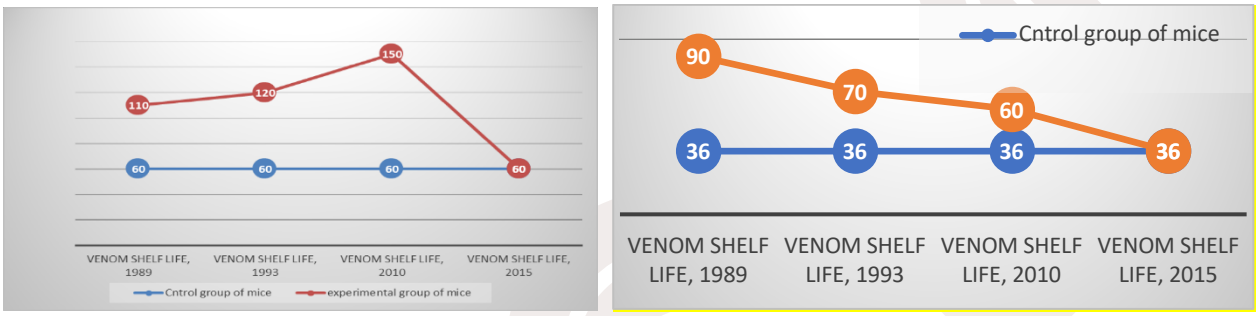


**Fig. 2. Diagram of the dynamics of survival of mice intoxicated with leopard viper venom at 6 mg/kg.**

Comparing the experimental data obtained by us with the results of literary sources, it should be noted that with an increase in the shelf life of the poison, a noticeable decrease in toxicity is noted, up to its complete loss, which is associated with a change in both the physicochemical properties and pharmacological activity, including the toxicity of the leopard viper venom.

**Conclusions:** 1. It was established that the introduction of poison with different shelf lives to white mongrel mice causes changes in the life expectancy of experimental animals compared to the control ones, which is manifested in a significant increase in the life expectancy of experimental groups of animals from 28 to 90 minutes. **2.** It was revealed that with an increase in the shelf life of the poison, a decrease in the toxicity of the poison is noted, which leads to an increase in the life expectancy of the experimental mice. 3. As a result of the experimental studies, the lethal dose LD50 of the studied samples of leopard viper venom with intraperitoneal administration to white mice was determined to be 3.0 mg/kg of body weight. 4. Based on the results of the studies, the lethal dose LD100 of the studied samples of dry venom of the blunt-nosed viper (Macrovipera lebetina obtusa) was 6.0 mg/kg of weight.

Thus, a regular decrease in the toxicity of the leopard viper venom samples was noted, which is manifested in the life expectancy of experimental animals. The results obtained must be considered both during storage and in the manufacture of preparations based on snake venom. It should be noted that the ability of snake venom proteins to exhibit selective toxicity, along with high specificity and affinity for cells and tissues, makes them suitable for practical use as pharmaceutical agents. Many components of snake venoms exhibit various useful pharmacological properties and can be used in the treatment of various diseases, such as cancer, hemophilia, cardiovascular, autoimmune, and neurodegenerative diseases.

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# IDENTIGICATION OF THE INFLUENCE OF THE SHELF LIFE OF THE VENOM OF THE BLUNT-NOSED VIPER (MACROVIPERA LEBETINA OBTUSA) ON ITS TOXICITY

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## SUMMARY

The presented article studies the lethal dose LD100 of the venom of the blunt-nosed viper (Macrovipera lebetina obtusa). This work aimed to identify the effect of the shelf life of the blunt-nosed viper venom on its toxicity. Venom samples with different shelf lives collected in 1989, 1993, 2010, and 2015 were used for the experiments. The experiments were conducted on 60 white outbred laboratory mice (males) weighing 18.0-22.0 g. As a result of experimental studies, it was found that the introduction of venom with different shelf lives to mice causes changes in the life expectancy of experimental animals compared to the control animals, which is manifested in a significant increase in their life expectancy from 28 to 90 minutes. It was found that with an increase in the shelf life of the venom, a decrease in the toxicity of the venom is noted, which leads to an increase in the life expectancy of the experimental mice. It has been experimentally revealed that with an increase in the shelf life of the poison, a decrease in the toxicity of the poison is noted, which leads to an increase in the life expectancy of the experimental mice. As a result of experimental studies, the lethal dose of the blunt-nosed viper venom $LD_{50}$ of the studied samples with intraperitoneal administration to white mice was determined to be 3.0 mg/kg of body weight. The lethal dose $LD_{100}$ of the studied samples of blunt-nosed viper venom was established, which (Macrovipera lebetina obtusa) corresponded to 6.0 mg/kg of body weight.

**Keywords:** venom, blunt-nosed viper, Macrovipera lebetina obtusa, toxicity, mice, $LD_{50}$, $LD_{100}$