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PHARMACOLOGICAL AND BIOCHEMICAL STUDIES OF VIPER VENOM (MACROVIPERA LEBETINA OBTUSA LINNAEUS, 1758) DEPENDING ON SHELF LIFE

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შაფიგა ტოპჩიევა [1], ლამია იილდიზ [2], ნანა გორგასლიძე [3]

გველგესლას შხამის ფარმაკოლოგიური და ბიოქიმიური კვლევები (Macrovipera lebetina obtusa Linnaeus, 1758) შენახვის ვადის მიხედვით

[1]აზერბაიჯანის რესპუბლიკის მეცნიერებისა და განათლების სამინისტროს ზოოლოგიის ინსტიტუტი; [2]ტოკატ გაზიომაშის უნივერსიტეტის ბაზისური სამედიცინო მეცნიერების/ფიზიოლოგიის დეპარტამენტი, თურქეთი; [3] თბილისის სახელმწიფო სამედიცინო უნივერსიტეტის სოციალური და კლინიკური ფარმაციის დეპარტამენტი, საქართველო

რეზიუმე

ნაშრომში წარმოდგენილია გველგესლას შხამის ბიოქიმიური და ფარმაკოლოგიური კვლევების ექსპერიმენტული მონაცემები (Macrovipera lebetina obtusa Linnaeus, 1758), შესაბამისად, 1989-2015 წლებში შეგროვებული ნიმუშებში ფოსფოლიპაზა A2-ის აქტივობის ცვლილებების დინამიკა, პროტეოლიზური აქტივობა (PA) და შხამის ნიმუშების L-ამინომჟავა ოქსიდაზას (AO) აქტივობა. ყველა შხამში აღმოჩენილია შემდეგი ფერმენტები: ჰიალურონიდაზა, ფოსფოლიპაზა A2, ნუკლეოტიდაზა, ფოსფოდიესტერაზა, დეზოქსირიბონუკლეაზა, რიბონუკლეაზა, ადენოზინტრიფოსფატაზა, ნუკლეოტიდ პიროფოსფატაზა, L-ამინომჟავა ოქსიდაზა (გარდა ზღვისა და ოპეპტოქსინებისა) ცილები სპეციფიკური თვისებებით და არაორგანული კომპონენტებით. L-ამინ ოქსიდაზას მრავალი ფუნქციური თვისება, როგორიცაა ციტოტოქსიკურობა, ანტიკოაგულანტი და ჰემორაგიული ეფექტები, ანტიბაქტერიული აქტივობა და რიგი სხვა ფიზიოლოგიური პროცესები. გველგესლას შხამის ფერმენტული აქტივობის დაქვეითება გამოვლინდა გრძელვადიანი შენახვის დროს 1989 წლიდან 2015 წლამდე პერიოდში. 2015 წელს შეგროვებული გველგესლას შხამის ნიმუშებში PA შემცველობა უფრო მაღალია 2.73, 2.0, 1.46, 1.71-ჯერ, ვიდრე შხამის ნიმუშებში, რომლებიც შეგროვდა 1989, 1991, 1993, 2010 წლებში, შესაბამისად.

The toxicity of venom is an integral characteristic and reflects the overall effect of the toxin on a living organism, while the enzymes of snake venoms have specific points of application and mechanisms of action [1,2].

Snake venoms are a complex mixture of organic and inorganic substances. Their main components are proteins and peptides of varying degrees of complexity, built from amino acids. These substances account for 65-85% of the weight of the dry residue of the poison. In addition, snake venoms contain free amino acids, fats, fatty acids, inorganic salts, and other substances [3,4].

The venom crystallizes when dried and, if properly stored, remains active for up to 26 years. The following enzymes were found in all venoms: hyaluronidase, phospholipase A2, nucleotidase, phosphodiesterase, deoxyribonuclease, ribonuclease, adenosine triphosphatase, nucleotide pyrophosphatase, L-amino acid oxidase (except for sea snakes) and exopeptidase, polypeptides (neuro- and hemotoxins), proteins with specific properties and inorganic components [5,6,7].

It should be noted that the wide range of peptides and proteins with different biological functions makes animal venoms a valuable source of new compounds, both for use in basic research and for the development of new drugs. The development and improvement of physicochemical and biochemical methods for identifying and standardizing snake venom will provide the pharmaceutical industry with a

high-quality and environmentally friendly, natural product, with a given toxicity and pharmacological activity.

Based on the above, the purpose of this work is to study the dynamics of changes in the activity of phospholipase A2, proteolytic activity (PA), and activity of L-amino acid oxidase (AO) of samples of poison, the venom of the viper (Macrovipera lebetina obtusa Linnaeus, 1758) with different storage periods, collected over the period time from 1989-2015.

Research results. The enzymatic activity of phospholipase A2, proteolytic activity (PA), and L-amino acid oxidase (AO) activity in standard samples of viper venom collected in 1989, 1991, 1993, 2010, and 2015 were studied.

The activity of the phospholipase A2 enzyme in the venom of the Transcaucasian viper was determined by the titrimetric method (**Table 1**). Statistical processing of experimental data was carried out using the Student's test.

Table 1. Phospholipase A2 activity in samples of viper venom (IU/mg)

Year of venom collection	*Enzyme activity (IU/mg)*
1989	30,5±1,8
1991	32,5±1,5
1993	34,6±0,9
2010	40,0±2.2
2015	42,0±1,8

The activity of phospholipase A_2 during storage since 1989 with an extension of the storage period decreased by 11.5 IU/mg compared to the venom sample collected in 2015. In all likelihood, as a result of biochemical changes, there is a decrease in enzyme activity from 100% to 72.61, 77.38, 82.38, and 95.24%, respectively, which must be taken into account when producing and storing preparations based on snake venom.

It has been experimentally established that the maximum PA value is observed in venom samples collected in 2015 and is 0.82 IU/mg. In samples of snake venom collected in 2015, the PA content is 2.73, 2.0, 1.46, and 1.71 times higher than in samples collected in 1989, 1991, 1993, and 2010, respectively (**Table 2**).

Table 2. Proteolytic activity (PA) and L-amino acid oxidase (AO) activity in viper venom samples

Year of venom collection	*PA, IU/mg*	*AO, IU/mg*
	M ±m	*M ±m*
1989	0,30±0.02	0.09±0.01
1991	0,41±0.01	0.11±0.02
1993	0.56±0,02	0.15±0.01
2010	0.70±0,03	0.26±0.02
2015	0.82±0,01	0.30±0.01

In venom samples collected in 2015, the L-amino acid oxidase activity was 0.30 IU/mg. In samples of viper venom collected in 2015, the content of AO is higher by 3.33, 2.73, 2.0, and 1.15 times than in samples collected in 1989, 1991, 1993, and 2010, respectively.

Conclusions

1. A decrease in the enzymatic activity of viper venom was revealed during long-term storage from 1989 to 2015 from 42 IU/mg to 30.5 IU/mg.

2. It was revealed that the duration of storage of viper venom has a significant effect on the enzymatic activity of the poison. With long-term storage of viper venom samples from the storage period from 1989, 1991, 1993 to 2010, a significant decrease in the activity of the phospholipase A2 enzyme is observed, which corresponds to 72.61, 77.38, 82.38, and 95.24%, respectively.
3. It has been established that in samples of viper venom collected in 2015, the PA content is higher by 2.73, 2.0, 1.46, and 1.71 times than in venom samples collected in 1989, 1991, 1993, and 2010, respectively.
4. The level of activity of L-amino acid oxidase (AO) was revealed. In venom samples collected in 2015, the L-amino acid oxidase activity is 0.30 IU/mg. In samples of viper venom collected in 2015, the content of AO is higher by 3.33, 2.73, 2.0, and 1.15 times than in samples collected in 1989, 1991, 1993, and 2010, respectively.

From the above, it follows that the average values of enzymatic activity of the venom collected in 1989 turned out to be significantly lower than the enzyme activity in samples of viper venom collected in 2015. The results of experimental data can be used in identifying, standardizing, and determining the pharmacological activity of viper venom.

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SUMMARY

The purpose of this work is to study the dynamics of changes in the activity of phospholipase A2, proteolytic activity (PA), and activity of L-amino acid oxidase (AO) of venom samples, venom of viper (Macrovipera lebetina obtusa Linnaeus, 1758) with different storage periods, collected over a while since

1989 - 2015 Enzyme activity in samples of Transcaucasian viper venom was determined by titrometric method.

Phospholipase A2 activity during storage since 1989 decreased by 11.5 IU/mg compared to the venom sample collected in 2015. In all likelihood, as a result of biochemical changes, there is a decrease in enzyme activity from 100% to 72.61, 77.38, 82.38, and 95.24%, respectively, which must be taken into account when producing and storing preparations based on snake venom.

In venom samples collected in 2015, the L-amino acid oxidase activity is 0.30 IU/mg. In samples of viper venom collected in 2015, the content of AO is higher by 3.33, 2.73, 2.0, and 1.15 times than in samples collected in 1989, 1991, 1993, and 2010, respectively. A decrease in the enzymatic activity of viper venom was revealed during long-term storage from 1989 to 2015.

It has been experimentally established that during long-term storage of viper venom samples from the storage period from 1989, 1991, 1993 to 2010, there is a significant decrease in the activity of the phospholipase A2 enzyme, which corresponds to 72.61, 77.38, 82.38, 95.24%, respectively.

It was revealed that in samples of viper venom collected in 2015, the PA content is higher by 2.73, 2.0, 1.46, and 1.71 times than in venom samples collected in 1989, 1991, 1993, and 2010, respectively.

The level of L-amino acid oxidase (AO) activity was determined. In venom samples collected in 2015, the L-amino acid oxidase activity is 0.30 IU/mg. In samples of viper venom collected in 2015, the content of AO is higher by 3.33, 2.73, 2.0, and 1.15 times than in samples collected in 1989, 1991, 1993, and 2010, respectively.

The results of experimental data can be used in identifying, standardizing, and determining the pharmacological activity of viper venom.

Keywords: viper venom, Macrovipera lebetina obtusa Linnaeus, enzyme activity, phospholipase A2, L-amino acid oxidases, proteolytic activity