SHAFIGA TOPCHIYEVA [1], SAMIRA SALAHOVA [2], LAMIYYA YILDIZ [3],
NANA GORGASLIDZE [4], RUMIYYA KARIMOVA [5]

BIOCHEMICAL AND PHYSICOCHEMICAL PROPERTIES OF HONEY BEE VENOM, APIS MELLIFER

[1]Institute of Zoology of the Ministry of Science and Education of the Republic of Azerbaijan, [2] Socar Polymer, Baku, Azerbaijan, [3]Tokat Gaziosmanpasha University, Medical Faculty, Department of Physiology, Turkey, [4]Tbilisi State Medical University, Department of Social and Clinical Pharmacy, Tbilisi, Georgia, [5]Azerbaijan Medical University, Baku

Doi: https://doi.org/10.52340/jecm.2025.04.23

შაფიგა თოფჩიევა [1], სამირა სალაჰოვა [2], ლამია ილდიზ [3],
ნანა გორგასლიძე [4], რუმია კარიმოვა [5]

„Apis mellifera“ ფუტკრის შხამის ბიოქიმიური და ფიზიკურ-ქიმიური თვისებები

[1]ზოოლოგიის ინსტიტუტი, აზერბაიჯანი; [2]სოკარ პოლიმერი, აზერბაიჯანი; [3]თოქათის ღაზიოსმანფაშას უნივერსიტეტი, თურქეთი; [4]თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი; [5]აზერბაიჯანის სამედიცინო უნივერსიტეტი

რეზიუმე

კვლევის მიზანი იყო ფუტკრის „Apis mellifera“-ს შხამის ბიოქიმიურ და ფიზიკურ ქიმიურ მახასიათებლებზე გარემოს დამაბინძურებლების გავლენის შესწავლა ფოსფოლიპაზა A2-ის აქტივობის მაგალითზე. ფუტკრის „Apis mellifera“ შხამის ნიმუშები, შეგროვდა გაზაფხულზე ქალაქ ბაქოს, სუმგაითისა და ზაქათალის მიმდებარე საფუტკრეებში. შხამში ფერმენტ ფოსფოლიპაზა A2-ის აქტივობა განისაზღვრა ტიტრიმეტრიული მეთოდით. ექსპერიმენტულად დადგინდა, რომ ფოსფოლიპაზა A2-ის ყველაზე დაბალი აქტივობა დაფიქსირდა ბაქოს მიდამოებში გავრცელებულ ფუტკრის შხამში (134,7 სე/მგ), სუმგაითის-155,7სე/მგ, ყველაზე მაღალი, კი ზაქათალას ნიმუშში (183,7სე/მგ, P<0,001). N-H, O-H, ≡C-H, S-H ჯგუფების IR-ანალიზით შთანთქმის სპექტრი 3675, 3420, 2963, 2918სმ-1 სიხშირის დიაპაზონშია. CH2 ჯგუფებში გაჯერებული ნახშირბადის ატომთან დაკავშირებული წყალბადის სპექტრი 1385სმ-1 სიხშირის დიაპაზონში გამოვლინდა. 1733, 1653 1295,1250სმ-1 სიხშირის დიაპაზონში, ასევე აღინიშნება კუმულირებული ბმების სპექტრები - C = N, C=C; C=O. ინფრაწითელ სპექტრში ნიმუშების ანალიზით მიღებული იქნა OH ჯგუფების ზოლი 617,525,419 სმ-1 დიაპაზონში და OH ჯგუფების დეფორმაციის ზოლი 1540 სმ-1 დიაპაზონში. შესაძლებელი გახდა ცხოველური წარმოშობის მრავალი ბიოლოგიურად აქტიური ნივთიერების გამოყოფა და მათი ბიოქიმიური, ფიზიკურ-ქიმიური, ფარმაკოლოგიური და ტოქსიკოლოგიური თვისებების შესწავლა. აპიტოქსინის ბიოქიმიური, ფიზიკოქიმიური თვისებების შესწავლას დიდი მნიშვნელობა აქვს როგორც შხამის, ასევე მათზე დაფუძნებული პრეპარატების შემუშავებისათვის.

Introduction. Natural animal venoms - their toxic and medicinal properties have been known to man since ancient times. Apitoxin is a venom produced by bees. It is a complex chemical compound, rich in protein substances, and has pharmacological effects. The quality, moisture content, protein analysis, and cytotoxicity of extracted apitoxin collected in different parts of Azerbaijan were compared with Artemia salina L. Both apitoxins showed significant differences (P>0.05) in terms of protein profile, with type 1 showing a higher abundance (77.8%) than type 2 (51.9%), and represented polypeptide chains, where more than 50% of their nitrogenous components were with a molecular weight of less than 10 kDa. Cytotoxicity analysis showed that the LD50 of the first type of apitoxin was 71.5μg mL-1, and that of the second type was 191.6μg mL-1. Thus, the area of accumulation of apitoxin in the collector affects the quality of the product, if the moisture and protein content comply with the standards recommended in specific legislation. [1,2,3]. Bee venom contains trace elements: phosphorus, copper, calcium, magnesium, and other elements. Bee venom consists of 18 amino acids. The enzymatic activity of bee venom is 30 times stronger than that of snake venom. Its activity is maintained for 7-8 years. The lead peptide

contained in bee venom melittin consists of 26 amino acids (50-55% of the serum substance of the venom) [4,5,6]. Bee venom contains enzymes - phospholipase A2, hyaluronidase, phosphatases, alpha-glucosidase, beta-galactosidase; toxic polypeptides and biogenic amines. The chemical composition of bee venom is complex; the main part of the venom is protein, high-molecular-weight (enzymes), and low-molecular-weight (peptides) substances. Bee venom contains microelements such as phosphorus, copper, calcium, and magnesium. The venom dissolves well in acids and water, does not dissolve in alcohol, withstands freezing and heating to 100-115°C, and is sensitive to sunlight. Bee venom is hygroscopic, and when stored in a loosely closed container, it loses its biological activity. When stored in a dark, cool place it can retain its pharmacological properties for up to 8 years, is sterile when diluted 1:50000, and is resistant to the effects of acids and alkalis. The lethal dose of bee venom for humans is about 0.2 g. For mice, a bee sting is toxic; they die after being stung by one bee. On average, a bee injects 0.3-0.8 mg of venom when stinging. LD50 of bee venom for white laboratory mice is 4 mg/kg of body weight. Based on the above, this work aimed to study the influence of environmental pollutants on the biochemical and physicochemical characteristics of honey bee venom. The research material was samples of bee venom (Apis mellifera L.), collected in the spring from bees living in apiaries in the vicinity of Baku and Sumgait. The titrimetric method determined the activity of the enzyme phospholipase A2 in the venom. A control experiment was conducted in parallel, where water was taken instead of venom. As a result of the conducted studies, it was revealed that the activity of the enzyme phospholipase A2 in the venom was determined by the titrimetric method. A control experiment was conducted in parallel, where water was taken instead of venom. The conducted studies revealed that the phospholipase activity in the venom collected in the vicinity of the cities of Baku, Sumgayit, and Zakatala was 134.7 IU/mg (Baku), 155.7 IU/mg (Sumgayit), and 183.7 IU/mg (Zakatala), respectively. During the analysis of the structural group of the venom and the interpretation of the infrared spectra, the absorption bands of individual functional groups of the venom biomolecules were determined. (Fig.1 1).

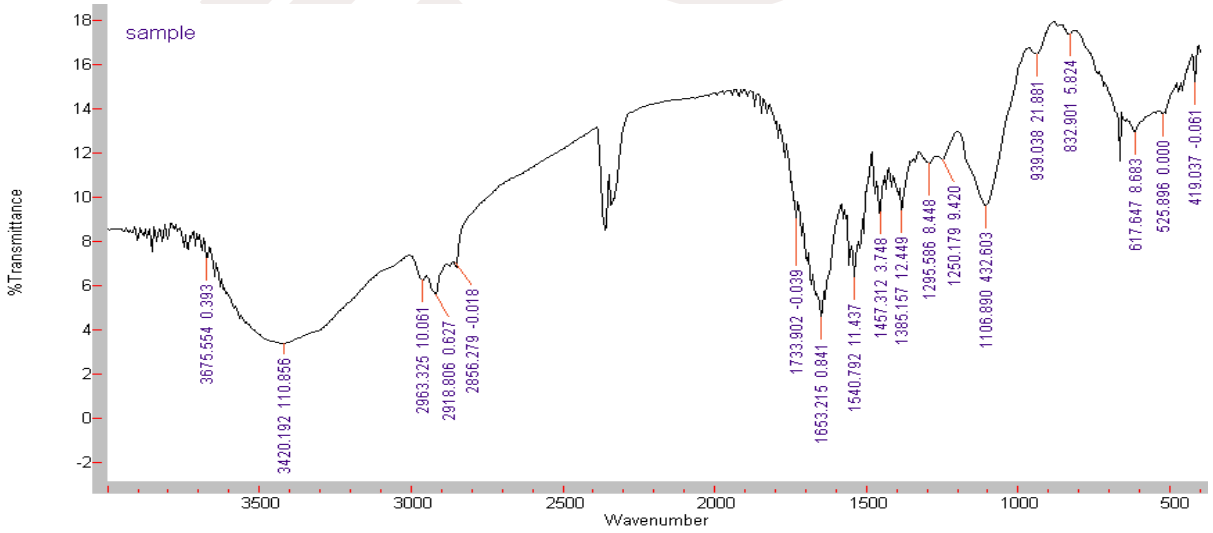

Fig. 1. IR transmission spectrum of honey bee Apis mellifera L. venom

Thus, the lowest activity of phospholipase A2 was established in the vicinity of the city of Baku (134.7 IU/mg), the highest in Zakatally (183.7 IU/mg, $P<0.001$). During the structural-group analysis and interpretation of the infrared spectra of the poison, the absorption bands of individual functional groups of the poison biomolecules were assigned. The absorption bands of the stretching vibrations of the N-H, O-H, ≡C-H, S-H groups in the frequency range of 3675, 3420, 2963, 2918cm-1 in the IR spectra of the

poison and the stretching vibrations of hydrogen associated with the saturated carbon atom in the CH_2 groups in the frequency range of 1385 cm^{-1} were identified (fig.2).

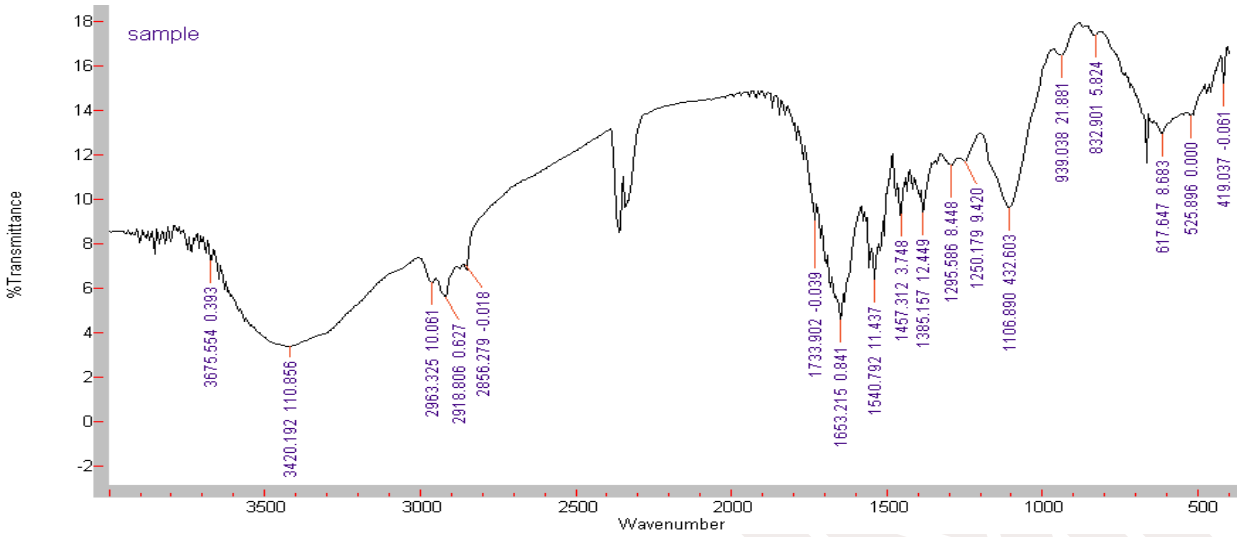

Fig. 2. IR transmission spectrum of honey bee Apis mellifera L. venom

Thus, IR absorption spectra of standard bee venom have been obtained and systematized. The obtained data can be used to establish the authenticity of venom both in its native form and in preparations based on it.

Conclusions: 1. It was experimentally established that the lowest activity of phospholipase A2 was found in the vicinity of Baku (134.7 IU/mg), the highest in Zakatally (183.7 IU/mg, P<0.001). 2. Revealed in the frequency range of 1733, 1653 1295,1250 cm-1, stretching vibrations of cumulated bonds are also noted - C = N, C=C; C=O. In the infrared spectra of the studied samples, stretching vibrations of OH groups in the range of 617,525,419 cm-1 and deformation vibrations of OH groups in the range of 1540 cm-1 were obtained.

The influence of honey bee waste products on physiological processes in the body of humans and animals is of considerable interest to clinicians. This is important not only for the definition and selection of rational, pathogenetic measures to combat the consequences of bee stings, but also for finding effective and improved, non-specific methods and means of therapy, as well as to use them as diagnostic and therapeutic drugs.

References

1. Topchiyeva Sh.A., Mehrabova M.A., Abiyev H.A. Investigation of electrophisical parameters of snake venom. The International conference on structural analysis of advanced materials, 2009; 1:57-58.
2. Krylov V.N. Bee venom - what is it. Beekeeping, 1993;(2):36-39.
3. Krylov V., Oshevensky L., Kurnikov G., Klemenova I. Cream based on wax with propolis and royal jelly in the treatment of dermatological diseases. Apimondia'99. Congress Vancouver, Canada. Proceedings, 1999; 36:249.
4. Чебышев Н.В., Вальцева И.А., Крылов В.Н., Кудрявцев С.В. Ядовитые животные суши и моря. Учебное пособие. М.: ММА им. И М.Сеченова, 1997, 60 с.
5. Baggio A., Gallina A., Dainese N., Manzinello C., Mutinelli F. et al. Gamma radiation: A sanitating treatment of AFB-contaminated beekeeping equipment. Apiacta, 2005, 40: 22-27.
6. Topchieva Sh.A., Yusifov R.Y., Mehrabova M.A. Influence of radiation on products of tu live of melliferous bee Apis Mellifere L. WSEAS International Conf. Recent researches in Chemistry, Biology, Environmental and Culture Montreux, Switzerland, 2011, p.99-103.

SHAFIGA TOPCHIYEVA [1], SAMIRA SALAHOVA [2], LAMIYYA YILDIZ [3], NANA GORGASLIDZE [4], RUMIYYA KARIMOVA [5]

BIOCHEMICAL AND PHYSICOCHEMICAL PROPERTIES OF HONEY BEE VENOM, APIS MELLIFER

[1]Institute of Zoology of the Ministry of Science and Education of the Republic of Azerbaijan, [2] Socar Polymer, Baku, Azerbaijan, [3]Tokat Gaziosmanpasha University, Medical Faculty, Department of Physiology, Turkey, [4]Tbilisi State Medical University, Department of Social and Clinical Pharmacy, Tbilisi, Georgia, [5]Azerbaijan Medical University, Baku

SUMMARY

This work aimed to study the influence of environmental pollutants on the biochemical and physicochemical characteristics of honey bee venom. When studying the activity of phospholipase A2, the research material was samples of honeybee venom (Apis mellifera), collected from bees in apiaries in the vicinity of the cities of Baku, Sumgait, and Zakatalli in the spring. The activity of the enzyme phospholipase A_2 in the venom was determined by the titrimetric method. As a result of the conducted studies, it was revealed that the activity of phospholipase in the venom collected in the vicinity of the city of Baku, Sumgait and Zakatalla is 134.7 IU/mg, 155.7 IU/mg and 183.7 IU/mg, respectively The absorption bands of the stretching vibrations of the N-H, O-H, ≡C-H, S-H groups in the frequency range of 3675, 3420, 2963, 2918cm-1 in the IR spectra of the poison and the stretching vibrations of hydrogen associated with the saturated carbon atom in the CH2 groups in the frequency range of 1385 cm-1 were identified. It was experimentally established that the lowest activity of phospholipase A2 was found in the vicinity of Baku (134.7 IU/mg), the highest in Zakatally (183.7 IU/mg, $P<0.001$). Revealed in the frequency range of 1733, 1653 1295,1250 cm-1, stretching vibrations of cumulated bonds are also noted - C = N, C=C; C=O. In the infrared spectra of the studied samples, stretching vibrations of OH groups in the range of 617,525,419 cm-1 and deformation vibrations of OH groups in the range of 1540 cm-1 were obtained. At present, it has been possible to isolate many biologically active substances of animal origin and study their biochemical, physicochemical, pharmacological, and toxicological properties. The study of biochemical, physicochemical properties of apitoxin is of great importance in developing conditions for radiation sterilization of both the venom and preparations based on them, which is the basis of the concept and strategy for the rational use of bioresources, one of the components of which in Azerbaijan is the honey bee.

Keywords: honey bee, venom, Apis mellifera, biochemical, physicochemical, phospholipase A_2