Research article

Open Access Journal of
Biomedical Science
ISSN: 2690-487X

The Study of Physicochemical Properties and Composition of Snake Autolysate Eryx Boas

Mirzohid Akbaraliev, Otkir Inagamov, Khusan Kamilov and Bakhtiyor Umarov*

Tashkent Research Institute of vaccines and Serum, Ministry of Health of the Republic of Uzbekistan

ABSTRACT

General data on the chemical composition, properties of snake organs and the history of their use in the of medical practice are presented. Considered drugs derived from snakes is continuing to be a valuable source of new drugs. Conducted biochemical and molecular biological, pharmacological studies (electrophoresis of proteins and nucleic acids, chromatography, immunological methods). A conclusion was made about the expediency of modifying the existing method, choosing the conditions for sample preparation or developing alternative methods for determining the authenticity of snake hydrolysates that are part of medicines.

KEYWORDS: Drugs; Snake protein hydrolysate; Eryxin; Amino acids; DNA; RNA; Toxicity

INTRODUCTION

The snake autolysate, as well as its individual components, is a complex of biologically active compounds, which is a valuable raw material for pharmaceutical science and industry. They are used in the production of anti-venom sera [1], as well as in the composition of drugs. Boiled sand snake meat is known to be recommended by ancient healers for many chronic inflammatory conditions. Eryxin - 1% aqueous hydrolyzate obtained from the biomass of snakes of the genus Eryx (boas), which includes a complex of low molecular weight biologically active substances, peptides, free amino acids, trace elements Ca, Mg, K, Fe, S, compounds containing -SH groupings, etc. [2].

"Eriksin" increases immunity and body defenses in the treatment of rheumatism, hepatitis, viral infections and tuberculosis. The drug is based on the meat of a snake of the genus "Eriks", that is, a sand boa, known for centuries in folk medicine in Central Asia. Pharmacological action is expressed in the fact that "Eriksin" stimulates T-cell immunity (T-lymphocytes). T-lymphocytes perform a number of important functions in the body, including anticoagulant properties that prevent thrombus formation by acting on plasma coagulation factors, inhibiting the appearance of fibrin filaments and helping to stop the growth of already formed blood clots, counteracting the effect of thrombin on fibrin. They also increase the effect of endogenous fibrinolytic enzymes on blood clots [3,4]. They are responsible for the manifestation of cellular immunological reactions, increase the content of immune lymphocytes in the spleen, lymph nodes and thymus; carry out reactions of transplantation immunity, providing rejection of transplanted tissues, while they function as cytotoxic cells that kill foreign cellular elements; carry out anti-cancer protection; provide resistance against some bacterial infections (tuberculosis, leprosy, malaria and others associated with intracellular parasitism of the pathogen) and enhance antiviral immunity [1,2]. The purpose of the research is to study the physico-chemical and pharmacological properties of the drug "Eriksin" obtained by the autolysis method from snake biomass.

MATERIALS AND RESEARCH METHODS

The total amount of proteins in the studied autolysates was determined several times according to the Lowry method [5]. The amount of total nitrogen in Eryxin solutions was determined by the Kjeldahl method [6]. Quantitative determination of free amino acids in the Eryxin preparation was carried out according to the method of Steven A., Cohen D [7]. Identification of FTC-amino acids was carried out on an Agilent Technologies 1200 chromatograph on a 75x4.6 Discovery HS C18 column. Determination of nucleic

Quick Response Code:

Address for correspondence: Bakhtiyor Umarov, Tashkent Research Institute of vaccines and Serum, Ministry of Health of the Republic of Uzbekistan

Received: March 27, 2023 **Published:** April 12, 2023

How to cite this article: Mirzohid A, Otkir I, Khusan K, Bakhtiyor U. The Study of Physicochemical Properties and Composition of Snake Autolysate Eryx Boas. 2023- 5(2) OAJBS.ID.000556. **DOI:** 10.38125/OAJBS.000556

acids was carried out by staining with ethidium bromide in agarose gel [8-11]. The study of acute toxicity of drugs was carried out according to the generally accepted method [12] on white mice of both sexes, weighing 18-21 g, 6 animals in each group, a total of 50 mice were used. The immunomodulatory activity of the drug "Eriksin" was studied by a biological method according to its ability "in vivo" to increase the weight of immune organs and the number of nucleated cells - NSC of the thymus, spleen, lymph nodes in mice.

RESULTS AND DISCUSSION

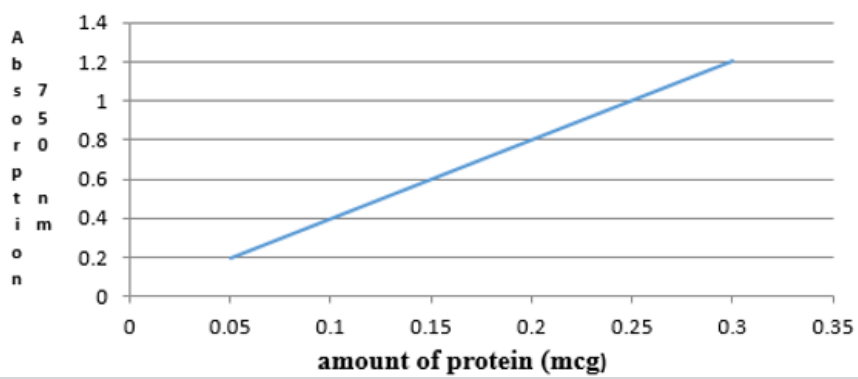

Figure 1: Results the total amount of proteins in the Eryxin preparation.

In the Figure 1 presents the results of the calibration curve of bovine serum albumin to determine the amount of total protein by the Lowry method in eryxin preparations. The protein content in the preparations had to be at least 5 mg/ml (calculated according to the calibration curve). To build a calibration graph, about 0.020 g (accurately weighed) of bovine serum albumin (TU 6-05-10-342-75, Reanal) was dissolved in water in a volumetric flask with a capacity of 100 ml. K 0.2; 0.4; 0.6; and 0.8 ml of the resulting solution was added water, respectively, to 1 ml, and then the analysis was carried out as indicated in the method. Comparing the number of proteins with known concentrations of proteins, the total amount of proteins in the studied preparations was obtained. The results obtained showed that in the studied samples the total amount of protein ranged from 22 mg/ml to 28 mg/ml. The amount of total nitrogen in Eryxin solutions was determined in accordance with the Kjeldahl method adapted during the development process [6]. In parallel, a control experiment was carried out, where distilled water was used instead of the drug. Based on the determined values, the total nitrogen content in the sample (N, mg/mL) was calculated according to the following formula:

$$N(\%) = (V\text{-}V0)\times 1,4/A$$

where 1.4 is the amount of nitrogen in mg, equivalent to 1 ml of 0.05 M sulfuric acid; V - volume of 0.05 M sulfuric acid during sample titration, ml; V0 - volume of 0.05 M sulfuric acid during titration of the control solution, ml; A is the volume of the drug sample to be burned, ml. Results by the Keldahl method, it was found that the quantitative content of nitrogen in the presented samples of "Eriksin" of various series ranged from 1.6% (N%) to 2.31% (N%). The preparation and determination of phenylthiocarbonyl (FTC) derivatives of amino acids was carried out according to the method of Steven A., Cohen D [6]. Identification of PTC amino acids was carried out on an Agilent Technologies 1200 chromatograph on a 75x4.6 Discovery HS C18 column. Table 1 presents data on the amino acid composition of the Eryxin preparation. Determining the concentration of DNA and RNA nucleic acids in the composition of protein hydrolysates in solution was determined by the spectrophotometric method at a wavelength of 260 nm. This means that in solutions of nucleic acids, the maximum photometric absorption is observed at 260 nm and directly correlates with the concentration of DNA or RNA. In our studies, an aqueous solution of the hydrolyzate was measured on a spectrophotometer at a wavelength of 260 nm, and to determine their purity, they were measured at 230 and 280 nm [8,10].

The spectrophotometric method for determining the concentration of nucleic acids is used at concentrations up to "2" OD. absorbances of the hydrolyzate at 260 showed very large numbers, often nucleic acid samples contain impurities of proteins and other organic substances. The absorbance ratio at 260 and 280 nm (260/280) is often used to evaluate the purity of a preparation. Pure DNA has an A260/280 ratio of about 1.8, an RNA sample without A260/280 impurities is about "2". In our analysis, the ratios 260/280 "0.82", 260/230 "0.32". From this we can conclude that the test solution contains very large amounts of protein and sugar impurities. The ratio of 260/280 in 100% protein solutions, the numbers "0.82" and "0.32" mean that in this solution the content of nucleic acids is equal to "0". Protein contamination in a nucleic acid solution cannot be determined by the ratio of 260/230. The 260/230 ratio is less sensitive when determining protein impurities in nucleic acid solutions: if the number is "2.00", then the protein % equals "0" and the percentage of nucleic acids levels off to 100%. Such differences are due to the higher value of the molar extinction coefficient of nucleic acids at wavelengths of 260 and 280 nm in comparison with proteins. Therefore, even for a protein solution of a relatively high concentration, the contribution to absorption at wavelengths of 260 and 280 nm is small. Protein contamination in a nucleic acid solution cannot be determined by the ratio of 260/230. Thus, all the presented data are consistent with our conclusions that the solutions we studied do not contain residues or impurities of nucleic acids.

For the reliability of our results, the determination of nucleic acids was stained with ethidium bromide. Ethidium bromide: has the appearance of dark red leaf-shaped crystals. The molar mass is 394.31 g/mol. Melts with decomposition at a temperature of 248-249 °C. It has the formula (3,8-diamino-5-ethyl-6-phenylphenanthridium bromide) - an organic compound, a fluorescent dye with the chemical formula $C_{21}H_{20}BrN_3$. It forms strong fluorescent complexes with polynucleotide chains, while it fluoresces only in double-stranded regions [8]. Fluorescence orange

with a spectral maximum at 600nm appears under ultraviolet illumination. When binding to DNA, the fluorescence intensity increases by about 20 times. Ethidium bromide is commonly used for nucleic acid detection in molecular biology laboratories. In the case of DNA, this is usually double-stranded DNA from PCR, restriction cleavage, etc. Single-stranded RNA can also be detected as it usually folds on itself and thus allows for local base pairing for dye intercalation. Detection usually involves a gel containing nucleic acids placed on an ultraviolet lamp. Actively interacts with the structure of DNA, unwinding its helix with the formation of cyclic structures, and is also able to twist the helix of the molecule in the opposite direction. It is used as an intercalating agent in molecular biology for the detection of nucleic acids, in particular, in the case of DNA electrophoresis in agarose gel. Agarose gel stained with ethidium bromide shows the presence and concentration of nuclea acid in this solution. In our studies, the EtBr dye was used to determine the presence of nucleic acids in the hydrolyzate under study. Nucleic acid staining was performed using a 1% agarose gel. EtBr was added to the gel prior to electrophoresis at a final concentration of 1.5% μg/mL, typically 0.5% is added according to the procedure. Electrophoresis was carried out at 40mA, 100V.

Table 1: The amount of free amino acids in the composition of the drug Eriksin.

	Name of Amino Acids	Number of Amino Acids	
		In the Sample mg/ml	Total Amount Per Formulation (mg/g)
1	Aspartic acid	0.00186	1.86044
2	Glutamine acid	0.250565	250.565
3	Serene	0.003205	3.205121
4	Glycine	0.020071	20.0712
5	Asparagine	0.020275	20.2752
6	Glutamine	0.06913	6.91304
7	Cysteine	0	0
8	Threonine	0.20316	203.2
9	Argenin	0.052503	53.503
10	Alanine	0.038044	38. 044
11	Proline	0.081256	81.256
12	Tyrosine	0.03867	39.0404
13	Valine	0.109025	109.026
14	Methionine	0.02879	28.7903
15	Isoleucine	0.050288	50.31
16	Leucine	0.072373	72.37258
17	Histidine	0	0
18	Ttryptophan	0.090445	90.45034

In the stained gel, the DNA and RNA bands are visible under UV light without being washed out of excess dye. Known DNA was applied to the first lane as a control and a stained DNA fragment was seen. In lanes 2-3, test samples were not stained with EtBr. Staining of DNA and RNA with EtBr was repeated several times, but it was seen that nucleic acids were not detected in the hydrolyzate under study, staining with EtBr did not occur. (Figure 2).

Figure 2: Electrophoresis of DNA samples with EtBr staining. 1-lane: DNA sample., 2-3 lanes: hydrolysate samples.

CONCLUSION

Thus, Eriksin is an environmentally friendly drug. This is an aqueous solution of a complex of biologically active substances (polypeptides and free amino acids), as well as trace elements obtained from the biomass of Eryx snakes (Latin boas). The pharmacological properties of the drug are that it stimulates T-cell immunity (T-lymphocytes). T-lymphocytes perform a number of important functions in the body. They are responsible for the development of cellular immunological reactions, increase the content of immune lymphocytes in the spleen, lymph nodes and thymus; carry out reactions of transplantation immunity, providing rejection of transplanted tissues, while they function as cytotoxic cells that kill foreign cellular elements; carry out anti-cancer protection; provide resistance against certain bacterial infections (tuberculosis, leprosy, malaria and others associated with intracellular parasitism of the pathogen) and enhance antiviral immunity [13]. Eryxin has a biostimulating effect, increases the body's resistance to the action of damaging factors. Activates the activity of the cardiovascular system. It has antiulcer effect, antispasmodic activity. Does not cause allergies, harmful side effects.

As a result, the effectiveness of eriksin in the complex treatment of rheumatoid arthritis, chronic persistent hepatitis, brucellosis, destructive pulmonary tuberculosis, and chronic obstructive bronchitis was established. A pronounced biostimulating effect was observed - an improvement in general well-being, mood, appetite, an increase in activity, normalization of sleep, etc. Along with the general eryxin, it has a specific therapeutic effect, contributing to the regression of pathological foci of inflammation, normalization or significant improvement in laboratory and biochemical parameters, as well as quantitative and functional parameters of the immune system.

SIDE EFFECTS

Side effects of eriksin appear only in the initial stage after its use in the form of a specific smell in the mouth, tachycardia, redness of the face, fever, mild headache.

REFERENCES

1. Asatov SS, Komorin AS, Karimov MS, Akbarov SV (2003) Hepatoprotective properties of eryxin in acute drug-induced hepatitis. Chemical Pharmaceutical Journal 2(37): 13-16.
2. Muminov SO, Khalilov US, Kurbanov DD, Kurbanova MT (2012) The effect of eryxin on the immunological resistance of women with CVD and CMV inflation. Bulletin of Vrach 1(3): 102-104.
3. Ubaidullaev AM, Uzakov GT, Akbarov SV (1999) Honey. Journal of Uzbekistan 6: 88-90.
4. Litvinov RI (2013) Molecular mechanisms and clinical significance of fibrinolysis. Kazan Medical Journal 94(5): 717-718.
5. Lowry OH, Rosenbrouch HG, Farr AL, Randall R (1975) Protein measurement with the folin phenol reagent. J Biol Chem 193(1): 265-275.
6. Kjeldahl J (1883) New method for the determination of nitrogen in organic substances. Zeitschrift für analytische Chemie 22(1): 366-383.
7. Steven A, Cohen DJ (1988) Amino acid analysis utilizing phenylisothi ocyanata derivatives. Jour Analytical Biochemistry 174(1): 1-16.
8. Maniatis T, Frich E, Sambrook J (1984) Methods of genetic engineering. Molecular cloning/per.
9. Umarov B, Boymatova M (2021) The taxonomic analysis and genetic biodiversity of nodule bacteria soybean (*Gycine max* (L.) *Merr*.) isolated from soils of Central Asia. Open Access Journal of Biomedical Science 4(5): 1162-1165.
10. Umarov BR (2022) Genetic biodiversity of nodule bacteria entering into symbiosis with soybean plants in the soils of Central Asia. 11th -International conference "ACB-2022" achievements and challenges in B. Baku, Azerbaijan 83-84.
11. Ibragimov AA (2020) Investigation of mechanism activity of antitumor and radio sensitizing activity of preparations K-26 and K-26w. American Journal of Biomedical and Life Sciences 8(5): 131-136.
12. Belenky ML (1963) Elements of a quantitative assessment of the pharmacological effect. Science, Leningrad, p. 146.
13. Akbaraliev MA (2022) Study of the immunotropic activity of purified Eryxin-1 and non-purified Eryxin-2 autolysate substance from snake biomass ERYX MILIARIS. Immunity pharmacy and vaccine 3: 61-66.