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Proteostasis collapse in rat's lung tissue as one of the effects of European adder (*Vipera berus subsp. berus* and *Vipera berus subsp. nikolskii*) envenomation

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ABSTRACT

Snakebites and their consequences are a growing concern around the globe. The mixture of enzymes, present in snake venom is a great challenge for a snakebite victim's body. European adder (*Vipera berus*) is considered as not very harmful snake due to the mild clinical symptoms of envenomation and rare cases of cardiovascular or pulmonary failure. Nevertheless, at the molecular level, many details of *V. berus* bite (including proteostasis instability) remain unclear. This work was aimed to determine the effect of Eastern Europe typical adder species *Vipera berus berus* and *Vipera berus nikolskii* envenomation on the protein homeostasis of rat's lung tissue. The decrease in total protein concentration, significant redistribution of main protein fractions (including proteolytically active), rise in middle-mass molecules and low molecular weight peptide concentration have been observed during *V. b. berus* and *V. b. nikolskii* envenomation, strongly indicating the proteostasis collapse and endogenous intoxication in lung tissue as an effect of European adder snakebite. Moreover, according to the results of experiments, the effect of *V. b. nikolskii* venom is more harmful to lung tissue protein homeostasis, comparing to *V. b. berus,* suggesting that the former may represent a greater danger to people. The results of this study may find applications in antivenom development and could be taken into consideration for proper snakebite treatment selection in order to avoid proteome complications in patients.

INTRODUCTION

Snakebite envenoming is a life-threatening condition that may induce pathological alterations in the victim's body and even lead to death. The European adder (*Vipera berus*) is widely distributed across Europe, being the most abundant viper species in this part of the World [1]. The venom of these creatures contains a mixture of molecules, mostly enzymes, peptides and non-enzymatic proteins. The former are responsible for the majority of the negative destructive effects in the organs and tissues of the envenomated body. Several proteomic studies have revealed that *Vipera berus* venom contains phospholipases A2, serine proteases, metalloproteinases and hyaluronidases. Other enzymes can be found in lesser quantities [2]. The important feature of *Vipera berus* venom is its hemotoxicity, due to the presence of serine proteases with thrombin-like activity and metalloproteinases that are able to disrupt tissues and blood vessels, causing hemorrhages [3].

Even though adder's venom contains a wide range of toxins, its bite rarely causes severe or systemic symptoms [2]. Common clinical manifestations of *Vipera berus* envenomation include pain, nausea and oedema [4]. In cases related to severe envenomation, hypotension also may occur [5].

Remarkably, snakebite usually induces respiratory system dysfunction, such as respiratory paralysis, hemorrhages and

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pulmonary edema [6], yet not many cases of lung failure after *Vipera berus* bite are described – only rare episodes of pulmonary edema and pulmonary embolism [1,7,8]. Still, molecular processes in lungs during envenomation remain unrevealed. It is known that protein homeostasis (*i.e.*, proteostasis) collapse may be an indicator for many respiratory system pathologies [9]. Accordingly, the aim of this study is to examine and compare the potential effects of Eastern Europe typical adder species *Vipera berus* berus and *Vipera berus nikolskii* envenomation on rat lung tissue proteostasis.

MATERIALS AND METHODS

Venom

Lyophilized *V. berus berus* and *V. berus nikolskii* crude venoms were obtained from V.N. Karazin Kharkiv National University (Kharkiv, Ukraine), kept at -20°C, dissolved in saline immediately before experiments, centrifuged at 10,000 g for 15 minutes, and the supernatant set aside fro further study.

Animals and maintenance conditions

The experiments utilized non-linear albino male rats (200-220 grams each) and followed the National Institute of Health Guidelines for the care and use of laboratory animals, as well as the European Council Directive on 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), and were conducted under the law of Ukraine of Feb. 21th, 2006 No. 3447-IV "On the Protection of Animals from Brutal Treatment". The animals were subjected to constant vivarium conditions of 20-24°C, 30-70% humidity, 12 h light/dark cycle. The total of 30 rats were divided into 3 groups: control, which was intraperitoneally injected by 0.5 ml saline solution (0.9 %), and 2 experimental groups, which were intraperitoneally injected by *Vipera berus berus* venom (LD50 – 1.576 µg/g) and *Vipera berus nikolskii* venom (LD50 – 0.972 µg/g), correspondingly. The chosen LD50 concentrations were based on the work of Shitikov et al. [10]. Animals were euthanized by cervical dislocation 24 hours after venom injection, and lung samples were taken afterwards.

Homogenates harvest

Homogenization procedures were performed at +4°C. One gram of lung tissue was homogenized in 50 mM tris-HCl buffer (pH 7.4, with 1 mM EDTA addition) in a 1:9 tissue-buffer ratio. Crude homogenate was centrifuged at 1500 g for 20 min, after which supernatant was collected, aliquoted and frozen in liquid nitrogen.

Protein quantification

Total protein contents in homogenates were measured according to Bradford assay [11] and expressed as mg of protein/gram of tissue.

Sodium dodecyl sulfate polyacrylamide electrophoresis and zymography

SDS-PAGE was performed according to standard procedure [12] using a vertical electrophoresis system (Bio-Rad, USA). Polyacrylamide concentrations in stacking and resolving gels were 4% and 12%, respectively, with electric currents of 19 mA and 39 mA applied, respectively. After separation, gels were stained with 2.5% Coomassie Brilliant Blue G-250 in 10% ethanol, 10% acetic acid and 15% isopropanol. Subsequently, gels were washed from excess dye by placing them in boiling water with 8% acetic acid addition.

Zymographic assay was carried out by polymerizing gelatin, collagen and fibrinogen in 12% resolving gels in a concentration of 1 mg/ml. In order to save enzymatic activity, samples were not heated before loading to the gels. After separation, SDS was washed out by soaking the gels in 2.5% Triton X-100 solution for 1 hour. The gels were then incubated for 12 hours in 0.05 M tris-HCl solution (pH 7.4) at +37°C. Staining and fixation of gels was undertaken according to standard SDS-PAGE protocol. The obtained results were analyzed using TotalLab – CLIQS Gel Image Analysis Software.

Middle-mass molecules content determination

Fraction of MMMs was separated according to the method proposed by Nykolaychyk [13]. All procedures were performed on ice, with 15 min incubation between each stage. Tissue homogenate samples were added to equal volumes of 1.2 M $HClO_4$ and centrifuged for 20 min at 1500 g. Afterwards, the collected supernatant was neutralized to pH 7 using 5 M KOH and then centrifuged for 20 min at 1500 g. Following this procedure, the obtained supernatant was diluted with 96% ethanol to the final concentration of 20 %. Optical density of samples was measured using spectrophotometer Smart Spec Plus (BioRad, USA) at 210, 238 and 254 nm (to visualize peptide bonds, low molecular weight peptides and middle-mass peptides, respectively). Peptide relative content was evaluated using a standard curve, based on N-carboxy-glycylglycine (0.26 kDa).

Size-exclusion chromatography

A Sephadex G15 column (Bio-Rad, USA) was employed in order to separate and quantify peptide fractions. The column was pre-equilibrated with 0.05 M Tris-HCl buffer (pH 7.4) containing 0.13 M NaCl, and the samples were loaded at a flow rate of 30 mL/hour. Molecular masses of the peptides were determined by calibration curve, obtained using standard mixture containing lysozyme (14.3 kDa), insulin (5.7 kDa), and vitamin B12 (1.35 kDa).

Statistical analysis

The results were analyzed and processed using TotalLab 2.01 and OriginPro v. 9.5 softwares. The data is presented as mean ± SEM. The significance of differences between groups was determined using one-way analysis of variances (ANOVA), differences were considered statistically significant when $*p < 0.05$.

RESULTS

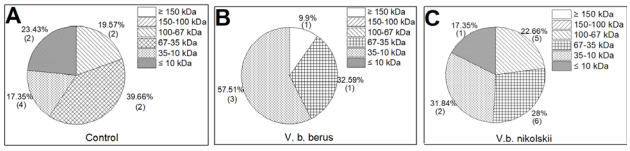
Total protein level alterations

The measurement of the total protein contents in lung tissue showed a prominent decrease of protein levels in rats envenomated by *Vipera berus nikolskii* – about 51.29±4.85 mg/g

of tissue, as compared with 65.01±1.56 mg/g in the control group. At the same time, after injection of *Vipera berus* berus venom, tissue protein levels decreased to 60.92±1.30 mg/g, which is a relatively slighter effect as compared to *Vipera berus nikolskii.*

Lung tissue protein qualitative analysis

Electrophoretic separation of distinct protein fractions was conducted in order to evaluate the lung tissue protein content changes more thoroughly (Figure 1).



The data represents the distribution (in %) of given protein groups. The number of fractions within each of protein groups is listed in the brackets

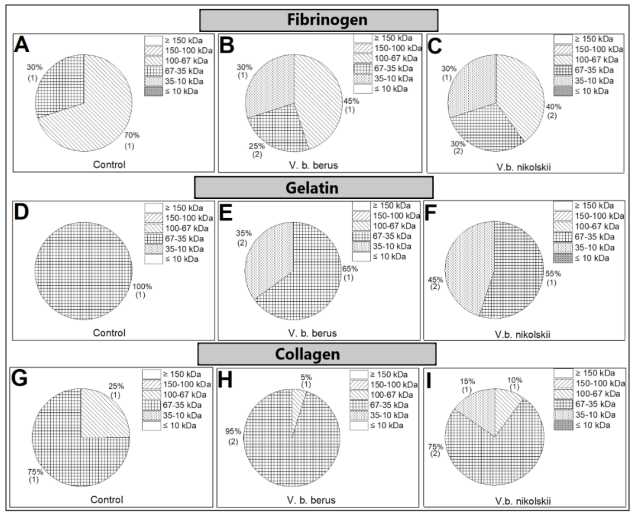
Figure 1. Protein profile of rat lung tissue in control group (A), and after *V. b. berus* (B) or *V. b. nikolskii* (C) envenomation

The results show a substantial redistribution of protein fractions during *V. b. berus* and *V. b. nikolskii* envenomation. There is a trend in protein formation, with middle- and low-molecular weight (mainly 10-35 kDa) proteins being mostly generated during envenomation (Fig. 1, B, C). At the same time, the number of fractions in each protein group also changes. Moreover, it should be noted that the effects of *V. b. nikolskii* (C) and *V. b. berus* (B) venom differ, and the former induces the formation of many protein fractions that are not present in the control group.

Evaluation of proteolytically active fractions presence in lung tissues

Tissue protein degradation during snakebite may be associated with both the enzymes, present in the venom, and the organism's own enzymes, as part of a proteostasis system. In this study, we conducted zymography assay with fibrinogen, gelatin and collagen as substrates with the aim to shed light on the proteolytic fractions in lung tissue and their involvement in proteolysis processes during envenomation (Figure 2).

As indicated in Figure 2, envenomation led to the formation of additional proteolytically active bands in all given substrates. Both *V. b. berus* and *V. b. nikolskii* envenomation brought about the formation of additional protein fractions (10-35 kDa) with fibrinogenolytic activity (Figures 2 B, C) and gelatinolytic activity (Figures 2 E, F). A new collagenolytic-active protein fraction also appears during *V. b. nikolskii* envenomation (Figure 2 I). After analyzing the molecular weights of these bands and their distribution, we assume that they might be the limited proteolysis products of native enzymes with higher molecular mass that are present in the control group (Figures 2, A, D, G). It is noteworthy that when compared with *V. b. berus* (Figures 2 B, E, H), *V. b. nikolskii* (Figures 2 C, F, I) envenomation induced a breakdown of relatively higher amounts of high-molecular weight enzymes, with simultaneous formation of more enzymatic fractions with lower molecular weight.



The number of fractions within each of protein groups is listed in the brackets

Figure 2. Distribution of main proteolytic groups (in %) in lung tissue of the control group and groups injected with *V. b. berus* and *V. b. nikolskii* by using fibrinogen (A, B and C respectively), gelatin (D, E and F respectively) and collagen (G, H and I respectively) as substrates

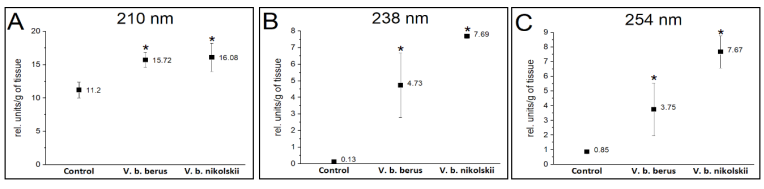
Contents of middle-mass molecules

Increased enzymatic activity in tissues during snake envenomation contributes to the formation of many middle- and low-molecular weight compounds, including peptides, nucleopeptides, misfolded protein fragments, etc. (the "middle-mass molecules" (MMMs)). These molecules can weight up to 5 kDa and are considered to be biochemical markers of endogenous intoxication [14,15]. In view of this, we examined the contents of MMM in the lung tissue of the envenomated rats, using a spectrophotometer at wavelengths of 210 nm, 238 nm and 254 nm in order to detect peptide bonds, low-weight peptides and middle-weight peptides, respectively (Figure 3).

As revealed in Figure 3, the relative contents of MMMs during envenomation by *V. b. berus*, and *V. b. nikolskii* in particular, have significantly risen. Accordingly, the levels of MMM_{238} have elevated approximately by 36.3 times after *V. b. berus* venom injection and by 59 times after *V. b. nikolskii* venom injection (Figure 3 B). The levels of MMM254 also have prominently risen during *V. b. berus*, and *V. b. nikolskii* envenomation – by 4.4 times and 9 times, respectively.

Identification of the main peptide fractions in envenomated lungs

The results of MMMs content determination showed a prominent leap in peptides concentration. To learn more



Results are presented as mean ± SEM (n=5); * p<0.05 *vs.* control group

Figure 3. Relative contents of MMMs measured at wavelengths of 210 nm (A), 238 nm (B) and 254 nm (C)

about this phenomenon, we conducted chromatographic separation of the main peptide fractions present in lung tissue during envenomation and then studied their molecular weights and relative concentration (Table 1).

Table 1. Lung tissue peptide composition following *V. b. berus* and *V. b. nikolskii* envenomation

Group	Fraction No.	Molecular weight, Da	Concentration, rel.units
Control	1	2147	0.091
	2	1782	0.063
	3	1309	0.121
	4	828	0.019
V. b. berus	1	2147	0.119
	2	1782	0.095
	3	1309	0.173
	4	1186	0.059
	5	828	0.042
V. b. nikolskii	1	2147	0.125
	2	1782	0.117
	3	1309	0.209
	4	1207	0.145
	5	828	0.067

Unique peptide fractions are highlighted in grey

An analysis of the size-exclusion chromatography indicates the presence of quantitative and qualitative changes in the lung tissue peptide pool during adder envenomation. It can be seen from Table 1 that concentrations of all peptide fractions have simultaneously increased. More important, however, is the appearance of peptide fractions with molecular weights of 1186 Da and 1207 Da that cannot be found in the control group at the time of *V. b. berus* and *V. b. nikolskii* envenomation, respectively.

DISCUSSION

In this work, we have aimed at determining the potential effects of European adder subtypes *V. b. berus* and *V. b. nikolskii* snakebite on rat lung tissue proteostasis and proteome stability. The results of total protein determination showed a reduction in lung tissue protein levels caused by *V. b. berus* and *V. b. nikolskii* venom. This protein degradation during snake envenomation can be explained by the activity of venom enzymes, metalloproteinases in particular [16], as well as by the activation of tissue matrix metalloproteinases (MMPs) that lead to cell disruption and extracellular matrix degradation [17]. The results obtained suggest that *Vipera berus nikolskii* venom has more proteolytic and overall tissue-destructive potential, which can be explained by it having a slightly different enzymatic profile, as compared to other *Vipera berus* subspecies [18].

Visible redistribution of protein fractions in lung tissue (Figure 1) may take place due to the uncontrolled and non-selective enzymatic degradation of cell components and extracellular matrix (ECM) proteins, including large proteins such as laminins, collagens etc. [19]. Changes in protein homeostasis is a normal process in organs and tissues, for example, during ECM remodeling. However, rapid protein composition alterations may be a sign of pathological processes within tissues. In the lungs, such changes can be induced by chronic pulmonary diseases such as chronic obstructive pulmonary disease, asthma, idiopathic pulmonary fibrosis, etc. [20]. The formation of higher amount of protein fractions, which are not present in the control group, may also suggest that *V. b. nikolskii* venom can cause relatively higher damage to lung tissue, as compared to that induced by *V. b. berus*. Moreover, the possible formation of degraded misfolded proteins triggers an inflammatory response that may aggravate the injury dealt to the tissue [21].

Results of zymography assay show the redistribution of proteolytically active protein fractions within lung tissue, as well as the appearance of new proteolytic fractions following *V. b. berus* and *V. b. nikolskii* envenomation (Figure 2). The potential threat of such "new" enzyme fractions could be in their immunity to allosteric inhibitors, since the site for the latter could be disrupted. Hence, they could increase the rate of proteolysis, thus causing more damage to the tissue. Additionally, uncontrolled proteolysis during envenomation could activate several enzyme precursors (*i.e.*, zymogens), already existing in tissue and cells [22]. Collagen and gelatin degradation is most likely a sign of the presence of matrix metalloproteinases (MMPs). Elevation of levels of enzymes from this group (particularly MMP-2 and MMP-9) can be observed in several lung diseases such as chronic obstructive bronchitis, emphysema, idiopathic pulmonary fibrosis etc. [23]. Uncontrolled activity of these enzymes can lead to ECM disruption and vascular injuries with further hemorrhages [24]. Given this fact, additional fibrinogenolytic activity in envenomated lungs only increases the extent of damage dealt.

Extremely high levels of low-weight peptides (Figure 3 B) and middle-weight peptides (Figure 3 C) are most likely a result of uncontrolled proteolysis and proteostasis collapse, since such peptides are usually degraded by exopeptidases. MMMs are the biochemical marker of endogenous intoxication and inflammation. Moreover, their activity is associated with the disruption of many biochemical processes and cascades [25]. Hence, the formation and accumulation of MMMs with consequent endogenous intoxication could be one of the many possible ways of harmful action of *V. b. berus* and *V. b. nikolskii* venoms.

The results of the peptide chromatographic separation studies of envenomated lung tissue demonstrate not only elevation in their concentration, but also the formation of additional peptide fractions that are absent in the control group (Table 1). Different molecular weights of these fractions can be explained by venom composition and protease mode of action. Although we have no information on the nature of these peptide fractions, potentially, they can exhibit bioactive properties. It is known that in some diseases, proteases can activate inactive peptides, which in turn may act as mediators and messengers in many biochemical processes and cascades [26]. For example, products of ECM proteins degradation, also called "matrikines" and "matricryptins", are able to facilitate inflammation processes, recruit immune cells, control apoptosis and inhibit the angiogenesis etc. [27]. Thus, it is possible that those unique peptide fractions, found during *V. b. berus* and *V. b. nikolskii* envenomation could also

have an impact on lung tissue homeostasis. However, precise determination of their properties and functions creates a problematic field for further studies.

CONCLUSIONS

To sum up, we have confirmed the harmful effect of *V. b. berus* and *V. b. nikolskii* envenomation on proteostasis in rat lung tissue. The results of total protein qualitative and quantitative analysis, proteolytic activity and middle-mass molecules content examination strongly evidence the activation of the destructive processes, that are triggered by adder venom. In the long term, such proteostasis collapse may lead to respiratory system complications. Furthermore, the results have revealed that comparing to *V. b. berus*, *V. b. nikolskii* venom possesses a far more harmful effect in lung tissue. This should be considered by clinicians working with snakebite cases. Over all, the results of this study may be applied in establishing more precise snakebite treatment planning, as well as in strategies of antivenom development.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Reading CJ. Incidence, pathology, and treatment of adder (*Vipera berus* L.) bites in man. *Emerg Med J.* 1996;13:346-51.
2. Siigur J, Siigur E. Biochemistry and toxicology of proteins and peptides purified from the venom of *Vipera berus berus*. *Toxicon X.* 2022;15:1-10.
3. Bocian A, Urbanik M, Hus K, Łyskowski A, Petrilla V, Andrejčáková Z, et al. Proteome and Peptidome of Vipera berus berus Venom. *Molecules.* 2016;21:1-13.
4. Hønge BL, Hedegaard SK, Cederstrøm S, Nielsen H. Hospital contacts after bite by the European adder (Vipera berus). *Dan Med J.* 2015;62:A5022.
5. Hamilton J, Kause J, Lamb T. Severe systemic envenomation following vipera berus bite managed with viperatab antivenom. *Wilderness Environ Med.* 2019;30:56-8.
6. Gnanathasan A, Rodrigo C. Pulmonary effects and complications of snakebites. *Chest.* 2014;146:1403-12.
7. Bart G, Pineau S, Biron C, Connault J, Artifoni M. Bilateral pulmonary embolism following a viper envenomation in France: A case report and review. *Medicine (United States).* 2016;95:1-3.
8. Hermansen MN, Krug AH, Tjønnfjord E, Brabrand M. Envenomation by the common European adder (Vipera berus): A case series of 219 patients. *Eur J Emerg Med.* 2019;26:362-5
9. Meiners S, Ballweg K. Proteostasis in pediatric pulmonary pathology. *Mol Cell Pediatr.* 2014;1:1-8.
10. Shitikov V, Malenyov A, Gorelov R, Bakiev A. "Dose-response" models with mixed parameters by the example of venom toxicity estimation of the common european adder vipera berus. *Principy Èkologii.* 2018;2:150-60.
11. Raksha N, Halenova T, Vovk T, Kharchenko O, Savchuk O, Samborska I, et al. Protein-peptide composition in the lungs of rats with hyperhomocysteinemia. *J Biol Res-Boll Della Società Italiana Di Biologia Sperimentale.* 2021;94:9858.
12. Raksha N, Halenova T, Vovk T, Beregova T, Maievska T, Tomchuk V, et al. Isolation and partial characterization of serine proteases from jellyfish of the Antarctic region. *J Appl Biol Biotechnol.* 2022;11:144-50.
13. Nykolaychyk B, Moyn V, Kyrkovskyy V. Method for determining of the peptide pool molecular. *Lab Case.* 1991;10:13-8.
14. Amiri A, Nikitina N, Stepanova L, Beregova T. Potential impact of cerium dioxide nanoparticles (nanoceria) on the concentration of c-reactive protein and middle-mass molecules after wound treatment in rats. *ScienceRise: Biol Sci.* 2019;1:14-9.
15. Denefil O. The rates of middle molecules in the blood in rats with different motor activity in alcoholic and non alcoholic exerimental hepatosis. *J Edu Health Sport.* 2021;11:385-95.
16. Herrera C, Escalante T, Voisin MB, Rucavado A, Morazán D, Macêdo JKA, et al. Tissue localization and extracellular matrix degradation by PI, PII and PIII snake venom metalloproteinases: Clues on the mechanisms of venom-induced hemorrhage. *PLoS Negl Trop Dis.* 2015;9:1-20.
17. Gutiérrez JM, Rucavado A, Escalante T, Herrera C, Fernández J, Lomonte B, et al. Unresolved issues in the understanding of the pathogenesis of local tissue damage induced by snake venoms. *Toxicon.* 2018;148:123-31.
18. Zinenko O, Tovstukha I, Korniyenko Y. PLA2 inhibitor varespladib as an alternative to the antivenom treatment for bites from nikolsky's viper Vipera berus nikolskii. *Toxins (Basel).* 2020;12:1-7.
19. María Gutiérrez J, Rucavado A. Snake venom metalloproteinases: Their role in the pathogenesis of local tissue damage. *Biochimie.* 2000;82:841-50.
20. Burgstaller G, Oehrle B, Gerckens M, White ES, Schiller HB, Eickelberg O. The instructive extracellular matrix of the lung: Basic composition and alterations in chronic lung disease. *Eur Resp J.* 2017;50:1601805.
21. Bouchecareilh M, Balch WE. Proteostasis: A new therapeutic paradigm for pulmonary disease. *Proc Am Thorac Soc.* 2011;8:189-95.
22. Twining SS, Simmons WH. Regulation of proteolytic activity in tissues. *Crit Rev Biochem Mol Biol.* 1994;29:315-83
23. Chakrabarti S, Patel KD. Matrix metalloproteinase-2 (MMP-2) and MMP-9 in pulmonary pathology. *Exp Lung Res.* 2005;31:599-621.
24. Warner RL, Beltran L, Younkin EM, Lewis CS, Weiss SJ, Varani J, et al. Role of Stromelysin 1 and Gelatinase B in experimental acute lung injury. *Am J Respir Cell Mol Biol.* 2001;24:537-44.
25. Mykolenko AZ. Changes of concentration of middle mass molecules in blood serum in the early days of isolated orthopedic trauma complicated by blood loss. *Deutscher Wissenschaftsherold.* 2016;2:16-8.
26. Schulte I, Tammen H, Selle H, Schulz-Knappe P. Peptides in body fluids and tissues as markers of disease. *Expert Rev Mol Diagn.* 2005;5:145-57.
27. Kisling A, Lust RM, Katwa LC. What is the role of peptide fragments of collagen I and IV in health and disease? *Life Sci.* 2019;228:30-4.