gleton Births. *PLoS ONE*. 2015;10(5):e0126743. https://doi.org/10.1371/journal.pone.0126743
3. Zherdev K. V., Pak L. A., Volkova M. O., Zubkov P. A., Chelpachenko O. B. Aktualnye napravleniya optimizatsii khirurgicheskogo lecheniya oporno-dvigatelnykh narusheny pri spasticheskikh formakh detskogo tserebralnogo paralicha. *Rossysky pediatrichesky zhurnal*. 2018;21(3):175-181.
4. Rogoveanu O., Tuțescu N., Kamal D., Alexandru D., Kamal C. [et al.] Correlations between risk factors and functional evolution in patients with spastic quadriplegia. *Journal of Medicine and Life*. 2016;9(2):170-176.
5. Malakhov O. A., Zherdev K. V., Trankovsky S. E., Malakhov O. O. Differentsialny podkhod k khirurgicheskoy korrektsii patologii tazobedrennykh sustavov u detey s DTsP. *Detskaya khirurgiya*. 2014;18(3):4-8.
6. Hägglund G., Alriksson-Schmidt A., Lauge-Pedersen H., Rodby-Bousquet E., Wagner P. [et al.] Prevention of dislocation of the hip in children with cerebral palsy: 20-year results of a population-based prevention programme. *Bone Joint J*. 2014;96-B(11):1546-1552.
7. Büyükavcı R., Büyükavcı M. A. Effects of ultrasound-guided botulinum toxin type-A injections with a specific approach in spastic cerebral palsy. *Acta Neurologica Belgica.* 2018;118(3):429-433. https://doi.org/10.1007/s13760-018-0929-5
8. Sharan D. Orthopedic surgery in cerebral palsy: Instructional course lecture. *Indian J. Orthop.* 2017;51(3):240-255. https://doi.org/10.4103/ortho.IJOrtho_197_16
9. Lamberts R. P., Burger M., du Toit J., Langerak N. G. A Systematic Review of the Effects of Single-Event Multilevel Surgery on Gait Parameters in Children with Spastic Cerebral Palsy. *PLoS ONE*. 2016;11(10):e0164686. https://doi.org/10.1371/journal.pone.0164686
10. Baklanov A. N., Kolesov S. V., Shavyrin I. A. Operativnoye lecheniye deformatsy pozvonochnika u patsiyentov s detskim tserebralnym paralichom. *Travmatologiya i ortopediya Rossii*. 2011;61(3):73-79.
11. Aranovich A. M., Popkov A. V., Shchukin A. A., Medvedeva S. N., Yevreinov V. V. [et al.] Rezultaty mnogourovnevykh odnomomentnykh operativnykh vmeshatelstv u patsiyentov s detskim tserebralnym paralichom. *Geny ortopedii*. 2013;(4):53-60.
12. Klinicheskiye rekomendatsii MZ RF «Detsky tserebralny paralich». 2016;34.
13. Heinen F., Desloovere K., Schroeder A. S., Berweck St., Borggraefe I. [et al.] The updated European Consensus 2009 on the use of Botulinum toxin for children with cerebral palsy. *Eur. J. Paediatr. Neurol*. 2010;14:45-66. https://doi.org/10.1016/j.ejpn.2009.09.005
14. Baranov A. A., Namazova-Baranova L. S., Kurenkov A. L., Klochkova O. A., Mamedyarov A. M. [et al.] Kompleksnaya otsenka dvigatelnykh funktsy u patsiyentov s detskim tserebralnym paralichom: ucheb.-metod. Posobiye. M.: Pediatr, 2014;84.
15. Wright F. V., Rosenbaum P., Fehlings D., Mesterman R., Breuer U. [et al.] The Quality Function Measure: reliability and discriminant validity of a new measure of quality of gross motor movement in ambulatory children with cerebral palsy. *Dev. Med. Child. Neurol.* 2014;56(8):770-778. https://doi.org/10.1111/dmcn.12453

About authors:

Fisenko Andrey Petrovich, MD, PhD, Professor, Director; e-mail: fisenko@nczd.ru

Chelpachenko Oleg Borisovich, MD, PhD, Traumatologist-orthopedist, leading researcher of the Laboratory of Neurology and Cognitive Health; tel.: +74991340786; e-mail: chelpachenko81@mail.ru

Pak Lale Alievna, MD, PhD, Leading researcher of the Laboratory of Rare Hereditary Metabolic Children Diseases; e-mail: lolitap@mail.ru

Volkova Maria Olegovna, PhD student; tel.: +74991340786; e-mail: ortopediya@gmail.com

Zubkov Pavel Andreevich, PhD student; e-mail: ortopediya@gmail.com

Zherdev Konstantin Vladimirovich, MD, PhD, Head of the Department of Neuroortopedics and Ortopaedics; tel.: +74991340786; e-mail: drzherdev@mail.ru

Ashrafova Ulviya Shakenovna, MD, Neurologist at the Department of Diagnostic and Rehabilitation Medicine of Children with Psychoneurological Pathology; tel.: +74991340169; e-mail: ashrafova@nczd.ru

Kirgizov Igor Vitalevich, MD, PhD, Professor, Head of the Pediatric Surgery Unit; tel.: +79057720953; e-mail: drkirgizov@yandex.ru

UDC 581.616.192
DOI – https://doi.org/10.14300/mnnc.2018.13091
ISSN – 2073-8137

ANTIOXIDANT AND HEMOLYTIC PROPERTIES OF DIFFERENT EXTRACTS FROM *PRUNELLA VULGARIS* L. LEAVES

Ohanyan A. Dj. [1], Shishkoyan N. J. [1], Kazaryan Sh. A. [1], Hovhannisyan A. A. [1], Tiratsuyan S. G. [1], Elbekyan K. S. [2], Koshel V. I. [2], Khodzhayan A. B. [2]

[1] Russian-Armenian University, Yerevan, Republic of Armenia
[2] Stavropol State Medical University, Russian Federation

АНТИОКСИДАНТНЫЕ И ГЕМОЛИТИЧЕСКИЕ СВОЙСТВА РАЗНЫХ ЭКСТРАКТОВ ЛИСТЬЕВ *PRUNELLA VULGARIS* L.

А. Ж. Оганян [1], Н. Дж. Шишкоян [1], Ш. А. Казарян [1], А. А. Оганесян [1], С. Г. Тирацуян [1], К. С. Эльбекян [2], В. И. Кошель [2], А. Б. Ходжаян [2]

[1] Российско-Армянский университет, Ереван, Республика Армения
[2] Ставропольский государственный медицинский университет, Российская Федерация

The plant *Prunella vulgaris* L. has long been used in traditional European and Asian medicine. Its extracts reveal immunoprotective, antitumor, antiinflammatory, and antiestrogenic features. We obtained aqueous and ethanol extracts from *P. vulgaris* L. leaves; carried out HPLC analysis; detected the total flavonoids content and the antiradical activity. The

effect of different extracts of *Prunella vulgaris* L. on the activity of superoxide dismutase of healthy donors' erythrocytes was studied. It was shown that the major component of ethanol extracts is rosmarinic acid. 50 % ethanol extract of *P. vulgaris* features high antiradical, antioxidant (SOD) and low hemolytic activity.

Keywords: Prunella vulgaris L. extracts, total flavonoid content, HPLC analysis; antiradical activity, erythrocytolysis, SOD

Растение *Prunella vulgaris* L. издавна используется в европейской, азиатской традиционной медицине. Его экстракты проявляют иммунопротекторные, противооухолевые, противовоспалительные, антиэстрогенные свойства. Были получены водные и этанольные экстракты листьев *P. vulgaris* L., проведен ВЭЖХ-анализ, определено общее содержание флавоноидов и антирадикальная активность. Исследовано действие разных экстрактов *Prunella vulgaris L.* на активность супероксиддисмутазы эритроцитов здоровых доноров. Показано, что мажорным компонентом этанольных экстрактов является розмариновая кислота. 50 % этанольный экстракт *P. vulgaris* обладает высокой антирадикальной, антиоксидантной (СОД) и низкой гемолитической активностью.

Ключевые слова: экстракты Prunella vulgaris L., содержание флавоноидов, антирадикальная активность, гемолиз эритроцитов, СОД

For citation: Ohanyan A. Dj., Shishkoyan N. J., Kazaryan Sh. A., Hovhannisyan A. A., Tiratsuyan S. G., Elbekyan K. S., Koshel V. I., Khodzhayan A. B. ANTIOXIDANT AND HEMOLYTIC PROPERTIES OF DIFFERENT EXTRACTS FROM PRUNELLA VULGARIS L. LEAVES. *Medical News of North Caucasus.* 2018;13(3):507-510. DOI – https://doi.org/10.14300/mnnc.2018.13091

Для цитирования: Оганян А. Ж., Шишкоян Н. Дж., Казарян Ш. А., Оганесян А. А., Тирацуян С. Г., Эльбекян К. С., Кошель В. И., Ходжаян А. Б. АНТИОКСИДАНТНЫЕ И ГЕМОЛИТИЧЕСКИЕ СВОЙСТВА РАЗНЫХ ЭКСТРАКТОВ ЛИСТЬЕВ *PRUNELLA VULGARIS* L. *Медицинский вестник Северного Кавказа.* 2018;13(3):507-510.
DOI –https://doi.org/10.14300/mnnc.2018.13091

ARA – Antiradical Activity
CAT – Catalase
DPPH – 2,2-Diphenyl-1-Picrylhydrazyl
HPLC – High-Performance Liquid Chromatography
GPx – Glutathione Peroxidase
GRx – Glutathione Reductase
OD – Optical Density
RA – Rosmarinic Acid
ROS – Reactive Oxygen Species
SOD – Superoxide Dismutase
TFC – Total Flavonoid Content

***Prunella vulgaris* L. (heal-all) is a herbaceous plant belonging to the *Prunella* genus of the *Lamiaceae* family [1], which has been used in traditional medicine for thousands of years. *Prunella vulgaris* L. has immunomodulatory, antioxidant, antiviral and many other properties. The active components of *Prunella vulgaris* L. extracts include flavonoids, triterpenoids, sterols and their glycosides, coumarins, organic acids, phytoncides, oils, saccharides, etc. The most important phytocomponents are phenolic compounds such as rosmarinic, ursolic and caffeic acids, tannins, rutin and quercetin, betulinic acid D-camphor, delphinidin, hyperoside, beta-carotene, etc. The flavonoids contained in *Prunella vulgaris* L. possess an important phytotherapeutic capacity [2, 3]. Rosmarinic acid has antioxidant and antiinflammatory properties, which prevent the development of reactive oxygen species (ROS) and lipid peroxidation.**

The etiology of many diseases implies oxidative stress [4, 5, 6, 7], whereas the condition required to maintain cellular homeostasis involves balance between the production of ROS (reactive oxygen species) or free radicals and their neutralization.

The enzymatic antioxidant system includes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx) [8, 9]. SOD – the first line of protection against free radicals – catalyzes the dismutation of the superoxide anion radical ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2).

The purpose of this work was to determine the antiradical, hemolytic activity of *Prunella vulgaris* L. extracts, and to evaluate erythrocyte superoxide dismutase.

Material and Methods. The active components were extracted from dry crushed leaves (1 g per 30 ml of extractant) with ultrasound (75W, 15 min in Ultrasonic Homogenizer, Sonic-150W, MRC, Israel). After that, the extracts were incubated on a shaker for 24 hours (60–70 rpm) and then centrifuged for 15 minutes at 3000 rpm on a Jouan GR412 centrifuge [10].

The total flavonoid content (TFC) was determined based on the flavonoids ability to chelate Al_3^+ atoms at 430 nm [11]. The antiradical activity of the studied extracts was based on the bleaching of the stable DPPH radical (Fluka) at 517 nm measured on a UV / Vis spectrophotometer (JENWAY 6405) [12].

The antiradical activity (ARA, %) was calculated following the formula below, taking into account the value of the residual absorption (correction factor – CF)

$$ARA\ \% = [(RA_{control} - RA_{sample}) / (RA_{control} - CF)] \cdot 100.$$

The extract activity was determined by the IC50 value, which indicated the amount of extract required to quench 50 % of DPPH radical in 1 ml of the solution, was determined based on dose-dependent ARA curves.

To determine the major components of the flavonoid class, a high-performance liquid chromatography (HPLC) method was employed using a Waters Alliance 2695 chromatograph with a spectrophotometric and diode array detector, as well as MassLynx data processing software. Separation was done on a C-18 column (250×4 mm, particle size – 4.5 nm) at an elution rate of 1 ml/min with the following gradient elution system: deionized water with 0.1 ml/l of 90 % orthophosphoric acid (solution A) – acetonitrile (solution B); 0–5 min linear solution A was adjusted from 10 % to 40 %, then to 50 % over the next 3 minutes and for the last 12 minutes, this ratio of solutions was maintained isocratically. Ethanol solutions of quercetin, rutin, apigenin, kaempferol at 365 nm, naringenin – 290 nm, genistein – 261 nm (all by Sigma company) were used as standards.

Erythrocytes were obtained from the blood of healthy people as well as the blood of patients affected with periodic disease. The optical density of the erythrocyte suspension was adjusted to 0.7 optical units (6.2×10^6 cells/ml) [9]. The erythrocyte resistance was evaluated by changing the optical absorption of the erythrocyte suspension at 680 nm for 10–15 minutes, the readings being taken every 30 seconds, on the Specord M400 spectrograph (Carlzeiss, Germany). The effect of *Prunella vulgaris* L. extracts (standardized by

rosmarinic acid) on erythrocyte resistance, was determined by the following formula:

$$P=Ax/(A0 - Ah)\cdot 100\ \%,$$

where Ax is the optical density (OD) of the sample after irradiation for x time; A0 – OD of unhemolyzed erythrocyte suspension; Ah – OD of the sample after full lysis, which does not reach the zero value because of hemichrome development.

The SOD activity was determined by the ability of the enzyme to inhibit adrenaline autooxidation detected by the adrenaline oxidation product with absorption at around 347 nm. The optical density of the solutions was recorded on a SF 26 spectrophotometer (Germany) at room temperature. SOD was obtained from erythrocytes hemolysate, and the activity was determined [13] subject to the formula

$$\text{SOD activity (CU)} = \frac{\%\ \text{inhibition}}{(100\ \% - \text{inhibition}\ \%)},$$

where the inhibition % (act. unit)=[1–(ΔD sample/ΔD control)]·100 %.

The statistical analysis was carried out using the Microsoft Office Excel ($p<0.05$).

Results and Discussion. A TFC analysis revealed that in most *P. vulgaris* extracts the indicator is quite high. The maximum TFC was found in 25 % of the *P. vulgaris* ethanol extract (24.75±0.7494 μg/g); it was lower in 50 % of the ethanol extract. Relatively low TFC was found in 96 % of ethanol, aqueous and 70 % of ethanol *P. vulgaris* extracts (Table). A HPLC analysis of extracts showed that the major component of ethanol extracts is rosmarinic acid (RA) (Fig. 1). The top high content of RA was identified in 50 % ethanol extract, which was 1.6 times as high as in the 96 % extract, and 1.1 times as high as in the 70 % extract. From the ARA point of view, the 70 % extract was the most active, while the least active was the 96 % ethanol extract.

Table

Total flavonoid content, RA, antiradical (IC_{50}) and SOD activity of *P. vulgaris* extracts

Samples	RAcontent (mg/g)	TFC (μg/g)	IC_{50}	SOD activity (%)
Aqueous extract	–	12.68±0.02472	7±0.05	50.7±1.1
25 % ethanol extract	–	24.75±0.7494	10.5±0.04	52.4±1.2
50 % ethanol extract	1.063±0.03	16.37±0.0826	0.36±0.002	18.7±0.9
70 % ethanol extract	0.982±0.01	12.28±0.31752	0.45±0.02	80.4±1.6
96 % ethanol extract	0.669±0.02	13.13±0.0192	0.65±0.01	22.2±0.6

Significant difference, $p<0.05$.

The results showed that the stable DPPH radical-neutralizing activity of all the extracts was of dose-dependent nature. However, there is no correlation observed between the TFC and the ARA of extracts, which may be due to the different qualitative composition of their flavonoids or to the presence of other compounds groups with antioxidant properties.

The hemolytic effect of extracts on erythrocytes was investigated straight after the addition of 0.1 ml of extract to 1 ml of the erythrocyte suspension in dark conditions, in the light without/with incubation. At dark incubation (60 min) the *P. vulgaris* extracts revealed no hemolytic activity. After 60 min incubation, the 50 % and 70 % of the *P. vulgaris* ethanol extracts led to partial hemolysis. The aqueous extract of *P. vulgaris* after 24 hours basically showed no hemolysis. The 25 % extract had insignificant hemolytic activity (erythrocyte resistance – 81 %), and the 70 % and 96 % *P. vulgaris* ethanol extracts after 24 hours resulted in complete hemolysis. In the light, the erythrocyte resistance remained 100 % for *P. vulgaris* aqueous extracts, whereas all the ethanol extracts resulted in destroyed red blood cells at different points (Fig. 2). The outcomes of the experiment suggest that *P. vulgaris* contains photosensitizers that get extracted with ethanol.

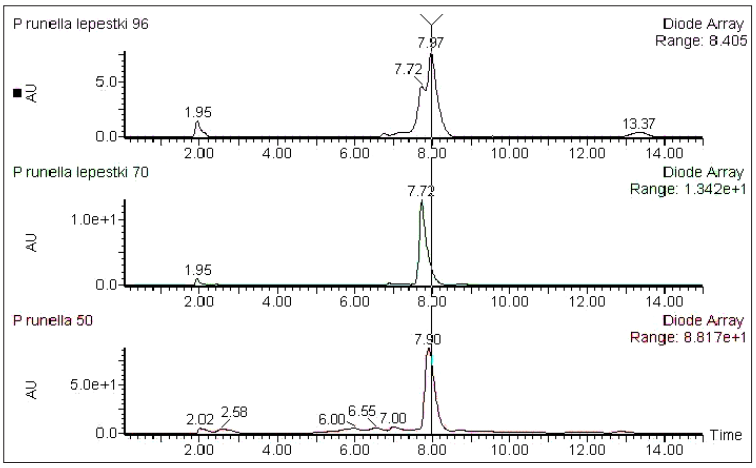

Fig. 1. HPLC analysis of *Prunella vulgaris* L. ethanol extracts

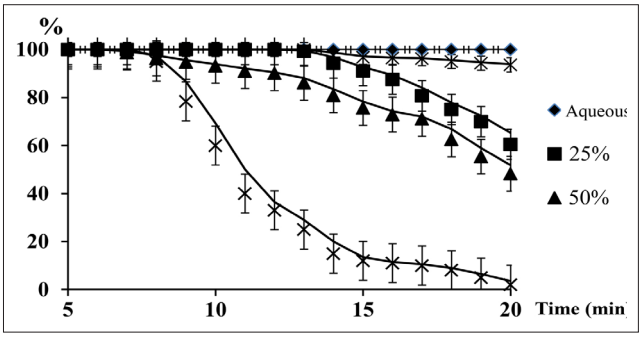

Fig. 2. Photohemolysis induced by *P. vulgaris* extracts

The results of the determining the erythrocytes SOD in the presence of *P. vulgaris* extracts showed that the inhibitory activity was higher in the 50 % ethanol extract (18 %), then in the 96 % extract (22 %), whereas the aqueous and the 25 % ethanol extract had it equal. An interesting thing was observed in the SOD activity in the presence of 70 % extracts (80.14 %, compared to control samples – 14.4 % higher inhibitory activity, yet much lower than in the 50 % and 96 % extracts (Fig. 3).

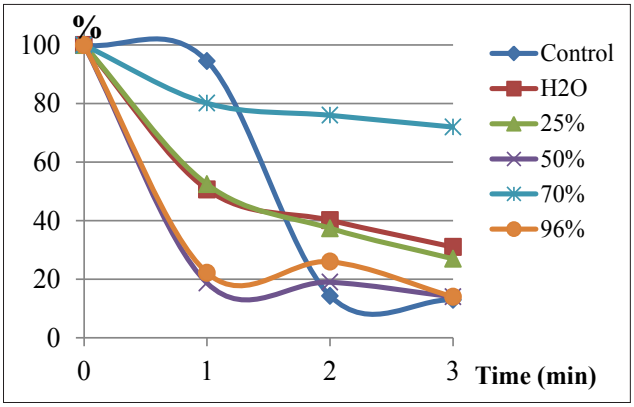

Fig. 3. Erythrocyte SOD activity in the presence of *P. vulgaris* extracts

With incubation for one hour, the endo- and exogenous systems of ROS neutralization revealed an almost similar low inhibitory activity, which may depend on the neutralization reactions through the incubation.

Conclusions. Therefore, the highest ARA was observed in the 50 % *P. vulgaris* ethanol extract, where the content of RA was maximal. The TFC in this extract is lower, which is indicative of the major contribution that RA – and not flavonoids – has in ARA. The high ARA level in the 25 % extract is manifested not through RA only, yet also through other active components. Low dark-period hemolytic activity was observed in the aqueous, in the 25 %, as well as in the 50 % extracts of *P. vulgaris* leaves. The 50 % and 96 % ethanol extracts have high SOD activity.

The outcomes obtained from the experiments described above could be used to summarize that high antiradical, antioxidant (SOD), and low hemolytic activity resides in the 50 % extract of *P. vulgaris*.

Disclosures:
The authors declare no conflict of interest.

References

1. Liu J. P., Feng L, Gu J., Wang R., Zhang M. [et al.] Simultaneous determination of ten characteristic antioxidant compounds for inhibiting cancer cell proliferation in Prunella vulgaris L. from different regions using HPLC-UV coupled with MS identification. *Analytical Methods.* 2014;9:3139-3146. https://doi.org/10.1039/c3ay41754a
2. Feng L., Jia X., Zhu M., Chen Y., Shi F. Chemoprevention by Prunella vulgaris L. Extract of Non-Small Cell Lung Cancer Via Promoting Apoptosis and Regulating the Cell Cycle. *Asian Pacific Journal of Cancer Prevention.* 2010;11:1355-1358.
3. Yu-Jin H., Lee E.-J., Kim H., Hwang K. *In vitro* antioxidant and anticancer effects of solvent fractions from *Prunella vulgaris* var. lilacina. *BMC Complementary and Alternative Medicine*. 2013;13:310. https://doi.org/10.1186/1472-6882-13-310
4. Gupta R. K., Singh N. Morinda citrifolia (Noni) alter oxidative stress marker and antioxidant activity in cervical cancer cell Lines. *Asian Pac. J. Cancer Prev*. 2013:14:4603-4606.
5. MacNee W., Tuder R. M. New Paradigms in the Pathogenesis of Chronic Obstructive Pulmonary Disease. *Proc. Am. Thorac. Soc*. 2009;6:527-531.
6. Browne S. E., Bowling A. C., Macgarvey U. Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. *Annals of Neurology*, 1997;41(5):646–653. https://doi.org/10.1002/ana.41041051
7. Khodzhayan A. B., Elbekyan K. S., Gevandova M. G., Shchetinin E. V. Impact of melatonin on immune system activity in rats under heavy metal poisoning. *Medical News of North Caucasus.* 2014;9(2):179-183. https://doi.org/10.14300/mnnc.2014.09051
8. Pacher P., Beckman J. S., Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol. Rev*. 2007;87:315-424. https://doi.org/10.1152.00029.2006
9. Halliwell B. Biochemistry of oxidative stress. *Biochem. Soc. Trans.* 2007;35:1147-1150. https://doi.org/10.1042/BST0351147
10. Cook N. C., Samman S. Review: flavonoids-chemistry, metabolism, cardioprotective effects and dietary sources. *Journal of Nutritional Biochemistry.* 1996;7(2):66-76. https://doi.org/10.1016/0955-2863(95)00168-9
11. Woisky R. G., Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control. *J. Api. Res*. 1998;37(2):99-105. https://doi.org/10.12691/jfnr-4-11-6
12. Vardapetyan H., Tiratsuyan S., Hovhannisyan A., Martirosyan A. Elucidation of DPPH radical cavenging, antibacterial and photodynamic activities of Hypericum perforatum extracts. *Biological Journal of Armenia*. 2012;64:111-116.
13. Sirota T. V. Use of Nitrosine Tetrazolium in the Adrenaline Autoxidation Reaction to Determine the Activity of Superoxide Dismutase. *Biomedical Chemistry*. 2013;59(4):399-410.

About authors:

Ohanyan Agapi Djivanovna, PhD student;
tel.: +37441931512; e-mail: agapi.ohanyan@rau.am

Shishkoyan Nona Djivanovna, student;
tel.: +37477097107; e-mail: shishkoyan1991@mail.ru

Kazaryan Shushanik Armenovna, teacher;
tel.: +37496260563; e-mail: shushanik.kazaryan@rau.am

Hovhannisyan Ashkhen Artashesovna, PhD assistant professor, Chair of department of the medical biochemistry and biotechnologies;
tel.: +37493620612; e-mail: ashkhen.hovhannisyan@rau.am

Tiratsuyan Susanna Gurgenovna, PhD, assistant professor;
tel.: +37491955587; e-mail: stiratsuyan@bk.ru

Elbekyan Karine Sergeevna, DBSc, Professor, Chair of department of the general and biological chemistry;
tel.: +79624007045; e-mail: karinasgma@inbox.ru

Koshel Vladimir Ivanovich, MD, DMSc, Professor, Head of the Department of Otorhinolaryngology with the course of PDE;
e-mail: lorfpdo@stgmu.ru

Khodzhayan Anna Borisovna, DMSc, Professor, Professor of the Department of Biology;
tel.: +78652353442; e-mail: kafedrabiologii@stgma.ru