ՀԱՅԱՍՏԱՆԻ ՀԱՆՐԱՊԵՏՈՒԹՅԱՆ ԳԻՏՈՒԹՅՈՒՆՆԵՐԻ ԱԶԳԱՅԻՆ ԱԿԱԴԵՄԻԱ
НАЦИОНАЛЬНАЯ АКАДЕМИЯ НАУК РЕСПУБЛИКИ АРМЕНИЯ
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ՀԱՅԱՍՏԱՆԻ ԿԵՆՍԱԲԱՆԱԿԱՆ ՀԱՆԴԵՍ

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ՀՀ ԳԱԱ «Գիտություն» հրատարակչություն

Լույս է տեսնում 1948 թվականից, հոդվածները հրատարակվում են հայերեն,

ռուսերեն կամ անգլերեն լեզուներով
Выходит с 1948 года, статьи публикуются на армянском, русском
или английском языках
Journal is published since 1948, the articles are published in Armenian,
Russian or English

Content

Ողջույնի խոսք

Հետխորհրդային տարիներին հիմնադրված Եվրասիա միջազգային համալսարանի ռազմավարական ծրագրում առանձնահատուկ նշանակություն է տրվել բարձրորակ կրթական գործունեության իրականացմանը և միջազգայնացմանը: Նպատակայնորեն ճիշտ ընտրված այս երկու թիրախային ուղղություններով տարված աշխատանքները տվել են իրենց դրական արդյունքները:

Հասարակության մեջ համալսարանը այսօր դասվում է ՀՀ բարձր վարկանիշ ունեցող, հավատ ներշնչող հաստատությունների շարքին:

Համալսարանի կայացման գործընթացը ուղեկցվել է նաև նոր մասնագիտական կրթական ծրագրերի ներդրմամբ: Առավելապես, միջազգային շուկայի պահանջները նկատի ունենալով, ներդրվել է նաև «Ֆարմացիա» մասնագիտությունը: 2022 թվականին ԵՄՀ -ն ունեցել է առաջին շրջանավարտները, ովքեր առավելապես մեզ բարեկամ Իրաքի Հանրապետության քաղաքացիներ էին:

Հատկապես միջազգային ուսանողների նկատմամբ ցուցաբերվել է հատուկ վերաբերմունք, արդյունքում գրեթե բոլորը ամփոփիչ քննություններին ցուցաբերել են բարձր առաջադիմություն, այնուհետև ճնշող մեծամասնությունը մասնակցել է Իրաքի Հանրապետությունում սերտիֆիկացման քննությունների և ստացել մասնագիտական աշխատանքի թույլտվություն:

Այսօր ուսանողի մասնագիտական, հետազոտական կարողությունների և հմտությունների զարգացումը համարվում է այն գլխավոր ծրագրային մեթոդական պահանջը, որը անհրաժեշտ է շրջանավարտներին ներկայանալու գործատուին որպես մասնագետներ:

Համալսարանը այդ ծրագիրը լիովին իրականացնում է:

Խնդիրը առավել հրատապ է բժշկական մասնագիտությունների, մասնավորապես՝ «Ֆարմացիա» մասնագիության պարագայում. այստեղ ուսանողի հետազոտական հմտությունները դիտվում են ոչ միայն որպես ծրագրային ելքային արդյունքների ապահովման միջոց, այլ նաև միջառարկայական և միջմասնագիտական խորը գիտելիքի ձեռքբերման պահանջ՝ նպաստելով բազմակողմանի հմտություններով օժտված մասնագետների պատրաստմանը:

Եվրասիա միջազգային համալսարանի Գիտական խորհուրդը և Ֆարմացիայի ամբիոնը, խորությամբ դիտարկելով այդ մասնագիտության գծով շրջանավարտների որակին ներկայացվող նոր չափանիշները և գործատուների կողմից ներկայացված պահանջները և այլն, գործադրում են լրացուցիչ ջանքեր՝ բարձրացնելու մասնագիտական ուսուցման որակը:

Այսպես, մասնագիտական պրակտիկաների տևողության մեծացման, լաբորատոր փորձարարական պարապունքների իրականացման, առանձին դասընթացների գծով միջազգային և հանրապետության լավագույն փորձագետ-դասախոսների ներգրավման ու միջազգային չափորոշիչները պահպանելու

միջոցով հնարավոր դարձավ ավարտական աշխատանքները դարձնել ուսանողական այնպիսի հետազոտություններ, որոնց մի մասը ամբիոնի որոշմամբ նպատակահարմար գտնվեց նաև հրատարակել Հայաստանի կենսաբանական հանդես պարբերականում:

Ուսանող-շրջանավարտի կատարած հետազոտությանը այս ձևով վերաբերվելը պետք է դարձնել ավանդույթ՝ շրջանավարտի ինքնահաստատման ձև, որը կարող է նրան օգտակար լինել նաև հետագա մասնագիտական գործունեության մեջ և դրական դեր խաղալ կարիերայի առաջընթացում:

Սուրեն Հեքիմի Օհանյան
Աշխ. գիտ. դոկտոր, պրոֆեսոր
Եվրասիա միջազգային համալսարանի ռեկտոր

Greetings

Higher professional education is in the stage of deep substantive reforms. The main prerequisite is the transition from the theoretically oriented education to the practical one.

The development of professional, research abilities and skills in the student is considered as the main educational program based methodological requirement, which is necessarily for graduates to be presented to the employer as a trained specialist.

The economy, in turn, is transitioning to the knowledge-based industries' development, which makes the provision of graduate research skills for universities as a prerequisite for the main reproduction of specialists.

The problem becomes more urgent in the case of medical professions, particularly pharmacy. Here, the student's research skills are seen not only as a reflection of program outputs, but also as a requirement for acquiring deeper interdisciplinary and interprofessional knowledge, contributing to the preparation of today's multi-skilled professionals.

Eurasia International University Scientific Council and the Chair of Pharmacy investigated deeply and basically the new standards presented to the quality insurance of graduates in that professional field and the requirements presented by employers, etc., made additional efforts to increase the educational quality.

By increasing the duration of professional practices, implementing laboratory experimental classes, involving international expert-lecturers in separate courses and applying international standards, it became possible to turn the theses works into student research and the significant part of them based on chair's decision, was qualified for publication in Armenian Biological Journal.

The emphasized consideration of the research performed by the student-graduate should be established as a tradition. Such work will form and generate self-establishment of the graduate, which can be in the future used during professional work and will trigger work progress.

Suren Hekim Ohanyan
Doctor of Geographical Sciences, Professor
Rector of Eurasia International University
Translated by Haykuhi Muradyan,
Head of Foreign Languages faculty at EIU.

THE FRAMES OF THE WORK OF PHARMACY DEPARTMENT AND SCIENTIFIC THESES OF THE STUDENTS

The aim of the "Pharmacy" educational program is to prepare qualified and professional pharmacists who will possess in-depth with practical and theoretical knowledge. Graduates of our program will be possessing with the critical as well as analytical way of thinking.

The graduates of our program are able to design the medicines, synthetize, evaluate the quality of the medicines, conduct the research in the field, be able easily and professionally the work in pharmacies, technological industrial companies, be educated and knowledgeable in the fields of drug management, economics as well GMP. We create a collaborative environment that meets both national and international quality assurance standards and market demand. Graduated students will be prepared as the specialists not only theoretically but also practically.

Our lectures are mostly experienced specialists trained in the best foreign universities, some of them are also leaders in the practical field, and this enables them, along with theoretical knowledge, to transfer their rich experience from the practical field to students.

During the last of BS as well as the MS programs the students are starting their rotations not only through the different pharmacy chains, drug industrial companies and clinics but also are attending the Scientific Institutes of the National Academy of Science of Armenia. Together with their Principal investigators the students are developing scientific theses work.

Graduates of 2022 defended their theses works and became not only specialist ready to overcome professional life challenges but also think analytically, scientifically and be creative in any difficult circumstances, guarantee the future development of any initiative. Most of the theses works are presented in this journal as short abstracts as well as short communications, articles.

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GRADUATES OF PHARMACY DEPARTMENT OF 2018-2022

Analytical comparison of the Ibuprofen tablets supplied from two different companies

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Abstract

Background. High performance liquid chromatography (HPLC) is one of the most important methods for the analyses of the medicines. The sensitivity of the method is allowing to detect even picomoles amounts of the existing compounds. The aim of the work was the investigation of the tablets of Ibuprofen, one of the most widely used medicine for the treatment of pain, inflammation etc. Both

products of the same medicine produced by two different companies were bought from Pharmacies of Armenia.

Methods. For determination of the maximums of absorption it was applied the spectrophotometric scanning by the utility of the Carry 60 (Agilent, Germany) spectrophotometer. Reverse-Phase HPLC was applied for the detection of Ibuprofen as well as the impurities and excipients. It was used Shimadzu LC system, which consists of the Controller CBM -20A, Pump A-LC-20AD, Autosampler –SIL-20 A, Oven, CTO-20A, PDA-SPD-M20A. For the reverse type of chromatography it was applied Column Waters Symmetry 300TM C18, with the pore size 5 mcm, the length and the diameter of the column was equal to 4.6x250 mm The number of the excipients was taken into the consideration from the leaf-list and after comparison with the European Pharmacopea, conclusions were made. There wasn't performed calculation regarding the absolute amount of medicines in tablets. The all analyses were comparative.

The conditions of the experiments were the following: flow rate- 1 ml/min, entire time of the experiment was equal to 20 minutes, isocratic flow supply for organic solution was equal to 80% and for inorganic solutions was equal to 20%, temperature for the analyses was increased up to 40C and the total injectable volume of the sample was 10 ul.

Results and conclusions. Ibuprofen has two maximums at 214 as well as 260 nm. Both companies- Company N1 as well as Company N2 provided the tablets with allowed by European Pharmacopeia % of the impurities. The number of impurities as well as excipients was larger in the tablets from Company N2 in comparison with Company N1. The amount of the Ibuprofen in comparison with accompanying products in Company N2 tablets was higher in comparison with Company N1 pills.

Key words: HPLC, Ibuprofen, comparison, analyses

Application of HPLC method for the comparison of the tablets of analgen provided by two different companies

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Background. HPLC (High Performance Liquid Chromatography) is a powerful separation method and must be able to resolve mixtures with a large number of similar analytes. It is a separation process in which the sample mixture is distributed between two phases in the chromatographic bed (column or plane). One phase is stationary while the other passes through the chromatographic lumen. The stationary phase is either a solid, porous, surface-active material in small-particle form or a thin film of liquid coated on a solid support or column wall. The aim of the work was comparison of products from two companies with each other without application of the absolute analytical method of calculation of the amount of the medicine in the tablets.

Methods. For the experimental analyses we used the Shimadzu LC system, which consists from the Controller CBM -20A, Pump A-LC-20AD, Autosampler –SIL- 20 A, Oven, CTO-20A, PDA-SPD-M20A. For the analyses it was also used the Column Waters Symmetry 300^{TM} C18, with the pore size 5 mcm, parameters of the column is the following 4.6x250 mm. For the delineation of the specific maximums of absorption of Analgen spectrophotometric method was applied (Carry 60, Agilent, USA). Flow rate equal to 1 ml/min was chosen as the best condition for obtaining of high-resolution chromatogram of Analgen.

Results and conclusions. The gradient type of elution for the detection of Analgen by RP-HPLC was the most effective one. The second Sample contained a greater number of impurities as well as excipients in comparison with Sample

1. We suggest, Sample 2 contains more amounts of the impurities in accordance to “Substances for pharmaceutical use (2034)” guideline.

Key words: HPLC, comparison, analyses, Analgen.

Comparison of the content of two tablets of Paracetanol produced by two different companies

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Background. High performance liquid chromatography (HPLC) is one of the main methods for the analyses of the medicines, detection of their impurities or the products of their degradation.

By changing of the column of the HPLC it is possible to change the type of the chromatography to gel-filtration, ion-exchange, affinity type or even reverse phase, which is appropriate for the separation of the compounds based on their hydrophilic as well as hydrophobic natures.

Accordingly, HPLC system is allowing separation of the compounds based on their molecular masses, surface ion charge, specific structure, etc.

The aim of the work was the comparison of two types of Paracetamol tablets produced by two companies. The results were comparative and not absolute.

Methods. In accordance with European Pharmacopeia, it was prepared the test solution for HPLC analyses by the following way: it was dissolved 50.0 mg of the substance, which was supposed to be examined in the solvent mixture and diluted to 5.0 mL with the solvent mixture.

For the experimental analyses we used the Shimadzu LC system, which consists from the Controller CBM -20A, Pump A-LC-20AD, Autosampler –SIL-20 A, Oven, CTO-20A, PDA-SPD-M20A. For the analyses it was also used the Column

Waters Symmetry 300TM C18, with the pore size 5 mcm, the parameters of the column were equal to 4.6x250 mm.

Results and conclusion. The existence of the compounds might change the range of the maximal absorption of Paracetamol. Paracetamol produced by Company N 1 contained more side products than Paracetamol, produced by Company N 2. In contrast to 254 nm at 210 nm the sensitivity of the method was higher for the detection of any existing impurities.

The total number of the excipients of Paracetamol produced by Company N 1 was equal to 5, whereas for the medicine, produced by Company N 2 - 7.

However, the number of peaks appeared after analyses of paracetamol at 254, produced by Company N 1 was equal instead of 6 to 9 peaks. Paracetamol produced by Company N 2, contains 7 excipients. The number of the appeared peaks were equal to 10, suggesting existence of one possible side product of syntheses.

Key words: HPLC, comparison, analyses, Paracetamol

Research of the main rules regarding pharmaceutical development of drug substance and drug product

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On the example of «Furacillin 0.02 % 200 ml solution for external use» drug product the main rules regarding pharmaceutical development of drug substances and drug products were studied and discussed. Section 3.2.P.2 Pharmaceutical Development of CTD for this drug product which is developed as an antimicrobial medicine against gram-positive and gram-negative bacteria was provided by six main parts. There were provided sections 3.2.P.2.1-3.2.P.2.6 of CTD, as well as some reference sections where more detailed information may be found. Reference sections provided by ICH were extracted and provided.

Key-words: Regulation, pharmaceutical development, Common Technical Document, section 3.2.P.2, reference sections.

Research and evaluation of stability data of drug products in agreement with Armenian drug law-regulations

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Based on the example of «Furacilin 0,02 % 200 ml solution for external use» drug product the main requirements toward CTD section 3.2.P.8.3 Stability data were extracted and discussed. Section is provided by three main parts: 3.2.P.8.3.1 (Results), 3.2.P.8.3.2 (Analytical procedures and 3.2.P.8.3.3 (Method Validations). Main differences the region specific and ICH general requirements were discussed. Reference sections of CTD, as well as their differences from 3.2.P.8.3 Stability data section were extracted as well. Applicable data according to time zero studies were calculated.

Key-words: Regulation, stability data, Common Technical Document, section 3.2.P.8.3, reference sections.

Research and evaluation of the main storage conditions and shelf lives of drugs as a part of their regulation process

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Research and discussion of Appendix B of ICH Q1E guideline were provided. Summary of the section 3.2.P.8 Stability of CTD has been discussed. Main reference sections were extracted and provided. Main differences between storage conditions of the drug products are provided. There were extracted applicable time points and frequency for the stability studies. Applicable data according to time zero studies were calculated for assay, pH, appearance, color, completeness of mass, etc. Acceptable shelf lives according to the available information are proposed.

Key-words: Regulation, storage conditions, Common Technical Document, section 3.2.P.8, time zero studies, shelf life, applicable data.

Evaluation of nonclinical data for anticancer pharmaceuticals as a part of their regulation process

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Discussion of the structures of CTD Modules 4 and 2.4 is provided, available information is extracted. Main differences between ICH guidelines S6, S9 and M3(R2) were extracted and provided. Mandatory studies and studies conducted on a case-by-case basis, requirements to the GLP certificate, main rules regarding nonclinical studies, as well as differences between nonclinical studies of well-known and new drug products were extracted. applicable statements and layout for Module 4 according to the rules were provided.

Key-words: Regulation, nonclinical studies, Common Technical Document, Module 4, ICH guidelines S6, S9 and M3(R2), GLP certificate, case-by-case studies.

The effect of solvent and pH on trypsin – polyphenolic compounds interaction

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Protease inhibitors are one of the most promising and investigated subjects for their role in pharmacological studies, biological functions, medical benefits and drug design. The digestive enzymes–polyphenolic compounds interactions can be discussed as inhibition of these enzymes. It is known that green tea is the source of antioxidants, particularly polyphenolic compounds. The inhibition of trypsin by polyphenolic compounds derived from green tea is extensively studied [1,2], whereas the inhibition of digestive enzymes, in particular trypsin by antioxidants derived from alpine herbal tea remains unexamined. Various physiological effects of herbal tea are well-reported such as germicidal, anti-inflammatory, sedative, analgesic effects etc.

In present work the complex formation between trypsin and polyphenolic compounds derived from thyme (*Serpylli herba*) extract is studied. The effects of solvents such as ethanol and acetonitrile, as well as pH of the media from acidic to basic on complex formation are revealed.

The obtained results show that the binding constant increases with the decrease of pH due to the increased activity of trypsin in acidic solution. On the other hand, in basic media conformational changes cause the decrease of availability of binding site for ligand. As a result, complex formation does not occur properly. In organic media trypsin deviates more from the crystal structure in the free state

or in the complexed form than in aqueous solution. This departure is greater in ethanol than in more polar acetonitrile media resulting in the higher binding strength in less polar ethanol compared with water and acetonitrile.

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Comparison and analyses of two tablets of Aspirin produced by two different companies

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Background. Aspirin is a drug that is very commonly used worldwide to treat many conditions, and it is considered as a class of non-steroidal anti-inflammatory drugs (NSAIDs). Aspirin contains an active substance known as acetylsalicylic acid. acid. Usually, aspirin is dispensed without a prescription, but sometimes a doctor must be consulted, and a prescription used to dispense it, especially in cases that require high doses of it, such as cases of pain relief.

Aspirin is used very commonly for the following two cases: pain reliever and antipyretic and anti-platelet aggregation compound. When a person is exposed to injury or infection, the body produces a substance called prostaglandin as a natural reaction of the body to resist this injury, and prostaglandins cause swelling and fever, and send pain signals to the brain, so the mechanism of action of aspirin in relieving pain and lowering body temperature depends on stopping the producing of prostaglandins.

Low-dose aspirin is useful in treating or preventing blood clots. In this case, aspirin acts as an antiplatelet in the blood vessels so that clots do not form, causing serious diseases such as heart attacks and strokes. This is one of the most common uses of aspirin.

Methods. For the experimental analyses we used the Shimadzu LC system, which consists of the Controller CBM -20A, Pump A-LC-20AD, Autosampler – SIL-20 A, Oven, CTO-20A, PDA-SPD-M20A. For the analyses it was also used the Column Waters Symmetry 300^{TM} C18, with a pore size 5 mcm, size

parameters of the column were the following 4.6x250 mm. Analyses of the medicines were made in comparative way and not absolute way.

The pore size 5 mcm, 4.6x250 mm.

Results and conclusion. Both medicines from the state point of the existence of the excipients are in the allowed frames of European Pharmacopeia for human use. The Aspirin purchased from Company N 1, except of the two main components contain one more ingredient with high area of the peak in contrast to the Aspirin produced by the Company N 2.

Key words: HPLC, analyses, comparison, Aspirin

Stability Studies of **Minoxidil Solution**

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Background. Minoxidil was the first introduced as an oral medication antihypertensive medication in the 1970s. Coincidentally, physicians observed hair regrowth and generalized hypertrichosis in balding patients, and the discovery of its common adverse event, hypertrichosis, led to the development of a topical formulation for promoting hair growth, which led to the development of a topical minoxidil formulation for treating androgenetic alopecia (AGA) first in male and then in female individuals.

The 2% minoxidil solution was first launched in the market in 1986, followed by the 5% solution in 1993. Despite its global acceptance for over 30 years, the mechanism underlying the hair growth-promoting effects of Minoxidil remains to be fully elucidated. To date, topical Minoxidil is the mainstay treatment for androgenetic alopecia and is used as an off-label treatment for other hair loss conditions. Despite its widespread application, the exact mechanism of action of Minoxidil is still not fully understood. The aim of the work was the evaluation of the stability of Minoxidil in the conditions of application of high temperature equal to 42 C as well as increase of the acidity.

Methods. Minoxidil was injected into the system of HPLC (High Performance Liquid Chromatography) in a volume equal to 10 ul as it is. The samples were incubated at about 40°C in thermostat (TC-80M, Russia) over the 7 days. For the experimental analyses we used the Shimadzu LC system, which consists from the Controller CBM -20A, Pump A-LC-20AD, Autosampler –SIL-20 A, Oven, CTO-20A, PDA-SPD-M20A. For the analyses it was also used the Column

Waters Symmetry 300TM C18, with the pore size 5 mcm, size parameters of the column were equal to 4.6x250 mm. The spectrophotometric methods were applied for the detection of the maximums of absorption of Minoxidil (Cary 60, Agilent, USA).

Results and conclusions. Accelerating stability test of Minoxidil in the conditions of 40C, incubation over 7 days didn't initiate any significant degradation processes, which were evaluated by the RP-HPLC. Stability of Minoxidil was also checked by the spectrophotometric scanning process from 190-1100 nm, which was more sensitive than the HPLC method. Acceleration of instability of Minoxidil was initiated by the decrease of pH up to 3.35 as well as increase of temperature of the water bath up to 100C. Scans of the boiled and acidated Minoxidil were slightly different. Baking or acidating of Minooxidil is slightly initiating the degradation of the medicines.

Key words: HPLC, analyses, Minoxidil, stability exeperiments

Comparative analysis of Residual Quantities of Imidazole in Natural Honey

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Background. Honey is one of the most significant foods since it contains many essential nutrients for the human body. Honey is made by bees, which are the primary producers of honey. Bees are identical to other organisms that can be infected with bacteria, fungi, or a variety of other illnesses and pathogens, that may threaten to many bees and consequently affecting natural honey production.

Veterinary medicines are used in the treatment of bees by beekeepers and are also considered one of the methods for reducing the annual death rate of bees due to diseases or other forms of infection, taking into account whether or not these medicines meet the European Union standards for veterinary medicines.

Chemical residues of veterinary medicines such as Imidazole derivatives, Terramycin, Apistan, Fumagilin,etc are used in the treatment of bees and are one of the issues that may cause an increase in the chances of bee population changes, or one of the problems that may cause issues at the consumer level, as well as in the event that the residues of the medicines are transferred to the beehives directly or indirectly through placing these veterinary drugs in the diet of bees.

The aim of the work was estimation of the existence of Imidazole group compounds: Clotrimazol as well as Metranidazol in the two samples of honey both form the main supermarkets in the capital city of Armenia-Yerevan.

Material and methods. For identifying and quantifying od imidazole derivatives, we used a modern technique to determine those residues in honey by HPLC. The experiment were based on dissolving of 200 mg of two different samples of honey separately in 5 ml of water in glass flask; 0.1 g of Metranidazole was dissolved in 5 ml of water in glass flask; 0.05 g of

Clotrimazole was dissolved in 5 ml of acetonitrile. For the experimental analyses we used the Shimadzu LC system, which consists from the Controller CBM - 20A, Pump A-LC-20AD, Autosampler –SIL-20 A, Oven, CTO-20A, PDA-SPD-M20A HPLC was conteining the column C18 with pore size 5mcm , size parameters of the column were equal to 4.6*250mm.

Results and conclusions. The analyses of honey were performed by HPLC system and wavelength equal to 237 nm and 220 nm were applied. The main peak of Clotrimazole in our applied conditions appeared at 2.2 minute. The peak of Metranidazole in settled by us conditions appeared at 0.8 minute. After addition of Metranidazole into the solution of honey it appeared on the chromatogram at 0,4 minute. After addition of Clotrimazole into the solution of honey it appeared on the chromatogram at 0,8 minute. The peak of honey was appearing later than mentioned medicines. Both samples of honey didn't contain Clotrimazole as well as Metranidazole.

Key words: honey, Imidazole, Metranidazole, Clotrimazole, HPLC

Circulation kinetics of albumin micro- and nano-particles as drug carriers

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Introduction. A successful drug delivery nanodevice should ideally meet the following criteria: drug retention; immune system evasion, extending circulation time; targeting to the diseased site while avoiding most healthy organs and make the drug available. The nanodevices may protect the drug from degradation, overcome unfavorable solubility and interactions, increase drug selectivity, improve drug absorption by facilitating diffusion through epithelial, improve intracellular penetration and distribution, and/or change the pharmacokinetic and drug tissue distribution profile.

The aim of this research project is to look into the kinetic circulation of albumin nanoparticles as drug carrier, as well as the behavior of nanoparticles in physiological and pathological conditions via analyzing the impact of particle size. In addition, we aim to evidence the efficacy of PRP-1 (Proline Rich Protein-1) /NP (nano-partciles) prominent compound, preserving the complicity of the BBB in the conditions of experimental stroke in comparison with the other groups.

Materials and methods. Albumin nano- and micro- particles were generated by the utility of Glutaraldehyde (40 %, Medisar, Armenia). Centrifugation was applied for obtaining of desired size of the particles, which was evaluated by means of TEM with the application of 5 % uranyl acetate. The oxidative phase of the brain damage was reflected by the injection of the

hydrogen peroxide into the brain parenchima. Circulation abilities of particles were defined by the utility of the albumin specific dye – Evans Blue.

Results and conclusions. We generated the particles with diameters from 200 nm – 3 microns. In vitro conditions it was noticed, that PRP along with KNaC4H4O6*4H2O is able to inhibit the activity of trypsin as the protease reflecting the abilities of blood proteases. During circulation experiments in animals we have noticed, the small size particles were circulated longer than large ones. PRP, NP as well as NP/PRP particles are protecting BBB from disruption after intracranial injection of hydrogen peroxide.

Key words: albumin particles, PRP, TEM, pharmacokinetics, experimental stroke

Improvement of the biological particles stability in blood stream by chemical modifications

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Background. The proper drug delivery systems and the efficient drugs lacks the side effects, are less toxic, might be used in low concentrations, don't interact with the other medically used compounds, have long circulation time, might be delivered into the damaged tissue directly after systemic administration. Reduction of the side effects, toxicity and high efficiency of the medicines might be reached based on the targeted drug delivery, when the medicinal compound is reaching the targeted tissue. This strategy is reducing the quantity of the utilized medicinal compounds as well as eliminating the side effects of the medicines. Coupling the compounds with the carriers in numerous cases extends the time window of the particles circulation in blood stream, making them even more applicable for the prophylactic utility.

Materials and methods. Albumin as well as covered with tannic acid particles were synthetized by the addition of Glutaraldehyde (40 %, Medisar, Armenia) step by step. The utility of different speeds of centrifugation allowed us to obtain the desired size of the particles. Stability of the particles was checked in the environment of trypsin, serine protease, mimicking the environment of blood plasma. Circulation experiments of tannic acid covered particles were performed in white laboratory rats. The particles were stained with specific to albumin dye – Evans Blue.

Results and conclusion. We obtained the tannin covered particles in vitro. Tannin covered particles are more stable over 48 hours in comparison with not

covered particles. Tannin covered particles are circulating longer than not covered albumin particles in blood stream of laboratory rats.

Key words: particles, tannic acid, stability, albumin, circulation.

New purification method of tannic acid

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Background. Tannic acid is a chemical compound belonging to the Tannins phenolic compounds, which are complex organic compounds with a carbohydrate structure and are found in a wide range of plants. These compounds are causing intensive initiation of multiple functions such as metabolism. Tannic acids might be playing the role of oxidation antagonists. Under the conditions of directly application on the surface of the skin tannic acids are preventing cold-induced sores, might be used against fever, blisters, diaper rash, impetigo, minor burns, and sunburn. They might be used to treat poison by iv infections, sore throats, tonsillitis, thinning gums, and ingrown toenails. However, more data are needed for clinical proofs of such effects. The aim of the work was purification of tannic acid from pomegranate peels for further evaluation of its binding abilities with phosphorus containing compounds.

Materials and methods.

The rude fraction of pomegranate peels was isolated from the fruit and sliced for further homogenation. Affinity-chromatography methods for purification of tannic acid were developed. Tannic acid sephadex G-50 conjugates- beads were serving for the affinity chromatography of phosphorus containing compounds.

Results and conclusion. We established the new method of tannins purification. It was developed the affinity chromatography method based on the high affinity

of albumin to tannins. Tannic acid molecules were easily detached during affinity chromatography by the eluent and were present in the highest concentration in the first fraction of collection. We also, created the affinity gel for the binding of the phosphorus containing molecules. Based on our investigations, 90 % of the phosphates from applied amount were binding with the tannic acid carrying gel

Key words: tannic acid, purification, chromatography, pomegranate peels.

Manufacturing process of Ibuprofen suspension: identification of risks and possible performance of the actions for establishment of the stability

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Background. Ibuprofen is a 2-phenylpropionic acid and the chemical relative of a group of substituted phenyl alkanoic acids prepared during the early 1960s. The drug Ibufenac, introduced in England and proven hepatotoxic, was shown before its removal from the market to have useful anti-inflammatory properties. This engendered a search for safer, equally potent drugs of the same general class, culminating in the introduction of Ibuprofen or 2 (4-isobutylphenyl) propionic acid. Ibuprofen was the first of three propionic acid compounds introduced into the United States, the others being fenoprofen and naproxen. It has weak but definite anit-inflammatory properties similar to those of Aspirin, but with considerably less adverse effect on the stomach. The drug has analgesic properties, probably related to its anti-inflammatory effect. It inhibits prostaglandin synthesis and has no effect on the adrenopituitary axis, making it a nonsteroidal agent. Ibuprofen has been shown to be effective in rheumatoid arthritis and osteoarthritis and is probably effective in ankylosing spondylitis, gout, and Bartter's syndrome. The aim of the work was the comparison of the stability of Mig, Nurofen products as well as Ibuprofen,

Methods. It was used spectrophotometer BK-UV1800PC (Biobase, China). The amount of Ibuprofen present in the sample was detected based on UV absorbance at the wavelength of maximum absorbance at about 218-222 nm, in comparison to the standard solution using dissolution medium (Phosphate buffer, pH 7.5) as a blank.

Results and conclusions. The degradation process of Mig and Nurofen change drastically based on their chemical abilities in comparison with Ibuprofen. The product viscosity of Mig and Nurofen products are higher than Ibufen, probably due to the Ibufen not homogeneous particles distribution in the sampling process. During the work we also concluded that when the storage conditions are changed (higher temperatures) the rheology of the suspension also was changed, and this affected the product stability. We also concluded, that Ibuprofen particles size differed in the Ibuprofen Product and that was smaller than in the other 2 products, which was the reason the particles remain suspended for the stability period ensuring homogeneous mix.

Key words: suspension, stability, ibuprofen, Mig, Nurofen

Description of the entire process of Sodium Diclofenac different pharmaceutical forms manufacturing processes from API synthesis till finished product. Demonstration through laboratory tests their advantages and differences regarding bioavailability

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Background. Diclofenac sodium is non-steroidal anti-inflammatory drug (NSAIDs) recommended for use in painful and in inflammatory rheumatic and certain non-rheumatic conditions. Its available in many dosages form which can be given orally, rectally, topically and intramuscular. Sodium diclofenac has short half-life which limits the potential for drug accumulation. The aim of this research is to demonstrate the bioavailability of different pharmaceutical forms (tablet, capsule, suppository) of sodium diclofenac through laboratory tests, dissolution is directly correlated with the absorption, different pharmaceutical forms have different dissolution rate, as faster the dissolution the absorption is also faster.

Methods. It was used spectrophotometer BK-UV1800PC (Biobase, China). The amount of Diclofenac present in the sample was detected based on UV absorbance at the wavelength of maximum absorbance at about 209 nm, in comparison to the standard solution using dissolution medium (Phosphate buffer, pH 7.5) as a blank.

Results and discussion. In accordance with the results dissolution of the capsules was occurring faster in comparison with the suppository as well we tablet type of Diclofenac formulations. Based on the spectrophotometric results suppositories were be dissolving during 30 minutes in blood stream, whereas in vitro this formulations of Diclofenac weren't dissolved. In vitro in contrast to tablet capsular formulations Diclofenac were dissolved for 56%.

Conclusion. In vitro dissolution represents the absorption process. Dissolution is as fast as absorption. Dissolution is directly correlated with the absorption. Different Pharmaceutical Dosage forms have different dissolution rates. At the laboratory (in vitro) performed dissolution test was able to demonstrate some idea about how the absorption is going on in each pharmaceutical form.

Key words: Diclofenac sodium, capsule, tablet, suppository, in vitro, dissolution.

Justification and demonstration through experimental test and literature, the main differances on coated and uncoated tablets.

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Background. An important preface to any discussion on coating must be—Why coat tablets? Since a broad range of pharmaceutical oral solid dosage forms are coated, important reasons include the following: 1. protecting the drug from its surrounding environment (particularly air, moisture, and light) to improve stability; 2.masking unpleasant taste and odor; 3. making it easier for the patient to swallow the product; 4. improving product identity, from the manufacturing plant, through intermediaries, and to the both healthcare workers and patients; 5. facilitating handling, particularly in high-speed packaging/filling lines, and automated counters in pharmacies, where the coating minimizes cross-contamination due to dust elimination; 6. improving product appearance, particularly where there are noticeable visible differences in tablet core ingredients from batch to batch; 7. reducing the risk of interaction between incompatible components. This would be achieved by using coated forms of one or more of the offending ingredients (particularly active compounds); 8. Improving product robustness because coated

products generally are more resistant to mishandling (abrasion, attrition, etc.).

The aim of the work was comparison of physical properties of uncoated and coated tablets based on the example of paracetamol.

Methods. It was investigated the drug release, as in repeat-action, as well as the delayed release (enteric coated) and sustained-release of the products. It was determined the amount of Paracetamol dissolved based on UV absorbance at the wavelength of maximum absorbance at about 243 nm, using dissolution medium (Phosphate buffer, pH 5.8) as a blank.

Results. That the dissolution rate of Paracetamol tablets at pH:5,8 is higher than the uncoated tablets at the same conditions. The differences could be because the coating materials are not soluble at that pH medium.

Conclusions. If we consider that the Paracetamol is absorbed at the pancreatic level at pH 5.8 the uncoated tablet will have better absorption and as a consequence faster analgesic action if it is administrated as uncoated tablet. If we consider that we want a prolonged action of the Paracetamol, we can use the coated form to ensure that those are going to be dissolved slowly and will be absorbed at lower rates form more long time.

Key words: paracetamol, coated, uncoated, tablet.

Antioxidant activity of Thymus Kotschyanus Boiss. & Hohen. extracts and essential oil

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Abstract

Medicinal plants are rich in therapeutically important secondary metabolites. In this paper, we are representing the phenolic content and the antioxidant properties of *Thymus kotschyanus* Boiss. & Hohen. decoction, ethanolic extract and different dilutions of essential oil. Based on the results we obtained from this study we concluded that decoction and 0.5% essential oil solution of *T. kotschyanus* have remarkable activity and can serve as a strong natural antioxidant.

Keywords: *Thymus kotschyanus*, essential oil, antioxidant activity, phenols.

Introduction. Medicinal plants are rich in therapeutically important secondary metabolites. The key advantages claimed for the therapeutic use of medicinal plants in various diseases are their economics, efficacy, and their safety, in addition to their availability. Because of these advantages, medicinal plants have been traditionally widely used in medicine. Among the various plants, *Thymus kotschyanus* Boiss. & Hohen. is traditionally used in Armenia by the vast majority of the population to treat numerous illnesses, however, it is not an officinal plant. The thyme has expectorant, antiseptic, antispasmodic and antifungal effects.

The contents and types of secondary metabolites differ depending on the locations of the plant. In general, essential oil (EO) of *T. kotschyanus* contains phenolic compounds such as thymol, carvacrol, linalool, α-terpineol, and geraniol (Tohidi et al., 2018). Many *in vitro* pharmacological experiments conducted over the last decade have shown clear pharmacological activity in both thyme EO and

plant extracts (Baharfar et al., 2015; Zakaryan et al., 2018; Ghasemi et al., 2020; Golkar et al., 2020).

In Armenia, traditionally fresh or dried thyme is used to flavour tea, cheese, curd, bean and meat dishes, sausages, sauces, salads, pickles, bread, spirits, etc. Thyme is an excellent honey plant, producing abundant nectar. The essential oil is used in the confectionery and canning industry, as well as in perfumery. The branches and roots have tanning properties. In dry hay, in the form of a partial mixture, thyme has a beneficial effect on the digestion of cattle. The plant is ornamental, it is widely used to make flower carpets and rock gardens (Nanagulyan et al., 2020).

The aim of the research is the screening of antioxidant activities of the *T. kotschyanus* plant aerial part extracts and EO and the determination of its extracts' total phenolic contents by spectrophotometry method.

Materials and Methods.

Plant Material. The species was collected from Vayots Dzor province of Armenia. Specimens were dried under natural conditions. Voucher specimens were deposited in the Herbarium of Yerevan State University (ERCB).

Decoction. Air-dried and powdered plant samples were heated with distilled water for 15 min in the water bath. The ratio of solvent to raw material is usually 20:1. After the incubation, the decoction was cooled for no less than 45 minutes at room temperature, filtered and added distilled water until the previous volume.

Obtaining of Extracts. Air dried samples (5.0g) were placed for 24 hours on magnetic stirrer with water-ethanol mixture (3:7(v/v), 50mL) for alcoholic extract preparation. After the incubation, extracts were filtered and used.

Hydrodistillation. Essential oils from aerial parts of *T. kotschyanus* were obtained by hydrodistillation using a Clevenger-type apparatus. The air-dried plant material (200.0 g) was placed in around-bottomed flask and was subjected to hydrodistillation for 3 h with 600 mL distilled water according to the European

Pharmacopoeia. The obtained oils were dried over anhydrous sodium sulfate and stored at 4°C before the analysis.

Determination of Total Phenolic Content. The content of total phenols was determined by spectrophotometry, with some changes, using gallic acid as standard (Vamanu, Nita, 2012; Gevorgyan et al., 2017).

Determination of Antioxidant Activity. The antioxidant activity (AOA) was determined by potentiometric measurements of change of ORP of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ mediatory system caused by antioxidants in extracts (Braynina et al., 2004; Gevorgyan et al., 2016).

Results and Discussions. EO was obtained by hydrodistillation using a Clevenger-type apparatus. The yield of EO of aerial parts in *T. kotschyanus* was 0.82% (w/w).

The total phenolic contents of the *T. kotschyanus* decoction and ethanolic extract were estimated through the Folin-Ciocalteu method. Gallic acid was used as standard for the calibration curve (Fig. 1). Total phenol content was expressed as milligrams Gallic Acid Equivalent (GAE) per mL of extract.

Total phenols of extracts ranged between 3.31±0.72 and 5.75±2.02 mg GAE/mL and decreased in the order of water>ethanol.

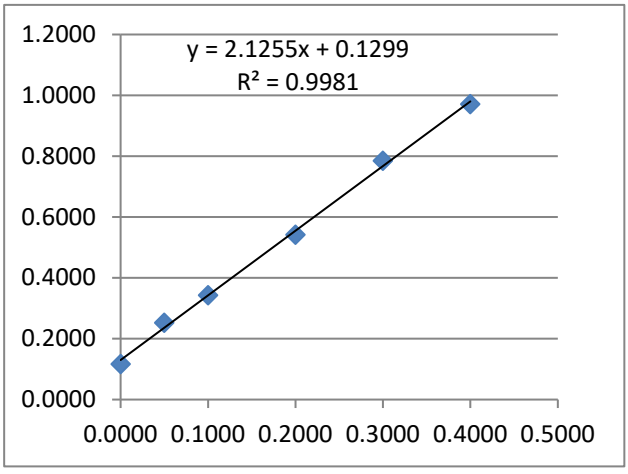

Fig. 1. Gallic acid calibration curve.

The AOA of *T. kotschyanus* was investigated for decoction and ethanolic extract and different dilutions of EO by potentiometric method of a change in Oxidation Reduction Potential (ORP) of $[Fe(CN)_6]^{3-}$ /$[Fe(CN)_6]^{4-}$ mediatory system. Results in Table 1 show the ORP and vitamin C equivalent values of *T. kotschyanus* decoction, ethanolic extracts and EO in different dilutions. Extracts show AOA as follows: 0.1 % essential oil > ethanolic extract > 0.25% essential oil > 0.5% essential oil > decoction.

Table 1.

Thymus kotschyanus essential oil and extracts redox potential and antioxidant activities equivalent in Vitamin C

Simple	***Redox potential mV***	***Vit. C x 10^{-4}g/l***
Ethanolic extract	234 ± 2.36	9
Decoction	225 ± 1.75	112
0.1 % essential oil	264.5 ± 4.95	-20
0.25% essential oil	215.5 ±16.26	26
0.5% essential oil	191.5 ± 2.12	79
Buffer	271	

The antioxidant agent is considered to be active against free radicals if the Vitamin C equivalent is $50x10^{-4}$g/l and more. So we can say that the ethanolic extract and 0.25% essential oil ethanolic solution have weak antioxidant activity and the 0.1% essential oil hasn't activity. The results indicate that *T. kotschyanus* 0.5% EO solution and decoction have higher activity and can serve as a strong natural antioxidant.

Conclusion. The yield of EO of aerial parts in *T. kotschyanus* was 0.82% (w/w). Our data showed that ethanolic extract of *T. kotschyanus* exhibited the highest amount of total phenols with values of *5.75±4.02* of GAE/mL. Based on the results of the determination of the AOA *T. kotschyanus* we concluded that decoction and 0.5% essential oil solution have remarkable activity and can serve as a strong natural antioxidant.

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Pharmaceutical analysis of Helichrisum Arenarium L.

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Introduction. Helichrysum arenarium L. Moench has an extended utility in European ethnomedicine as a medicinal plant that's possessing with cholagogue, choleretic, hepatoprotective, and detoxifying activities [1].

Helichrysum is a herbaceous perennial plant belonging to the Asteraceae family. Its natural distribution areas range includes Central, Eastern and South Eastern Europe, the Northern Balkans, West Siberia, Central Asia, Mongolia and China [2].

The beginning of the genus name is derived from Greek phrases “helios”, that means sun, and “chrysos”, that means gold, what refers to the shiny-golden color of flowers. The predominant biologically active compounds of Helichrysi arenarii inflorescentia are flavonoids, with chalcone isosalipurposide, and flavanones salipurposide, prunin and naringenin as dominant constituents, whilst different compounds found in extraordinary quantity are phtalides, carotenoids, essential oil and yellow pigments: α-pyron derivates which includes arenol and homoarenol [1].

Growth and development processes of H. Arenarium populations might be influenced by the weather conditions; the potential fertility is correlated significantly with the precipitation amount. The amount of biomass and its seasonal distribution depends on weather conditions and the number of plant species coexisting in the analyzed phytocoenoses [3].

Flowering time: July–October. The species occurs in both natural and anthropogenic communities. Sandy Everlasting is also an ornamental plant supplying material for dry bouquets [3].

In addition, the essential oil from the flowers of Helichrysum species, Immortelle, have been used as a cosmetic agent [4].

There are 3 types of the drug in the trade: dry flower heads (Helichrysi arenarii flos), fluid extract, typically received through extraction with water-ethanol or glycerin and dry extract of flower heads (Extractum florum Helichrysi arenarii siccum), typically received from fluid extract. The flower heads are especially used for decoctions, whilst dry extracts are extensively used for the manufacturing of galenic preparations in the form of capsules and tablets. These forms available in the marketplace are especially represented through Russian preparations; the most popular one is “Flamin tablets”. Besides the unsustainable collection, the primary hazard issue that threatens species subsistence is the transformation of natural habitat to agricultural land (orchards, vineyards) and regions for the cultivation of fast-growing tree species (types of poplar, pine, and acacia). Protection rules triggered a need to increase a new efficient developing era for sandy everlasting, which can restore the species for it use in phytotherapy [1].

Methods and materials:

Helychrysum arenarium seeds were collected in Tavush region, Dilijan, during the flowering period (July, 2021). The identification of plant was carried out at the Department of Pharmacognosy, plant flowers were deposited and are available at the Herbarium of the Institute of Botany, National Academy of Sciences of Armenia, Yerevan.

Crude drug in advance was prepared by the special technique for examination by the "Microscopical examination technique" based on article, (NPh XIII, ex. 2, p. 277-285). Tendering hot method was used. The grounded seeds were boiled in 5% of alkaline (NaOH) for 5 minutes.

Than the solution was removed and the raw material was washed and was left in water. Soaked pieces of seeds were placed between subjective glass and cover by in advance the water-glycerin mixture (1:1) drop was applied. Examination was carried out by Microscope ISOBASE EW10X/20 lens.

Extractible materials were organic, biological active, low molecular and secondary metabolic products. Determination of extractible matter was carried out without application of the fixing method for the determination of quantitative

constituents of biological active substances (Pharmacopea XIII,1.5.3.0006.15. ex II, 2015).[5]

One gram of raw material was added to 50ml 50% alcohol solution. The mixture was boiled 30 min on water bath connected with condenser. After cooling, it was filtrated, the amount of liquid extract was measured, then dried on water bath in porcelain bowel. Carefully collected powder of dry extract was weighted.

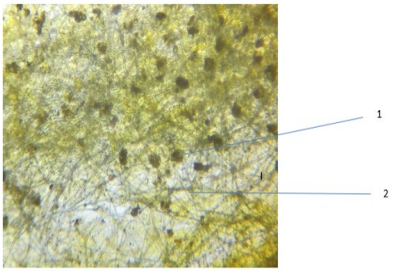
Just 3 grams of powdered plant was transferred into a weighing bottle. The sample was subjected to a temperature of 105° C for four hours followed by cooling in desiccators and weighted (Brazilian Pharmacopoeia V, 2010). The drying process was calculated from the point when the temperature in the drying shelf was 105 ^{0}C. The operation was repeated twice, for 2 hours and 30 minutes. The results of three determinations were evaluated in terms of weight percentage using the equation (Pharmacopea XIII,1.5.3.00067.15. ex II, 2015).

Three grams of the powder was transferred to porcelain crucibles which were previously calcined, cooled and weighed. The samples were charred in a muffle furnace at 450 ºC for 6 hours. After cooling in a desiccator, they were weighed on an analytical scale. This procedure was repeated twice, for 4 hours and 2 hours. The amount of ash was calculated based on methods mentioned in Pharmacopeian. (Pharmacopea XIII,1.5.3.0006.15. ex II, 2015).

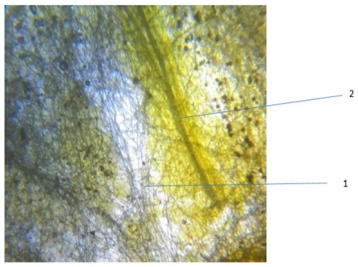
Results and discussion:

Raw material identification by the microscopical study considers very high in contemporary Pharmacognostic analysis. Particularly, more than two major distinguishing features of the plants are supposed to be identified in microscopic field. In order to obtain the objective evaluation of the main anatomical

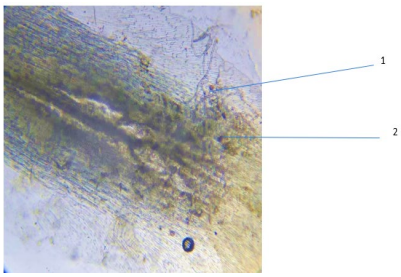
distinguishing features of raw material during the investigation Helichrysum is



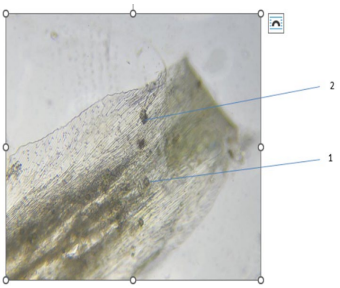
Picture 1. Upper surface of epidermis. *1. Hairs,2. Essential oil glands.*



Picture 2. Lower surface of epidermis. *1.Hairs, 2. Vascular tissue.*



Picture 3. Tubular flower. *1.Bristles, 2. Flagelleted hair.*



Picture 4. Wrapper leaf. *1.Glands, 2. Flagelleted hair.*

supposed to be collected during fruiting period.

Everlasting flower leaves microscopic slides are presented by the pictures 1,2. Many essential oil glands were visible on to the upper surface of leaves. Three types of hairs were noticeable: unicell simple hairs, knee shape simple hairs, which cells of which are generating corner, and headed hairs with oval shape head. The vascular tissue was visible well, especially along the central vein. The tracheas had the oval shape and spiral. Also, high density of hairs was noticeable. Stomatas were marked on upper and lower surfaces.

Helichrysum flowers microscopic pictures are presented by the picture 3, 4.

On the flowers, especially at the ends of the teeth, numerous glands are visible on a short unicellular stalk. Glands are situated by the 1-2 rows, multi-tiered, often with a small cuticle. The edge of the teeth of the flowers is fringed, consists

2

of papillary outgrowths of the epidermis and at the base there is a row of mechanical cells. At the apex of the ovary there is a tuft of large, fragile, rough bristles sticking up or slightly deflected.

Table 1. Commodity indicators of Helychrisum (%, X ± S_E)

Commodity indicators	*Extractable matter*	*Total ash*	*Moisture*
Dry raw material	14%± 0,05	7.67% ± 0,02	11.33% ± 0,12

Conclusions

Anatomical - distinctive features of Helychrisum raw material of Armenian flora have been revealed and the herb specie is identified

Within the framework of preliminary standardization, commodity indicators of raw material were approved. Total ash was equal to 7.67 ± 0.02%.

Alcohol extract of medicinal raw materials contained 14 % of extractible matter.

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Pharmaceutical analysis of Leonurus Cardiaca L.

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Introduction. Leonurus cardiaca L. (motherwort in English, Echte Herzgespann in German, agripaume in French) is a Lamiaceae family perennial herb. The plants can reach a height of one meter, with hollow aerial stalks emerging from the rhizomes. The leaves are palmately lobed and have stiff hairs on them. Flowers are pink and about 1 cm long, arranged in 10-20 clusters in the leaf axils of the last 10-15 knots. The herb, which originated in Asia and southeastern Europe, is now widely used for therapeutic purposes all over the world. L. cardiaca was a good candidate for developing alternative treatments in both traditional eastern and modern medicine because of its potential application in treating of a variety of cardiac disorders as well as female-specific afflictions. Apart from its traditional medical usage, motherwort is used as a seasoning in a variety of vegetable soup recipes, particularly lentil or split pea soups, or as a garnish (1).

Tachyarrhythmia, and a variety of other cardiac diseases have all been treated with L. cardiaca in traditional medicine, treatment of neurological, cardiac, and gynecological problems, as well as thyroid dysfunctions, are some of the other known therapeutic applications. Its antioxidant, antibacterial, and anti-inflammatory capabilities, as well as its therapeutic benefits on digestive diseases and bronchial asthma, are all supported by scientific research. In L. cardiaca, different classes of secondary metabolites have been discovered. Flavonoids (e.g., flavonols like quercetin and rutin, flavones like genkwanin and apigenin, and their glycosides), phenylethanoid glycosides (e.g., verbascoside and lavandulifolioside), iridoids (e.g., harpagide), labdane diterpenes (e.g., forskolin), etc (2) are present in the herb.

Motherwort contains monoterpenes, diterpenes, triterpenes, nitrogen-containing chemicals, phenylpropanoids, flavonoids, phenolic acids, volatile oils, sterols, and tannins, as well as volatile oils, sterols, and tannins. Internally,

preparations of the herb have traditionally been used to treat nervous heart issues and digestive problems. They've also been used externally in wounds and skin inflammations, as well as for bronchial asthma, climacteric symptoms, and amenorrhoea. The herb and its formulations have mildly negative chronotropic, hypotonic, and sedative effects. Its antibacterial, antioxidant, anti-inflammatory, and analgesic properties, as well as its effects on the heart and circulatory system, have all been proven in pharmacological tests. Clinical investigations have shown that it possesses with sedative and hypotensive properties (3).

Methods and materials:

Leonurus cardiaca herb was collected in Kotayk Province t.Tsaghkadzor, during the flowering period (June , 2021). The identification of plant was carried out at the Department of Pharmacognosy, plant herbs were deposited and are available at the Herbarium of the Institute of Botany, National Academy of Sciences of Armenia, Yerevan.

Crude drug was prepared previously by the special technique for examination by means of "Microscopical examination technique" based on article, (NPh XIII, ex. 2, p. 277-285). Tendering hot method was used. The grounded seeds were boiled in 5% of alkaline (NaOH) for 5 minutes.

Than the solution was removed and the raw material was washed and left in water. Soaked pieces of seeds were placed between subjective glass and cover glass. In advance the plant was covered be water-glycerin mixture (1:1) drop. Examination was carried out by Microscope ISOBASE EW10X/20 lens.

Extractible materials were organic, biological active, low molecular and secondary metabolic products. Determination of extractible matter was carried out. The fixing method for determination of quantitative constituents of biological active substances (Pharmacopea XIII,1.5.3.0006.15. ex II, 2015) (4) was applied.

One gram of raw material was added to 50ml 50% alcohol solution. It was boiled 30 min on water bath connected with condenser. After cooling, it was filtrated,

measured the amount of liquid extract, then dried on water bath in porcelain bowel. Carefully collected powder of dry extract was weighted.

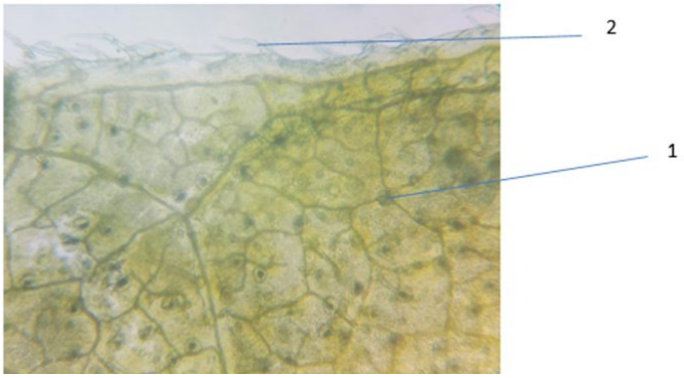
Just 3 grams of powdered plant was transferred to a weighing bottle. The sample was subjected to a temperature of 105° C for four hours followed by cooling in desiccators and weighted (Brazilian Pharmacopoeia V, 2010). The drying process was calculated from the point when the temperature in the drying shelf was equal to 105 ^{0}C. The operation was repeated twice, for 2 hours and 30 minutes. The results of three determinations were evaluated in terms of weight percentage of the samples amounts using the equation presented in the citation (Pharmacopea XIII,1.5.3.00067.15. ex II, 2015).

Three grams of the powder was transferred into the porcelain crucibles which were previously calcined, cooled and weighed. The samples were charred in a muffle furnace at 450 ºC for 6 hours. After cooling in a desiccator, they were weighed on an analytical scale. This procedure was repeated twice, for 4 hours and 2 hours. The amount of ash was calculated using methods of Pharmacopeia. (Pharmacopea XIII,1.5.3.0006.15. ex II, 2015).

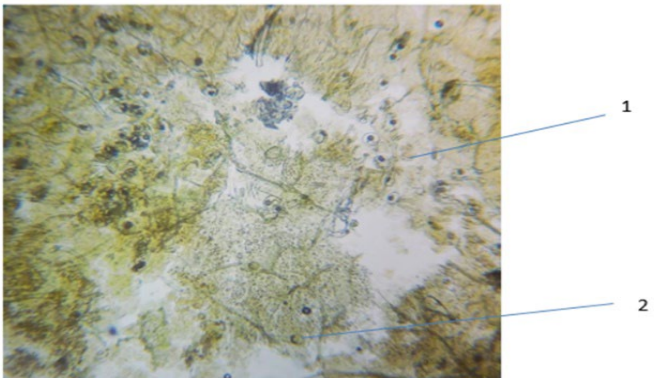
Results and discussion:

In contemporary Pharmacognostic analysis application of the raw material identification microscopical method is valued very highly. Particularly, more than two major distinguishing features carried out in that fields. In order to obtain the objective evaluation of the main anatomical distinguishing features of raw material during the investigation Leonurus Cardiaca L. is supposed to be collected during fruiting period.

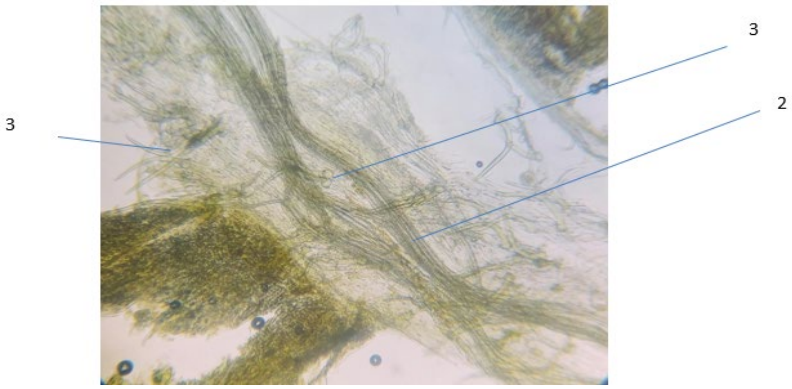
The leaves microscopic slides are presented by the pictures 1,2:



Picture 1. Upper surface of epidermis. *1. Essential oil glands, 2. Covering trichomes.*



Picture 2. Lower surface of epidermis. *1. Headed trichomes, 2. Vascular tissue, 3.Simple trichomes.*



Picture 3. Features of leaves. *1.Essential oil glands, 2. Stomata.*

Microscopical examination of upper surface of leave showed than epidermal cells with sinuous sidewalls are situated in the higher density on the underside. The vascular tissue was well visible, especially along the central vein. Vessels were spirally and annularly thickened. Numerous various trichomes were marked. Headed trichomes were 1-2 cell stalk with 1-2 cell large or small spherical head. Covering trichomes were uniseriate, whole or fragmented.

Three-five celled simple trichomes were noticeable. The trichomes at the places of attachment were dilated, the walls were noticeably thickened, the surface of the hair was warty.

Table 1 Commodity indicators of Leonurus (%, $X \pm S_E$)

Commodity indicators	*Extractable matter*	*Total ash*	*Moisure*
Dry raw material	16.45%± 0,05	7.67% ± 0,02	11.33% ± 0,12

Conclusions

Anatomical - distinctive features of Leonurus as the raw material of Armenian flora have been revealed and identified.

Within the framework of preliminary standardization, commodity indicators of raw material were approved. Total ash was equal to 7.67 ± 0.02%, humidity - 11.33 ± 0.12%.

Alcohol extract of medicinal raw materials contained 16.45% of extractible matter.

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Pharmaceutical analysis of Linum Usitatissimum L. grawing in Armenian flora

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Introduction. Linum usitatissimum or flaxseed is widely grown around the World. Flax blooms are pale blue, and the fruit capsules are loaded with little brown seeds. Flax oil can be extracted from the seeds, which are one of the greatest plant sources of the n-3 polyunsaturated fatty acid (PUFA) a-linolenic acid (ALA). Flaxseed is becoming more popular as a functional diet source since it offers the largest level of essential fatty acids from a plant source /1/.

Linseed yields seed is a rich source of both non-edible and edible oil. Industrial oil is an important ingredient in the manufacture of paint, varnish and linoleum. Edible linseed oil is used for human consumption and contains alpha-linolenic acid (ALA), a polyunsaturated fatty acid has nutritional and health benefits. Aside from ALA, linseed is becoming increasingly popular as a nutritional and functional food in the Western world, because of high content of therapeutic health promoting substances such as fatty acid, soluble and insoluble fiber and lignans, and its suitability for use in bread, breakfast cereals, muesli bars and other food products /2/.

Linseed was employed as a source of edible oil and high-grade lighting oil in peasant communitiessince it contained roughly 40% oil. The tall stems are taken before the seed matures, and the fibers for spinning are recovered from them. Traditionally, they were dried first, then submerged (wetted) in water to allow the pectin connecting the fibers to other cells and tissues of the stem to decompose (retting). The fibers (averaging 4 cm in length) were separated by hammering (breaking) and combingafter the stems were retted /3/

According to the scientists the various properties were attributed to L. usitatisimum including antioxidant, immunomodulatory, anti-inflammatory, antimicrobial, antiprotozoal, insecticidal, analgesic, anti-hyperlipidemia, anti-

hyperglycemic, anti-tumor, wound healing and Feticidal activities. There is also information related with disease prevention and healing properties of the flax. Diseases like GI disorders, cardiovascular, urogenital, respiratory diseases and some neurological syndromes were mentioned to be treated by Flax/1/.

Methods and materials:

Linum usitatissimum seeds were collected in Aragatsotn region v. Yernjatap, during the fruiting period (October , 2021). The identification of plant was carried out at the Department of Pharmacognosy, Plant seeds were deposited and are available at the Herbarium of the Institute of Botany, National Academy of Sciences of Armenia, Yerevan.

Crude drug were prepared by the special technique for examination and analyses by the utility of the "Microscopical examination technique" based on the protocol of the article (NPh XIII, ex. 2, p. 277-285). Tendering hot method was used. The grounded seeds were boiledin in 5% of alkaline (NaOH) for 5 minutes. Than solution was removed and the raw material was washed and was left in the water. Soakedpieces of seeds were put between subjective glass and cover glass along with addition of water-glycerin mixture (1:1) drop on top of the herb. Examination was carried out by Microscope ISOBASE EW10X/20 lens.

Extractible materials were organic, biological active, low molecular and secondary metabolic products. Determination of extractible matter was carried out. The fixing method for the determination of quantitative constituents of biological active substances was applied /4/.

One gram of raw material was added into 50ml 50% alcohol solution. Boiled 30 min on water bath connected with condenser. After cooling, it was filtrated, measured the amount of liquid extract, then dried on water bath in porcelain bowel. Carefully collected powder of dry extract was weighted (Pharmacopea XIII,1.5.3.0006.15. ex II, 2015)

Just 3 grams of powdered plant was transferred into a weighing bottle. The sample was subjected to a temperature of 105° C for four hours followed by cooling in desiccators and weighted (Brazilian Pharmacopoeia V, 2010). The drying process was calculated from the point when the temperature in the drying

shelf was equal to 105 0C. The operation was repeated twice, for 2 hours and 30 minutes. The results of three determinations were evaluated based on the weight percentage evaluation using the equation presented in Pharmacopea XIII,1.5.3.0007.15. ex II, 2015.

Three grams of the powder was transferred to porcelain crucibles which was in advance calcined, cooledand weighed. The samples were charred in a muffle furnace at 450 ºC for 6 hours. After cooling in adesiccator, they were weighed on an analytical scale. This procedure was repeated twice, for 4 hoursand 2 hours. The amount of ash was calculated using Pharmacopeian methods (Pharmacopea XIII,1.5.3.0005.15. ex II, 2015)

One gram of whole and 1g crushed raw material was placed in a two different graduated cylinder with a division value equal to 1 ml. The raw material was moistened with 1ml of alcohol, water was added until the mark and the mixture as thoroughly shaken every 10 minutes for1 hour to evenly wet the raw material. After 24 hours, the volume occupied by mucilage was measured and the swelling index was calculated using following formula: /5/.
(Pharmacopea XIII,1.5.3.0008.15. ex II, 2015).

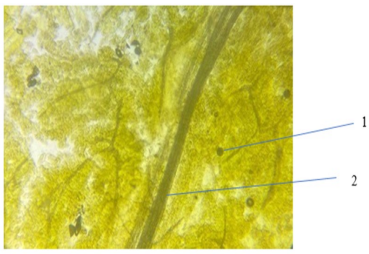
$$\boldsymbol{SI = \frac{Xi - Xf}{Xi} \times 100}$$

Where` Xi -initial volume of mucilage in graduated cylinder, ml,

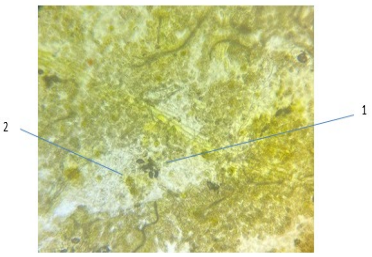
m_2- final volume after hydration, ml.

Results and discussion:

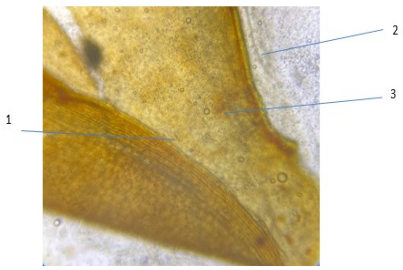
In contemporary Pharmacognostic analysis application of the raw material identification microscopical method is valued very highly. Particularly, more than two major distinguishing features carried out in that fields. In order to obtain the objective evaluation of the main anatomical distingiushing features of raw material during the investigation Linseed was collected in flowering period.
Valuable features of the leaves of Linum Usitatissimum L. are presented by the Pictures 1 and 2.



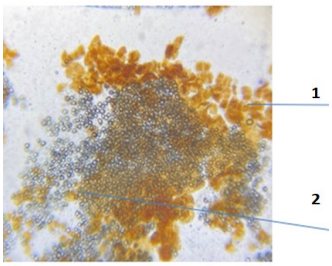
Picture 1. Upper surface of leaves *1. Druses, 2. Elements of vascular tissue.*



Picture 2. Lower surface of leaves. *1.Stomata, 2. Druses.*



Picture 3 Elements of seeds peel after maceration. *1.Parencimal cells, 2. Mechanical tissue, 3. Musilage containing cells.*



Picture 4. Grounded seeds microscopic picture. 1. *Quadrangular cells, 2. Musilage containg cells.*

The examination of upper surface of leaves epidermis showed than cell walls were thick, polygon. There were well visible conducting (vascular) tissue's elements on the central vein. Vessels were spiraliform, inthe form of a helix. Prismatic and round shape druses from inorganic materials were also distinguished. A lot of actinocite stomatas were visible on to the lower surface of leave. Also noticeable few coloredcells were seen. As a result of softening mucilage containing cells walls tissue were thickening.

Epidermis consist of big, quadrangular cells, covered by thick layer cuticule, filled musilage. Underthe epidermis noticeable two layers of parenchimal cells exist. Under that layer the mechanichal tissue with thickened, lignified yellow cells were visible. The most inter layer consisted from quadrangular dark yellow cells.

Musilage containing transparent, dense round shape cells were expressed and visualized. There were noticed thick walled parenchymal cells filled with pigment.

Table 1 Commodity indicators of Linseed (%, $X \pm S_E$).

Commodity indicators	*Extractable matter*	*Total ash*	*Moisure*	*Index swelling*
Dry raw material	1.65%± 0,05	5.67% ± 0,02	12.33% ± 0,12	66.67%± 0,05 11.11%± 0,05

Conclusions

Anatomical - distinctive features of Linseed raw material of Armenian flora has been revealed and identified as the plant species.

Within the framework of preliminary standardization, commodity indicators of raw material were approved. Total ash was equal to 5.67 ± 0.02%, humidity 12.33 ± 0.12%, index swelling 66.67% ± 0.05%.

Alcohol extract of medicinal raw materials contained 11.11% of extractible matter.

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Pharmaceutical analysis of Peucedanum Officinale L.

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Peucedanum officinale has been known as a medicinal plant since at least 17th century and features in the herbals of Nicholas Culpeper (in whose day it was more plentiful, for he records it as growing abundantly on Faversham marshes) and John Gerard. Culpepper records the additional common names hoar strange, hoar strong, (compare German "Haarstrang", meaning hog's tail) brim stone wort and sulphur wort. Essential oils smell great, reduce stress, treat fungal infections, and help you sleep. They are concentrated extractions from plants. A process called distillation turns the “essence” of a plant into a liquefied form for many medicinal and recreational uses. There’s a wide variety of essential oils available. Some are valued for their pleasing aroma. Others claim to have powerful healing properties. But their potency can have side effects, you must be aware of (1).

Peucedanum is a herbaceous perennial plant in the family Apiaceae found mainly in Central Europe and Southern Europe. Native to Southern Europe and Asia Minor, fennel is cultivated in temperate regions worldwide and is considered an invasive species in Australia and parts of the United States. All parts of the plant are aromatic and used in flavouring, and the bulblike stem base of Florence fennel (variety azoricum) and the blanched shoots are eaten as a vegetable. The essential oils of different Peucedanum species are composed of monoterpenes and sesquiterpene hydrocarbons, oxygenated sesquiterpenes, aliphatic alcohols, and esters The seeds and extracted oil are suggestive of anise in aroma and taste and are used for scenting soaps and perfumes and for flavouring candies, liqueurs, medicines. Peucedanum is traditionally used as a vegetable in the East Asia region, but it is sometimes used as a medicine for headaches, colds, neuralgia, rheumatoid arthritis and other inflammatory diseases P. japonicum has been reported to contain coumarins, phenolic compounds, chromones, polyacetylenes,

inositols and steroid glycosides. P. *japonicum* has anti-obesity , antiplatelet ,antiallergic , antioxidative and anti-inflammatory properties (2).

Methods and materials:

Peucedanum herb was collected in Aragatsotni province v.Byurkan, during the flowering period (May , 2021). The identification of plant was carried out at the Department of Pharmacognosy, plant herbs were deposited and are available at the Herbarium of the Institute of Botany, National Academy of Sciences of Armenia, Yerevan.

Crude drug in advance was prepared by the special technique based on "Microscopical examination technique" article, (NPh XIII, ex. 2, p. 277-285). Tendering hot method was used. The grounded seeds were boiled in 5% of alkaline (NaOH) for 5 minutes.

Than solution was removed and the raw material was washed and was left in the water. Soaked pieces of seeds were placed between subjective glass and cover glass. In advance water-glycerin mixture (1:1) drop was placed on top of the herb. Examination was carried out by Microscope ISOBASE EW10X/20 lens (3).

Extractible materials were organic, biological active, low molecular and secondary metabolic products. The fixing method for determination of quantitative constituents of biological active substances (Pharmacopea XIII,1.5.3.0006.15. ex II, 2015) was applied (4).

One gramm of raw material was added into 50ml of 50% alcohol solution. It was boiled for 30 min on water bath connected with condenser. After cooling, the mixture was filtrated, the amount of liquid extract was measured, then dried on water bath in porcelain bowel. Carefully collected powder of dry extract was weighted.

Just 3 grams of powdered plant was transferred to a weighing bottle. The sample was subjected to a temperature of 105° C for four hours followed by cooling in desiccators and weighted (Brazilian Pharmacopoeia V, 2010). Drying process was calculated from the point when the temperature in the drying shelf was 105 ^{0}C. The operation was repeated

twice, for 2 hours and 30 minutes. The results of three determinations are evaluated in terms of weight percentage on the sample amount using the equation (Pharmacopea XIII,1.5.3.00067.15. ex II, 2015).

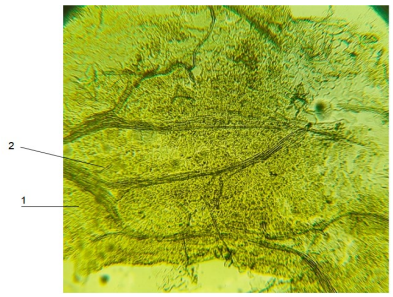
Three grams of the powder was transferred to porcelain crucibles which werepreviously calcined, cooled and weighed. The samples were charred in a muffle furnace at 450 ºC for 6 hours. After cooling in a desiccator, they were weighed on an analytical scale. This procedure was repeated twice, for 4 hours and 2 hours. The amount of ash was calculated using Pharmacopeian methods. (Pharmacopea XIII,1.5.3.0006.15. ex II, 2015).

Results and discussion:

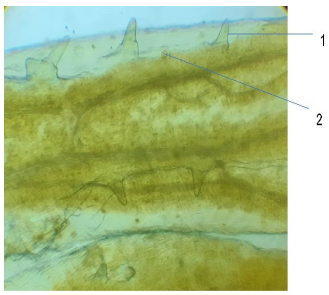
Identification of raw materials based on microscopical research is highly demandable in contemporary Pharmacognosy.

Pharmacognostic analysis. Particularly, more than two major distinguishing features are supposed to be identified in the microscopy fields. In order to obtain the objective evaluation of the main anatomical distingiushing features of raw material during the investigation Peucedanum herb was collected in flowering period.

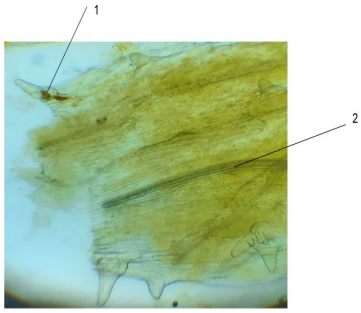
Peucedanum leaves microscopic slides are presented by the pictures 1 and 2.



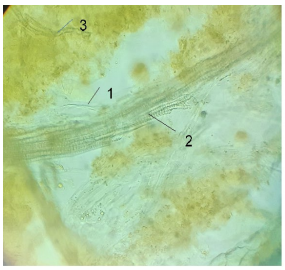
Picture 1. Upper surface of epidermis 1.Stomatas, 2. Simple trichomes.



Picture 2. Upper surface of epidermis.
1.Con shape trichomes, 2. Simple trichomes.



Picture 3. 1.Essential oil containing simple trichome. 2. Vessels of conductive tissue.



Picture 4. 1.Simple trichome. 2. Ring shape,spiral vessels of xylem, 3. Simple multicellular trichome.

Microscopical examination of upper surface of leave showed existence of epidermal cells with sinuous sidewalls, especially on the underside. Simple hairs were noticeable in small number. Conical hairs were well visible with their base (attachment) cells and in some places were noticeable few glandular hairs. Drops of dark essential oil are sometimes noticeable in conical hairs. Xylem vessels of the conductive system were well visible. Epidermal mesophyll cells with thick walls and containing small prism crystals of calcium oxalate were identified.

Table 1 **Commodity indicators of Peuceanum (%, X ± S_E)**

Commodity indicators	*Extractable matter*	*Total ash*	*Moisure*
Dry raw material	5.2± 0,05	12.84% ± 0,02	13.58% ± 0,12

Conclusions

- Anatomical - distinctive features of Peucedanum Officinale L. raw material of the Armenian flora have been revealed and the plant species were identified.

- Within the framework of preliminary standardization, commodity indicators of raw material were approved: total ash 12.84 ± 0.02%, humidity 13.58 ± 0.12%.
- Alcohol extract from medicinal raw materials contains 5.2 % of extractible matter.

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Pharmaceutical analysis of Hypericum Perforatum L.

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Hypericum perforatum, also known as St. John's wort, hypericum or millepertuis is a member of the family Hypericaceae and a herbaceous native perennial plant of Europe, Western Asia, and northern Africa. Nowadays it has a worldwide distribution. The crude drug, called herba hyperici, consists of the upper aerial parts of the plant collected just before or during the flowering period. Hypericum perforatum has been utilized as a therapeutic plant for quite a long time, for the treatment of external and internal disorders. Externally, oily preparations of the plant may be applied to treat minor burns, wounds, skin inflammation, and nerve pain. Internally, it is indicated for the treatment of anxiety and mild to moderately severe depression. It competes for status as a standard antidepressant therapy and is the only herbal alternative for synthetic antidepressants. Hypericum perforatum contains several classes of biologically active compounds. These constituents often vary in its concentration, due to genetic variation within the species and/or adulteration, ecological factors, time of harvesting, preparation and processing of sample material and storage conditions, such as exposure to light, time of harvesting. Important bioactive components are concentrated in buds, blossoms, and tips of twigs. Despite this variation, it is known that around 20% of the plant extract is comprised of bioactive compounds [1].

The main active components of St. John's wort are thought to be hypericin and hyperforin. St. John's wort also contains other common plant constituents (e.g., flavonoids and flavonoid derivatives, xanthone derivatives, amentoflavone, biapigenin, volatile oil) which are able to possess with antidepressant effects. Studies have suggested that St. John's wort acts via inhibition of the reuptake of serotonin, dopamine, and noradrenaline, along with activation of gamma-amino-butyrate and glutamate receptors. At high dosages, hypericin is a monoamine

oxidase inhibitor; however, these effects have not been demonstrated with the consumption of St. John's wort at dosages recommended for the treatment of depression. The absorption and elimination of hypericin extract have been investigated in healthy volunteers [2].

Methods and materials:

Hypericum perforatum herbs were collected in Gegharquniq region v. Sevan, during the flowering period (July, 2021). The identification of plant was carried out at the Department of Pharmacognosy (EIU), plant herbs were deposited and are available at the Herbarium of the Institute of Botany, National Academy of Sciences of Armenia, Yerevan.

Crude drug previously in advance was prepared by the special technique for examination with the utility of "Microscopical examination technique", which was developed based on article, (NPh XIII, ex. 2, p. 277-285). Tendering hot method was used. The grounded seeds were boiled in 5% of alkaline (NaOH) for 5 minutes.

Then solution was removed and the raw material was washed and was left in the water. Soaked pieces of seeds were placed between subjective glass and cover glass by adding water-glycerin mixture (1:1) drop. Examination was carried out by Microscope ISOBASE EW10X/20 lens.

Extractible materials were organic, biologically active, low molecular and secondary metabolic products. The fixing method for determination of quantitative constituents of biological active substances (Pharmacopea XIII,1.5.3.0006.15. ex II, 2015) [3] was applied.

One gram of raw material was added into 50ml 50% alcohol solution. Boiled 30 min on water bath connected with condenser. After cooling, it was filtrated, measured the amount of liquid extract, then dried on water bath in porcelain bowl. Carefully collected powder of dry extract was weighted.

Just 3 grams of powdered plant was transferred to a weighing bottle. The sample was subjected to a temperature of 105° C for four hours followed by cooling in desiccators and weighted (Brazilian Pharmacopoeia V, 2010). Drying process was calculated from the point when the temperature in drying shelf was

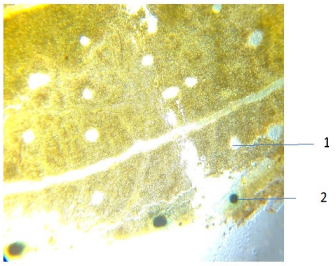
105 ^{0}C. The operation was repeated twice, for 2 hours and 30 minutes. The results of three determinations were evaluated in terms of calculation of the difference between the weight percentage and sample amount using the equation mentioned in the reference (Pharmacopea XIII,1.5.3.00067.15. ex II, 2015).

Three grams of the powder was transferred to porcelain crucibles, which were previously calcined, cooled and weighed. The samples were charred in a muffle furnace at 450 ºC for 6 hours. After cooling in a desiccator, they were weighed on an analytical scale. This procedure was repeated twice, for 4 hours and 2 hours. The amount of ash was calculated using Pharmacopeian methods. (Pharmacopea XIII,1.5.3.0006.15. ex II, 2015).

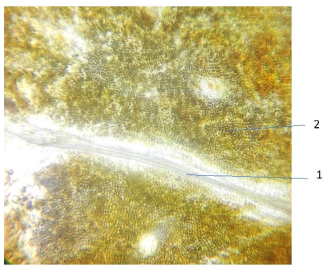
Results and discussion:

Determination of raw material by means of microscopical study is highly demandable in contemporary Pharmacognostic analysis. Particularly, more than two major distinguishing features of the herb materials are supposed to be delineated in the fields . In order to obtain the objective evaluation of main anatomical distinguishing features of raw material during the investigation Hypericum is supposed to be collected in flowering period.

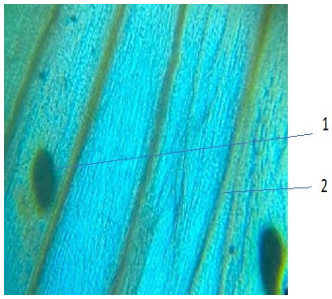
Important features of Hypericum leaves microscopic slides are presented by the pictues 1 and 2.



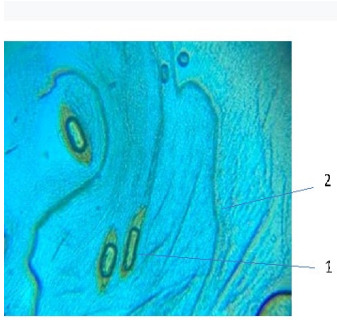
Picture 1. Epidermis of the upper side of the leaf. 1. Colorless recepticale, 2. Pigmented recepticale, 3.Stomata.



Picture 2. Lower surface of leaf epidermis. 1.Stomata, 2. Vein.



Picture 3. 1.Colored recepticals, 2.Vascular tissue.



Picture 4. 1.Essential oil. 2.Vascular tissue.

Large rounded, oval receptacles of two types are noticeable in the mesofile and throughout the plastic sheet: colorless, translucent and colored, with brownish-violet pigment. Stomata were found out only on lower surface of leaf. The epidermis of the lower and upper sides of the leaf consisted of tortuous cells in outline with a pronounced bead-like thickening.

The Hypericum flowers microscopic essentials are presented by the pictures 3 and 4.

Table 1 Commodity indicators of Hypericum (%, $X \pm S_E$)

Commodity indicators	*Extractable matter*	*Total ash*	*Water loss*
Dry raw material	14%± 0,05	10.33% ± 0,02	7.67% ± 0,12

Conclusions

Anatomical - distinctive features of Hypericum raw material of the Armenian flora been revealed and identified as the plant species.

Within the framework of preliminary standardization, commodity indicators of raw material were approved. Total ash 10.33%± 0.02%, humidity 7.67% ± 0.12%.

Alcohol extract from medicinal raw materials contains 14% of extractible matter.

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Potential targeting of the stroke by means of the layered nano- and micro-particles

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Abstract

Background. Targeted type of delivery of the medicines is the most effective way to treat diseased tissues. We propose, in experimental settings the efficient delivery of Proline Rich Peptide (PRP; novel compound, serving for the neuroprotection), PP (general activator of Phosphoribosyl Pyrophosphate Synthase -1 (PRPS-1; EC=2.7.6.1), regulative enzyme of purines and pyrimidines syntheses) as well as allopurinol, inhibitor of Xanthine Oxidoreductase (XOR, which includes Xanthine Oxidase; XO (EC=1.17.1.4) and Xanthine Dehydrogenase XDH (EC 1.17.1.4), the regulative enzyme of purine catabolism) by means of PEG-ylated and not PEG-ylated albumin nanoparticles will possibly stimulate fast regeneration and protection of the brain tissue in the experimental stroke settings.

Methods. For determination of the size of the particles it was used electron transmission microscopy technique (Philips CM-100 TM, USA). The loading and the controlled release of the medicines was evaluated by the colorimetric methods (Cary 60, Agilent, USA). Circulation abilities of loaded albumin particles were compared with PEG-ilated particles.

Results. By the utility of the modified desolvation method, we were able to create the particles from <100 nm to < 2 microns diameter. The sedimentation methods allow us to control the size of the obtaining particles. PEG-ilated particles are more stable in water as well as in the trypsin environment. Release of most of the medicines predominantly occurred during day $3t^h$; the effect was stronger in not PEG-ilated partciles.

Conclusion. We created layered with the different medicines PEG-ylated and not PEG-ylated particles, which might target different pathological stages of experimental stroke development.
Key words: albumin particles, PEG, dexamethasone, allopurinol, phosphates, PRP, in vivo.

Introduction

Medicines, after appearance in blood stream, might adhere to the proteins by not specific formation of the bonds. After attachment with the native carriers medicines or chemical compounds retain their long circulation time. It was shown, albumin particles attached to the medicines decrease the activation of the complement, reduce the macrophage-related phagocytosis as well as prevent binding of the compounds to the IgG (Peng et al., 2013).

It was shown, the albumin nanoparticles might prevent formation of the hemorrhages (Liu et al., 2021). Meantime, albumin as the main protein preserves the oncotic pressure of blood, prevents extensive development of the brain edema in the settings of the experimental stroke (Belayev L et al., 2005). Unfortunately, the clinical trials are evidencing about the exacerbating effect of the albumin due to the increase of the pulmonary dysfunctions after stroke, which was increasing the percentile of the deaths (Martin RH et al., 2016). However, clinical trial results might be reviewed for the corrections related with the doses, time of the treatment as well as the formation of the specific group of the patients, who didn't previously developed pulmonary dysfunctions. Also, by injection of the albumin particles instead of free albumin we don't strongly increase the oncotic pressure of blood, consequently might not be facing previously detected side effects of stroke patients.

We propose, the attachment of the medicines to the albumin particles will prolong circulation time of the compounds. From the other hand, albumin in the limited extent will preserve the oncotic pressure of blood and prevent the enlargement of brain edema in case of its utility in the settings of experimental stroke.

In the frames of our investigations we included the preparation of the albumin nanoparticles with the diffused saturation with the several medical compounds: allopurinol (as the inhibitor of Xanthine Oxidoreductase (XOR; EC=1.17.1.4) and preventer of ROS generation), inorganic phosphate groups (as the activators of PRPS-1 enzyme (EC=2.7.6.1), which is regulative and responsible for the syntheses of purines and pyrimidines), Proline Rich Protein-1 (PRP-1), which is, in accordance to our investigation, is diminishing the degradation processes of the NPs (Nano Partciles) because of the wide proteases' inhibiting abilities and serving as the neuroprotective agent. Also, created by us particles include dexamethasone, which is able to diminish the aggressive inflammation processes after stroke.

To create the protective shells for the particles, we decided to cover them with PEG (poly ethylene glycol-6000). We suggest, PEG shells are able to hide the peptides from blood proteases.

In our previous experiments, we have shown, the stimulation of the purine and pyrimidine metabolism will trigger the processes of the recovery after stroke (Danielyan et al., 2019). Moreover, similar results are possible to obtain, if to inhibit the purines catabolism via the inhibition of the key regulative enzymes, XOR (Danielyan and Simonyan, 2017).

Allopurinol, the classical inhibitor of XOR, is preventing formation of the free radicals as well as stimulating the cells proliferation in vitro (Danielyan and Kevorkian, 2011).

Stimulation of the activity of the other enzyme, such as PRPS1 (PRPS-1; EC=2.7.6.1), by regulation of the purines as well as pyrimidine syntheses, is promoting the cells division and even neurogenesis (Danielyan et al., 2019).

Galoyan AA and co- authors for the first time discovered the novel neurohormones-like peptides from neurosecretory granules of human and bovine neurohypophysis (Galoyan, 2004). Most of them are rich with the naturally very rare structural amino acid-Proline. Such phenomenon is very well documented for the family of the peptides synthesized in salivary glands (Carlson, 1993). "Members" of newly discovered family of neuropeptides were numbered by

Galoyan AA (Gladkevich et al., 2007) and co-authors from PRP-1 to PRP-4 and contain 15, 14, 13, and 10 amino acids, respectively. Moreover, identical peptide (PRP-5) was identified on the peptide map of human hypothalamus (Galoyan et al., 2007). According to mass spectral analysis the molecular mass of human PRP-5 is 1560.5 Da. Three amino acids in the human PRP differ from the bovine analog (Gladkevich et al., 2007). Localization of bovine PRP for the first time was revealed via immunohistochemistry. It was demonstrated that PRP exists in the magnocellular neurons of the nucleus supraopticus (NSO) in parvocellular neurons concentrated in rostral and medial subdivision of the nucleus paraventricularis (NPV) of the rat. Also, it was shown that PRP immunoreactivity is higher in the NSO than in the NPV (Galoyan et al., 2007). According to authors these peptides are generated by proteolysis of the C-terminal neurophysin-vasopressine associated glycoprotein and along with vasopressin and oxytocin might be transferred from the hypothalamus to the neurohypophysis by axonalal transport (Gladkevich et al., 2007). Physiological range of the PRP effects is wide, which might be explained by it's neurohormonal nature of generation and neuroprotective abilities (Galoyan et al., 2007; Gladkevich et al., 2007). Biological effects of them predominantly are depended on the doses, time, initial state of the organism and roots of injection, administration into the organism (Gladkevich et al., 2007).

It is proved, the life terms of the small peptides in blood stream is very short. Thus, we have decided to prolong PRP-1 circulation time in blood stream by coupling it with the albumin particles. Also, the single, long term acting injection might diminish the dose of the peptide as well as will reduce the possible treatment expenses.

We propose, the layered with four different compounds: PRP, PP, allopurinol, dexamethasone, albumin particles, protected with the PEG shell, will circulate longer and possibly will target different stages of experimental stroke: cell death, inflammation, generation of ROS, regeneration.

Methods

Transmission electron microscopy. Albumin microcarriers and nanoparticles were analyzed with TEM (Electron Transmission Microscopy,). A drop of the sample was placed on the polyvinyl formal-coated 200-mesh copper grids for 2 min, and then washed with Milli-Q water. The grids were stained with 2% uranyl acetate. The stain was wicked off using a filter paper and the grids were dried. Grids were analyzed at 25 000 magnification. The nanoparticles were generated based on the transmission electron microscopy (TEM) results (Figure 1B), (Philips CM-100 TM, USA).

Generation of the albumin particles. Five hundred microliters of 10% bovine albumin (Arpimed, Armenia) was dissolved in 1 ml of water. Glutaraldehyde (GA; 10 uL, 40 %, Medisar, Armenia; GA) was added along with the 1 ml of lithium carbonate solution into the mixture (n = 6). This mixture was incubated for 24 h. The mixture was centrifugated at 14000 RPM for 30 minutes. The precipitate was dissolved in 1 ml of water. The particles were washed from the remaining parts of the GA 3 times. The remaining part of the precipitate was dissolved in 1000 μl of saline. The schematic representation of the particles preparation is presented by the **Figure 2**.

Determination of the proteins. Determination of the proteins were performed based on the Lowry method. The absorption was determined by means of the spectrophotometer (Agilent, Cary 60, USA) at λ=730 nm **(Lowry et al., 1951)**.

Determination of non-organic phosphates. Solution for the phosphates detection was containing the concentrated sulfuric acid, 4% ascorbic acid, 0.3% anthimony potassium tartrate and sodium molybdate (4.5%). The absorption was detected by the Cary 60 Spectrophotometer (Agilent, USA) at 700 nm wavelength (Danielyan and Chailyan, 2020).

Determination of allopurinol concentration. The 0.1 M hydrochloric solution of allopurinol has two peaks of the absorption. The absorption was detected by the Cary 60 Spectrophotometer (Agilent, USA) at 250 nm wavelength (Belikov, 2009).

Determination of dexamethasone. The ethanol solution of dexamethazome has two points of the absorptions. The absorption was detected by Cary 60 Spectrophotometer (Agilent, USA) at 235 nm as the maximal point of absorption (Belikov, 2009).

Measurement of zeta potential of the particles.

Measurement of zeta potential was performed by the utility of modified Stolz, S. method (Stotz, 1978), where under the conditions of high electrical pressure (V=7 V) the particles were moving in the 0.1M PBS with pH=7.4. The movies in of the movements of the particles in the cell counter chamber were taken under the trinocular light microscope (Boeco, Germany). Calculations were performed based on the following equation (1):

$$\xi = \frac{\mu\ (6\pi\eta)}{\varepsilon} \quad (1)$$

where **ξ is the Zeta potential (mV), μ-mobility $cm^2/V*t(s)$, η-** viscosity(Pa * t(s)) of 0.1 M PBS, Ɛ-permittivity (F/m or $F \cdot m^{-1}$) of water (DELGADO and GONZÁLEZ-CABALLERO, 2005).

Statistical analysis of the results. Data are expressed as mean ± SEM. Statistical significance between control and experimental groups were analyzed by One-way analysis of variance (ANOVA) or student t-test. Results were accepted as the statistically significant, when $p<0.05$.

Vertebrate Animals. The all-animal study experiments were performed based on the IACUC policies and animal care standards, regulations adopted by the Armenian Ethical Committee of Institute of Biochemistry named after H Buniatian, National academy of Science of Republic of Armenia. Standard procedures and protocols: anesthesia, euthanasia, animal surgery and collection of blood were performed based on the above-mentioned regulations. The animals, white laboratory rats, were anesthetized by administration of Pentobarbital 2 mg/ 100 mg of weigh, injected I.P, using a hypodermic needle. With this dosage,

animals are normally anesthetized within 5 min. Depth of anesthesia were determined by squeezing the animals' leg with forceps. Absence of movement in response to this procedure was indicate sufficient anesthesia. If insufficient, additional doses of the anesthesia were given. During surgery cranial and rectal temperature continuously were controlled as these parameters reflect the normal animal physiological state. After 7 days animals were deeply anesthetized and euthanized by the cervical dislocation. All post-operative observations were documented.

Results

Detection of the efficiency of the bound medicines with the particles. The first set of the experiments was devoted to the evaluation of the percentile of the bound with the particles compounds. After the final preparation the particles were sedimented at G=14000 for 30 minutes and in the supernatant the mentioned above compounds were measured

Results of the binding experiments were the following: 100% ±5,30% binding for PRP, 34,33%±1,20% binding of the inorganic phosphates, 60,61%±2,00% - for the dexamethasone and 62.32%±3,80% for allopurinol.

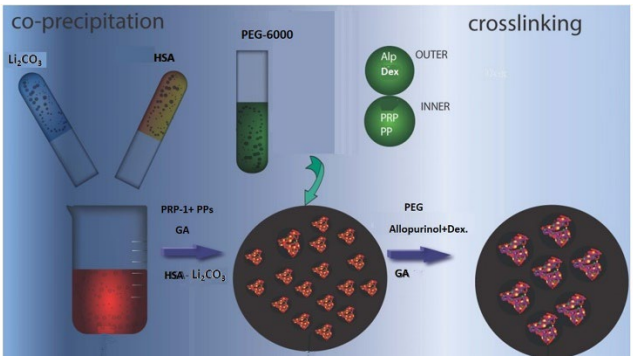


Figure 1. A. Formation of the albumin nanopartciles by the utility of CCD method (Chaiwaree et al., 2020). First stage of the formation. GA as well as the ions of Li+ are used for the polymerization and NP formation. B. During the second stage to the ready and washed from GA albumin particles, containing PRP-1 and phosphates was added 6000 units containing PEG as well as new portion of GA. Along with the PEG it was added dexamethasone as well as allopurinol.

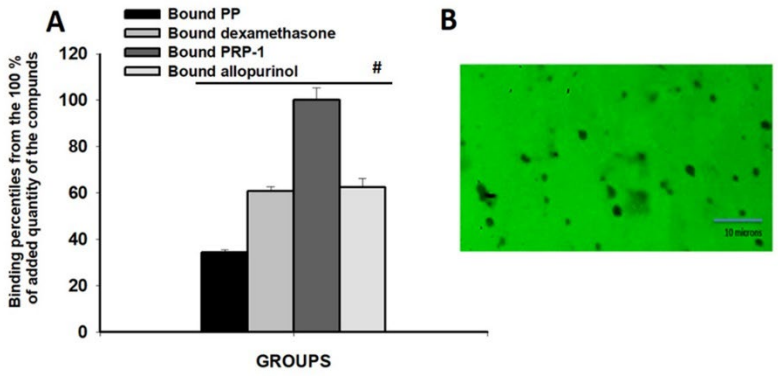


Figure 2. A. Binding of the medical compounds as well as inorganic phosphates with the albumin particles. There was used the Cary 60 spectrophotometer with the scanning abilities range from 190-1100 nm. PRP-1 unbound quantity was measured by the application of the Lowry method. The real unbound quantity was determined after normalization with the control particles sample, which didn't contain any peptide, however was degrading over the time in the aqueous solution. The absorption of the unbound proteins was measured at 730 nm. Ethanol solution of dexamethasone absorption was chosen to serve for the detection of its quantity. Experimentally it was delineated 235 nm as the maximum of its absorption. The allopurinol was dissolved in the 0.1 M hydrochloric acidic solution based on the scans, was measured at the 250 nm wavelength. Inorganic phosphates after colorimetric reaction proceeding were measured ate the 700 nm of the wavelengths. ANOVA-ONE-WAY was applied for the delineation of the statistical significance of differences between the groups. **B. Determination of the size as well as the shape of the particles for the further utility of them for in vivo and in vitro experiments.** It was applied the 25000 times magnification. Philips CM-100 TM Electron Microscopy was used for the above mentioned purpose.

Particles degradation experiments in the environment of the buffer as well as trypsin. In the second set of the experiments there were used 2 different environments for the detection of the degradation processes of the particles covered and not covered with PEG. The incubation of the particles was performed over the 72 hours. Maximum of the proteins' quantity diffused into the solutions was detected during the 3^{th} day of incubation.

Albumin particles degraded during the first 24 hours in water and trypsin environment accordingly 1) $1,5^{e-3}\pm7,0^{e-4}$; $0,102\pm5,314^{e-3}$ mg/ml of the protein solution. PEG-ylated partciles degraded in water/trypsin solutions with the

release of the following amount of the PRP-1 1) $7,0^{e-3}$ $\pm 1,6^{e-3}$; $0,031\pm 4,068^{e-3}$ (**Fig. 3A**). During the second day albumin particles degraded with the release of PRP-1 equal to $0,0367\pm 3,3189^{e-3}$ and $0,1229\pm 4,9900^{e-3}$, whereas the PEG-ylated micro and nanopartciles degraded with the PRP-1 and protein release equal to $0,0249\pm 6,4687^{e-3}$ and $0,1031\pm 1,8441^{e-3}$; mg/ml (**Fig. 3B**). During the 3rd day the quantity of the peptide and proteins in 2 different solutions were equal to $0,0500\pm 3,3189^{e3}$,$0,1800\pm 4,9900^{e-3}$, $0,0150\pm 3,3189^{e-3}$,$0,1000\pm 4,9900^{e-3}$ mg/ml ($p<0.05$ in the groups of degradations during the first and second days), (**Fig. 3C**).

Dexamethasone along with the allopurinol was chemically bound with the surface of the PEG-covered and not covered particles. As it is clear from the **Figure 3D,** even surface bound dexamethasone was released into the environment more slowly for the PEG-coved albumin vs albumin particles. The statement was true for the water as well as the trypsin environments over 3 days. During the first day dexamethasone was released from the albumin particles in water and trypsin solutions in the quantity equal to 0,5843±0,0223 vs 1,0227±0,0987 mg/ml and from PEG-ylated particles 0,2230±0,1170 vs 0,4933±0,0500 mg/ml; during the 2nd day 0,5457±0,0363 vs 0,4647±0,05 mg/ml in comparison with the PEG-ylated type 0,2533±0,0227 vs 0,35±0,20 mg/ml; at the day 3th 0,8250±0,0477 vs 1,2653±0,0647 mg/ml in comparison with the PEG-ylated type 0,4493±0,0421 vs 0,6883±0,0623 mg/ml.

Despite, technically allopurinol was attached to the surface of the particles; however, the most quantity of it was released during the second and in 3th days (**Figure 3 G,H,I**). During the first day from albumin particles in the water and trypsin solutions the quantity of the allopurinol per ml was equal to 0,1059±0,0119, 1863±0,0274 mg in comparison for the PEG-ylated particles in the same types of the environmental solutions $0,0625\pm 6,9214^{e-3}$; $0,1608\pm 6,0^{e-3}$ (**Figure G**). Over the second day in the same groups the allopurinol quantity was equal to the $0,0894\pm 4,9286^{e-3}$; $0,0757\pm 5,0^{e-3}$ vs $0,091\pm 4,8571^{e-3}$; 0,0689±0,01 and, during the 3rd day 0,0917±0,0113; 0,0824±0,0100 vs 0,0800±0,0145;$0,0700\pm 8,5714^{e-3}$ (**Figure 3 H,I**). In-group statistical calculations

are evidencing about the significant decrease of allopurinol release for albumin vs PEG-ylated particles.

As it was mentioned in the project, we proved, none organic phosphates are able to promote regeneration of the neuronal tissue in vivo as well as in vitro.

Regeneration of the tissue after stroke is the late post stroke stage. Thus not organic phosphates during our preparations were included into the core part of the albumin particles, to be released at the later stages of the particles degradation.

Based on our experiments, we concluded, not organic phosphates's release was noticed in the trace amounts in all groups (**Figure 3J,K,L**). During the first day the phosphates quantity in ml of the aqueous supernatant vs trypsin environment for albumin vs PEG-ylated partciles was equal to $2{,}2778^{e-4}\pm 9{,}8952^{e-6}$; $3{,}2778^{e-4}\pm 5{,}8952^{e-6}$ vs $3{,}3333^{e-5}\pm 1{,}3799^{e-5}$; $4{,}3333^{e-5}\pm 1{,}3799^{e-5}$; for the second day $3{,}3889^{e-4}\pm 5{,}3099^{e-5}$; $6{,}4444^{e-4}\pm 8{,}0^{e-5}$ vs $1{,}8333^{e-4}\pm 1{,}9446^{e-5}$; $5{,}3889^{e-4}\pm 5{,}3099^{e-5}$ and for the 3th day $5{,}6111^{e-4}\pm 8{,}3163^{e-5}$; $7{,}6111^{e-4}\pm 8{,}3163^{e-5}$ vs $6{,}1667^{e-4}\pm 4{,}8718^{e-5}$; $2{,}8889^{e-4}\pm 9{,}0420^{e-5}$.

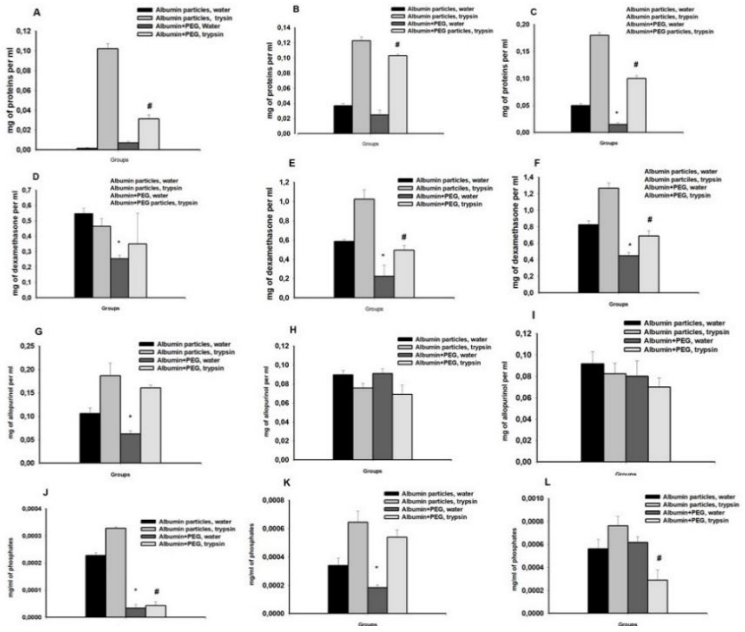
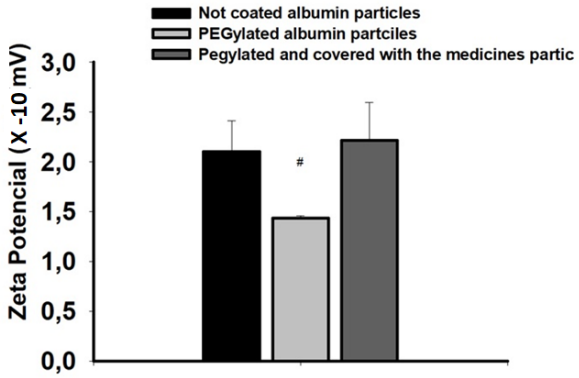


Figure 3. A., B, C. Degradation of the PEG-covered vs not covered albumin particles in the environment of the water and presence of the protease- trypsine. Binding process was determined by the utility of Lowry method for the measurement of the proteins. After centrifugation for 30 minutes at G=14000, in the supernatant, the quantity of the proteins was measured. It was used Cary 60 spectrophotometer (Agilent, USA). The student t-test was used for the determination of the significance of the results in the groups. The results were accepted statistically significant, when $p<0.5$ (n=10 per group). P value between covered with PEG and not covered particles in the environment of trypsin was
Less than 0.001 during day 1. For day two – between the same groups $p<0.05$. During day 3 between Peg-ylated and
Not PEG-ylated groups of the particles in water environment $p<0.05$, for trypsin – $p<0.02$.

D,E,F. Degradation and release of the dexamethasone from the PEG-covered vs not covered albumin particles in the environment of the water and presence of the protease- trypsine. After centrifugation for 30 minutes at G=14000, in the supernatant the quantity of the dexamethasone was measured at 235 nm wavelength. It was used Cary 60 spectrophotometer (Agilent, USA), ($p<0.05$ in the groups of degradations during the first and second days).
Dexamethasone release from the PEG-covered and not covered particles during day 1 in water environment was less than 0.05, in trypsin dyrig day 2 less than 0.05, during day 3 in water less than 0.05 and in trypsin less than 0.05 as well.

G,H,I. Degradation and release of the allopurinol from the PEG-covered vs not covered albumin particles in the environment of the water and presence of the protease- trypsine. After centrifugation for 30 minutes at G=14000, in the supernatant the quantity of the allopurinol was measured at 250 nm wavelength. It was used Cary 60 spectrophotometer (Agilent, USA).
The value of p for allopurinol release during day 1 was less than 0.05 for PEGylated and not PEGylated particles.

J,K,L. Determination of the not organic phosphates release from the PEG-covered vs not covered albumin particles in two environment: waster vs trypsin containing. Coloring solution was containing the concentrated sulfuric acid, 4% ascorbic acid, 0.3% anthimony potassium tartrate and sodium molybdate (4.5%). The absorption was detected by the Cary 60 Spectrophotometer (Agilent, USA) at 730 nm wavelength.
Statistically significant results were noticed between the groups of phosphates release for day 1 (in water and trypsin solutions between albumin and covered with PEG particles related groups – $p<0.002$, $p<0.002$), 2 (in water between albumin and covered with PEG particles related groups – $p<0.05$), 3 (in trypsin solution between albumin and covered with PEG particles related groups – $p<0.05$).
Evaluation of zeta potential of the particles. To delineate the aggregation as well as the stability phenomena of particles we measured the zeta-potential in phosphate buffer (pH=7.4). For control group of the particles, not covered with PEG the zeta potential was equal to 2,1±0,31(x-10) mV, for the group of protected PEG-ylated particles - 1,44±0,02 (x-10) mV, whereas for the PEG-ylated and loaded with medicines - 2,22±0,38(x-10) mV (p between control group and PEGylated particles <0.05, t-test, **Figure 4**).
Figure 4. Measurement of zeta potential for the particles. The electrical field with the



7 V electrical pressure was created by the electrodes, placed on the surface of the cells counter chamber, filled with the 0.1 M PBS with pH=7.4. The movements of the particles were taped with the further counting of the electrophoretic mobility. Every group was containing lot less than 8 samples (n=8). Results were accounted as the statistically significant when $p<0.05$ (student t-test).

Discussion

Human serum albumin is able to bind medicines as well as the peptides through non-covalent interactions. Amino acidic, thiol as well we carboxy groups might facilitate the tight interaction with the atoms of the ligand (Fasano et al., 2005).

Different methods of the albumin particles preparations are suggested, which includes the emulsion/solvent extraction, polyelectrolyte complexation/complex coacervation method, electrospray technique, nano spray drying, desolvation method, self-assembly. Except of the desolvation method, the others might induce formation of the large scales and sized of the particles.

Emulsion is a mixture of two or more immiscible liquids wherein one or more of the liquids are dispersed into another liquid (Yang et al., 2007). By taking into the consideration, that proteins are amphoteric with multiple charged functional groups, they can be made cationic or anionic by adjusting various factors such as the pH of the protein. As soon as the proteins become charged they can interact with the other types of the charged molecules. The pH-dependent electrostatic interaction between proteins and other polymers, such as DNA can be used to design stable biocompatible nanoparticles (Truong-Le et al., 1998).

Nano spray drying is a technique used in processing of the nanoparticles in liquid samples. Liquid samples are sprayed into chambers where heated nitrogen and carbon dioxide gas flow in the direction of spray from the nozzle (Oliveira et al., 2013). Micelles can be formed when proteins are dissolved in a solution with exceeding critical micelle concentration and the temperature, which is critical for the solution to form nanosized aggregates (Batrakova et al., 2006).

The desolvation method allows the synthesis of nanoparticles through a simple process of adding desolvating agents, such as ethanol and acetone, to protein solutions containing drugs. Desolvating agents change the protein structure and reduce the solubility of the protein, thereby leading to the formation of precipitation in the form of protein nanoparticles (Zhao et al., 2015).

In our current experimental we used desolvation method with the following crosslinking with the GA and surface modifications, performed with the PEG. By this methodological move we made not too stable core and dense chemically stable shell.

Interestingly, some literature data are evidencing about the possibility of the crosslinking the protein for the formation of the particles by the utility of the N-ethyl-N- (3-dimethylaminopropyl) carbodiimide (EDC) or polyethyleneimine (PEI), which led to the production of spherical particles having an average size of approximately 300 nm. The authors are evidencing, charge was controlled and was varying from -15 to +30 mV. The value of the charge was more prominent and vivid, when the number of the crosslinking's was elevated. Also, the same group showed the more crosslinkings more medicines might be entrapped into the particles. We adjusted the GA quantity to be added by our previous experiments, where the size of the particles also was sensitive to the quantity of crosslinker (Aganyants et al., 2015).

In the binding experiments the content of the inorganic phosphates included into the particles compositions was lower in comparison with the other types of the medicinal compounds. It might be explained based on the fact that utility of GA is not sufficient for the formation of the chemical bonds between the phosphates and the albumin.

The other compounds were able to bind in comparison with the 100 % of the added their amount for at least of 60 %.

As it is clear from our TEM microscopy results, we generated the micro as well as the nanopartciles with the diameter lower than 100 nm and not more than 1-2 microns.

During degradation experiments, as it was expected, the maximal amount of the proteins was released in the presence of trypsin. PEG-ylated particles were more protected from the impact of water as well as from the influence of the trypsin.

Interestingly, dexamethasone was disattaching from the surface of the particles most prominently during the 3rd day and in the emphasized extent in the presence of trypsin.

In case of allopurinol, which is in accordance to our scheme of the experimental design was attached on the surface of the PEG, was releasing also in the extended way from the surface of the particles in the presence of trypsin.

The quantity of the phosphates was lower in comparison with the medicines attached to the surface of the particles as well as diffusely entrapped into them due to the low binding with the albumin during the preparation procedure.

Based on the results, obtained after measurement of zeta-potential, it was clear, PEG-ylation of the particles was decreasing the positive voltage, probably due to the coverage of the charged amino acids as well as due to the increase in the diameter. Loading and coating the PEG-ylated particles was restoring the charge.

The entire design of the controlled released of the particles was made based on the knowledge about the stages of the stroke disease development. On the surface of the PEG-particles there was attached dexamethasone as well as allopurinol. The first stage after the damage of the tissue belongs to the processes of the inflammation as well as formation of the free radicals. Dexamethasone is the compound to suppress activity of the immune system whereas allopurinol, by serving as the classical inhibitor of XOR is able to prevent formation of the XOR products, which are free radicals as well as uric acid. Moreover, in accordance to our results, allopurinol is able to prevent degradation of the purines and stimulate regenerative processes in the brain tissue (Danielyan and Kevorkian, 2011).

The inner layers of the particles were including inorganic phosphates, which might serve not only for the generation of the ATP but also might activate the PRPS-1, which is the key regulative enzyme for the syntheses of the purines as well we pyrimidines.

Thus, after degradation of the core part of the particles PRP-1, as the neuroprotective agent, as well as phosphates will serve for the protection of the brain cells and will stimulate the processes of the regeneration.

We were able to generate PEG-ilated layered particles, which might be applicable for the treatment of the experimental stroke.

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We state, the conflict of the interests doesn't exist between of the authors, the results are original and have been never submitted for publication.

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«ՀԱՅԱՍՏԱՆԻ ԿԵՆՍԱԲԱՆԱԿԱՆ ՀԱՆԴԵՍ» -ի
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