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INTERNATIONAL PRACTICES ON GMP IN PHARMACEUTICALS

Abstract

Pharmaceutical science encompasses meticulous processes, from initial pharmacological testing to distribution and pharmacovigilance. Each stage demands unwavering precision and adherence to regulatory standards to ensure the quality and safety of medicinal products. This article delineates the multifaceted landscape of pharmaceutical regulations, focusing on Good Manufacturing Practice (GMP) standards as a cornerstone of quality assurance. GMP standards, encompassing GxP principles, mandate adherence to rigorous protocols throughout the drug lifecycle. Within the European Union (EU), GMP standards are codified in directives and regulations governing medicinal products for human and veterinary use. Volume 4 of Eudralex serves as a compendium of EU regulations, providing comprehensive guidelines for pharmaceutical manufacturing.

Mutual Recognition Agreements on GMP inspections between the EU and other jurisdictions aim to facilitate trade and regulatory cooperation, exemplifying international collaboration in pharmaceutical oversight. Recent agreements between the EU and the US FDA streamline inspection processes, reducing redundant inspections and expediting market access for pharmaceutical products.

Moreover, electronic systems such as EudraGMDP enhance transparency and regulatory compliance within the pharmaceutical industry. By evaluating international GMP practices, this article underscores the importance of harmonizing regulatory standards for Good Manufacturing Practices globally.

In conclusion, adherence to GMP standards not only ensures the quality of pharmaceutical products but also plays a pivotal role in the development of pharmacy practices worldwide.

Keywords: *Pharmacy, GMP, Eudralex, EMA, FDA, IRIS, Eudra GMDP*

Introduction

Pharmaceutical science is a very broad concept and requires strict responsibility and precision at every step. Here, each step refers to the stages from initial pharmacological testing of a potential medicinal product to mass production, documentation procedures for registration and sales authorization, presentation to the customer in a pharmacy, and even monitoring the side effects and adverse effects of the drug. Of course, these stages are divided into many steps, each of which is small and detailed, and proceeds in appropriate sequences. Each of these parts has special nuances that ultimately focus on the quality of the drug and are important to pay attention to. These nuances are complexed and grouped in the form of standards, rules, and regulations. The most important standards encountered and required to be followed in pharmaceutical activities include GxP

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standards, which require strict adherence to relevant regulations at all stages of drug preparation and delivery from the manufacturer to the consumer. Standards such as GMP - Good Manufacturing Practice (1), which covers the production process, GDP - Good Distribution Practice, which is especially important for wholesale enterprises, GLP - Good Laboratory Practice (2), which regulates laboratory processes, GPvP - Good Pharmacovigilance Practice, which serves to collect and process information about the additional and side effects caused by the drug in the body after taking it (3), GCP - Good Clinical Practice, which controls the quality and accuracy of clinical practices (4), and GDocP - Good Documentation Practice, which ensures compliance of submitted documents with the rules (5), can be the most important examples of GxP (Ronninger, 2012: 3). It is important to comply with each of these regulations, and at the same time, when importing pharmaceuticals and biologically active food products to most countries, including Europe, Azerbaijan, it has been accepted as a strict requirement that manufacturing enterprises have only GMP certificates among those listed. For this reason, compliance with GMP requirements, and obtaining and periodically renewing the GMP certificate are at the top of the list of duties of every pharmaceutical manufacturing enterprise (Doneski, 2023: 2).

GMP standards are regulated by legal requirements, including Directive 2001/83/EC and Directive (EU - European Union) 2017/1572 relating to medicinal products for human use; Directive 2001/20/EC and Regulation (EU) 536/2014 for investigational medicinal products; Directive 91/412/EEC and Regulation (EU) 2019/6 for veterinary medicinal products; active substances used for humans include Regulation No. 1252/2014 (1, 8). Directive 2003/94/EC applies to both human and investigational medicinal products. The principles and guidelines of Good Manufacturing Practices defined in the Commission Directives 2003/94/EC and 91/412/EEC are reflected in Volume 4 of Eudralex, otherwise known as "The rules governing medicinal products in the European Union". This volume consists of an introduction and 4 parts, and these parts are composed of different chapters and appendices (8). Part I contains "Basic requirements for medicinal products" and consists of 9 chapters approved between 2013 and 2015.

Part II, "Basic requirements for active substances used as starting materials", came into force in August 2014. Part III includes documents related to GMP about quality management, risk assessment, batch control, export, shipping, and marketing of pharmaceutical and medicinal products. In addition to the documents mentioned, Part II of Volume 4 of "The Rules Governing Medicinal Products in the European Union" includes various appendices. Annex 1 of sterile medicinal products, Annex 2 of biologically active substances and medicinal products for human use, Annex 3 of radiopharmaceuticals, Annex 4 of all veterinary medicinal products except immunological veterinary medicinal products, Annex 5 of immunological veterinary medicinal products, Annex 6 of gaseous medicinal products, Annex 7 of plants origin medicinal products, Appendix 9 liquids, creams and ointments, Appendix 10 pressurized and metered dose aerosols for inhalation, Appendix 13 medicinal products for investigational purposes, and Appendix 14 are designed to control the production of products obtained from human blood or plasma.

Also, Annex 8 Sampling of Starting and Packaging Materials, Annex 11 Computerized Systems, Annex 12 Use of Ionizing Radiation in the Manufacture of Medicinal Products, Annex 15 Qualification and Validation Processes, Annex 16 Qualified Person Certification and Batch Release, Annex 17 Parametric Release, Annex 19 retention and reference samples, and Appendix 21 sets out the requirements for the import of medicinal products.

Part IV of the 4th volume of the "The rules governing medicinal products in the European Union" is composed of "Guidelines for Good Manufacturing Practice specific to Advanced Therapy Medicinal Products" (9).

To overcome trade barriers, the EU has signed Mutual Recognition Agreements on GMP inspections with regulatory authorities located outside its borders. These agreements aim to allow EU authorities and their counterparts to trust each other's GMP inspections, waive batch testing of products entering their territory, and share information on inspections and quality defects. Mutual Recognition Agreements on GMP have been signed between the EU and Australia, Canada, Israel,

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Japan, New Zealand, Switzerland, and the USA, covering medicinal products intended for human and animal use.

On May 30, 2023, the US Food and Drug Administration (FDA) announced that Austria, Belgium, Bulgaria, Denmark, Estonia, Finland, France, Greece, Hungary, Ireland, Luxembourg, the Netherlands, Poland, Portugal, Slovenia, and Spain has confirmed that its national authorities are equivalent to the United States in terms of inspection capacity, capabilities and general procedures for Good Manufacturing Practices for veterinary products. At the same time, the EU has recognized the US FDA as the equivalent competent authority for Good Manufacturing Practices for veterinary products. Later, on September 26, 2023, the US FDA recognized the Swedish authority, and on November 28, 2023, the Latvian authority. This means that the EU and US FDA can rely on the results of pharmaceutical GMP inspections carried out in the parties' respective territories, and there is no longer a need to repeat inspections of manufacturing sites.

The EU also has Mutual Recognition Agreements equating Good Manufacturing Practices for medicinal products for human use with Croatia, Italy, Malta, Czech Republic, Romania, Lithuania, Cyprus, Germany, and Slovakia, including countries for veterinary products. The list of countries with agreements based on human and veterinary drugs is reflected in the Sectoral Annex to the EU-US Mutual Recognition Agreement together with the relevant dates. It is mentioned in the Sectoral Annex that the agreements valid for medicinal products intended for humans have entered into force since July 2019. For veterinary medicines, this document entered into force on May 11, 2023, when the EU and the US signed Joint Sectoral Committee Decision No. 2536/2023. The recognition of verifiability and comparable procedures makes bringing medicines to market faster and cheaper, reduces administrative burdens and costs from re-inspections, and allows manufacturers in other countries to focus more.

The mentioned agreements are based on audits of the Joint Audit Program in all EU Member States and close technical cooperation between the European Medicines Agency (EMA), the US FDA, and the Commission. Currently, the evaluation by the competent authorities of the EU continues according to the schedule agreed with the US. The US FDA has set a target date of July 2024 for the completion of the assessment of the inspection capacity of all EU authorities for veterinary medicines. The batch testing waiver will only come into effect when all EU veterinary authorities are recognized by the US. From that point on, veterinary products imported from the US will no longer need to be re-tested for quality control before being placed on sale in the EU. As a continuation of the technical cooperation between the EU and the USA, it is planned to expand the scope of the Sectoral Annex in the direction of vaccines for humans and even plasma-derived medicinal products at a later stage. These topics are currently on the agenda of the meetings of the EU-US Trade and Technology Council (Lopez-Navas, 2022: 8).

An agreement was signed between the EU and Japan on 18 July 2018 to expand the range of medicines for which they recognize the results of inspections at each other's manufacturing sites, which is considered a continuation of the first agreement signed on 29 May 2004. The full scope of the said agreement includes chemical pharmaceutical products, homeopathic medicines (as long as they are accepted as medicines in Japan and subject to GMP requirements), vitamins, minerals, and herbal medicines (if they are considered medicines on both sides); includes certain biological pharmaceuticals, including immunological agents and vaccines, active pharmaceutical ingredients (APIs) for any of the above categories, and sterile products belonging to any of the above categories. This agreement serves both parties to rely on Good Manufacturing Practice inspections in each other's territories, to waive batch testing of drugs entering Japan from EU countries and vice versa, and to share information on inspections and quality defects.

Directive 2011/62/EU amending Directive 2001/83/EC introduced EU-wide rules for the import of active pharmaceutical ingredients. Article 46b(2) established that APIs can only be imported if accompanied by written approval from the competent authority of the exporting third country stating the similarity to the GMP and control standards adopted by the EU (8).

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As GMP procedures are strictly and precisely implemented in the EU, various electronic systems have been established to facilitate the work of manufacturers. For example, marketing authorization holders and applicants should use the EMA's IRIS system to communicate with the EMA regarding GMP inspections required by the EMA's scientific committees (11).

EudraGMDP is a publicly available EU database containing production and import permits, registration of active ingredient manufacturers, GMP certificates, and declarations of non-conformity. After inspecting the production site, the EU authorities enter the GMP certificate or statement of non-conformity into the EudraGMDP database (6, 12).

Conclusion

Thus, in this article, international practices on GMP were evaluated, and such information should be analyzed and correctly applied for the further development of Good Manufacturing Practices in our country. This characteristic will play a large role in the development of not only Good Manufacturing Practices but also general pharmacy in our country.

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EXPERIMENTAL STUDY OF CHANGES IN MORPHOLOGICAL PECULIARITIES OF THE EOSINOPHILIC LEUKOCYTE IN THE LUNG UNDER THE INFLUENCE OF THE MACROPHAGE MIGRATION INHIBITOR FACTOR

Abstract

The research investigated on observation of eosinophilic leukocytes in the lungs of white laboratory rats under the effect of a Macrophage migration inhibition factor (MIF) by light and electron microscopy. The object of the study was 60 white male rats subdivided into 3 groups: control, placebo (saline injection), and main (MIF injection). The eosinophilic leukocytes are detected by specific cytoplasmic eosinophilic granules and ultrastructurally visible distinctive osmiophilic granules. The subpopulations of indicated cells in the lungs were identified as "stromal-interstitial", within "BALT", "perivascular" and "bronchial". It was found that in the experiment, the administration of MIF induces the migration of eosinophilic leukocytes into the alveoli and respiratory tract. It is necessary to note that the introduction of MIF has a gradual effect on the quantification and functional activity of eosinophilic leukocytes in the lungs of laboratory white rats: an increase in the first 2-7 days and stabilization at a higher level in the next 15-30 days. At least two ways of the effect of MIF on eosinophilic leukocytes have been evaluated: 1) by direct reception of MIF and 2) by indirect mechanisms. After injection of MIF, eosinophilic leukocytes probably have a regulatory effect in various microzones of the lung. The obtained results show that research in this direction may be promising in modeling immunopathological lung diseases.

Keywords: *eosinophilic leukocytes, MIF, morphology, lungs*

Introduction

The "Macrophage migration inhibition factor" (MIF) refers to proinflammatory cytokines (Song, Xiao, Dekker, Poelarends, Melgert, 2022: 105; Kong, Chen, Lan, 2022: 4908). As well as in recent years has been widely studied its significance role in acute and chronic inflammation (Shen, Tang, Zhu, Huang, 2021: 6; Du, Hao, Ma, Liu, 2022:01; Wen, Zhu, Zhang, Yang, Gao, Li, Yang, Liu, Tang, 2022: 01), sepsis (Toldi, Nemeth, Hegyi, Molnar, Solymar, Farkas, Alizadeh, Rumbus, Pakai, Garami, 2021: 8051), shock (Patel, Yamada, Oliveira, Stiehler, Zechendorf, Hinkelmann, Kraemer, Stoppe, Collino, Collotta, Ferreira, Pillmann, Sordi, Marzi, Relja, Marx, Martin, Thiemermann, 2022: 11), autoimmune (Bilsborrow, Doherty, Tilstam, Bucala, 2019: 1) and oncological pathologies (Charan, Das, Mishra, Chatterjee, Varikuti, Kaul, Misri, Ahirwar, Satoskar, Ganju, 2020: 1; Noe, Mitchell, 2020:1). The anti-glucocorticoid effects of the MIF have already been proven (Fan, Kao, Yang, Gu, Harris, Fingerle-Rowson, Bucala, Ngo, Beaulieu, Morand, 2014: 2059).

There are some explorations on its role in angiogenesis, carcinogenesis, and tissue regeneration (Zhang, Zhu, He, Fan, Deng, Hong, Liang, Zhao, Li, Zhang, 2019: 12641). The peculiarities of MIF expression have been studied in kidneys, skin, pituitary gland, etc. organs, as well as alteration caused by MIF injection (Li, Tang, Lv, Wang, Yang, Tang, Huang, He, Zhou, Huang, Klug, Meinhardt, Fingerle-Rowson, Xu, Zheng, Lan, 2019: 3867; Ding, Li, Wu, Lv, Yu, Hao, 2022: 18558; Shimizu, 2005: 65).

The target of MIF is macrophages (neutrophilic leukocytes), macrophages, dendritic cells of lymphoid clusters and nodes, microglial cells (glial macrophages), plasma cells, tissue basophils, some populations of lymphocytes, as well as smooth muscles, fibroblastic line cells, endothelium, covering and glandular epithelial cells (Calandra, Roger, 2003: 791).

The changes caused by MIF in the lungs are still poorly understood, although it is supposed that it has a pathogenetic role in bronchial asthma and obstructive pulmonary diseases (Rossi, Haslett, Hirani, Greening, Rahman, Metz, Bucala, Donnelly, 1998: 2869; Florez-Sampedro, Soto-Gamez, Poelarends, Melgert, 2020: L1184). One of the targets of the action of MIF may be eosinophilic leukocytes located in different organs (Rosenberg, Dyer, Foster, 2013: 9). MIF may be important in the pathogenesis of allergic diseases and helminthiasis, affecting the differentiation, functional activity and migration of eosinophilic leukocytes; the participation of eosinophilic leukocytes in this process has also been determined (Bozza, Lintomen, Kitoko, Paiva, Olsen, 2020: 15).

The results of numerous studies in clinical conditions are contradictory about the population of eosinophilic leukocytes in the lungs, as well as in experiments (Kowalski, Weller, 2016: 607-617; Blanchard, Rotenberg, 2009: 81-121).

The analysis shows that the topography, light microscopic, and ultrastructural features of eosinophilic leukocytes of the lungs are insufficiently systematized (23, 24). Thus, the following aspects of the morphology of these cells in the lungs are currently superficially studied:

- Appropriate his topography, frequency of occurrence, and morphometric indicators;
- Topographic, density-quantitative, and morphological changes in eosinophilic leukocytes that may be caused by the introduction of MIF;
- The direction, severity, and dynamics of changes in the population of eosinophilic leukocytes in the post-injection period of MIF.

The aim of the study.

The purpose of this research was a comprehensive study of the morphological features of eosinophilic leukocytes in the lungs of white laboratory rats in norm and after intravenous administration of MIF.

Materials and methods of research.

The investigation was conducted on 60 adult male white rats of an unknown breeding line, physically active, weighing between 200 and 250 g. The experimental animals acquired from the Scientific Research Center of Azerbaijan Medical University were kept in standard vivarium conditions, as well as with approved health certificates. The Macrophage Migration Inhibitor factor (MIF Rat, ProSpec (Protein-Specialists) CYT-193) was obtained from ProSpec-Tany TechnoGene Ltd. (Ness-Ziona, Israel) and dissolved in saline solution. The experimental animals were divided into 3 following groups: 1) 12 rats served as the control group, without any intervention; 2) 12 rats serving as compared group (placebo), were once receiving an intravascular injection of 0.3ml saline; 3) 36 rats served as the main experimental group, were receiving also the once intravascular injection of MIF diluted in saline solution (1.39µg MIF dissolved in 0.3ml saline). At 2 hours, 2 days, 3 days, 7 days, 15 days, and 30 days post injection (both saline and MIF), the animals were decapitated under anesthesia and excluded from the experiment. The investigated tissue samples were taken from the right (4 pieces from each lobe) and left (around the portal and peripheral zone) lungs (Fig.1).

During the experiments were followed the principles of the declaration of the European Union on animals used for experimental and other scientific purposes (Guidelines for accommodation and care of animals (article 5 of the convention) approved by the multilateral consultation, 2006: cons. 123).

The samples were placed in 4.0% buffered formalin for histopathological examination, then embedded in paraffin, while for electron-microscopic analysis fixed in buffered 2.5% glutaraldehyde + 2% paraformaldehyde + 4% glucose + 0.1% picric acid solution and embedded in Araldite- Epon mixture. The obtained semithin sections from Araldite-epon blocks in thickness 0.5-1.0µm were stained with double polychromatophil staining (solution "A" - 0.5% methylene blue,

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0.5% azur-II, 0.5% bura, and solution"B" - 5% alcohol, 0.1% alkaline fuchsin) (D'Amico, 2005: 207), as well as the 3-5µm sections purchased from paraffin blocks stained with hematoxylin and eosin. Studies at the light-optical level were carried out in stereometric mode under an Axio Scope A14 (Carl Zeiss) microscope with an Axiocam ERc5s video camera.

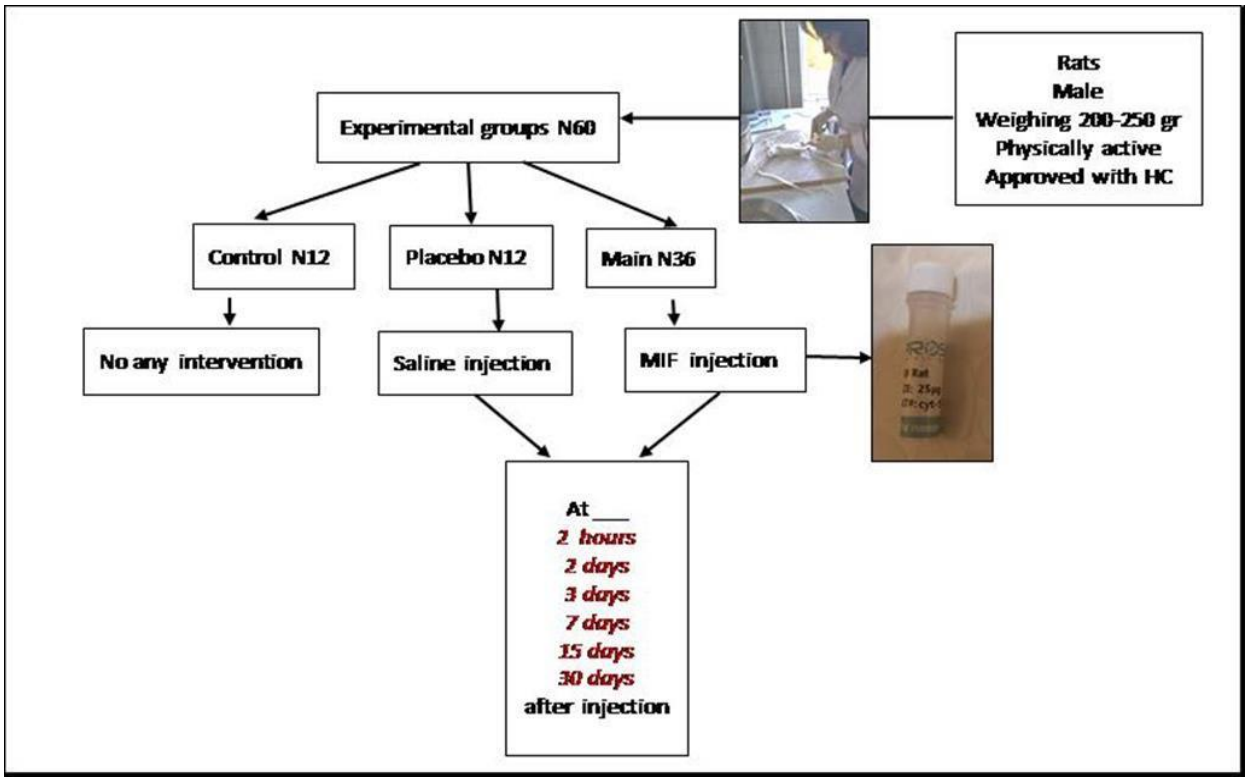

Figure 1. Experimental protocol of research work where male rats administered by saline, MIF, and animals were excluded from the experiment at various times, as well as the obtained information, was compared by data of control group rats.

After examination of the semithin section, the interested areas of the Araldite-Epon blocks were pointed for studying under TEM – transmission electron microscope (JEM-1400, Japan). Gained from the same blocks ultrathin sections 50-60 nm thick were first stained with 2.0% uranyl-acetate, then with 0.6% pure lead-citrate prepared in a 0.1N solution of NaOH and were analyzed at a voltage of 80.0 kV on TEM.

During conducting the experiments and interpreting their results were used to appropriate and methodological recommendations (Rosenberg, Dyer, Foster, 2013: 9 and 23, 24). Also, the morphometric analysis of objects was performed.

The average mathematical value of quantitative indicators and its average error (M ± m) were calculated, and the statistical reliability of differences at the accuracy level P=0.95 ($p<0.05$) was evaluated by the t-criterion (27). The p_1 is determined in comparison with the norm, and p_2 is compared with the placebo group (injections of saline solution).

Research results and their discussion.

In control white rats, eosinophilic leukocytes were found in the stroma of the lung, especially in the portal region (root, hilium); along the neurovascular bundles in the fibrotic tissue line; in the stromal septa of various zones; around microvessels; in bronchus-associated lymphoid tissue (BALT); in the wall of medium and large diameter bronchi. They are not found in the bottom of the alveoli, and in small bronchi, they are observed individually.

As seen in Fig. 2, a diagram that shows the distribution of the eosinophilic leukocyte population in the lungs of the control white rat, depending on the histological location and counted the percentage of eosinophil quantification.

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The stromal-interstitial eosinophilic leukocytes presented is 31,4%; around and within the wall of vessels known as perivascular is 26,2%; within BALT is 22,1%; in the wall and around of bronchi named as bronchial is 20,3%.

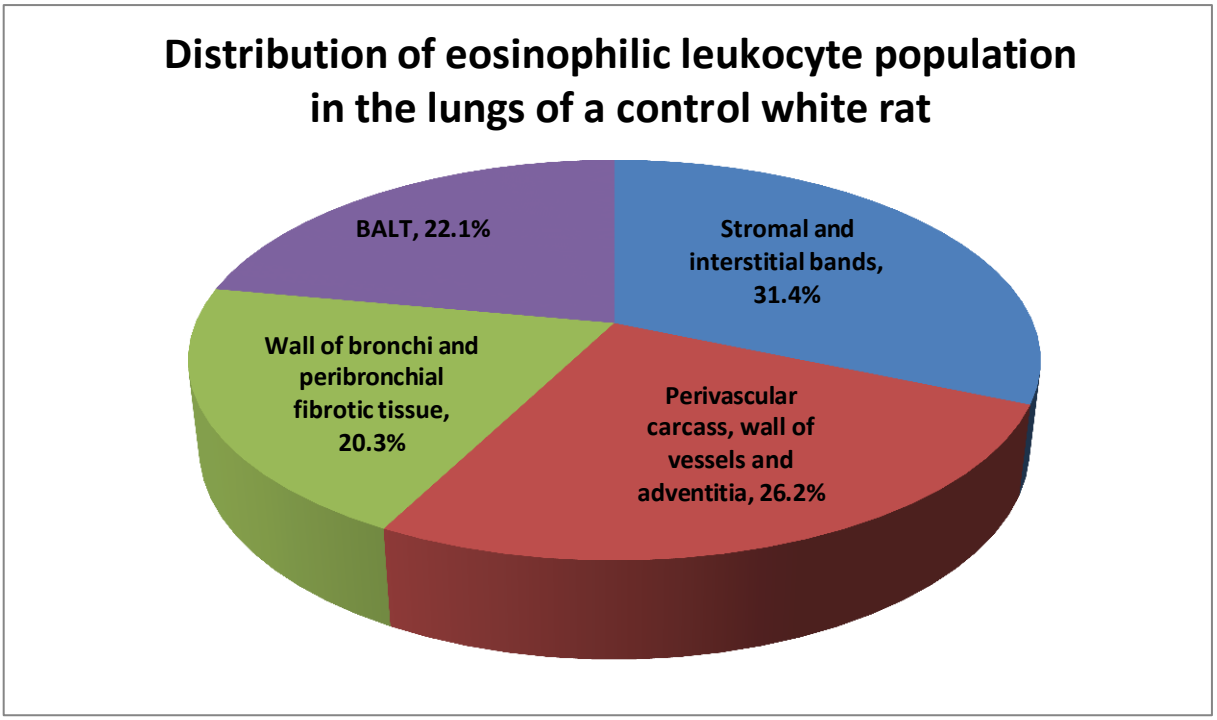

Figure 2. Distribution of eosinophilic leukocyte population in the lungs of a control white rat.

The main criterion for determining eosinophilic leukocytes at the light-optical level was accepted the localization of an eosinophilic substrate with cytoplasmic aggregates in cells as a granular or grossly diffuse nature.

However, the eosinophilic structures observed in the lumen of the bronchi, and sometimes in the bottom of alveoli, were evaluated as "degenerative eosinophilic leukocytes" and "degenerative eosinophilic corpuscles", which is quite consistent with the generally accepted approach (Erjefält, Persson, 2000: 2074, and 23, 24).

During electron microscopic analysis, the specific ultrastructural characteristic element determining of eosinophilic leukocytes were taken as the electron-dense osmiophilic granules, however along with them were also taken into account the general shape of these cells, the shape of the nucleus, the composition of cytoplasmic organelles, the presence of residual bodies and manifestations of exocytosis (degranulation, extrusion) (Muniz, Weller, Neves, 2012: 281-288; Klion, Ackerman, Bochner, 2020: 179 and 24).

On light and electron microscopic examination (Fig.3) eosinophils located in different regions of lungs singly or in groups of 2-3 cells and detected by their nuclear shape and eosinophilic granules presenting in cytoplasm. The eosinophilic leukocytes are 9.0-13.6 µm wide (11.2±0.8 µm), irregularly contoured, sometimes with 1-2 cytopodia of 0.4-1.0 µm (visible on electronogram in Fig.3B, C, D), they have only of simple support type connections with surrounding cells.

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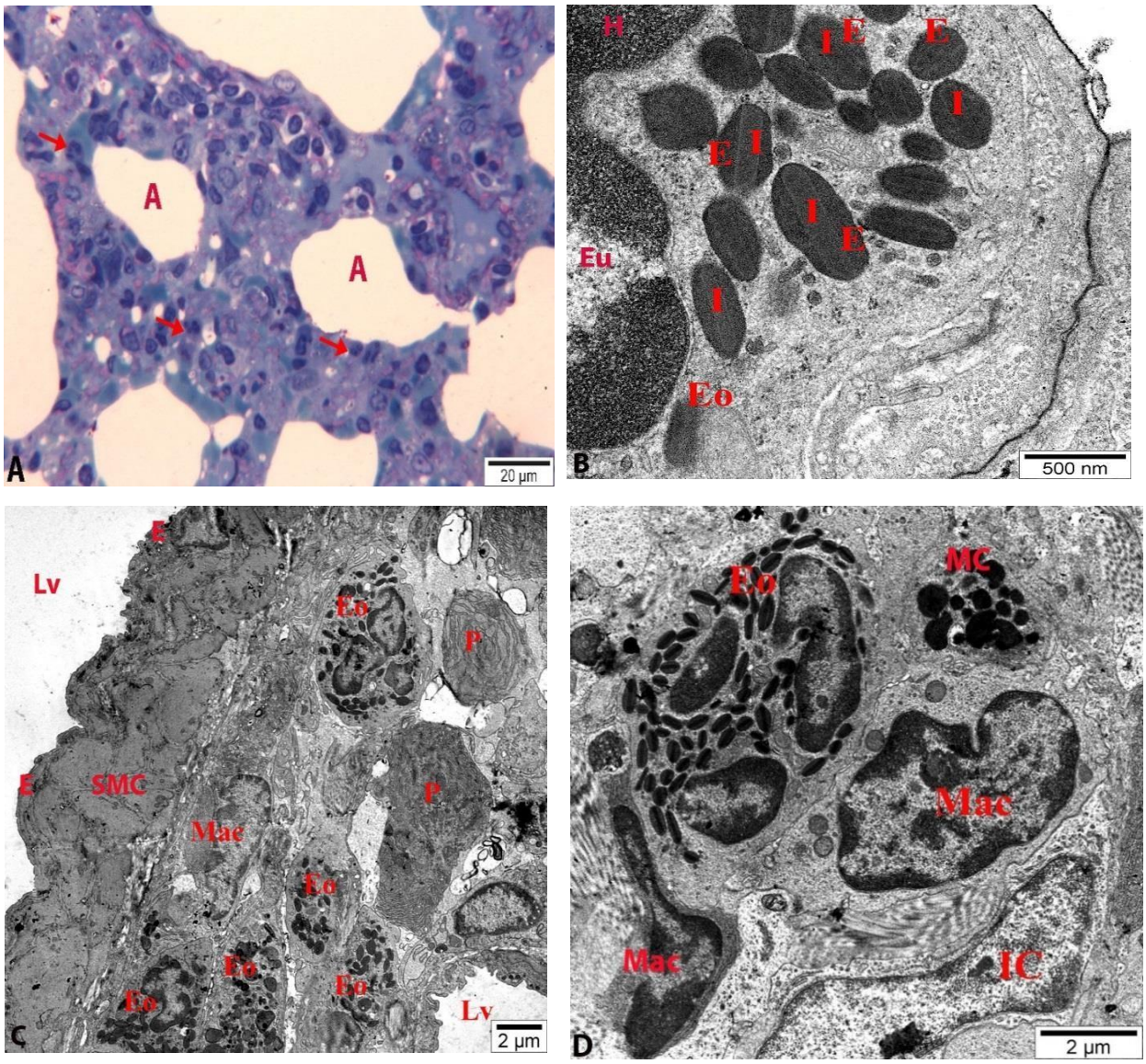

Figure 3. Microscopic presentation of eosinophilic leukocytes of intact white laboratory rats. A – localization within the interstisial stroma (shown by red arrow), between alveoli (indicated by A); B – characteristic eosinophilic cytoplasmic osmiophilic granules in oblong shaped, containing enzymes arranged in internum (I) and externum (E), accumulation of heterochromatin (H) and euchromatin (Eu) in nucleus; C – eosinophils situated in groups within tunica adventitia of the arterial vessel (Lv-lumen of vessels, SMC-smooth muscle cells, E-endothelial cells) and contacts with some connective tissue cells (Mac-macrophages, P-plasma cells), d – interaction of eosinophils within stroma with connective tissue cells (MC-mast cells, IC-interstitial cell). A – semithin section, double polychromatophil staining, scale bar 20µm; B–D - ultrathin section, uranyl acetate and pure lead citrate staining, scale bars B-500nm, C, D -2µm.

Under electron microscope observed more simple support and tight junction between them, but direct fixation contacts are not determined (Fig.3C). We consider that the absence of such contacts is important in the displacements of those cells between vessel and tissue, also within tissue, as well as in the performance of specific functions of them.

Eosinophilic leukocytes were not found in the floor of alveoli and bronchioles in intact white rats, which may indicate that mentioned cells does not freely migrate into the airways due to the

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absence of relevant inducers and pathogenic factors. No significant difference was noted in the incidence of eosinophilic leukocytes in the right and left lungs.

Ultrastructurally, on Fig. 3B showed eosinophilic leukocytes in the lungs have eu- and heterochromatin (indicated by Eu and H) accumulations in the nucleus, as well as clearly visible synthesis and secretion microsystems in the cytoplasm, specific osmiophilic granules, primary and secondary lysosomes, sparsely scattered cytoskeletal elements; the specific osmiophilic granules with oblong shape contain crystal like electron dense center internum (shown in fig by I) surrounding by less electron dense externum (marked in fig by E). Around of the tunica adventitia of arterial vessels (Fig.3C) placed the different cells, such as eosinophils, macrophages, plasma cells. The former cells are distinguished from the neighbour cells by their nuclear shape, cytoplasmic spesific granules and different electron-optical density. On Fig.3D demonstrated interaction of eosinophils with macrophages, mast cells, interstisial cells within interstisium of lung.

The administration of MIF did not significantly change the localization of the studied cells in the lungs. Compared with control (p_1>0.05) and placebo (p_2>0.05) groups, the number of eosinophilic leukocytes were unreliable increased at 2 hours after injection of MIF. However, during the experiment, the total population and relative numbers of livestock showed continuous growth, and then stabilization at a higher level. So, compared with control the increase in the number of eosinophilic leukocytes was 3.3% 2 hours after the injection of MIF, 28.4% after 2 days, 24.4% after 3 days, 18.5% after 7 days, 12.6% after 15 days, and 7.4% after 30 days. The introduction of saline solution had no significant effecton animals of the placebo group, therefore, compared with the control, the increase in the number of corresponding cells was only 0.1-0.5% (Fig. 4).

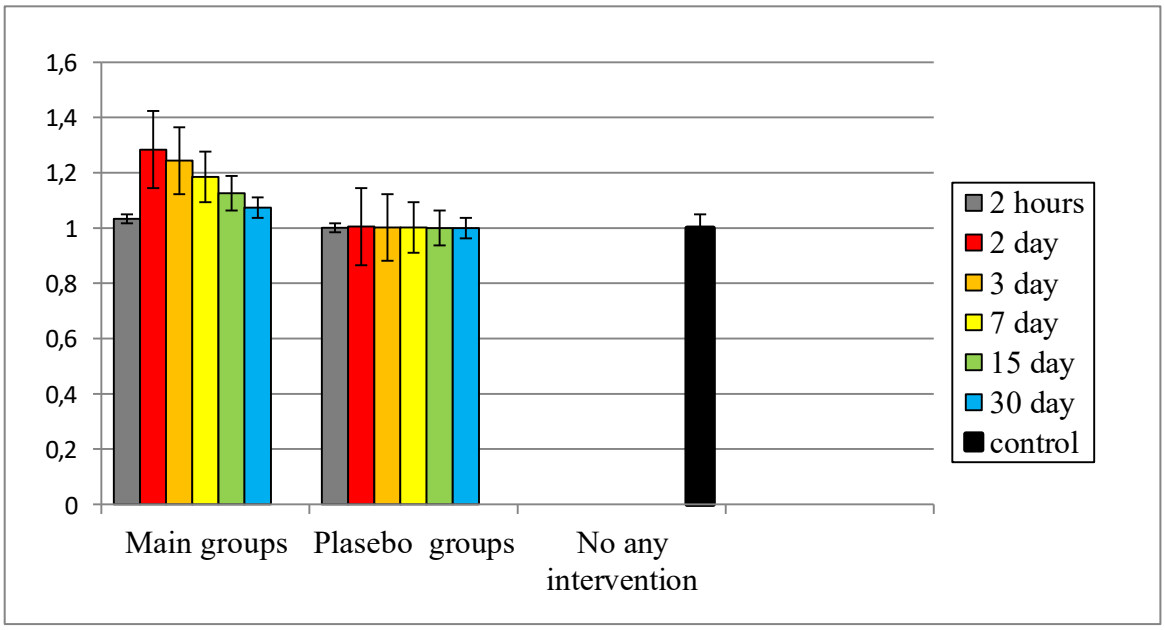

Figure 4. Dynamics of changes on quantification of eosinophils in lung tissue sections of white laboratory rats (in arbitrary units; a.u.). On diagram compared the data of eosinophils of rats that intravenously administred with MIF in main groups (the data of indicated cells shown with different color related to time of experiment), a saline solution in placebo groups, as well as the data of animals in control group, which hadn't done any intervention.

In conclusion, we note that during the experiment - on the 2nd, 3rd and 7th days after the injection of MIF - the total number of eosinophilic leukocytes elevated significantly (p_1<0.03 and p_2<0.05). For the further, on the 15th-30th day administration of MIF, the initially detected elevation is replaced by relative stabilization.

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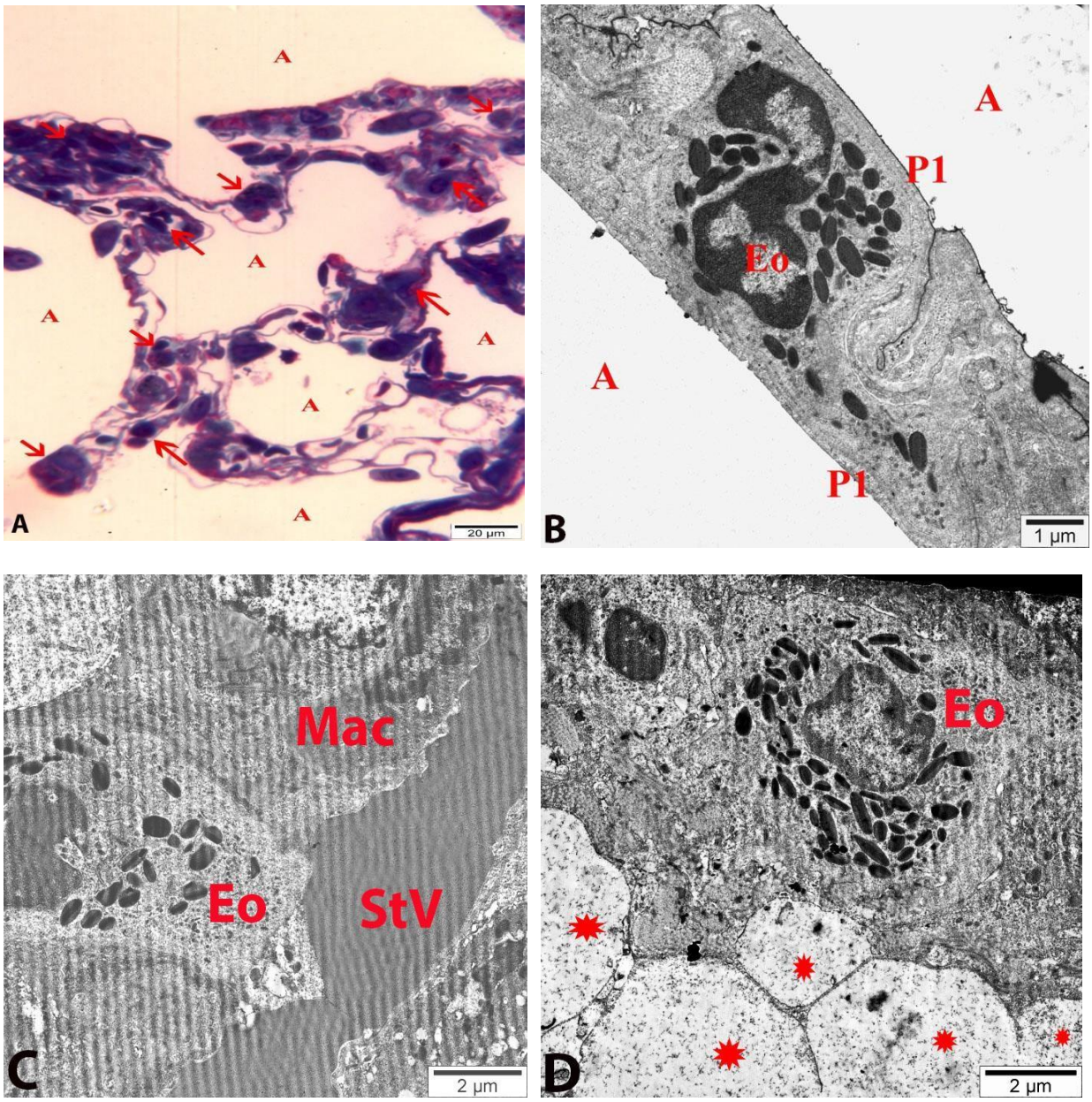

Figure 5. – Topographic features of eosinophil leukocytes in the lungs on days 2-3 after MIF injection. A – migration eosinophils (shown by red arrow) to the alveolar lumen (marked by A) and bottom on 2nd day; B – localization in the aero-hematic barrier on 2nd day (P1-pneumocyte 1, A-alveoli,); C – close contact with a stagnant microvessel (indicated by StV) and macrophages (Mac) on 3rd day; D – localization in the edematous interstitium (indicated by red star) on 3rd day. A – semithin section, double polychromatophil staining, scale bar 20µm; B–D - ultrathin section, uranyl acetate and pure lead citrate staining, scale bars B-1µm, C, D -2µm.

In parallel, eosinophilic leukocytes were observed around the alveoli, in the aerohematic barrier and in the bottom of the alveoli, which was not typical for the control and placebo groups. During this period (2-7 days after injection), the analyzed cells were found around the alveoli, in the aerohematic barrier, on the alveolar floor and in the lumen of the alveoli. Especially on the 2nd and 3rd days, eosinophilic leukocytes are located very close to stagnant, closed microvessels with degenerated endothelial cells, and microfields of perivasal plasmorrhage or form contacts with such microvessels (Figure 5).

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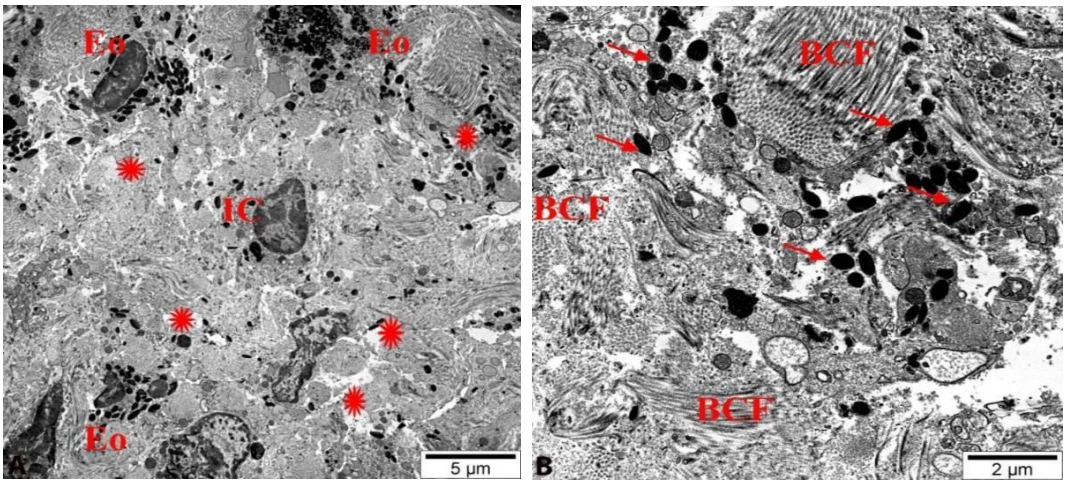

Figure 6. – Exocytosis (degranulation, extrusion) of specific osmiophilic granules from eosinophilic leukocytes. A – day 7 after MIF injection; B – day 30 after MIF injection; specific granules (shown byred arrow) between interstitial cells (IC), within edematous tissue (marked by red stars) and bundle of collagen fibers (BCF), background of fibrosis. Ultrathin section, uranyl acetate and pure lead citrate staining, scale bars shown on electronograms.

During the experiment, from the 2nd hour to the 15th day after injection were monitored the ultrastructural manifestations of extrusion of specific osmophilic granules and lysosomal exocytosis of eosinophilic leukocytes in the lungs. This is especially evident on the 7th-15th day within fibrous tissue around the small bronchi, in interstitial areas near to increased fibrous strands (Figure 6). It should be noted that there are no sources in the literature on extrusion and lysosomal exocytosis of these cells under the influence of MIF. However, as not yet been found the systematic information on the functional determination of these processes and their role in inflammation, fibrosis caused by injection of MIF into the lungs, but our research in this direction continues.

On the 15th and 30th days after the injection of MIF the eosinophilic leukocytes migrated between collagen bundles within stroma and in the peribronchial regions, where specific osmophilic granules are extruded, which is not typical for the group of control and placebo groups (injection of saline solution) (Figure 6).

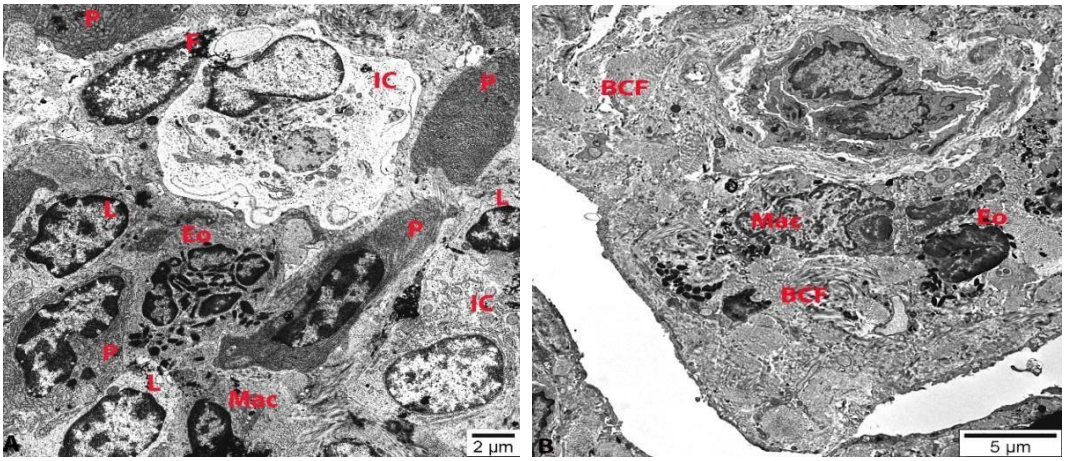

Figure 7. – An electronogram presented the cooperation of immune-competent cells within the stroma of lung tissue after administration of MIF. Relatively numerous contacts between eosinophilic leukocytes (Eo) and macrophages (Mac), lymphocytes (L), plasma cells (P), interstitial and fibroblasts (F). A – 3rd day after MIF injection; B – Day 7 after MIF injection. On the 7th day after injection clearly visible bundles of collagen fibers (BCF) around congested vessels and cells. Ultrathin section, uranyl acetate, and pure lead citrate staining, scale bars shown on electronograms.

It is possible that eosinophilic leukocytes induce fibrosis in stroma due to the extrusion of biologically active substances with a paracrine effect, but it is necessary to investigate in general the possible physiological/pathophysiological effects of exocytosis (extrusion) of specific granules of eosinophilic leukocytes.

After the injection of MIF condensed the cell complexes formed by eosinophilic leukocytes with lymphocytes, macrophages, tissue basophils, and perivasal adventitial cells, and also increased the frequency of simple and specialized contacts between cells. We evaluated this feature as "cooperation of eosinophilic leukocytes with other immunocompetent cells" (Figure 7.).

The dynamics of "increase-stabilization" is also observed in the number of immunocompetent cells, including eosinophilic leukocytes: first an increase (2-7 days), and then a relative stabilization (15-30 days).

In clusters of formed elements, which are sometimes observed in the lumen of blood vessels, were not detected eosinophilic leukocytes in any of the experimental groups, although these cells were found, as a rule, around small and medium-diameter vessels in various segments of the samples of lungs.

Thus, it can be considered that the introduction of MIF causes quantitative changes in the population of eosinophilic leukocytes in the lungs (increase and subsequent stabilization). At the same time, we determined the light microscopic and ultrastructural manifestations of changes in the functional activity of the studied cells. Presumably, this effect is realized in at least two ways: 1) Direct effect on "target" cells based on the direct reception of MIF in eosinophilic leukocytes. Considering the information that eosinophilic leukocytes have such MIF receptors as CD74 and CXCR4 (M. Bozza et al, 2020: 15), the probability of such an effect is high. 2) The effect of MIF injection on eosinophilic leukocytes through indirect mechanisms. We believe that intermediate rings, i.e. specialized lymphocytes, tissue basophils, activated macrophages, as well as fibroblast cells, are also involved in this mechanism; we have discovered that the signs of this may be polymorphism and violation in the number of cooperative cell complexes, including eosinophils.

As shown by some authors (Akuthota, Weller, 2012: 113-119; Rosenberg et al., 2013: 9-22), the results of the current study prove the prospects of a comprehensive study of eosinophilic leukocytes in the analysis of the prognosis, pathogenesis, clinical course of the lung disease related to its inflammation, fibrotic-obstructive and cancers.

Conclusion

1. The total population of eosinophilic leukocytes in the lungs of intact white laboratory rats consists of at least 4 distinct subpopulations: 1) "stromal interstitial", 2) within "BALT", 3) "perivascular" and 4) "bronchial".
2. In the experiment, the introduction of MIF stimulates eosinophilic leukocytes of the lungs.
3. During the experiment, the administration of MIF induces (accelerates) the migration of eosinophilic leukocytes into the alveoli and in the lumen of the respiratory tract.
4. Eosinophilic leukocytes interact with tissue basophils, macrophages, specific dendritic cells and certain types of lymphocytes on 2nd-7th days after the injection of MIF, also with myocytes and adventitial cells on 2nd-3rd days, and demonstrated intensive functional cooperation on 7th - 15th days with fibroblastic cells, which is manifested by an increase in the number of corresponding cellular contacts, elevated level of morphological polymorphism and the high extrusion rate of specific osmophilic granules.

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IMPLEMENTED REFORMS ON PHARMACOVIGILANCE IN AZERBAIJAN

Abstract

Pharmacovigilance, an essential procedure in healthcare, encompasses the identification, assessment, and prevention of adverse drug effects and other undesirable outcomes. Despite rigorous clinical trials conducted before medication release, some side effects may remain unidentified until after widespread usage. The National Pharmacovigilance Center of the Republic of Azerbaijan, operating under the Ministry of Health, plays a pivotal role in overseeing pharmacovigilance procedures. Citizens experiencing medication ineffectiveness or adverse effects are encouraged to report to the Analytical Expertise Center (AEC), facilitating thorough review and appropriate action. The AEC ensures patient safety by promptly addressing newly identified side effects and advising on reporting any adverse reactions, including those from traditional or alternative medicines. An increase in adverse event reports prompts governmental investigations, while strict confidentiality measures safeguard personal data. The AEC's systematic monitoring and evaluation, coupled with adherence to international standards and legal frameworks, contribute to enhancing drug safety. Regular updates on medication safety information are disseminated to healthcare professionals and the public, promoting informed medication use. Pharmacovigilance regulations, laws, and guidelines govern the AEC's operations, ensuring compliance and efficacy. Through collaborative efforts with healthcare institutions, the AEC conducts training to strengthen pharmacovigilance practices. Recruitment of pharmacovigilance representatives within medical facilities follows stringent criteria, emphasizing competence and timely reporting obligations. Overall, the AEC's vigilant oversight and proactive measures serve to optimize medication safety and uphold public health standards in Azerbaijan.

Keywords: *Pharmacovigilance, pharmacy, Analytical Expertise Center, The State Agency for Compulsory Medical Insurance TABIB, PSMF, PSUR, RMP, GPvP*

Introduction

Pharmacovigilance is a procedure for identifying, assessing, and preventing drug side effects and other unfavorable outcomes (1, 2). Comprehensive clinical trials are conducted to examine medications before they are released into the pharma market. Nevertheless, it's possible that some of the side effects of the medication haven't been identified despite widespread or prolonged use (Al Meslamani, 2024: 1). Side effects are an unanticipated response that the body experiences when taking the medicine as directed by the manufacturer. Each drug's container includes an insert that lists potential side effects under the "Side effects" section (Lakshmipathy, 2024: 7). It is possible to demonstrate these effects such as nausea, headaches, vomiting, etc (Dubey, 2024: 3).

The National Pharmacovigilance Center of the Republic of Azerbaijan controls the procedures for pharmacovigilance and was determined as the Analytical Expertise Center (AEC) under the

Ministry of Health. Should a notification be received regarding the ineffectiveness of a medication purchased from a pharmacy, the citizen may apply the medication (in its original packaging) and the pharmacy receipt to the "One Window" division of the Ministry of Health's Analytical Expertise Center. The Center's relevant departments review applications that are forwarded from there. Reporting of medication and vaccine additional reactions can also be submitted online by individuals and healthcare providers using the link https://primaryreporting.who-umc.org/en.

AEC realizes the procedures for the reduction of patient risk when a new drug side effect is identified for the safe usage of the medication by the patient. At the same time it advises reporting any possible negative effects noticed when using medications and other medical supplies (such as traditional, herbal, and alternative medicines). Specifically, it is imperative to report any unexpected effects that are noticed while using strictly restricted pharmaceuticals (6). If there is a spike in applications for the same drug series after people report side effects or medicine's ineffectiveness, the government may decide to launch an investigation. The institution complies with paragraph 19.2 of the "Pharmacovigilance Regulation of Medicines" (7) to guarantee the confidentiality of personal data. The Center thoroughly examines the information provided to identify any side effects and ensure using the medication is as safe as possible. The typical assessment of the offered data leads to several actions including the compilation of new safety information about the medication for patients and healthcare professionals, modifications to the package insert's instructions, restrictions on usage scheduling, recalls of the medication, etc. Periodically, these data are posted on the Analytical Expertise Center's official website. Between January 1 and August 31, 2022, AEC was notified of 115 medication side effects. One notification was sent from the patient, 68 from pharmaceutical companies, and 46 from healthcare professionals. On the Analytical Expertise Center's official website (www.pharma.az), citizens can search for the required drug by name, obtain the package insert with the instructions for use, and familiarize themselves with any extra effects (Harmark, 2008: 7).

The Analytical Expertise Center Pharmacovigilance department's activities are based on the systematic monitoring of side effects and benefit/risk ratios to ensure the safe use of medicinal products. This involves gathering, registering, evaluating, archiving, and establishing relationships between parties to take necessary measures to minimize any harm that drugs may cause. The appropriate steps are then taken to reduce the potential harm the medications may cause. The following are the actions taken to achieve this goal:

- Monitoring, registration, assessment, archiving about side effects, and data transfer to the World Health Organization's Uppsala Drug Monitoring Center;
- Keeping an eye on drug safety alerts by visiting the official websites of global health centers, looking into appeals related to safety from registration holders, evaluating the benefit/risk based on this data, and implementing the appropriate safety precautions for drugs that are registered or in the process of being registered in Azerbaijan.
- Reviewing and implementing the necessary actions after the assessment of the risk management plans (RMP) and Periodic Safety Update Reports (PSUR);
- Assembling the list of medications subject to special control;
- Supplying healthcare personnel with information;
- Compilation of the system for pharmacovigilance;
- Taking the required actions to promote health professionals' impromptu information sharing to optimize the pharmacovigilance system's implementation;
- Organization pharmacovigilance training (6);

The following laws and rulings have a direct bearing on Azerbaijan's pharmacovigilance system (9):

- Law of the Republic of Azerbaijan "On Medicines" No. 208-IIIQ dated December 22, 2006 (10);
- Law of the Republic of Azerbaijan on amending the Law of the Republic of Azerbaijan "On Medicines" No. 979-VIQD dated July 14, 2023 (11)

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- “Medication Recall Rules” approved by Decision No. 460 dated November 27, 2019 (12)
- “Pharmacovigilance Regulation of Medicinal Products” by Decision No. 503 dated December 25, 2019 (7);
- Decision on the approval of the "Instruction on Good Pharmacovigilance Practice" No. 39 dated August 25, 2020 (13).

The Pharmacovigilance rules contain general provisions, basic concepts, the main dossier of the pharmacovigilance system, the periodically updated safety report (PSUR), the risk management plan (RMP), general requirements for the implementation of pharmacovigilance, the license holder, medical and pharmaceutical staff in the field of pharmacovigilance, treatment, and prevention covers the tasks of enterprises.

At the same time, it includes the structure of the main dossier of the pharmacovigilance system, its supplement, the periodically updated safety report, the risk management plan; includes the updating of the main dossier of the pharmacovigilance system, its form, accessibility, and storage location, the content of notifications about side effects of medicinal products, notifications, data, reports to be submitted to the institution and their evaluation, registration and data storage, audit and final provisions.

Instruction on Good Pharmacovigilance Practice contains general provisions, basic concepts, requirements for quality system, pharmacovigilance system master file (PSMF), inspection, audit, risk management system, management and reporting of side effects of medicinal products, periodically updated safety report (PSUR), signal management process, post-registration safety studies, safety information, risk minimization measures, special control (additional monitoring) and pharmacocontrol documents (RMP, PSMF and PSUR) include the procedure of implementation of expertise.

A collection of documents known as the main dossier of the pharmacovigilance system - The Pharmacovigilance System Master File (PSMF) provides a detailed description of the pharmacovigilance system that the license holder employs in relation to the safety of one or more pharmaceutical products; a regularly updated safety report (PSUR) that evaluates the drug's benefit-risk ratio and is created by the license holder in the format and at the time specified at the stage following the drug's state registration; a risk management system's complete explanation can be found in the risk management plan (RMP).

At the same time, AEC organizes the trainings together with various institutions. With the collaboration of the Analytical Expertise Center of the Ministry of Health of the Republic of Azerbaijan and the Union of Management of Medical Territorial Units (TABIB), a "Basic training on pharmacovigilance" was organized. The participants in the training were given a practical demonstration of the World Health Organization's International Drug Monitoring Program, the Republic of Azerbaijan's current pharmacovigilance system and legislation, processing notifications to the WHO "VigiFlow" system, and coding data in "VigiFlow," "VigiLyze," and "VigiBase."

Pharmacovigilance representatives in medical facilities are recruited within the TABIB by the State Agency for Compulsory Medical Insurance and acceptance of them is based on various criteria such as higher medical or pharmaceutical education and practice related to medicine or pharmaceutical activity. This person's duties include setting up an internal pharmacovigilance system and notifying the AEC via VigiFlow online software of any side effects that arise when using medication within 15 calendar days. Within 48 hours, serious adverse medication reactions must be reported to AEC using the VigiFlow online software.

The recruiting process consists of an interview and a test phase. The medical institutions get successful candidates from both phases and make an appointment. If all the requirements are met the person is employed.

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Conclusion

Finally and in general, it can be noted that systematic implementation of pharmacovigilance procedures underscores its critical role in safeguarding public health by identifying, assessing, and mitigating adverse drug effects. The establishment of the National Pharmacovigilance Center of the Republic of Azerbaijan, functioning as the Analytical Expertise Center (AEC) under the Ministry of Health, serves as a central hub for receiving and scrutinizing reports of medication ineffectiveness and negative reactions. Prompt action is taken upon identifying new side effects to minimize patient risk and ensure medication safety. Furthermore, collaboration with global health organizations and adherence to stringent regulations, such as the "Pharmacovigilance Regulation of Medicinal Products," underscore Azerbaijan's commitment to maintaining pharmaceutical safety standards.

The recruitment and training of pharmacovigilance representatives within medical facilities, overseen by the State Agency for Compulsory Medical Insurance, further strengthens the country's pharmacovigilance infrastructure. This concerted effort towards pharmacovigilance underscores Azerbaijan's dedication to promoting safe medication usage and protecting public health.

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CHEMISTRY

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PHYSICO-CHEMICAL PROPERTIES OF COMPLEX SALTS FORMED BY HEXADECANE, OCTADECANE AND CIS-9-OCTADECENE ACIDS WITH MONOETHANOLAMINE

Abstract

In the article, the results of the study of the quaternary ammonium salt formed by hexadecanoic acid, a monobasic carbonic acid, with monoethanolamine, in laboratory conditions, in distilled, drinking, and sea waters contaminated with Balakhani oil with different degrees of mineralization, are given. The surface activity property of the products of different concentrations of this complex was studied using a tensiometer, the element composition was calculated by calculation, and the composition and structure were confirmed by infrared spectroscopy. The result of investigating the composition of the complexes formed by hexadecanoic acid with monoethanolamine by infrared spectroscopy proves that the reaction proceeds according to the scheme indicated in the article.

As a result of the research, it was determined that the mass share of carbon in the salt obtained on the basis of hexadecanoic acid and monoethanolamine is 68%, the share of hydrogen is 12.6%, the share of oxygen is 15%, and the share of nitrogen is 4.4%. The salt obtained on the basis of hexadecanoic acid and monoethanolamine shows high surface activity by reducing the surface tension at the water-air interface from 71.98 mN/m to 30.9 mN/m. The synthesized reagent has strong oil-collecting and oil-dispersing properties. With the application of this complex, it is possible to remove thin layers of oil from the water surface. The obtained complex is well soluble in ethyl and isopropyl alcohols.

Keywords: *oil collection, oil dispersion, surface tension, surfactant, carbonic acids*

Introduction

One of the most serious environmental problems facing humanity today is the pollution of the world's oceans. Examples of these sources of pollution include tankers carrying oil, accidents during oil extraction and transportation. Oil stains on the surface of the water lead to the deterioration of water quality and the balanced connection of the upper water layers with the atmosphere, disrupting the effect of oxygen on living things. Surfactant substances (SAM) used to remove thin layers of oil from the water surface are divided into oil dispersants and oil collectors (Abbasov, 2002: 6-10; Ahmadova, Abilova, Rahimov, Asadov, Ahmadbayova, 2018: 205, 416-422;

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Asadov, Rahimov, Ahmadova, Huseynova, Ahmadbayova, 2017: 229-235; Asadov, Ahmadova, Rahimov, Abilova, Nazarov, Zubkov, 2017: 3297-3305; Asadov, Huseynova, Rahimov, Ahmadova, Zubkov, 2017: 244, 533-539; Asadov, Ahmadova, Rahimov, Abilova, Zargarova, Zubkov, 2018: 21, 247-254; Asadov, Ahmadova, Rahimov, Huseynova, Quliyev, Aliyev, Agamaliyeva, Ahmadbayova, 2019: 861-870; Asadov, Ahmadova, Rahimov, Huseynova, Suleymanova, Ismailov, Zubkov, Mammadov, Agamaliyeva, 2018: 550, 115-122; Asadov, Ahmadova, Zubkov, Rahimov, 2018: 214-219; Humbatov, Dashdiyev, Asadov, Askerov, Hasanov, 2001: 448; Rozen, 2004: 444; Rustamov, Abbasov, Mamedova, Piriev, 2008: 717).

Methodology of the experiment. Hexadecanoic acid is a white crystalline, odorless, saturated monobasic carbonic acid with general formula $C_{15}H_{31}COOH$, relative molecular mass 256.4 g/mol, melting point 62.9 °C, boiling point 351 °C, insoluble in water.

Monoethanolamine (MEA) is a colorless, clear, viscous liquid with a molar mass of 61.1 mol/g and an ammoniacal odor. The surface activity of substances was determined at the air-water interface using a KSV Sigma 702 (Finland) tensiometer using a Du Nui ring.

Conduct of Research.

The reaction between hexadecanoic acid and MEA was carried out in laboratory conditions in a 1:1 molar ratio at 65°C for 1 day with intensive stirring.

The scheme of the reaction is as follows:

$C_{15}H_{31}COOH + NH_2C_2H_4OH \rightarrow$

$[C_{15}H_{31}COO^- N^+ H_3C_2H_4OH]$

In the salt obtained on the basis of hexadecanoic acid and MEA, the mass share of carbon is 68%, the share of hydrogen in ash is 12.6%, the mass share of oxygen is 15%, and the mass share of nitrogen is 4.4%.

Results and their discussion.

The IR spectrum of the salt formed by hexadecanoic acid with MEA was recorded on FT-IR, Spectrum BX and ALPHA (Bruker) spectrometers using a KBr disk. In the IR-spectrum of the obtained complex, C-H valence at 2848, 2915, cm^{-1}, COO-valence in complex ether fragment at 1405, 1533 cm-1, C-H strain at 1464 cm^{-1}, 2552, 2636 cm^{-1} There are bands of N+-H oscillations. The values of specific electrical conductivity of aqueous solutions of different concentrations of salts formed by MEA of hexadecane, octadecane and cis-9-octadecene acids were determined with the help of a conductometer (picture 1). As can be seen from Figure 1, as the concentration of SAMs in the solution increases, the values of specific electrical conductivity increase.

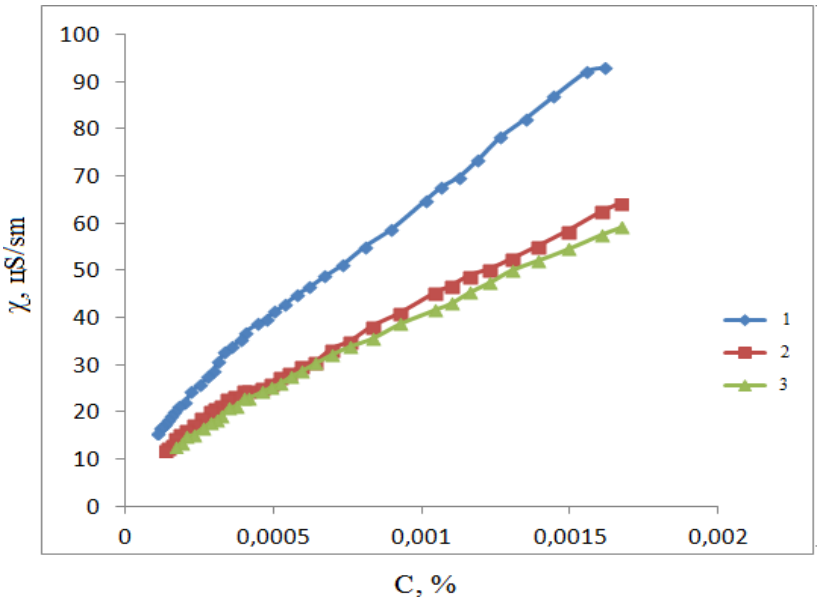

Figure 1. Electrical conductivity curves of hexadecane (1), octadecane (2) and cis-9-octadecene (3) salts formed by MEA

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Surface tension isotherms were constructed based on the surface tension values measured by the tensiometric method (Fig. 2)

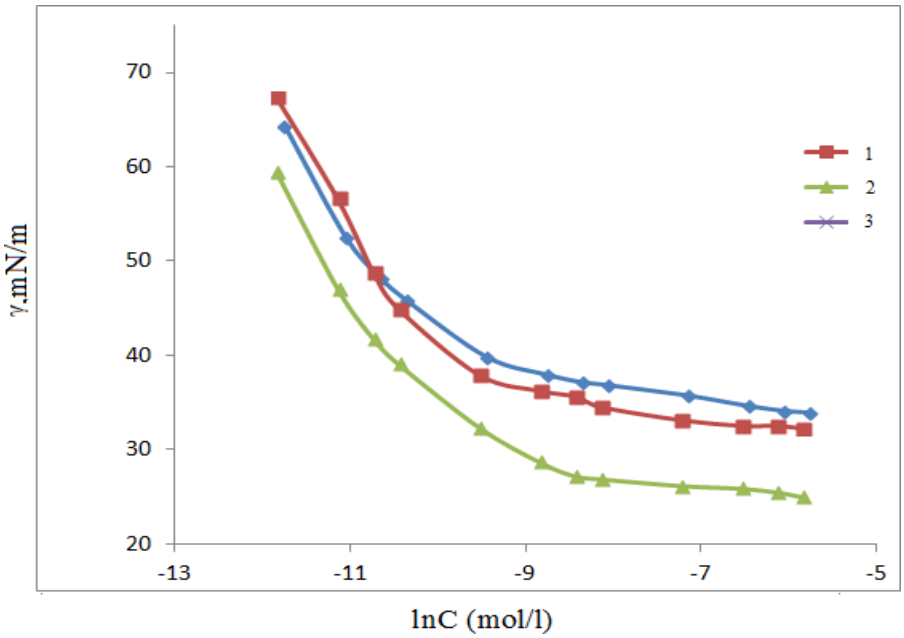

Figure 2. Electrical conductivity curves of salts of hexadecane (1), octadecane (2) and cis-9-octadecene (3) acids formed by MEA

Based on this picture, the dγ/dlnC value was determined by a graphical method (5). The surface tension at the water-air boundary in a non-reagent environment is equal to 72.0 mN/m. Electrical conductivity curves of salts of hexadecane, (1), octadecane (2), and cis-9-octadecene acids (3) formed by MEA, respectively stabilization of surface tension values occurs at 34.0, 32.2, 25.0 mN/m. The colloid-chemical parameters of the synthesized SAMs were calculated according to the equation given in (5) and the results are listed in table 1.

Table 1. Colloid-chemical parameters of salts formed by MEA of hexadecane, octadecane and cis-9-octadecene acids.

SAM	KMQ×10^{-3}, mol·dm^{-3}	G_{max}×10^{-10}, mol·sm^{-2}	A_{min}×10^{-2}, nm^2	γ_{KMQ}, mN·m^{-1}	π_{KMQ}, mN·m^{-1}	pC_{20}	ΔG_{mis}, kC/mol^{-1}	ΔG_{ad}, kC/mol^{-1}
1	1.57	1.99	83.6	34.7	37.3	4.77	-25.69	-44.47
2	1.45	1.78	93.6	32.5	39.5	4.73	-25.89	-48.14
3	1.46	2.37	69.9	25.9	46.1	4.53	-25.87	-45.31

Note: KMQ is the critical micelle formation density, γKMQ is the surface tension of the solution during KMQ, Γmax is the maximum adsorption, Amin is the minimum surface area of the polar group, πKMQ is the surface pressure or efficiency, pC20 is the efficiency value, ΔGmis is the enthalpy change during micelle formation. , ΔGad is the enthalpy change during the adsorption process.

Complexes formed by hexadecane and heptadecano acids with TEA were studied as an oil collecting and oil dispersing agent in cleaning the water surface clouded with an oil layer with a thickness of 0.17 nm. The effectiveness of this reagent was studied in the laboratory on waters with different degrees of mineralization using the Balakhani light oil sample. The reagent was used both in its pure form and in the form of a 5% aqueous solution. The reduction of the area of the primary oil layer due to the penetration of the reagent into oil-contaminated waters determines its effectiveness. The oil accumulation coefficient is a quantity that characterizes this effect. K is

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calculated as the ratio of the initial area of the oil layer to the area of the oil spot formed by the effect of the reagent.

Table 2 – Research results of oil collection and oil dispersing ability of hexadecanoic acid and monoethanol complex (Balakhan oil; thickness 0.17 mm).

The case of giving the reagent to the surface of the oil	Distilled water		Drinkable water		Sea water	
	τ, time	K(K_D,%)	τ, time	K(K_D,%)	τ, time	K(K_D,%)
Undiluted product	0-24 48-72 72-96	8.0 9.0 9.0	0-24 48-72 72-96	9.7 9.1 9.1	0-24 48-72 72-96	Disp. 70%
5% aqueous dispersion	0-24 48-72 72-96	9.6 9.0	0-24 48-72 72-96	Disp.	0-24 48-72 72-96	Disp. 70%

As a result of the research, it was determined that the undiluted and 5% solution of the reagent shows oil-dispersing properties in seawater. The reagent keeps its effectiveness for 4 days.

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IN VITRO GERMINATION OF POLLEN OF SOME *LONICERA* L. SPECIES

Abstract

The morphological structure of flowers of *Lonicera* L. species, the study of their biology, and observations show that the studied species are mainly cross-pollinated plants. Bees, ants, and other insects visit flowers for nectar, and thus they are considered one of the main pollinators of the plant. Pollen viability is an important indicator in the study of the generative development of plants. Pollen viability can be objectively evaluated using the method of germination in an artificial food medium. There is little literature data on pollen germination of *Lonicera* L. species, based on which, the fertility and sterility of pollen, and the effect of concentrations of different substances on pollen germination were studied.

Keywords: *ornamental plants, introduction, Lonicera L., pollen, pollen tube*

Introduction

Lonicera L. species are mainly distributed in the temperate and subtropical regions of the northern hemisphere. Most species grow in the thickets of broadleaf, coniferous, mixed, and montane forests of Eurasia and North America, some species are found in the tropical forests of Southeast Asia. 200 species of genus Lonicera L. are known in the world flora. About 140 species are cultivated in dendrological collections of botanical institutions of different countries. Representatives of this family are mainly shrubs and lianas, rarely grasses. The leaves are mostly opposite, evergreen, semi-evergreen or deciduous. These plants are distinguished by their characteristic tubular, bell-shaped flowers. A flower is a complex reproductive organ that carries out the reproduction processes of angiosperms. According to most authors, a flower is a specialized, growth-limited, non-branching organ that ensures the realization of the sexual process resulting in the formation of spores and gametes, the formation of seeds and fruits. The main parts of the flower are the receptacle, petal, sepal, stamens, and pistil (Mammadov, 2011: 49).

The studied plants are mainly cross-pollinated plants, sometimes self-pollination also occurs. The pollen cells of the stamens vary greatly in size, color, and shape. Inside it is the solid cytoplasm and it contains fat, starch, sugar, and other similar substances. While still in the pollen nest, during germination, the pollen cell nucleus divides into 2 cells: a vegetative and a generative cell. During germination, the vegetative cell produces the pollen tube, and the generative cell produces 2 sperm (Ibrahimov, 2004: 187).

In a natural case, the dust grain falls into the pistil's mouth to germinate, and it contains nutrients and biologically active substances for normal germination. This substance attaches the pollen grain to the mouth of the pistil and provides nutrients for its germination (Humbatov, 2017: 567).

The biomorphological characteristics of the studied species of Lonicera L. are shown below (Mammadov, 2015: 291).

Lonicera Caucasica Pall.

It is a low shrub with a height of up to 3 m, an upright trunk, and branches covered with gray bark. Leaves are simple, entire, glabrous, narrowed towards the base, up to 8 cm long. The flowers

are located in pairs or bunches in the axils of the leaves, they are fragrant and nectar. Fruits are black, spherical, paired in leaf axils.

Lonicera fragrantissima Lindl. & Paxton

The height of the plant is 2-3 m, it is an upright bush with many branches. The leaves are dark green, up to 8 cm long and 3.5 cm wide. The flowers are yellowish-white in color and have a nice fragrance. Each pair of flowers is about 1 cm long. The flowers attract attention before the plant is fully leafed. This plant is considered "The harbinger of spring".

Lonicera japonica Thunb.

The flowers are white, and yellow in color and have a sweet vanilla scent. The leaves are ovate or ovate-oblong in shape. The base of the leaf blade is heart-shaped. Leaf length is 3-8 cm. Young leaves are hairy on both sides, older leaves are smooth. The fruits are black and shiny.

Lonicera korolkowii Stapf.

It is a 3 m tall, soft hairy, branched, decorative, deciduous shrub. The length of the leaves reaches 3 cm, they are oval or elliptic. The flowers are pink and arranged in pairs. The fruits are spherical, bright orange-red, and remain on the branches until autumn.

Lonicera maackii (Rupr) Herd. – Maaka doqquzdonu

The height of the bush is 4m. Dark green leaves are 5-8 cm long, ovate or elliptic. The flowers are white and 2 cm long. The flowers are located in pairs, the white flowers of the plant later turn yellow or pale. The berries are bright red, and balloon-shaped.

Lonicera tatarica L.

It is a shrub with a height of 1.5-2.5 m. The bark of the trunk is grayish and peels off gradually. Its leaves should be 6 cm long and 3 cm wide and ovate. The flower is large, located in pairs in the leaf axil, the petals are pink or dark pink. The fruit is 6-8 mm in diameter, red or yellowish, spherical, ripens in August, and is located on the branches in pairs, free or slightly contiguous.

Results and Discussion.

One of the important parts of the flower is the stamen. This is called androecium (A). Each stamen consists of three parts: a thin stalk below, a pair of sacs above, and the part that connects the pollen sacs to the stalk. The first of these is called the filament, the second is the anthers or pollen, and the third is the dam (İbrahimov, 2004: 184). To study the viability of pollen and pollen germination of 6 species of *Lonicera* L., stamens of flowers with buds ready to bloom were used. Pollen viability was studied by acetocarmine staining method (Retina, 1981: 75). Determination of the fertility and sterility of pollen was carried out using the dye-acetocarmine, in 10 fields of vision for 4 repetitions. (Chelak, 1989: 31).

First, the anthers were removed from the stamens. Then conditions are prepared for the germination of pollen tubes. At this time, a drop of distilled water is poured on the object glass through a pipette. Pouring a drop of pre-prepared solution on it with a pipette and shaking the anthers with the tip of tweezers, pollen is added to the plant and then covered with a covered glass. (Gaziyev, 2017: 195).

Sucrose (30%), and boric acid (0.0005%) concentrations were used to prepare the nutrient medium (Romanyuk, 1986: 53).

The edges of the cover glass are vaselined so that the glass does not move and the liquid does not evaporate. A chamber with humid conditions created for rapid germination of pollen is placed in the thermostat together with the object. The temperature in the thermostat is raised to 20-25^0 C (Gasimov, 2010: 196). Germination was determined 24 hours after sowing in 5-6 field of view with 100x magnification of the microscope. When the pollen germinates, that is, when the pollen tube is formed, it is necessary to make sure that the edge of the tube corresponds to the ruler of the micrometer. After that, during one day, the growth of the pollen along the ruler is observed and how many mm it grows is determined.

The effects of substances with different concentrations on the germination of pollen of Lonicera L. species were analyzed and determined. Pollens were germinated in petri dishes with a thin layer

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of water at the bottom at a temperature of 25°C (Golubinski, 1974: 280). Pollen tubes larger than the diameter of germinated pollen are formed.

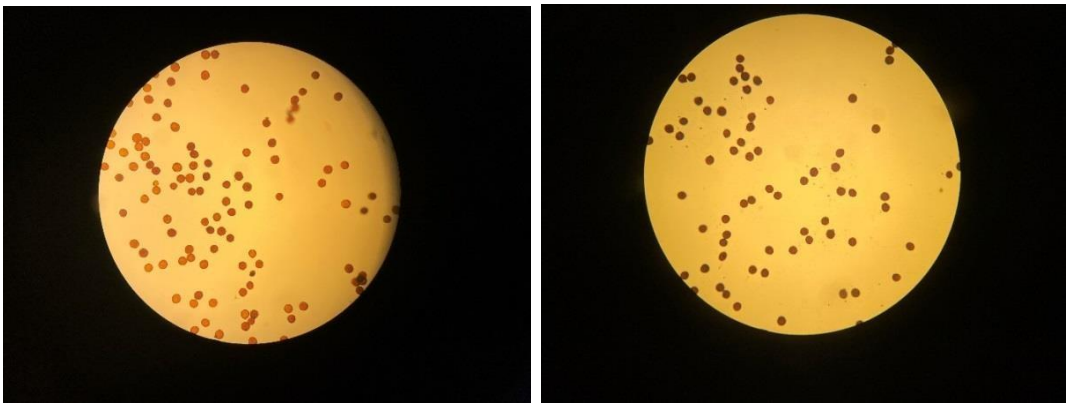
In the first part of the study, the pollen of Lonicera L. species was divided into 2 groups according to their viability:

Group I - pollen with high viability

(L. fragrantissima, L. japonica, L. tatarica, L. korolkowii)

Group II - pollen with moderate viability

(L. caucasica, L. Iberica)



a *b*

Figure 1. Pollen of L. iberica (a) and L. fragrantissima (b).

In the second part of the experiment, we studied pollen germination in pure sucrose solutions and the effect of adding agar to the nutrient medium (Romanyuk , 1987: 74). Pollen germination begins with the development of the pollen tube. Usually, germination begins with the outward growth of the pollen cell from the pores on the exine. A nutrient medium is essential for pollen grain growth. The processed protrusion becomes elongated and becomes a pollen tube (Tutayuq, 1967: 242). Germination percentage and pollen tube length were observed in 30% sucrose solution (Table 1). Pollen of *Lonicera* L. species germinates poorly in pure sucrose solutions. Comparing the germination of pollen of the above species in sucrose solutions with the addition of 1% agar-agar, it was found that the percentage of germination is higher than in the previous medium, and the pollen tubes are 2-5 times longer. The addition of boric acid to the composition of the nutrient medium has a stimulating effect on the germination of pollen and the growth of pollen tubes at a concentration of 0.0005% of boric acid. (Table 2).

Figure 2. L. tatarica. germination of the species in sucrose solution (with the addition of 1% agar-agar)

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Table 1.
Germination results of freshly collected cornflower pollen in sucrose solutions (with the addition of agar-agar (1%)).

№	Species	Optimal concentration of sucrose (%)	Pollen Germination (%)	Pollen tube length (mm)
1	*L. caucasica*	25	25	1,63
2	*L. fragrantissima.*	25	65	3,01
3	*L. japonica*	30	70	2,15
4	*L. korolkowii*	25	48	3,07
5	*L. maackii*	25	45	1,75
6	*L. tatarica*	25	75	5,15

Table 2.
Effect of boric acid on pollen germination of *Lonicera* L. species.

№	Species	Control		Boric acid		
		pollen germination (%)	pollen tube length (mm)	optimal density (%)	pollen germination (%)	pollen tube length (mm)
1	*L. caucasica*	23	1,63	0,005	27	1,75
2	*L. fragrantissima.*	65	3,01	0,005	66	3,51
3	*L. japonica*	70	2,15	0,005	73	2,75
4	*L. korolkowii*	49	3,08	0,005	59	3,58
5	*L.maackii*	45	1,75	0,005	48	1,86
6	*L. tatarica*	52	5,11	0,005	62	5,57

When the concentration of boric acid exceeds 0.01%, it inhibits the germination of pollen of *Lonicera* L. species. After 4-5 removal of Lonicera L. pollen samples from a desiccator for storage and planting at 20°C, changes in humidity have a negative effect on viability.

Conclusion

During determination of fertility and sterility of studied *Lonicera* L. species, the protoplasm of pollens with normal viability is stained dark red, while sterile pollens without viability remain unstained or take on a light yellow color.

An experiment on pollen germination and storage of *Lonicera* L. species showed that better pollen germination was observed in a nutrient medium compared to the germination of freshly collected pollen. To determine their viability, sucrose, agar-agar, and boric acid were used to increase the germination percentage. When stored for a long time, increasing the amount of boric acid and sucrose in the nutrient medium during the germination of plant pollen is appropriate. Determining the optimal concentrations of sucrose showed that the pollen of *Lonicera* L. species germinated better when the concentration of sucrose solution in the nutrient medium was 30%.

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