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BIOINDUSTRY IN SOUTH KOREA: AN ANALITICAL OVERVIEW AND MAIN PERSPECTIVES OF ITS DEVELOPMENT

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This article provides an analytical overview of South Korea's bioindustry, examining its current status, achievements, and challenges. The article covers various aspects of the economy, including innovation opportunities, government policies, economic impact, and social change. The analysis identifies key achievements, including South Korea's leadership in biopharmaceutical production and innovation, and addresses challenges such as global competition and climate change. It outlines key future development prospects, highlighting the potential of digital healthcare, personalized medicine, and green biotechnology to position South Korea as a leader in the bioeconomy. It also highlights lessons that can be learned from South Korea's experience and their applicability to other countries, including Ukraine and Poland, that are seeking to strengthen their bioeconomy and bioindustry potential. Considering the strategic approach of South Korea, this study aims to identify valuable ideas for promoting innovation, ensuring sustainable development and positioning the bioindustry as a driving force for national economic growth. The analytical analysis of this work concerns the coverage of the current state of industrial production and areas of scientific research in classical biotechnology to improve existing and develop new bioengineering technologies, which are aimed at developing the strategic importance of the bioindustry for achieving sustainable development of the country.

Aim. The article purposed to analyze the current state, key achievements, challenges, and prospects of the South Korean bioindustry, focusing on obtaining valuable knowledge and practices that can be adapted to promote the development of the bioindustry in Ukraine and Poland.

Materials and Methods. Methodological analysis and abstract-logical method of generalizing the criteria for assessing the formation, development, and integration of biotechnological production into the structure of the global output of safe products, as well as bioproducts for improving health and rejuvenation.

Results. The article describes the current state of key industries based on the Korean bioindustry classification code KS J 1009: biopharmaceutical, biochemical, and bioenergy, biofood, bioenvironmental, biomedical equipment, and bioinstrument & bioequipment, bioresource, and bioservice industries. Each sector is characterized by distinct dynamics of growth, levels of innovation implementation, and integration into national and global bioeconomic strategies.

Conclusion. The analysis highlights both the technological achievements and structural priorities shaping South Korea's modern bioindustry.

Key words: South Korea, bioindusry, bioeconomy, sustainable development, innovations, investments, standards.

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Bioindustry has become a cornerstone of modern economic development, combining advanced technologies with sustainable solutions to address global challenges in healthcare, agriculture, and environmental protection. Automation and data-driven processes are increasingly being implemented in biotechnology. They are expected to radically improve the predictability and reproducibility of research and manufacturing outcomes by 2040 through the creation of new bioengineered products, as well as groundbreaking innovations such as mRNA vaccines against cancer and CRISPR-based therapies, improvements in safe delivery systems such as lipid nanoparticles for gene editing for clinical use, etc. Today, the US is the leader in the world of biotechnology products, with a share of almost 50%. However, this depends on the specific data source and year. According to Statista, in 2021, the United States accounted for nearly 59% of the global value of biotechnology. Still, there is a reality of competition between the United States and China in the field of biotechnology. South Korea is also a major player in the global biotechnology market, with a growing presence in the biopharmaceutical sector. However, its share is much smaller than that of the United States. South Korea stands out as one of the world's leading countries in this field, leveraging its material resources, advanced technological base, strong government support, and innovation-driven economy. Over the past two decades, the country has made significant strides in the production of biopharmaceuticals, the use of bioresources and the creation of biomaterials, the investment development of bioenergy, the scaling up of model bioengineering research and pilot developments, establishing itself as a key player in the global bioeconomy, which uses biological resources to produce goods, services, and processes. South Korea has significant potential for bio-based industries, particularly in health, food, and biomaterials.

Global economic growth is expected to remain at 2.8% in 2025, unchanged from 2024. The global biotechnology market will reach approximately USD 2.18 trillion by 2025, up from USD 1.95 trillion in 2024. According to the Biotechnology Industry Outlook 2025 by StartUs Insights (https://www.startus-insights.com), the bioindustry is expected to continue to expand, potentially reaching USD 5.90 trillion by 2034, with a CAGR of 11.6% from 2025 to 2034. In this context, analyzing the structure and priorities of South Korea's bioindustry becomes particularly important, as the country represents one of the most rapidly advancing and innovation-driven markets in Asia. Understanding its strategies, investment trends, and technological focus provides valuable insights into the future trajectory of the global bioeconomy and highlights potential models for sustainable growth, international cooperation, and commercialization of biotech innovations.

Considering South Korea's strategic approach to the development and implementation of biotechnology, this study aims to examine the current state, achievements, and challenges of the bioindustry, identify valuable ideas for promoting innovation, ensuring sustainable development, and positioning the bioindustry as a driving force for national economic growth. Special attention is given to the applicability of South Korea's experience in shaping effective bioeconomic policies and fostering international competitiveness in the biotechnology sector.

Materials and Methods

The work used methodological analysis and abstract-logical method to generalize the criteria for assessing the formation, development and integration of bioengineering biotechnologies and the biotechnological process into the bioindustry with a number of transnational companies, corporations and firms for the production of bioproducts and the provision of services for the use of bioprocesses, taking into account that the main trends in the modern development of the world bioeconomy include globalization, informatization, transnationalization. To process the information, methods of searching for scientific literature, statistical analysis, systematization, comparison, and generalization of information, and processing of the obtained data were used. Analysis of own developments and their generalization regarding the assessment of the sectors of classical biotechnology and cellular and genetic engineering for the formation and development of the bioindustry in South Korea, the use of the biotechnological process in various productions of target bioproducts according to world standards in accordance with the recommendations, requirements and standards with the development of patents of the Eurasian Patent Organization, as well as the creation of a number of promising biotechnologies, the combination of which will constantly vary depending on specific practical

tasks, the development of technical conditions for their production, in particular with regard to the bioindustry of Ukraine.

Results and Discussion

In the 21st century, in the context of changes and transformations in the global economy, the bioindustry is one of the key sectors that determines the technological competitiveness of countries and contributes to their sustainable development. South Korea or the Republic of Korea (hereinafter referred to as Korea) demonstrates the development of the bioindustry, investments in biotechnology research, and active international cooperation [1]. The country's bioorientation is due to strategic state support, developed scientific and research infrastructure, a high level of technological integration, and export orientation to global markets.

The development of the bioindustry in Korea is due to both global challenges and the strategic planning of the country's government. Several stages of the formation of the bioindustry in Korea can be distinguished.

I. The initial stage (1970–1980) is characterized by the development of fermentation and fermentation technologies for the production of traditional products (soy sauce, fermented beverages, etc.) and the emergence of the first research institutes in the field of biotechnology.

II. The stage of the formation of the bioindustry (1990s), which began with the emergence of the first companies specializing in the production of biopreparations and the creation of a basic plan for the promotion of biotechnology (Biotech 2000, Bio-21) in 1994.

III. The stage of expansion and globalization (2000–2010), which is characterized by integration into the global bioindustry, increasing export potential, and the development of bioenergy.

IV. Modern stage (2010s–present). Today, the bioindustry is one of the strategic directions of economic development in Korea and is supported through national programs. It is expected that the bioindustry will solve a number of urgent problems of today, such as the phenomenon of an aging society, depletion of fossil fuel resources, outbreaks of new infectious diseases, water shortages, and global warming [2, 3].

As of 2023, Korea's bioindustry revenue was USD 25.6 billion [4], accounting for 1.7% of global bioindustry revenue. In the same year, the export value of products and services in Korea's bioindustry amounted to KRW 11.6 trillion, equivalent to USD 8.9 billion [5–7]. According to forecasts [4], by 2030, the bioindustry revenue will grow more than 3 times and will reach USD 81.6 billion. The top 10 companies of the Korean bioindustry are given in Table 1.

Samsung Biologics Co Ltd, **Celltrion Inc** are leaders of the Korean bioindustry, as well as prominent players in the global bioindustry [8, 9]. Logos of the top 10 companies of the Korean bioindustry are given on the Fig. 1.

The basis for harmonious cooperation, communication, and coordination of Korean bioindustry producers is the Korea Biotechnology Industry Organization (KoreaBIO) with its logo (Fig. 2). The organization was established on the basis of 3 associations in 2008: Bioindustry Association Korea (BAK), Korea Biotechnology Research

Fig. 1. **Logos of the top 10 Korean biotech companies**

* Author's compilation.

Fig. 2. **Logo of the Korea Biotechnology Industry Organization**

Table 1

The top 10 Korean biotech companies by market capitalization in 2024

No.	Company	Foundation year	Market cap, mln USD	Main products
1.	Samsung Biologics Co Ltd	2011	45,180.00	Development, manufacturing of antibody-drug conjugates (ADCs) and mRNA; CDMO-services.
2.	Celltrion Inc	2002	27,129.00	Biosimilars, novel biopharmaceuticals and monoclonal antibodies (MCAs); CDMO-services.
3.	Alteogen Inc	2008	10,860.00	Development and commercialization of novel biologics (long-acting biobetters) such as ADCs, biosimilars etc.
4.	Yuhan Co Ltd	1926	6,327.00	Dietary supplement, biopharmaceuticals, CDMO-services. Development of active pharmaceutical ingredients (APIs).
5.	SK Biopharmaceuticals Co Ltd	1993	6,184.00	Development of innovative pipeline in CNS, metabolic disorders (epilepsy, myotonia etc).
6.	LigaChem Bioscience Inc	2006	2,740.00	Development of novel biopharmaceuticals, production of medical devices and supplies.
7.	Sam Chun Dang Pharm Co Ltd	1943	2,486.00	Dietary supplement, biopharmaceuticals, over the counter drugs (OTC), APIs; CDMO-services.
8.	Hanmi Pharm Co Ltd	1973	2,446.00	Biopharmaceuticals, dietary supplements and CDMO-services.
9.	SK Bioscience Co Ltd	2018	2,273.00	Biopharmaceuticals, vaccines and infusion solutions.
10.	Daewoong Pharm Co Ltd	1945	1,166.00	Biologics, chemicals, OTC and medical devices; CDMO-services.

* Developed by the authors based on [8].

Association (KBRA), and Korea Bio-venture Association (KoBioVen). KoreaBIO is intended to promote technological development, industrialization, and development of the national economy according to Article 38 of the Industrial Development Act of the Ministry of Trade, Industry, and Energy [10].

The enormous potential of Korean biotech companies is determined by government support and developed infrastructure. And the sector's growth to become a competitive and innovative global force — underpinned by a dynamic economy, a highly skilled workforce, and significant government and commercial infrastructure investment — is bringing major opportunities for international companies [11].

The biotechnology classification code — KS J 1009: Classification of the bioindustry (set by the Korean agency for technology and standards (KATS) in 2008) — was developed to classify bioindustry into sectors, facilitate the collection of statistical data, and shape public policy. According to the standard, the bioindustry is divided into eight sectors:

1. biopharmaceutical industry;
2. biochemical and bioenergy industry;
3. biofood industry;
4. bioenvironmental industry;
5. biomedical equipment industry;
6. bioinstrument and bioequipment industry;
7. bioresource industry;
8. bioservice industry [2, 3, 12].

In the context of the development of the bioindustry, this standard is aimed at ensuring quality, safety, and efficiency in the production of bioproducts in various bioindustrial sectors. Among these sectors, the biopharmaceutical industry stands out for its exceptional support, as evidenced by the highest level of investment in research and development (R&D) in the bioindustry. In 2023, it amounted to about KRW 3.7 trillion (equivalent to USD 2.7 billion) [13].

Fig. 3. **Biopharmaceutical market size of South Korea in 2012–2023**
* Developed by the authors based on [19].

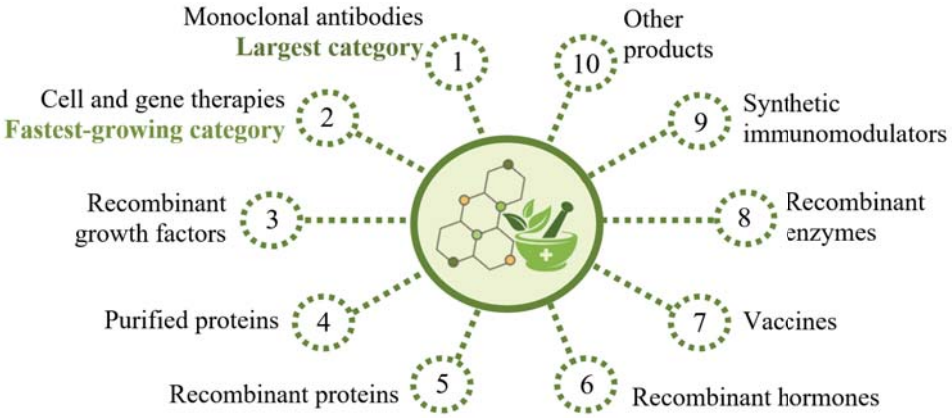

Fig. 4. **Product types of Korea's biopharmaceutical industry**
* Developed by the authors based on [20].

In 2023 Korean bioindustry had 1293 enterprises, including 328 biopharmaceutical, 261 biochemical and bioenergetic, 264 biofood, 89 bioenvironmental, 138 biomedical equipment, 60 bioinstrumental and bioequipment, 27 bioresource, 126 bioservice (including bioservices in other production sectors that integrate bioobjects and/or bioprocesses into their operations) [14]. Each of the areas is characterized by achievements and challenges that need to be overcome for the sustainable development of Korea's bioindustry [12, 14, 15].

Biopharmaceutical industry

It's focused on production and R&D of biopharmaceuticals: bioantibiotics, biologically manufactured low-molecule medicines, hormones, vaccines, therapeutic antibodies and cytokines, blood products, cell-based and gene therapeutics, biomaterial-based medicine, veterinary biopharmaceuticals, etc. The industry also includes biological diagnostic products, generics, and biosimilars.

In 2022, the Korean biopharmaceutical industry ranked 12th in the world by market capacity [16–18]. The graph (Fig. 3) shows a gradual growth since 2015. The decrease in market volumes in 2022 is associated with a reduction in demand for "COVID-19-products", increased regulation of clinical trials and genetic research, a fall in share prices of industry leaders such as Celltrion Inc. and Samsung Biologics Co. Ltd, and the global economic downturn.

There are nine dominant types of bioproducts on the Korean biopharmaceutical industry market (Fig. 4): MCAs (1), cell-based and gene therapeutics (2), recombinant growth factors (3), purified (4) and recombinant (5) proteines, recombinant hormones (6), vaccines

Fig. 5. **Korean biopharmaceutical companies**

* Author's compilation.

Fig. 6. **Logo of the Korea pharmaceutical and biopharma manufacturers association**

(7), recombinant enzymes (8), synthetic immunomodulators (9) [20].

Leading Korean biopharmaceutical companies are (Fig. 5): Samsung Biologics Co Ltd, Celltrion Inc, Alteogen Inc, Hanmi Pharmaceutical Co Ltd, SK Bioscience Co Ltd, GC Pharma Co Ltd, Chong Kun Dang Pharmaceutical Co Ltd, KoreaVaccine Co Ltd, Daewoong Pharmaceutical Co Ltd, D&D Pharmatech Inc, Boryung Biopharma Co Ltd, and Eubiologics Co Ltd [13].

The companies have their specialization and areas of leadership. Samsung Biologics Co., Ltd is a global leader in providing CDMO services due to its large-scale production capacity and technology. Celltrion Inc. is distinguished by the production of biosimilars, in particular Remsima™ — the first biosimilar of MCAs in the world, approved by the FDA. Hanmi Pharmaceutical Co., Ltd focuses on providing biopharmaceuticals for the treatment of metabolic and autoimmune disorders, infectious diseases, oncology, and rare diseases. It also produces OTCs and dietary supplements. SK Bioscience Co Ltd, KoreaVaccine Co Ltd, Boryung Biopharma Co Ltd, and Eubiologics Co Ltd specialize in making vaccines. D&D Pharmatech develops drugs for the treatment of neurological disorders, such as Alzheimer's and Parkinson's disease. GC Pharma Co., Ltd produces plasma protein therapeutics and biopharmaceuticals. Daewoong Pharmaceutical Co Ltd develops drugs for regenerative medicine and chronic diseases [10, 13].

Investments in the development of Korea's bioindustry are made by the state, private entrepreneurs, and foreign companies. The Korean government provides subsidies to support clinical trials, works on the legislative and regulatory aspects of the bioindustry, and creates a regulatory framework in accordance with current needs. In particular, in August 2019, it adopted the Act on advanced regenerative medicine and advanced biological products (ARMAB), which significantly simplifies the introduction of innovations in the bioindustry [21]. At the same time, the implementation of laws to regulate the activities of manufacturers ensures the quality of bioproducts and the introduction of innovative technologies into the industry. In 1945, the Ministry of Health and Welfare (MoHW) facilitated the creation of the Korea Pharmaceutical and biopharma manufacturers association (KPBMA) with its logo (Fig. 6). The Association represents the interests of Korean pharmaceutical companies, ensures partnerships of manufacturers with related and international organizations, and government agencies. In 2025, KPBMA includes 174 domestic pharmaceutical firms and 21 multinational companies, among which 24 are biopharmaceutical [22].

The revival of investment activity by foreign companies indicates the level of and confidence in the Korean biopharmaceutical industry. Investment agreements provide a stable income stream while reducing the risks and costs of full-scale drug development.

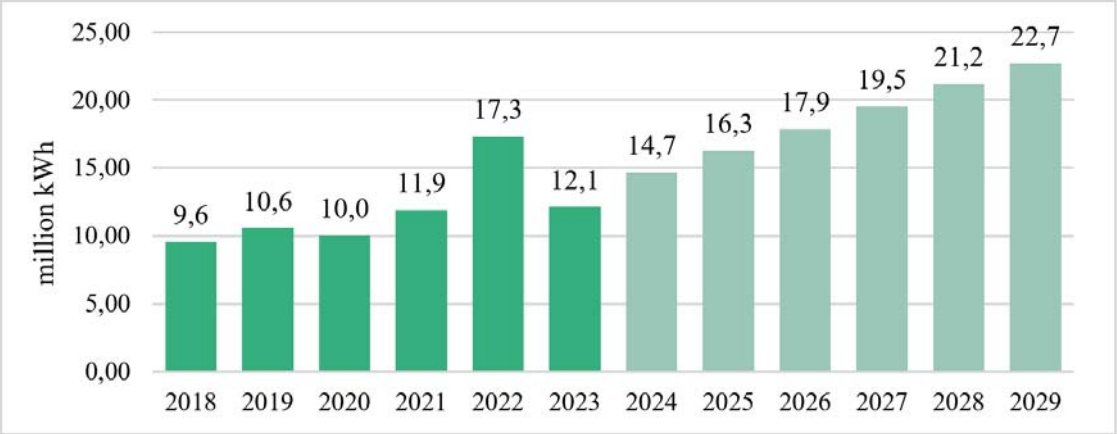

Fig. 7. **Bioenergy market size of South Korea in 2018–2023 and forecast to 2029**
* Developed by the authors based on [23].

Among the information on foreign cooperation and investment in 2025 are the following:

• Alteogen In announced a licensing agreement with UK-based MedImmune (AstraZeneca) to develop subcutaneous (SC) formulations for three of AstraZeneca's IV-administered cancer drugs.

• OliX Pharmaceuticals Inc announced a global licensing agreement with USA-based Eli Lilly to complete the 1st phase of clinical trials of a candidate drug OLX702A.

• Genome & Company signed a licensing deal last month with UK-based Ellipses Pharma for its immuno-oncology drug candidate GENA-104.

Among the results of cooperation in 2025 are the completion of the third phase of trials of the subcutaneous form of the drug Keytruda in collaboration with Alteogen Inc and Merck & Co the completion of the third phase of trials of combination therapy with the drugs Leclaza from Yuhan Co Ltd and Rybrevant from Johnson & Johnson [7].

Unfortunately, as of 2025, no examples of direct Ukrainian-Korean and Polish-Korean commercial projects or investments in the biopharmaceutical industry have been found.

Biochemical and bioenergy industry

It's focused on production and R&D of chemicals: biopolymers, enzymes, and reagents for research and industry, biocosmetics, biological agrochemicals, etc. The essential sub-sector is biofuel and bioenergy products, which form the basis of the country's energy security.

The bioenergy industry is developing rapidly. In 2024, the Korean bioenergy industry produced 14.67 billion kilowatt-hours of electricity. The market is expected to grow by 9.13% by 2029 (Fig. 7). Despite this, the volume of Korean bioenergy on the global market remains relatively modest [23]. Therefore, Korea is increasingly investing in bioenergy, which reflects its commitment to sustainable development and reducing greenhouse gas emissions in its energy sector.

The industry's main products include biopolymers, biocosmetics, and biofuels. Leading Korean biochemical and bioenergy companies are (Fig. 8): SK Chemicals Co Ltd, Orient Bio Inc, Anygen Co Ltd, Elecseed Pty Ltd, CheilJedang Bio Co Ltd, Beauty of Joseon Ltd, GS Caltex Co Korea South-East Power Co., Ltd.

Orient Bio Inc and CJ Bio Co Ltd are producers of a wide range of biochemicals and reagents. Anygen Co Ltd is a bio-venture company and specializes in the development of peptides and their biomaterials. Beauty of Joseon Ltd develops and produces different types of biocosmetics.

There is particular interest in bioenergy. It is expressed both in government support and in the desire of manufacturers to follow trends and introduce new approaches to energy production on an industrial scale. The country's government is actively promoting the development of biohydrogen production. On February 5, 2021, the Hydrogen Economy and Hydrogen Safety Management Act ("Hydrogen Act"), the world's first hydrogen law, came into force in Korea. After that, Hanwha Group, Hyosung Group, Hyundai Group, SK Group and Posco International, and other companies invested USD 38 billion to stimulate the country's hydrogen economy by 2030. The participating companies plan to increase hydrogen production and consumption many times over by 2030. In

addition to biohydrogen, entrepreneurs are introducing the production of other types of biofuels from various types of raw materials or industrial waste to support Korea's energy security. In Fig.9, bioenergy facilities in Korea in 2024 are marked.

Jungrang Water Recycling Centre is a leader in biogas production with a daily capacity of 5,112 m^3 of biogas. The leaders in the generation of electricity from municipal waste are: waste gasification demo-plant by plasma, owned by GS Platech (capacity of 50 kW, Cheongsong city); waste-to-energy plant, owned by NowOn Technologies Pvt Ltd (capacity of 281 thousand tons per year, Seoul city); landfill gas-to-energy plant, operated

Fig. 8. **Korean biochemical and bioenergy companies [10]**
* Author's compilation.

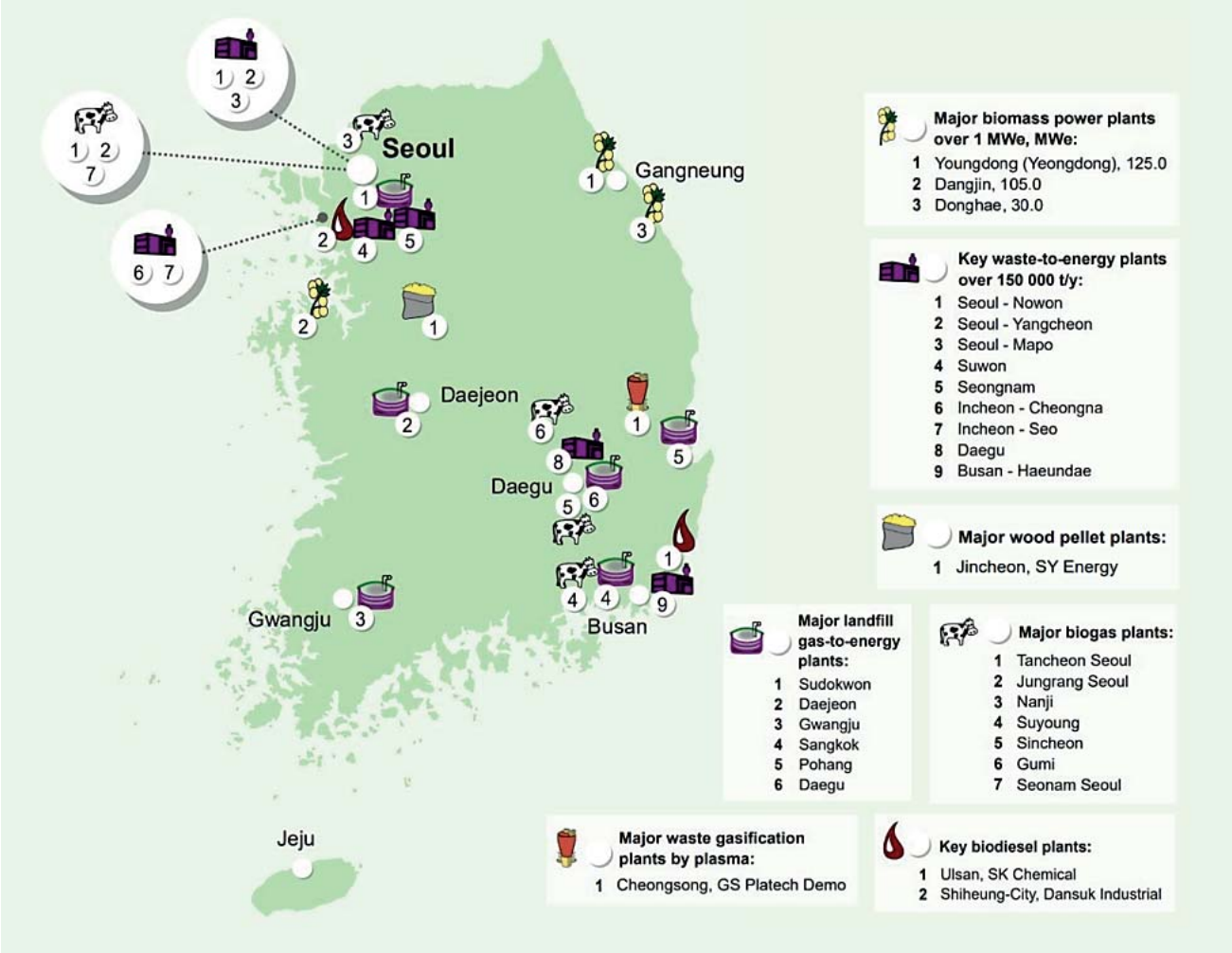

Fig. 9. **Korea's bioenergy infrastructure facilities in 2024**
* The development from website of the research project Advanced Energy Technologies [24].

Table 2

Comparison of bioenergy production and forecast of its annual growth rate in 2025

No.	Country	Bioenergy production, billion kWh	CAGR,%
1.	South Korea	16.28	8.68
2.	Poland	11.09	2.02
3.	Ukraine	0.70	1.40

* Developed by the authors based on [8].

by Sudokwon Landfill Site Management Corporation (processes about 18 thousand tons, Incheon city) and wood pellet enterprise, owned by SY Energy Co Ltd (300 thousand tons of pellets per year, Jincheon city) [24].

The leading manufacturers of biodiesel are SK Energy Co Ltd, S-Oil Co, GS Caltex Co and Hyundai Oilbank Co Ltd [24–26]. The enterprises are working on increasing production capacities and expanding export opportunities in the field of sustainable aviation fuel (SAF). This activity is correlated with a SAF expansion strategy of Korea's Ministry of Trade, industry and energy and the Ministry of land, infrastructure and transport. The aim is to capture 30% of the global blended SAF export market [26]. Among these enterprises, HD Hyundai Oilbank Co Ltd stands out for its use of the supercritical process, which involves the use of high temperatures and pressures without a catalyst. This technology expands the raw material base for biodiesel production, as it allows the use of inedible, difficult-to-process raw materials, such as palm oil from residues [25].

The above-listed production facilities are part of Korea's strategy to decarbonize the energy sector and increase the use of renewable energy sources, aimed at achieving carbon neutrality, strengthening energy security. and stimulating innovation in the field of the green economy. In the comparative context of bioenergy in Korea, Poland, and Ukraine, the former is the undisputed leader (Table 2).

The issue of energy security and further economic recovery is particularly acute and relevant for Ukraine in the context of the Russian-Ukrainian war. Due to damage to the infrastructure, the country needs reliable energy sources. Bioenergy reduces dependence on traditional fuels (coal, oil, and gas) and contributes to the decentralization of the energy system, which is critically important in the context of military operations. In the long term, bioenergy also contributes to the ecological modernization of the country, the reduction of greenhouse gas emissions, and harmonization with European environmental standards, which is an essential component of Ukraine's European integration course. Thus, the development of bioenergy is becoming not only a tool for survival in crisis conditions, but also a strategic direction for the recovery and sustainable growth of the state. Based on these factors, cooperation in the bioenergy industry between Ukraine and Korea is being intensified. In particular, in 2023, representatives of the Ministry of Energy of Ukraine and the Ministry of Land, Infrastructure and Transport of the Republic of Korea confirmed mutual interest in further cooperation in hydropower, nuclear industry, and renewable energy [27].

In 2024, a framework intergovernmental agreement was signed with Korea, according to which the Ukrainian economy will receive USD 2.1 billion in soft loans from the Korean Economic Development Cooperation Fund (EDCF). The priority area for the development of future cooperation between Ukrainian and Korean businesses is green metallurgy and the development of modern energy infrastructure. Posco International will actively participate in Ukraine's recovery projects. One of its upcoming projects is the construction of a cogeneration power plant in Odessa using RDF (refuse-derived fuel). This initiative, worth USD 106 million, will be implemented in cooperation with EDCF [28]. The payback period of the project is estimated at 6.5 years.

By the agreement, there is an opportunity to involve The Fund's finances in state initiatives and recovery projects from The Unified Project Portfolio. Under favorable conditions, the scope of this agreement will expand to the private sector, the number of projects will increase, and the size of investments will increase.

Korea is the largest Asian investor in Poland. According to the National Bank of Poland, in 2018, the value of South Korean investments amounted to EUR 978.8 billion. However, as of 2025, no examples of direct Polish-Korean commercial projects or

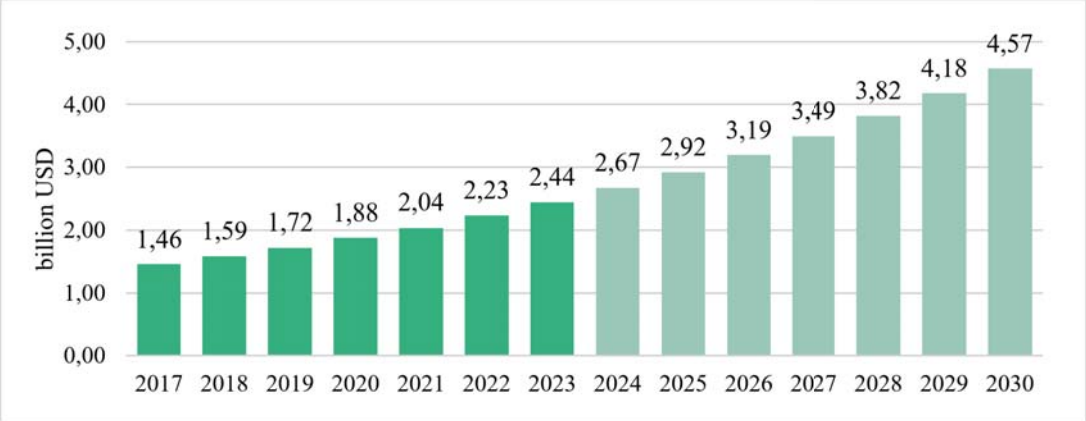

Fig. 10. **Functional health foods market size of South Korea in 2017–2023 and forecast to 2030**
* Developed by the authors based on [31].

Fig. 11. **Korean biofood companies**
* Author's compilation.

investments in the biochemical and bioenergy industry have been found. Given Korea's interest in building a hydrogen economy and the potential of Poland and Ukraine in bioenergy, future cooperation between the states and the exchange of technologies will contribute to their sustainable development and decarbonization of economies for environmental safety. Thus, Poland and Ukraine have the potential for leadership in European bioenergy, provided that regulatory barriers are overcome and investment is actively attracted [29].

Biofood industry

It's focused on production and R&D of functional health foods (e.g., eubiotics, nutraceuticals), fermented foods, food additives (dietary supplements), feed additives, and other biofoods. The industry receives active support from the government, stimulating exports and global popularization of Korean cuisine [30].

Priority is given to creating functional health foods, alternative protein sources (vegetable), as well as environmental technologies in production and packaging. In 2023 Korean functional health foods market reached a revenue of KRW 3.19 trillion or USD 2.44 billion (Fig. 10). The expected CAGR to 2030 for this branch is 9.4%, which is one of the most significant indicators in the world.

Leading Korean biofood companies are (Fig. 11): CJ CheilJedang Co Ltd, Daesang Co Ltd, SeaWith Inc, Haerim Fucoidan Ltd, Greengene Inc, Simple planet Inc, Bereum Co Ltd, Advanced Protein Technologies Co [10].

CJ CheilJedang Co Ltd produces amino acids for humans and animals and functional foods with probiotics, enzymes, fiber, and other bioactive components (including probiotics and postbiotics under its brands BiomeNrich™ and WellNrich™). It works in the direction of molecular cuisine, experimenting with cooking processes at the molecular level, enzymes, stabilizers, and emulsifiers of biological origin. The company's brand Bibigo™ popularizes Korean dishes such as mandu, kimchi, etc.

Functional foods are also produced by Simple Pplanet Inc and probiotics and postbiotics by Bereum Co Ltd.

Daesang Co Ltd specializes in the production of fermented foods, food additives, and amino acids. It is widely known for its fermented products such as miso and soy sauce.

Haerim Fucoidan Ltd focuses on products containing seaweed fucoidan, which is used in pharmaceuticals, dietary supplements, and cosmetics.

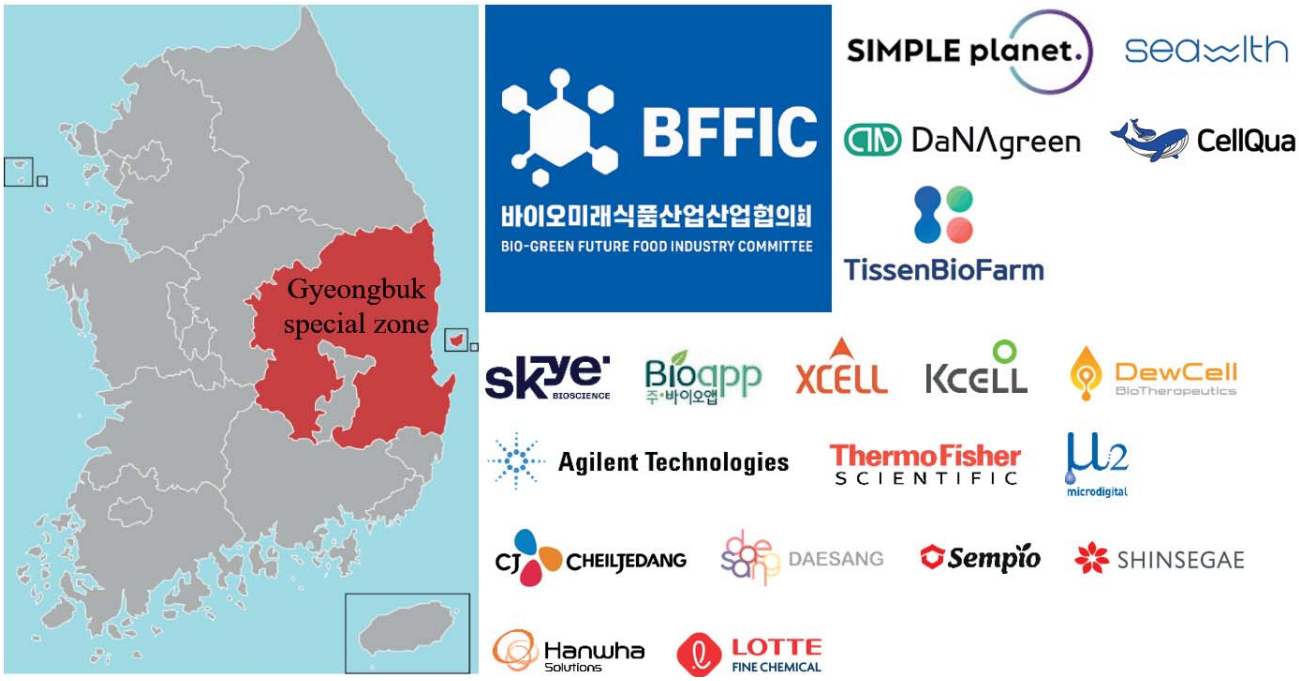

Fig. 12. **Gyeongbuk special zone on the map. Logo of Bio Future Food Industry Association and logos of its members**

* Developed and compilated by the authors based on [36].

SeaWith Inc specializes in the production of cultured meat under the brand name Welldone™. The company aims to create a sustainable alternative to traditional livestock farming, reducing its environmental impact and ensuring ethical protein production.

Greengene Inc. is working on a chloroplast gene editing technology that improves the absorption of carbon dioxide during photosynthesis. The method helps to increase the nutritional value of products (GREENedit™) by increasing the synthesized plant protein.

The innovativeness and multi-vector nature of the developments of the enterprises mentioned above indicate their desire for sustainable development of the state, overcoming the global food crisis, energy challenges, and environmental problems [30]. Support for innovative solutions in industry is provided by scientific institutions, organizations, associations (scientific and technological base), and the government (regulatory support). Thus, in 2018, the Bio-Green 21 Project was founded in cooperation with academies, research centers, industry, and the Rural Development Administration. The goal of the project is to ensure the global competitiveness of Korean agrobiotechnologies by creating an infrastructure for research and its use for the development of new technologies [32, 33].

In 2024, the Bio Future Food Industry Association (BFFIC) was established to support and develop the cultivated meat market. The association consists of 33 companies, including biofood industry innovators (Simple Planet Inc, SeaWith Inc, TissenBioFarm Co Ltd, CellQua Inc), well-known food conglomerates (CJ CheilJedang Group, Daesang Group), nutrient media developers, production process developers, etc. To facilitate the BFFIA initiative, the Korean government has designated the Gyeongbuk cell-cultured foods regulatory-free special zone (RFSZ) (Fig. 12), free from legal obstacles and intended for the creation of industrial facilities for the production of cultured meat.

In the first phase of the project, companies will create banks of highly purified animal cells and establish quality and safety standards for these products. The second phase includes a demonstration of mass production and commercialization using 3D scaffolding and molecular cuisine technologies to improve the taste and texture of the final product.

The Gyeongbuk special zone is designed to accelerate the commercialization of cultured meat and will expire in 2028. During the project, the government will work on a regulatory framework to remove legal obstacles to developers and establish standards for such products [34–35].

Korea is witnessing the dynamic development of new areas of the biofood industry, in particular in the field of functional nutrition, molecular gastronomy, catering, fusion cuisine, and smart farming. Some of the products produced within these segments are already presented on the markets of Ukraine and Poland, which indicates a growing interest in this area. Both countries show a high interest in the development of biotechnology in the agro-industrial complex, in particular bioingredients, dietary supplements, and innovative food solutions. Ukrainian and Polish manufacturers express their readiness to cooperate with Korean companies, in particular by supplying specialized products for the needs of the innovative gastronomy segment, which meets modern global trends in sustainable nutrition.

In particular, on 2023 July 15, as part of the visit of the Korean President to Ukraine, issues of security and economic cooperation between the countries were discussed [37]. Among the promising areas of cooperation in the bioindustry, the parties are considering the introduction of nnovative farming and organic farming into the agro-industry and the development of environmentally friendly technologies for building a modern city and its infrastructure, which concerns the next sector of the bioindustry [38–40].

Regarding the raw material base for the bioindustry, an important initiative is the use of new methods of classical biotechnology and bioengineering in the agro-industry.

Bioenvironmental industry

It's focused on production and R&D of bioenvironmental products and services: biological treatment agents and systems for treatment and recycle, materials and equipment for bioimmobilization, measuring apparatus and services for environmental pollution and assessment. Key areas include: biological wastewater treatment, soil bioremediation, organic waste processing, air biofiltration, green biotechnologies in the urban environment (e.g., phytoremediation, vertical farms, ecological facades with moss and microalgae), etc.

According to the analyzed sources of information, the most significant growth, development, and investments are biological wastewater treatment (CAGR = 7.2%), bioremediation (CAGR = 12.1%), and vertical farming (CAGR = 21.0%) (Fig. 13) [41–44].

Modern biotechnological solutions, in particular biological wastewater treatment, bioremediation, and vertical farming, play a key role in ensuring environmental safety, increasing economic efficiency, and contributing to the sustainable development of the state through the rational use of resources and minimizing environmental impact.

Biological wastewater treatment is an essential phase for reducing anthropogenic pressure on the environment, as it ensures safe disposal and reuse of water resources. Among the methods of biological wastewater treatment, aerobic methods dominate, providing 53% of the industry's revenue in 2024. Interest in these methods is confirmed by scientific works, research, and development of Korean scientists [42, 45, 46]. Anaerobic and anoxic methods are less common [42].

In turn, bioremediation is aimed at deeper removal of contaminants in soils and water, which complements the ecological restoration system. In 2021, in situ, soil bioremediation provided 56.36% of the industry's revenue [43]. Toxic compounds in soil and groundwater pose a threat to human health and nature. In particular, an accidental oil spill is a significant environmental disaster, which disrupts the complex networks of biotic and abiotic interactions of ecosystems. Bioremediation, as a set of bioprocesses and interactions between microorganisms, is a soft and cost-effective approach to removing organic pollutants [47].

The final link in the ecological coexistence of nature and industry is the use of green biotechnology in the urban environment, in particular, vertical farming. It allows for the effective use of purified resources, ensuring food security for society, and creating a closed, environmentally friendly cycle for sustainable agricultural production in urbanized conditions.

Leading Korean bioenvironmental companies are (Fig. 14): EcoBio Holdings Co Ltd, KERT Korea Environmental Restoration Technology Co Ltd, NeoEcogene Co Ltd, N.Thing Inc, Farm8 Co Ltd [48].

EcoBio Holdings Co Ltd is engaged in the business of waste recycling. At the same time, KERT Co Ltd specializes in soil purification, which can treat oil pollution, heavy metals, and complex contaminated soil.

N.Thing Inc and Farm8 Co Ltd specializes in vertical farming.

In the post-war period, the development of the bioenvironmental industry in

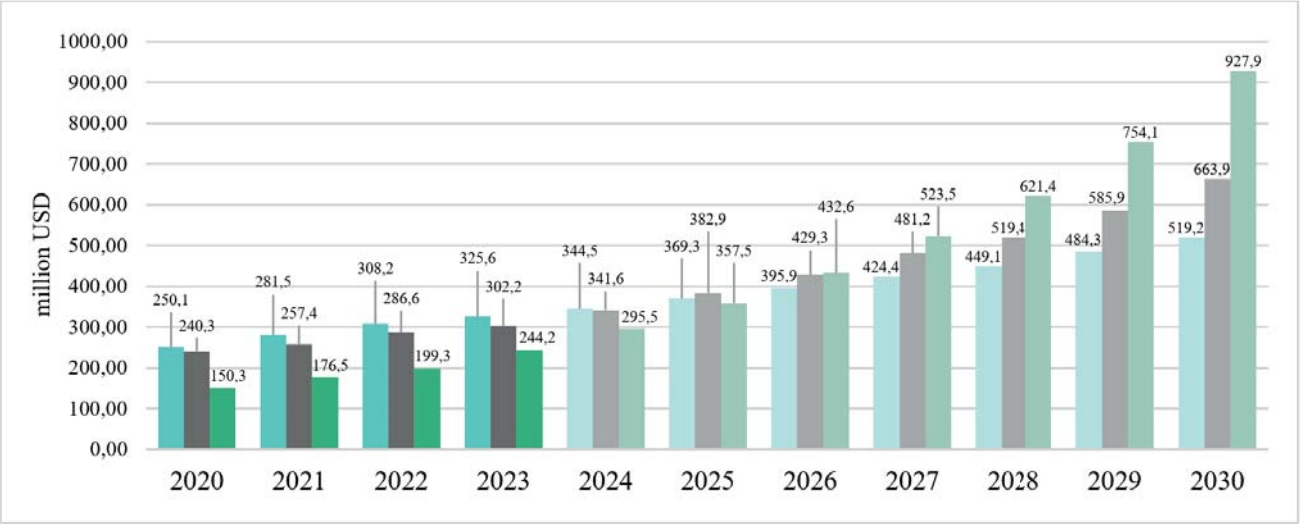

Fig. 13. **Aspects of bioenvironmental industry: biological wastewater treatment ( ), bioremediation ( ) and vertical farming ( ) market size of South Korea in 2020–2023 and forecast to 2030**
* Analised, developed, and compilated by the authors based on [41–44].

Fig. 14. **Korean bioenvironmental companies**
*** Author's compilation.**

Ukraine will be of utmost importance for ecological restoration, food security, energy independence, and sustainable economic growth. Large-scale destruction of the natural environment, soil degradation, pollution of water resources, and the atmosphere as a result of hostilities will require the use of biotechnological solutions for environmental remediation (cleaning) and restoration of ecosystems. One of the key areas will be biological wastewater treatment and bioremediation — technologies that allow using safe biological methods to eliminate soil, water, and air pollution. Vertical farming will ensure local, environmentally friendly food production with minimal use of land resources, which is especially relevant in conditions of destroyed infrastructure and potentially dangerous zones.

Other promising areas include:

- Composting and reuse of organic waste to create fertilizers and biogas;
- Phytoremediation — the use of plants to clean the environment;
- Production of biopolymers and biodegradable materials from renewable raw materials;
- Creation of energy-efficient ecological buildings using bioinsulation materials;
- Agroecology and organic farming — to restore degraded soils and reduce chemical loads.

The development of these areas will allow Ukraine not only to restore its ecological potential but also to become a regional leader in the field of green bioeconomy.

South Korean companies are interested in participating in projects for the development of a smart city and infrastructure in Ukraine. One of such potential projects is the transformation of Truhaniv Island in Kyiv into an environmentally friendly innovation park with research laboratories and residential real estate. But so far this is just an idea [38].

Biomedical equipment industry

It's focused on production and R&D of biomedical equipment, biosensors, biomarkers, and *in vitro* diagnostics. Biomedical devices are designed for therapeutic and diagnostic purposes and cover a wide range of technologies: from advanced imaging systems and surgical instruments, implantable devices (pacemakers and insulin pumps), to biometric

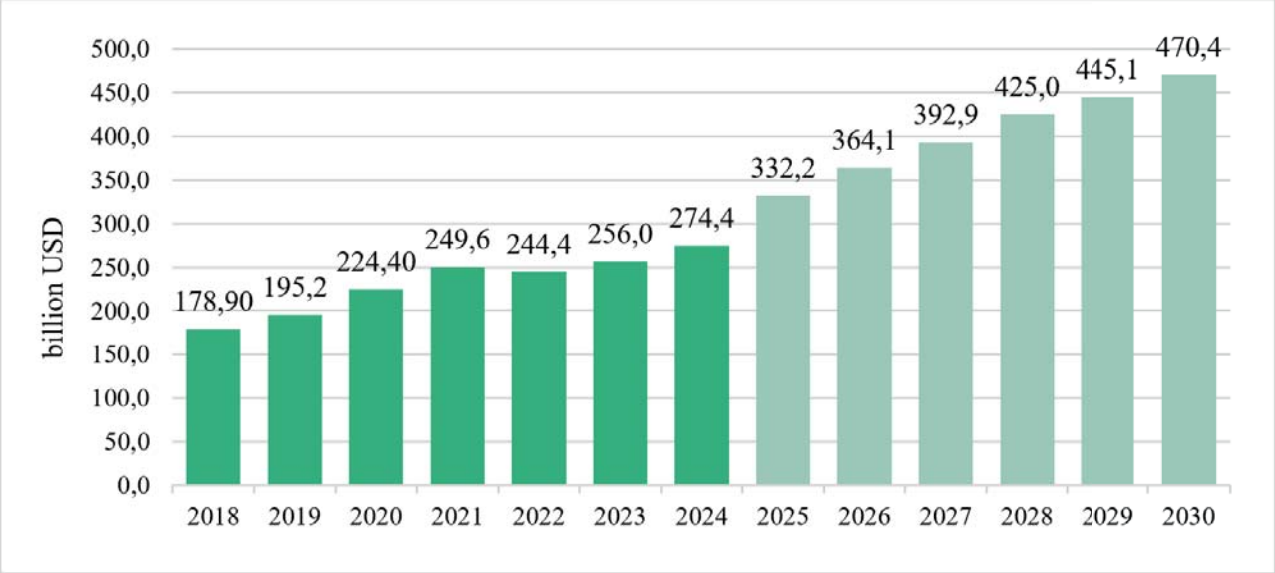

Fig. 15. **Biosensors market size of South Korea in 2018–2022 and forecast to 2030**
* Developed by the authors based on [49].

Fig. 16. **Korean companies that provide biomedical equipment**
* Author's compilation.

sensors and biosensors for continuous health monitoring.

Since rapid diagnostics play a vital rolerole in preventing the spread of infectious diseases (such as the coronavirus disease caused by SARS-CoV-2), the biosensor medical device industry has excellent investment potential. The growth of the sector is also due to rapid urbanization, technological progress, and increasing disposable incomes, especially in developing economies. In 2024, the Korean biosensors market generated a revenue of USD 274.4 million. It is expected that by 2030 its CAGR will be 9.6% (Fig. 15) [49].

Korea's biosensor market, as a leader in the electronics industry, shows significant growth potential due to technological advancements, growing consumer demand, and changing regulatory frameworks. As the market matures, product innovation and digital transformation are expected to drive its expansion. Growing interest in sustainable and environmentally friendly solutions will drive product demand. Sales are expected to shift towards high-quality, premium products, contributing to rising disposable incomes and the dominance of quality over quantity. Government initiatives that promote industrial modernization and international trade partnerships will further expand growth opportunities.

Among the leading Korean biomedical equipment manufacturers (Fig. 16): MiCo BioMed Co Ltd, GeneMatrix Inc, KogeneBiotech Co Ltd, Genomictree Inc, Huinno Inc, Dx&Vx Co Ltd, Calth Inc, Inogenix Inc.

MiCo BioMed Co Ltd, GeneMatrix Inc, KogeneBiotech Co Ltd, Calth Inc, and Inogenix Inc. specialize in the development and production of molecular diagnostics.

Genomictree Inc focuses on the development of innovative *in vitro* molecular diagnostics technologies. The company is working on the discovery of DNA methylation biomarkers for early diagnosis of diseases such as colorectal cancer.

Huinno Inc develops biodevices for monitoring cardiac activity, such as smart watches and patches, equipped with artificial intelligence technology for analyzing biometric signals

Dx&Vx Co Ltd offers multiomics solutions for personalized medicine, including the development of vaccines and strains for treatment.

The Ukrainian biosensor market is in a stage of active development, focused primarily on scientific research and prototyping. The country has already developed dozens of effective biosensors suitable for use in various fields, including medical diagnostics, agro-industry, and environmental monitoring. At the same time, the widespread implementation of such technologies is hampered by limited funding, lack of production capacity, and complex licensing procedures.

Among the leading companies working in this area, BIOsens stands out — the developer of a mobile analyzer for the rapid detection of mycotoxins in grain crops. This solution has significant practical significance for farms and contributes to increasing food security. The BIONANOSENS project, dedicated to analytical biotechnology and innovative management, was also an important initiative. Despite the difficult conditions caused by the pandemic and war, its implementation confirmed the high potential of Ukrainian science in the field of biosensors.

Thus, although the development of the market requires overcoming a number of challenges, Ukraine has significant prerequisites for becoming an essential player in the field of biosensor technologies. With state support and investment, scientific developments can turn into effective commercial solutions with great applied value for healthcare, the agricultural sector, and the environment.

Bioinstrument and bioequipment industry

It's focused on production and R&D of equipment for analysis, synthesis, and manufacturing of genes, proteins, peptides, cell cultures, and other bioproducts and bioprocesses.

Bioservice industry

It's focused on providing R&D and/or manufacturing services, processing treatment & warehousing services, biodiagnostic services, and consulting cooperation. Bioservices in Korea is a key component that supports research, development, and innovation in the biotechnology sector. This industry focuses on providing specialized services and technologies necessary for the successful development of biotechnology, pharmaceuticals, medicine, agro-industry, and other related fields. Due to the wide range of bioservices that bioindustry enterprises can provide, we propose to conditionally divide the types of bioservices into two categories: production-related bioservices and intellectual bioservices or practical and intellectual support.

Production-related bioservices, as the name suggests, include services related to the development, research, analysis, quality control, and production of bioproducts.

Practical support includes:

– Contract research organization services (CROs), which provide processes for the development and research of bioproducts, improving their quality, optimizing their production technology, etc. CROs can also consist of the study of laboratory and toxicological properties of the product and the use of genetic and cellular engineering to modify bioproducers and bioprocesses:

– Contract manufacturing organization services (CMOs), which provide processes for the mass production of bioproducts to order: from the preparation of raw materials to the filling and packaging of the finished product, as well as its quality control (molecular diagnostics, biosensor analysis, physicochemical and microbiological expertise, certification according to GMP/GLP/ISO);

– A separate category can be distinguished, contract development and manufacturing organization services (CDMOs), which provide the entire cycle of commercialization of bioproducts from development and research to mass production;

– Contractual provision of auxiliary production processes (CP — biopurification of water, industrial effluents, air zones, and territories, bioremediation, etc.), supply of consumables, reagents, and equipment for scientific institutions and companies.

Intellectual support includes:

– biodata processing, genetic analysis services (study of hereditary diseases, personalized treatment, disease screening, use of CRISPR-Cas9, for modification of genetic material, as well as use of next-generation sequencing (NGS) technologies for genome analysis), development of algorithms for bioanalytics and diagnostics, IT solutions for the bioindustry (electronic laboratory journals, management systems, etc.);

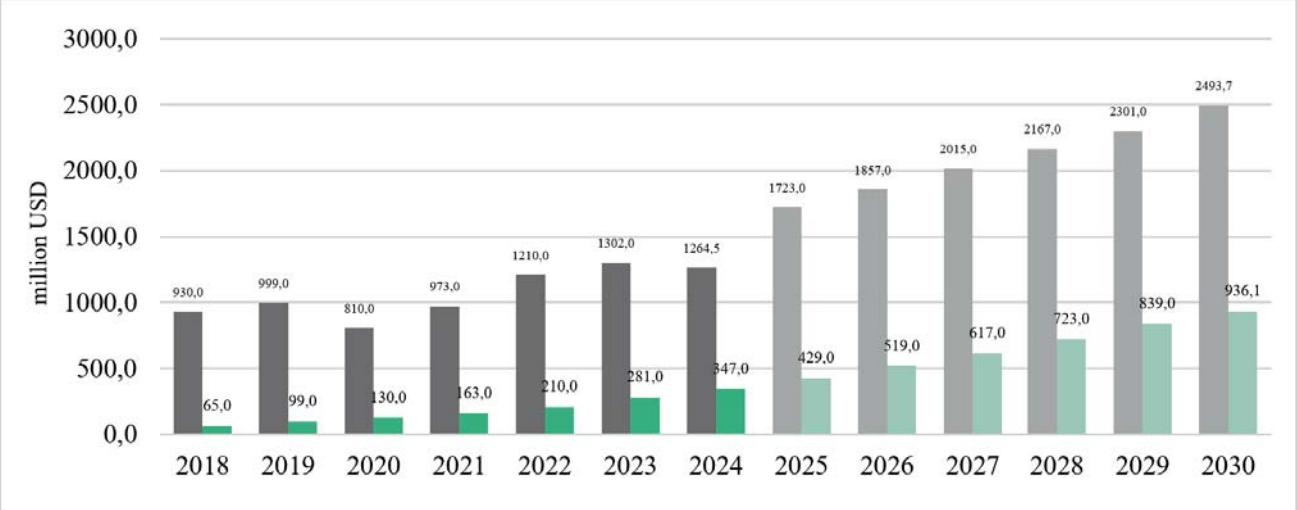

Fig. 17. **Biopharmaceuticals contract manufacturing market size ( ) and biosimilars contract manufacturing market size ( ) of South Korea in 2018–2023 and forecast to 2030**

* Analised, developed, and compiled by the authors based on https://surli.cc/kthelk.

Fig. 18. **Korean bioservice companies**

* Author's compilation.

– bioproject consulting, business planning, and support of startups, and training, training of qualified personnel.

The development of the bioservice industry is evidenced by the dynamics of the growth of the contract manufacturing of bioproducts. In particular, in 2024, contract manufacturing of biosimilars more than doubled in the period 2018–2021, and is projected to grow 9 times by 2030 (Fig. 17). Rapid growth will also be observed in the larger market of contract manufacturing of biopharmaceuticals by 2030.

Among the leading Korean companies in the bioservices industry are (Fig. 18): Samsung Biologics Co Ltd, Celltrion Inc, Clinomics Inc, Woojung Bio Inc, SpMed Co Ltd, Binex Co Ltd, and Invites Biocore Co Ltd.

Strict adherence to international standards such as GMP, ISO, and GLP (Good Laboratory Practice) ensures high-quality services. Korea is one of the world's leading bioservices centers, providing services to companies in the US, EU, and other regions. Bioservices ensure the effective implementation of innovations in the bioindustry and medicine, and contribute to the rapid development of scientific research, the commercialization of innovations, and increasing the country's global competitiveness. Thanks to infrastructure, government support, and highly qualified specialists, Korea is consolidating its position as a worldwide leader in bioservices [50]. In particular, the development of the bioservice industry is supported by the research base of scientific institutions. Over the past decade, Korea has become a global clinical center. For example, in 2017, 213 clinical trials of biopharmaceutical products (Fig. 19) were approved [8, 16].

Innovation. Korea has a very high level of academic achievement. The country ranked 6th out of 133 countries surveyed in the 2024 Global Innovation Index. The country ranks 4th in the world in terms of the number of patents granted to Korean citizens both domestically and abroad, behind Japan and China in the region. The country also has a higher number of active patents than the world average, indicating a favorable environment for innovation in the country [24].

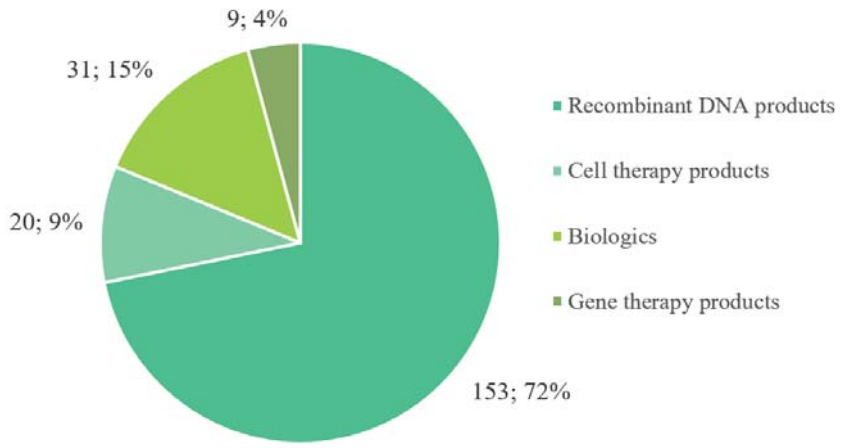

Fig. 19. **Number of approvals for clinical trials of biopharmaceuticals in 2017**
* Developed by the authors based on [16].

Among the research institutions, organizations, and associations, the following should be mentioned:

1. The Korea Drug Research Association (KDRA) was founded in 1986 as a non-profit organization. KDRA represents innovative Korean companies focused on research and development. The association supports the development of new, improved drugs by promoting information exchange and outsourcing [51].

2. The Korea Pharmaceutical and Bio-Pharma Manufacturers Association (KPBMA) has played a key role in the development of the pharmaceutical industry in Korea since its establishment in 1945. KPMA's objectives are to develop new drugs through R&D and supply of pharmaceutical products [18].

3. The Korea Research Institute of Bioscience & Biotechnology (KRIBB) is a leading research center in Korea that conducts basic and applied research in bioscience and biotechnology. It was founded in 1985 in Daejeon. The institute operates under the auspices of the Korea Institute of Science and Technology (KIST), is funded by the Korean government, and specializes in research on new biomaterials, development of environmentally friendly biotechnology, new energy sources, and biofood innovations [52].

4. Korea Research Institute of Chemical Technology (KRICT) is a leading state-owned research institute in South Korea, which is engaged in the development of advanced chemical technologies for industry, ecology and medicine. Founded in 1976, Daejeon. Key research areas: green chemistry, new generation chemical materials, pharmaceutical, and medicinal chemistry [53].

5. Korea Institute of Oriental Medicine (KIOM) is a leading research institution that researches and develops traditional oriental medicine, in particular Korean (Hanbang). The institute was founded in 1994, Daejeon. Key projects concern acupuncture, cerebrovascular diseases, and complicated diabetic diseases [54].

6. The National Cancer Center of Korea (NCC) is Korea's leading medical institution specializing in cancer prevention, treatment, research, and education. The center plays a key role in the national cancer control strategy and in implementing advanced technologies to improve cancer treatment [55].

7. The Mogam Biotechnology Research Institute (MBRI) is the first government-approved non-profit research organization established in 1984. It specializes in the development of innovative biologics. The institute plays a key role in the development of Korea's pharmaceutical industry by contributing to the creation of new drugs and therapies [56].

The logos of the leading research infrastructure organizations are shown in Fig. 20.

Technoparks and infrastructure

The Korean government is developing the bioindustry by promoting and supporting **bioclusters**, taking into account their regional characteristics. To encourage the development of a regional innovation system and ensure balanced national growth, a biocluster system was introduced in 1998, establishing bioindustry complexes in each region. There are 18 bioclusters (Fig. 21) according to the "Third master plan for

Fig. 20. **Logos of the leading research infrastructure organizations in South Korea**
* Author's compilation.

fostering and supporting pharmaceutical and bio industries", jointly announced by Korean government ministries on March 24, 2023 [57–59]. The main of them are:

– Biomedical R&D Cluster (Seoul City);
– Bio Cluster (Incheon City);
– High-tech Medical Complex (Chungcheongbuk Province);
– Bio Venture Town (Daejeon City);
– Biomedical Cluster (Hwasun County);
– Natural Product and Medical Instrument Cluster (Gangwon Province);
– Gyeonggi Bio-center (Gyeonggi Province);
– Pangyo Techno Valley (Gyeonggi Province);
– High-tech Medical Complex (Daegu City) [59].

The bioindustry in Korea is regulated by several key government agencies that ensure control, safety, and development of the industry. At the same time, the government is trying to support the development of the bioindustry as much as possible. Ministry of Food and Drug Safety Ministry of Food and Drug Safety (MFDS) is the main regulatory body for the bioindustry and its products. The ministry sets standards for production and testing; it monitors the long-term results of the use of innovative therapies. Under its jurisdiction is the control of vaccines, drugs, genes, and cell therapy products. Each regional center has its own Food and Drug Safety (FDS) units or offices that are engaged in the regulation and approval of products, including medicines, herbs, and other similar products in South Korea. To control the quality and safety of bioindustry production, the experience of the FDA and the units that continue the FDA's control function at the national level for entering the international market are taken into account [57].

Ministry of Science and ICT (MSICT) promotes research and development in the fields of genetic engineering, bioinformatics, artificial intelligence in medicine, and the development of bioenergy. The ministry funds research projects and promotes international cooperation in this area.

The Ministry of Health and Welfare (MoHW) focuses on the implementation of biotechnology in the healthcare system. It develops the legal framework for the integration of regenerative medicine into medical practice and monitors its impact on public health.

There are also specialized regional development agencies in various regions of Korea that promote clustering of companies and research centers, creating conditions for the growth of the bioindustry at the local level. The coordinated work of all these institutions ensures effective regulation and active development of the bioindustry [57].

All production scientific state institutions affect the harmonious, sustainable development of the state.

Based on the experience of the Korean bioindustry, we can identify the "three pillars" for the sustainable development of the bioindustry:

– research institutions, organizations, associations;
– industrial associations of entrepreneurs;
– government support for innovative solutions and response to trends and developments.

To strengthen cooperation between representatives of the Ukrainian bioindustry and foreign colleagues, it is necessary to develop a clear strategy for its development. The key provisions of the Strategy should include:

1. Initiating government dialogue. Launch an intergovernmental platform for dialogue in the field of biotechnology (at the level of the ministries of economy, health, and science). Include bioindustry issues in bilateral economic forums.

2. Supporting scientific exchange. Promote academic partnership between the Ukrainian NAS, Ukrainian universities (e.g., KNU, NUFood Technologies, Lviv Polytechnic NU), and Korean universities (e.g., KAIST, POSTECH). Create scholarships/grants for internships and joint research in biotechnology, pharmaceuticals, and agrobio.

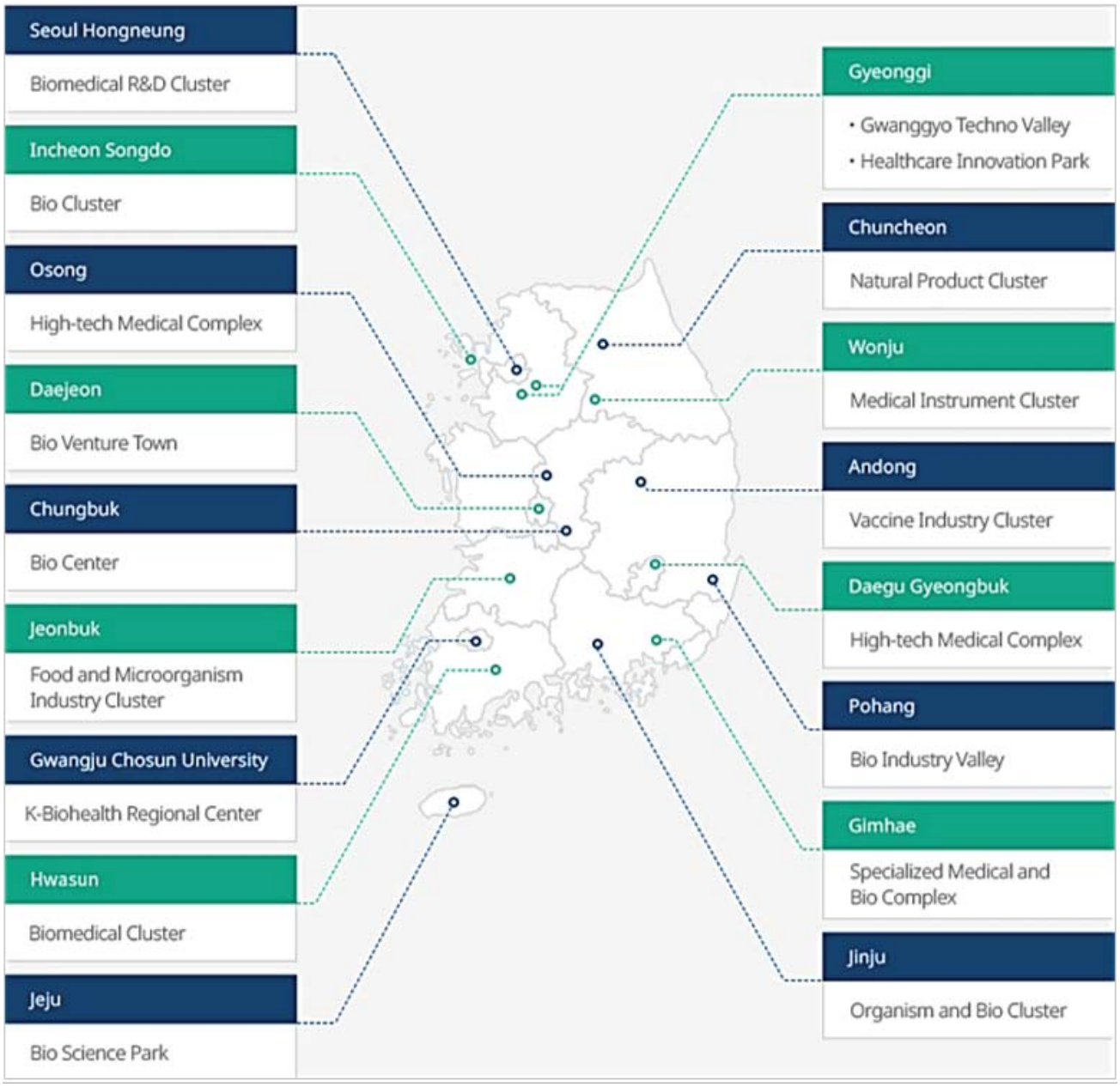

Fig. 21. **Bioclusters in South Korea**
* The development from Invest KOREA platform website [59].

3. Attracting investors. Organize roadshows for Korean investors in Kyiv/Seoul with presentations of Ukrainian R&D startups and the potential of pharmaceutical production. Offer preferential conditions for the creation of production clusters in medical or agrobiotechnologies.

4. Develop pilot projects (e.g., DS or vaccines) to reduce risks and demonstrate yourself as a reliable partner.

Conclusions

Korea's bio-industry is rapidly developing, becoming one of the key sectors of the country's economy. Thanks to government support, innovative technologies, and a favorable business climate, Korea has established itself as a global leader in biotechnology, biopharmaceuticals, and functional foods. Government initiatives such as the Bio-21 strategy and Bio-vision have become the basis for the growth of the industry. Through funding, the creation of bioclusters, and legislative reforms, the Korean government

is stimulating the development of both large corporations and small startups.

Key achievements of the Korean bioindustry include:

1) Development of biopharmaceuticals. Korea is a leading player in the production of biologics such as monoclonal antibodies (MABs), vaccines, and other drugs. Leading Korean companies such as Samsung Biologics Co., Ltd, and Celltrion Inc. occupy an essential place in the global market.

2) Functional foods. Thanks to advanced technologies, Korean companies are creating products that not only meet nutritional needs but also contribute to improving health.

3) Innovation in manufacturing. The development of cultured meat, bioplastics, and animal protein substitutes demonstrates an environmentally friendly approach to product manufacturing.

The role of the bioindustry for the sustainable development of Korea is decisive, as it contributes to the achievement of environmental, economic, and social goals of sustainable development:

– environmental sustainability, which involves the development of bioenergy and the production of biomaterials. By 2050, the country plans to transition to carbon neutrality and reduce dependence on fossil fuels through the use of biofuels (bioethanol, biodiesel) and biogas;

– economic development, which is a result of supporting biotechnology startups and scientific research through government grants and investments;

– social initiatives, such as supporting food security and environmental policies.

The bioindustry is the foundation of the country's transition to a green economy. The government has identified biotechnology as one of its national development priorities, seeing it as a tool for diversifying the economy, creating jobs, and ensuring technological leadership in the global market. Overall, the bio-industry has become a driver of Korea's economic growth, contributing to the transition from traditional industry to a high-tech knowledge economy. It is also an essential factor in strengthening the country's international image as a center of innovation and a leader in the field of sustainable development.

The dynamic growth of the food bioindustry in South Korea, driven by innovations in functional foods and sustainable production technologies, has created favorable conditions for the expansion of adjacent sectors. One of the most rapidly developing areas within this framework is the dietary supplement industry, which leverages biotechnological advancements to meet growing consumer demand for health-promoting products. Building upon the foundations of food biotechnology, the production of nutritional supplements in South Korea integrates scientific research, natural ingredients, and stringent quality control to offer targeted solutions for various physiological needs and lifestyle challenges.

Given the increasing relevance of this sector, a more detailed analysis of South Korea's dietary supplement industry is considered a promising direction for further research. It will be addressed in a subsequent publication.

Author contributions

A.D.: conceptualization, study design, data collection, drafting the manuscript, and original draft preparation. O.S.: critical revision, manuscript editing, translation editing, and language polishing. T.K.: language polishing, supervision, reviewing, and final approval of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Lee, D-H. (2015). Bio-based economies in Asia: Economic analysis of development of bio-based industry in China, India, Japan, Korea, Malaysia and Taiwan. *International journal of hydrogen energy*, 41(7), 4333–4346. https://doi.org/10.1016/j.ijhydene.2015.10.048.
2. Kim, M. J. (2021). Locational determinants of biotechnology firms in Seoul metropolitan area [Submitting a master's thesis of Engineering, SNU]. S-Space: Seoul National University Institutional Repository. URL: https://s-space.snu.ac.kr/handle/10371/178115.
3. Lee, J. H., Kim, J. W., Hyeon, B-H. (2019). Creation of system dynamics in an uncertain and complex market: the case of Korea's evolving biopharmaceutical industry. *Asian journal of innovat. & policy*, 8(2), 180–207. https://doi.org/10.7545/ajip.2019.8.2.180.

4. Horizon Databook: website. (2023). South Korea biotechnology market size & outlook, 2023-2030. URL: https://www.grandviewresearch.com/horizon/outlook/biotechnology-market/south-korea
5. Statista Market Insights: website. (2023). Export volume of the biotechnology industry from South Korea from 2016 to 2023. URL: https://www.statista.com/statistics/694041/south-korea-production-biotechnology-industry-exports/.
6. Federal reserve bank of St. Louis: website. (2025). South Korean won to U.S. dollar spot exchange rate. URL: https://fred.stlouisfed.org/series/AEXKOUS.
7. Park, J., Park, S. (2025). S. Korea's biotech sector kicks off 2025 with multi-billion-dollar deals. *The Chosun Daily*: online source. URL: https://www.chosun.com/english/industry-en/2025/03/19/7O6IMVBVBBEIROXF2DQHJWSL6M/.
8. GlobalData: website. (2025). Top 10 pharma companies in South Korea by market capitalization. URL: https://www.globaldata.com/companies/top-companies-by-sector/healthcare/south-korea-companies-by-market-cap/.
9. Companies Market Cap: website. (2025). Largest biotech companies by market capitalization. URL: https://companiesmarketcap.com/biotech/largest-companies-by-market-cap/.
10. KoreaBIO: website. (2023). URL: https://www.koreabio.org/en/.
11. Lee, S., Rhee, Y. E. (2025). South Korea: a new global hub for biopharma. *Intralink company*: website. URL: https://www.intralinkgroup.com/en-GB/Latest/Intralink-Insights/February-2025/South-Korea-a-new-global-hub-for-biopharma.
12. KBioIS: website. (2025). Bioindustry classification code KS J 1009 (바이오산업 분류코드): Korea technology and standards agency. URL: https://www.kbiois.or.kr/portal/intro/categoryInfoPage.do.
13. Statista Market Insights: website. (2023). Value of research and development (R&D) investment in biotech industry in South Korea in 2023, by sector. URL: https://www.statista.com/statistics/717658/south-korea-r-and-d-investment-in-bioindustry-by-sector/.
14. Jang, S-H., Yeon, J-H. (2023). The relation between bio-industry performance and innovation capacity — focusing on the korean bio-industry. *Circular business model innovation for a circular bioeconomy*, 15(9), 7116. https://doi.org/10.3390/su15097116.
15. Lim, D. S. (2009). Biotechnology industry, statistics and policies in Korea. *Asian biotechnology and development review*, 1(2), 1–27.
16. Korea biomedicine industry association: website. (2018). Biopharmaceutical industry in Korea. URL: https://www.kobia.kr/e_sub03/sub01.php.
17. Statista Market Insights: website. (2024). Size of the biopharmaceutical market in South Korea from 2012 to 2022. URL: https://www.statista.com/statistics/780916/south-korea-biopharmaceutical-market-size/.
18. Korea Pharmaceutical and Bio-Pharma Manufacturers Asociation: website. (2024). Industry overview. Statistics. URL: https://www.kpbma.or.kr/eng/industry/statistics.
19. Statista Market Insights: website. (2024). Size of the biopharmaceutical market in South Korea from 2012 to 2022. URL: https://www.statista.com/statistics/780916/south-korea-biopharmaceutical-market-size/.
20. Prescient & Strategic Inteligence: website. (2024). Biopharmaceuticals market size & share analysis (2024–2030). URL: https://www.psmarketresearch.com/market-analysis/biopharmaceuticals-market
21. Kim, D-S., Bae, S. J. (2022). Impact and challenges of enactment for advanced regenerative medicine in South Korea. *Frontiers in bioengineering and biotechnology*, 10 (2022). https://doi.org/10.3389/fbioe.2022.972865.
22. Shin, K. S., Choy, M. K., Lee, C., Park, G. (2019). Government R&D subsidy and additionality of biotechnology firms: the case of the South Korean biotechnology industry. *Sustainability*, 11(6), 1583. https://doi.org/10.3390/su11061583.
23. Statista Market Insights: website. (2024). Bioenergy — South Korea. URL: https://www.statista.com/outlook/io/energy/renewable-energy/bioenergy/south-korea.
24. Advanced Energy Technologies: website. (2024). Energy industry in South Korea. URL: https://aenert.com/countries/asia/energy-industry-in-south-korea/.
25. Choi, S-C. (2024). HD Hyundai Oilbank ventures into bioenergy sector as new growth driver. The Korea economic Daily Global edition: online source. URL: https://www.kedglobal.com/petrochemicals/newsView/ked202411290012.
26. Sun, D. (2024). South Korea's GS Caltex supplies SAF to Japan. Argus: online source. URL: https://www.argusmedia.com/en/news-and-insights/latest-market-news/2609741-south-korea-s-gs-caltex-supplies-saf-to-japan.
27. Rebuild Ukraine: 5th Internetional exhibition-conference. (2023). Ukraine and the Republic of Korea to deepen cooperation in attracting investments to restore the energy sector. URL: https://rebuildukraine.

in.ua/blog/ukraine-and-the-republic-of-korea-to-deepen-cooperation-in-attracting-investments-to-restore-the-energy-sector.
28. Ministry of economy of Ukraine. (2025). Ukraine and Korea to develop green metallurgy and energy projects. URL: https://www.kmu.gov.ua/en/news/ukraina-spilno-z-respublikoiu-koreia-rozvyvatyme-proekty-v-haluziakh-zelenoi-metalurhii-ta-enerhetyky.
29. Derewenda, F. (2023). Can Poland and Ukraine lead Europe in bioenergy? *Ceenergynews*: online source. URL: https://ceenergynews.com/ukraines-energy-future/can-poland-and-ukraine-lead-europe-in-bioenergy/.
30. Kim, H. S., Kwak, S-S. (2020). Crop biotechnology for sustainable agriculture in the face of climate crisis. *Plant Biotech. Reports*, 14, 139–141. https://doi.org/10.1007/s11816-020-00619-4.
31. Horizon Databook: website. (2023). South Korea functional foods market size & outlook. URL: https://www.grandviewresearch.com/horizon/outlook/functional-foods-market/south-korea.
32. ISAAA.Inc: website. (2018). Agricultural Biotechnology Research Center of South Korea Officially Launched. URL: https://www.isaaa.org/kc/cropbiotechupdate/article/default.asp?ID=16372
33. Park, S-H., Cho, J-I., Kim, Y-S., Kim, S-M., Lim, S-M., Lee, G-S., Park, S-C. (2018). National program for developing biotech crops in korea. plant breeding and biotechnology, 6, 171-176. DOI: 10.9787/PBB.2018.6.3.171
34. FoodBusinessAfrica: website. (2024). South Korea launches Bio Future Food Industry Association. URL: https://www.foodbusinessafrica.com/south-korea-launches-bio-future-food-industry-association/.
35. Cultivated X: website. (2024). Bio Future Food Industry Association launches in South Korea to boost the cultivated meat sector. URL: https://cultivated-x.com/meat/bio-future-food-industry-association-south-korea-cultivated-meat-sector/.
36. Pangyo Techno Valley: website. (2024). Korea biotechnology industry organization launches 'Bio future food industry council' to explore sustainable solutions for alternative material supply. URL: https://www.pangyotechnovalley.org/base/board/read?boardManagementNo=21&boardNo=2112&menuLevel=2&menuNo=53.
37. Radio "Svoboda". (2023). Zelensky met with the President of South Korea: they talked about the peace formula and cooperation. URL: https://www.radiosvoboda.org/a/32504846.html.
38. 8allocate: website. (2019). From smart farming to R&D outsourcing: why more South Korean businesses look to Ukraine. URL: https://8allocate.com/blog/from-smart-farming-to-rd-outsourcing-why-more-south-korean-businesses-look-to-ukraine/.
39. Ohorodnyk, N., Svarchevska, O., Shved, O., Kupka, T. (2023). Main aspects of the manufacturer of organic products in Ukraine. *Biotechnologia Acta*, 16(1), 40–50. https://doi.org/10.15407/biotech16.01.040.
40. Diia: website (2024). How Biofach 2023 helped ukrainian organic watermelons and melons enter the EU markets. URL: https://www.export.gov.ua/history-of-success/how-biofach-2023-helped-ukrainian-organic-watermelons-and-melons-to-enter-the-eu-markets.
41. Bonafide Research: website. (2023). South Korea water and wastewater treatment market. URL: https://www.bonafideresearch.com/product/6309398516/south-korea-water-and-wastewater-treatment-market.
42. Grand View Research: website. (2024). Biological wastewater treatment market: South Korea. URL: https://www.grandviewresearch.com/horizon/outlook/biological-wastewater-treatment-market/south-korea.
43. Grand View Research: website. (2024). Bioremediation market: South Korea. URL: https://www.grandviewresearch.com/horizon/outlook/bioremediation-market/south-korea.
44. Grand View Research: website. (2024). Vertical farming market: South Korea. URL: https://www.grandviewresearch.com/horizon/outlook/vertical-farming-market/south-korea.
45. Park, J., Shin, K., Lee, H., Choi, S., Kim, G., Depuydt, S., De Saeger, J., Heynderickx, P. M., Wu, D., Asselman, J., Janssen, C., Han, T. (2023). Evaluating ecotoxicological assays for comprehensive risk assessment of toxic metals present in industrial wastewaters in the Republic of Korea. *The Science of the total environment*, 867, 161536. https://doi.org/10.1016/j.scitotenv.2023.161536.
46. IHE Delft: website. (2022). Sang-Yeob Kim earns PhD research on wastewater treatment new type of oxygen delivery system. URL: https://www.un-ihe.org/sang-yeob-kim-earns-phd-research-on-wastewater-treatment-new-type-oxygen-delivery-system.
47. Lee, Y., Jeong, S. E., Hur, M., Ko, S., Jeon, C. O. (2018). Construction and Evaluation of a Korean Native Microbial Consortium for the Bioremediation of Diesel Fuel-Contaminated Soil in Korea. *Frontiers*

in microbiology, 9, 2594. https://doi.org/10.3389/fmicb.2018.02594.
48. Seoulz: website. (2023). The rise of smart vertical farms in Korea: the future of farming. URL: https://www.seoulz.com/the-rise-of-smart-vertical-farms-in-korea-the-future-of-farming/.
49. Grand View Research: website. (2024). Biosensors market: South Korea. URL: https://www.grandviewresearch.com/horizon/outlook/biosensors-market/south-korea.
50. Bioindustrial Services website. URL: https://bioindustrialservices.co.za/.
51. Korea Drug Research Association website. URL: https://www.kdra.
52. Korea Research Institute of Bioscience & Biotechnology website. URL: https://www.kribb.re.kr.
53. Korea Research Institute of Chem. Technology website. URL: https://www.krict.re.kr/.
54. Korea Institute of Oriental Medicine website. URL: https://kiom.re.kr/.
55. National Cancer Center of Korea website. URL: https://www.ncc.re.kr/.
56. Mogam Biotechnology Research Institute website. URL: http://www.mogam.re.kr/eng.
57. Ministry of Education, Science and Technology presentation: Biotechnology in Korea. URL: https://english.moe.go.kr/main.do?s=english.
58. Korea Technopark Association website. URL: http://www.technopark.kr/.
59. Invest Korea website. Pharma & Bio. URL: https://www.investkorea.org/ik-en/cntnts/i-309/web.do.

БІОПРОМИСЛОВІСТЬ У ПІВДЕННІЙ КОРЕЇ: АНАЛІТИЧНИЙ ОГЛЯД ТА ОСНОВНІ ПЕРСПЕКТИВИ ЇЇ РОЗВИТКУ

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Подано аналітичний огляд біоіндустрії Південної Кореї, її поточний стан, досягнення та виклики. Висвітлено різні аспекти економіки, зокрема й інноваційні можливості, державну політику, економічний вплив та соціальні зміни. Визначено ключові досягнення, лідерство Південної Кореї у виробництві та інноваціях біофармацевтики, а також розглянуто такі проблеми, як глобальна конкуренція та зміна клімату. Окреслено ключові перспективи майбутнього розвитку, підкреслюючи потенціал цифрової охорони здоров'я, персоналізованої медицини та зеленої біотехнології для позиціонування Південної Кореї як лідера в біоекономіці. Висвітлено уроки, які можна винести з досвіду Південної Кореї, та їхню застосовність до інших країн, зокрема України та Польщі, які прагнуть зміцнити свій потенціал біоекономіки та біоіндустрії. Враховуючи стратегічний підхід Південної Кореї, це дослідження має на меті визначити цінні ідеї для сприяння інноваціям, забезпечення сталого розвитку та позиціонування біоіндустрії як рушійної сили національного економічного зростання. Висвітлено сучасний стан промислового виробництва та напрямів наукових досліджень у класичній біотехнології для вдосконалення існуючих і розроблення нових біоінженерних технологій, що спрямовані на розвиток стратегічного значення біоіндустрії для досягнення сталого розвитку країни.

Мета. Аналіз поточного стану, ключових досягнень, викликів та перспектив південнокорейської біоіндустрії, зосереджуючись на отриманні цінних знань та практик, які можна адаптувати для сприяння розвитку біоіндустрії в Україні та Польщі.

Матеріали й методи. Методологічний аналіз та абстрактно-логічний метод узагальнення критеріїв оцінки формування, розвитку та інтеграції біотехнологічного виробництва у структуру світового випуску безпечної продукції, а також біопродуктів для покращення здоров'я та омолодження.

Результати. Описано сучасний стан ключових галузей промисловості на основі корейського класифікаційного коду біопромисловості KS J 1009: біофармацевтична, біохімічна та біоенергетична, біохарчова, біоекологічна, біомедичне обладнання, а також біоінструменти та біообладнання, біоресурсна та біосервісна галузі. Кожен сектор охарактеризовано різною динамікою зростання, рівнями впровадження інновацій та інтеграції в національні та глобальні біоекономічні стратегії.

Висновок. Аналіз висвітлює як технологічні досягнення, так і структурні пріоритети, що формують сучасну біоіндустрію Південної Кореї.

Ключові слова: Південна Корея, біоіндустрія, біоекономіка, сталий розвиток, інновації, інвестиції, стандарти.

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SUNFLOWER SEED HUSK BIOCHAR: SYNTHESIS AND TOXICITY RISK ASSESSMENT

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Aim. Searching for efficient biocompatible sorbents that possess zero neurotoxicity is an actual task. Biochars are auspicious carbon materials for the adsorption of heavy metals in the environment, wastewater, and also in human organisms.

Methods. Biochar from sunflower seed husk (SB) was synthesized by pyrolysis at 800 °C without special functionalization. Neurotoxicity risk of SB was assessed in an animal model using presynaptic nerve terminals isolated from rat cortex (synaptosomes).

Results. It was shown in radiolabelled experiments that SB did not change the synaptosomal ambient levels of the excitatory neurotransmitter L-[3H] glutamate and inhibitory neurotransmitter [^{3}H] GABA within the concentration range 0.25–1.0 mg/ml. In the fluorimetric experiments using the dye JC-1, SB at a concentration of 1.0 mg/ml did not change the mitochondrial membrane potential of the nerve terminals.

Conclusions. SB demonstrated the absence of neurotoxicity signs and high biocompatibility, and therefore, SB has the potential to be used as an adsorbent in biotechnology and medicine.

Key words: agricultural waste; sunflower seed husk; biochar; non-functionalized carbon materials; neurotoxicity risk; glutamate; GABA; presynaptic terminals, brain.

Usage of agricultural waste for the synthesis of carbon materials based on “green” principles is a very promising stream in modern biotechnology as well as environmental sciences and management [1]. Among carbon materials, biochar, a black solid carbon-rich substance, has been attracting a lot of attention for various applications due to its unique specific characteristics and low-cost production. Biochar is produced by pyrolysis,

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i.e, heating organic materials in an oxygen-free or low-oxygen environment. Biochar possesses a large specific surface area, high cation exchange capacity, high porosity, has functional groups at the surface, and is stable in different media (generally in the absence of oxidizers) [2, 3]. The above properties may vary depending on the type of biomass used as a starting material, temperature, heating rate, residence time of the pyrolysis formulation, and the modification technique [4].

Many studies have reported the considerable adsorption efficiency of various biochars in the removal of multiple contaminants [5]. The adsorption mechanisms are different and depend on pollutant physical and chemical properties and biochar surface characteristics, and include binding by electrostatic interaction, ion exchange, adsorption on surface or pore filling, and chemical binding with the formation of insoluble compounds [5–7]. Literature data describe biochars as the eco-friendly, cheap, multipurpose materials with high potential for recovering soil efficiency, managing toxic metals and organic pollutants, which could be employed as a green alternative to conventional sorbents [2, 3, 7]. However, biochar can have toxic effects on living organisms as harmful substances may be present in it [8]. Biochar contaminants can be both organic and/or inorganic. They can be byproducts of pyrolysis during biochar production and/or components of the feedstock that remain more concentrated after the pyrolysis process [8].

Among different organic agricultural waste materials, sunflower seed husks recently appeared in the mainstream of biotechnological research. Different condition of synthesis of biochar from sunflower seed husks and numerous applications of this biochar were proposed in numerous studies and were reported in the literature. In particular, sunflower seed husks were pyrolyzed at 450 °C, and the produced biochar was tested for its ability to filter out NO gas. It was found that the sunflower seed husk biochar could be proposed as a low-cost and effective solution for reducing air pollution with NO in industrial and urban environment [9].

Biochar was considered as an additive for improving soil [10]. It was revealed that biochar improved soil parameters, and soil enrichment with sunflower husk biochar increased CH_4 oxidation, favouring to reduction of CH_4 exhaust [10]. In another study, biochar produced from sunflower husks had a significant effect on soil respiration, soil water flux, and soil temperature [11].

A promising approach for eliminating various contaminants from wastewater was proposed using bacterial consortium-biochar composites [4]. These composites exhibited higher remediation capacity with respect to lead and phenol than the sum of bacterial consortium and biochar *per se*, showing thus the synergistic adsorptive capability of biochar and bacterial consortium [4]. In another study, sunflower seed husk biochar was used for the purification of water contaminated with cadmium [12].

H_3PO_4-treated sunflower seed husk biochar effectively adsorbed antibiotics tetracycline, ciprofloxacin, ibuprofen, and sulfamethoxazole from the aquatic environment [3].

Taking into account the above mentioned facts, the aims of this study were:

1) to synthesize and characterize biochar from sunflower seed husks; and

2) to assess its toxicity and biocompatibility for further biotechnological and medical application as adsorbent, measuring the ambient levels of the excitatory and inhibitory neurotransmitters L-[^{3}H] glutamate and [^{3}H] GABA, respectively, and the mitochondrial membrane potential in isolated rat cortex nerve terminals (synaptosomes). The activated carbon studied herein is referred to as the sunflower seed husk biochar (SB).

Methods and Materials

Synthesis of SB

The preparation of biological material was carried out according to the established procedure: sunflower seed husk was thoroughly ground using a laboratory mill until a homogeneous powdery mass was obtained. Direct pyrolysis of sunflower waste was carried out in two stages by heat treatment in argon. At the first stage, the crushed sunflower seed husk material was subjected to thermal destruction at a temperature of 600 °C for 3 hours. At the second stage, the resulting pyrolyzed residue was mixed with sodium hydroxide and kept for 3 hours at a temperature of 800 °C. After completion of the heat treatment, the carbon material (SB) was washed with distilled water until a neutral pH value was reached.

Experiments using brain nerve terminals

Ethics

Males, Wistar rats, were kept at 22–23 °C in a quiet temperature-controlled room in the vivarium of the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine. Rats were supplied with dry food pellets and water *ad libitum*. Experiments involving animals were carried out in accordance with the Guidelines of the European Community (2010/63/EU), Scientific Requirements and Research Protocols; Research Ethics Committees of the Declaration of Helsinki; and ARRIVE Guidelines for reporting experiments involving animals [13, 14], and Ukrainian laws and policies. The protocols of the experiments were approved by the Animal Care and Use Committee of the Palladin Institute of Biochemistry (Protocol # 1 from 10/01/2024). The overall number of animals was 12.

Isolation of nerve terminals from the rat cortex (rat cortex synaptosomes)

The synaptosomes were isolated from the rat cortex. The rat cortex was homogenized in the following solution: sucrose 0.32 M; HEPES-NaOH 5 mM, pH 7.4; EDTA 0.2 mM. All procedures were carried out at + 4 °C. The isolation was carried out as described by Cotman with minor modifications [15–18]. The standard saline solution in the synaptosome experiments was as follows: NaCl 126 mM; KCl 5 mM; MgCl2 2.0 mM; NaH2PO4 1.0 mM; HEPES 20 mM, pH 7.4; and D-glucose 10 mM. Protein concentrations were recorded as described by Larson [19].

The ambient level of L-[^{3}H] glutamate in synaptosomes

The synaptosome suspension with a concentration of 2 mg of protein/ml was preincubated at 37 °C for 10 min; after that, the suspension was loaded with L-[^{3}H] glutamate at 37 °C for 10 min. The synaptosome suspension was diluted with 10 volumes of the standard saline solution, and centrifuged at10,000×g for 20 s. The pellets were suspended in the standard saline solution up to a concentration of 1 mg protein/ml. The ambient L-[^{3}H] glutamate level was monitored in 125 µl aliquots with a concentration of 0.5 mg of protein/ml The aliquots were preincubated for 8 min, and after that, SB was added, and the suspension was incubated at 37 °C for 0 and 6 min. Then the suspension was centrifuged at 10,000 × *g* for 20 s. The value of the ambient L-[^{3}H] glutamate level was recorded in the supernatant and pellets (preliminary treated with SDS, 100 l of 10% SDS stock solution) using the liquid scintillation counting with Sigma-Fluor® High Performance LSC Cocktail and counter Hidex 600SL (Finland). The experimental data were from "n" independent experiments with different synaptosome preparations.

The ambient level of [^{3}H] GABA in synaptosomes

The synaptosome suspension with a concentration of 2 mg of protein/ml was preincubated at 37 °C for 10 min; after that, the suspension was loaded with [^{3}H] GABA in the standard saline solution at 37 °C for 10 min. Throughout all experiments, 100 µM aminooxyacetic acid was added to the incubation media. Then, the synaptosomes were diluted with 10 volumes of the standard saline solution, centrifuged, and the pellets were suspended in the solution up to a concentration of 1 mg of protein/ml. The aliquots were preincubated for 8 min, and after that, SB was added and further incubated at 37 °C for 0 and 5 min; after that centrifuged at 10,000 × *g* for 20 s [20]. The value of the ambient [3H] GABA level was monitored in the supernatant aliquots using the liquid scintillation counting with Sigma-Fluor® High Performance LSC Cocktail and the counter Hidex 600SL (Finland). The experimental data were obtained from "n" independent experiments with different synaptosome preparations.

The mitochondrial membrane potential in synaptosomes

The cationic fluorescent membrane-permeable dye JC-1, a mitochondrial membrane potential assay kit, was applied to measure the mitochondrial membrane potential in synaptosomes in the control and in the presence of SB. Synaptosome suspension with the concentration of 0.15 mg of protein /ml was incubated in a stirring cuvette; after that, JC-1 at a concentration of 5 µM was added and preincubated at 37 °C for 10 minutes; and then the fluorescence spectra were measured at a 485 nm excitation wavelength and from 510 to 610 nm emission wavelengths. The ratio of the fluorescence intensity at 590 versus 530 nm was used in the calculations. The fluorescence measurements were carried out using a spectrofluorimeter, Hitachi 650-10S, and Shimadzu RF-6000.

Statistical analysis

The experimental results were expressed as the mean ± SEM of *n* independent experiments. One-way ANOVA was used with the accepted significance $P < 0.05$.

Materials

HEPES, EGTA, EDTA, salts of the analytical grade, High Performance LSC Cocktail were obtained from Sigma, USA; L-[^{3}H] glutamate and [3H] GABA were from Revvity, Waltham, MA, USA.

Methods

Scanning electronic microscopy (SEM) images were obtained using the FEI Inspect Instrument at 20 kV. Samples were placed on a carbon film without any special treatment. Analysis of the surface of the biichar was performed using energy-dispersive X-ray spectroscopy (EDX) using an Apollo XL SDD EDAX instrument.

Results and Discussion

SB characterization

SEM studied the morphology of the biochar prepared from sunflower seed husk. The material contained particles of irregular shape (Fig. 1) with a wide size distribution (10–100 μm). A large number of voids were observed, which could be due to the partial preservation of the cellular microstructure of the material of biological origin. Such heterogeneous particle morphology and the presence of voids of different sizes can influence the sorption properties of the resulting material, as well as the kinetics of the processes occurring on its surface.

The element composition of the surface of the SB sample was analyzed using energy-dispersive X-ray spectroscopy. The carbon content (Fig. 2) was expectedly the highest and was equal to 89.5%, and the oxygen content was 8.3%. Other typical biogenic elements were also present in small amounts: magnesium (1.0%), silica (0.8%), and calcium (0.4%). These elements apparently originate from the starting material used for pyrolysis.

Toxicity assessment of SB using rat brain nerve terminals: measurements of the ambient levels of *L*-[3H] glutamate and [3H]GABA as well, as the mitochondrial membrane potential

Neurotoxicity risk assessment of SB was carried out in the nerve terminal preparations according to its influence on the ambient levels of the key excitatory neurotransmitter L-[^{3}H] glutamate and the inhibitory neurotransmitter [^{3}H] GABA. The changes in the ambient level of the neurotransmitters reflect the plasma membrane disintegration and changes in the transportation of neurotransmitters [21, 22]. It was proven that SB within the concentration range 0.25–1.0 mg/ml did not change the ambient levels of L-[^{3}H] glutamate and [^{3}H] GABA in the nerve terminal preparations (Fig. 3). Therefore, it can be concluded that SB was biocompatible and did not demonstrate any neurotoxic signs at these concentrations.

In the following sets of experiments, the possible effect of SB on the mitochondrial membrane potential was assessed in the

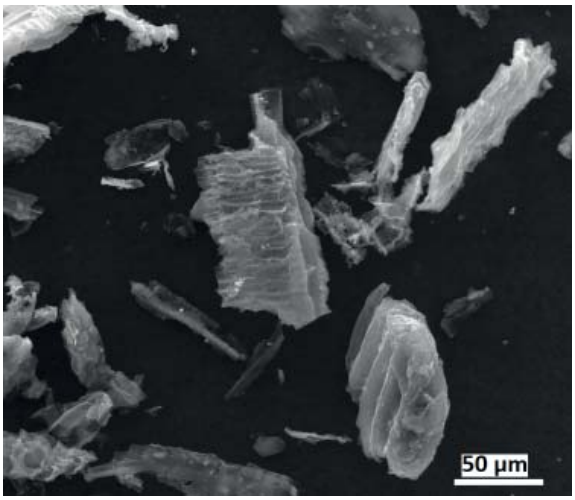


Fig. 1. **SEM microphotograph of SB**

nerve terminals. The measurement was performed using the cationic carbocyanine fluorescent dye JC-1. It was revealed that SB at a concentration of 1 mg/ml was inert regarding modulation of the synaptosomal mitochondrial membrane (Fig. 4). Therefore, SB (1 mg/ml) did not depolarize the mitochondria membrane in nerve terminals. These fluorimetric data entirely corresponded to the above results obtained using L-[3H] glutamate and [3H]GABA (Fig. 3).

It was found that SB did not change the ambient levels of neurotransmitters and the mitochondria membrane potential, thus it can be concluded that SB did not have neurotoxicity signs and was biocompatible.

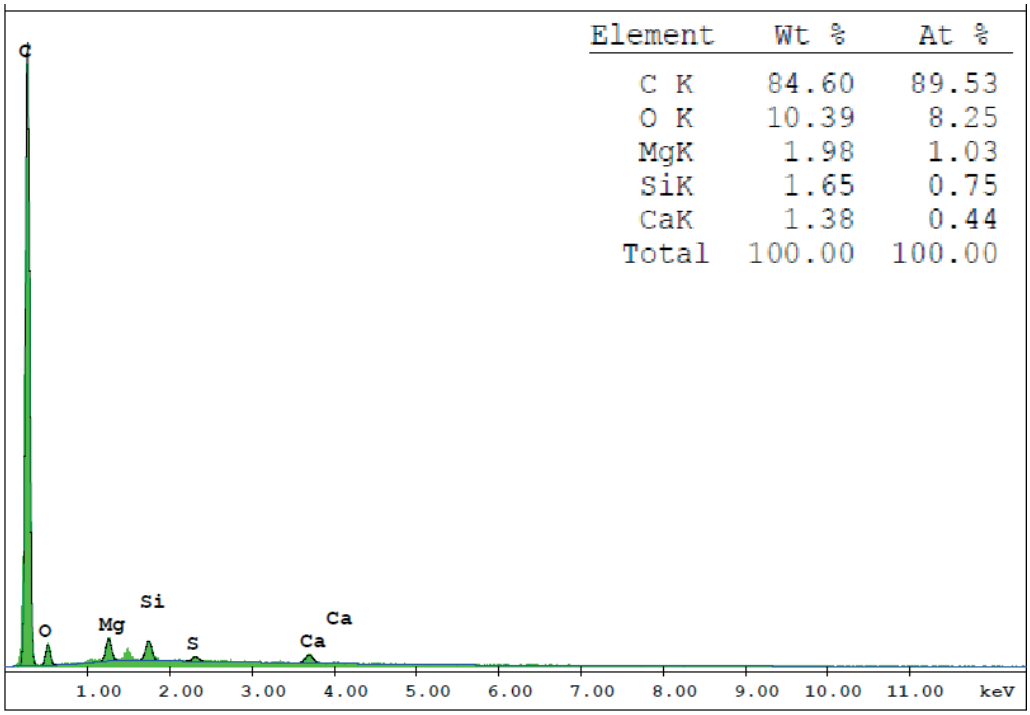
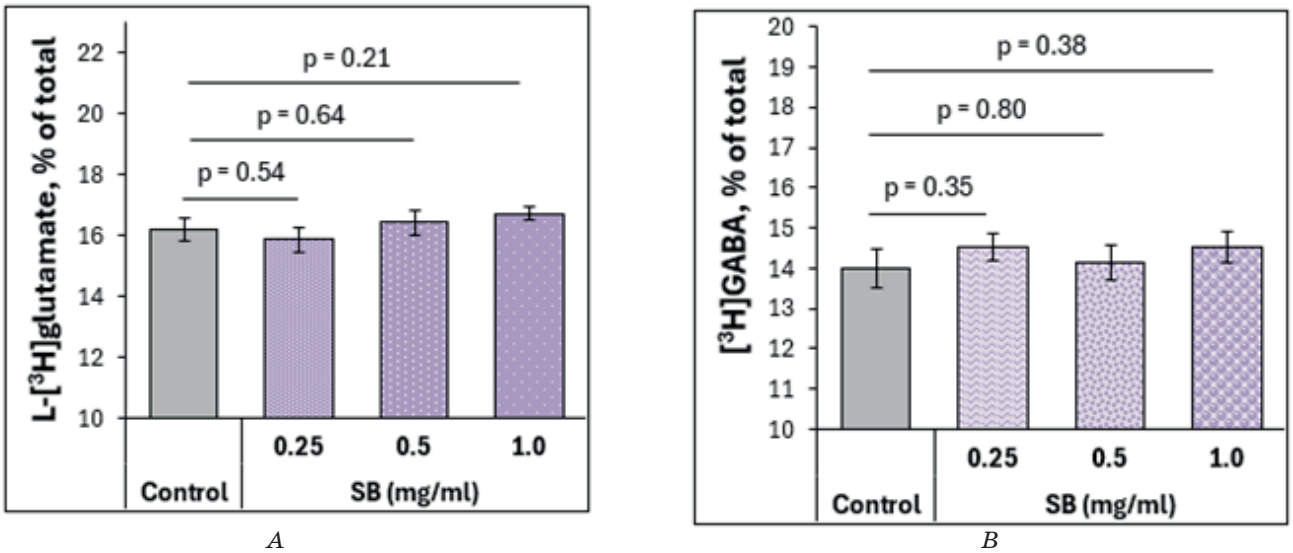


Fig. 2. **Energy-Dispersive X-ray Spectroscopy (EDX)** and element composition of the surface of SB



A *B*

Fig. 3. **The ambient levels of neurotransmitters *L*-[^{3}H] glutamate (*A*) and [3H]GABA (*B*) in the nerve terminals in the presence of SB (0.25–1.0 mg/ml)**

Note. Data are the mean ± SEM. $n = 12$.

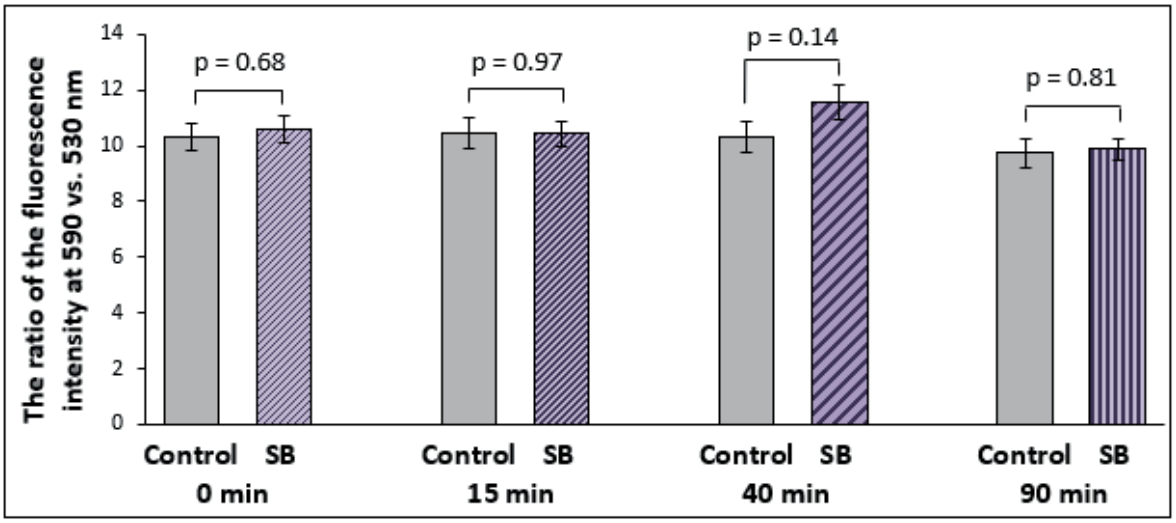


Fig. 4. **The mitochondrial membrane potential of the nerve terminals in the presence of SB (1.0 mg/ml) was measured using the cationic membrane-permeable dye JC-1 and the fluorescence intensity ratio at 590 vs. 530 nm**

Note. JC-1 at a concentration of 5 μM was added to the synaptosome incubation media (0.15 mg of protein/ml); incubated in the dark at a temperature of 37 °C for 10 min; after that SB aliquots were added to the cuvette; and the JC-1 fluorescence was recorded at 0, 15, 40, 90 min time point at an excitation wavelength of 485 nm and emission wavelength from 510 to 610 nm. Data are the mean values ± SEM. $n = 12$.

Due to these features, we believe that SB has high potential for its application as an adsorbent in biotechnology and medicine.

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Competing interests

The authors declare no financial and non-financial competing interests exist.

Author Contributions

A. V. Terebilenko, M. O. Ivanytsya, D. O. Mazur, Ya. I. Kurys — Chemical methodology, Investigation, Data curation, Formal analysis; N. V. Krisanova, N. G. Pozdnyakova — Conceptualization, Biochemical methodology, Investigation, Data curation, Formal analysis, Writing — review & editing, Writing — original draft, Project administration; M. V. Dudarenko, A. O. Pastukhov, R. V. Sivko, L. M. Kalynovska, M. M. Driuk — Methodology, Investigation; T. A. Borisova, S. V. Kolotilov — Conceptualization, Data curation, Formal analysis, Writing — review & editing, Writing — original draft.

REFERENCES

1. Omar, R. A., Talreja, N., Chuhan, D., Ashfaq, M. (2024). Waste-derived carbon nanostructures (WD-CNs): An innovative step toward waste to treasury. *Environmental Research*, 246, 118096. https://doi.org/10.1016/j.envres.2023.118096
2. Yaashikaa, P. R., Kumar, P. S., Varjani, S., Saravanan, A. (2020). A critical review on the biochar production techniques, characterization, stability and applications for circular bioeconomy. *Biotechnology Reports*, 28, e00570. https://doi.org/10.1016/J.BTRE.2020.E00570
3. Nguyen, T. K. T., Nguyen, T. B., Chen, W. H., Chen, C. W., Kumar Patel, A., Bui, X. T., Chen, L., Singhania, R. R., Dong, C. Di. (2023). Phosphoric acid-activated biochar derived from sunflower seed husk: Selective antibiotic adsorption behavior and mechanism. *Bioresource Technology*, 371, 128593. https://doi.org/10.1016/J.BIORTECH.2023.128593

4. El-Gamal, E. H., Rashad, M., Saleh, M. E., Zaki, S., Eltarahony, M. (2023). Potential bioremediation of lead and phenol by sunflower seed husk and rice straw-based biochar hybridized with bacterial consortium: a kinetic study. *Scientific Reports*, 13(1), 21901. https://doi.org/10.1038/s41598-023-49036-x
5. Zhao, M., Dai, Y., Zhang, M., Feng, C., Qin, B., Zhang, W., Zhao, N., Li, Y., Ni, Z., Xu, Z., Tsang, D. C. W., Qiu, R. (2020). Mechanisms of Pb and/or Zn adsorption by different biochars: Biochar characteristics, stability, and binding energies. *Science of The Total Environment*, 717, 136894. https://doi.org/10.1016/J.SCITOTENV.2020.136894
6. Qasem, N. A. A., Mohammed, R. H., Lawal, D. U. (2021). Removal of heavy metal ions from wastewater: a comprehensive and critical review. *Npj Clean Water*, 4(1), 36. https://doi.org/10.1038/s41545-021-00127-0
7. Ambaye, T. G., Vaccari, M., van Hullebusch, E. D., Amrane, A., Rtimi, S. (2020). Mechanisms and adsorption capacities of biochar for the removal of organic and inorganic pollutants from industrial wastewater. *International Journal of Environmental Science and Technology*, 18(10), 3273–3294. https://doi.org/10.1007/S13762-020-03060-W
8. Godlewska, P., Ok, Y. S., Oleszczuk, P. (2021). THE DARK SIDE OF BLACK GOLD: Ecotoxicological aspects of biochar and biochar-amended soils. *Journal of Hazardous Materials*, 403, 123833. https://doi.org/10.1016/j.jhazmat.2020.123833
9. Sunflower Seed Husk Biochar: A Sustainable Solution for Reducing Nitric Oxide Pollution – Biochar Today. (n.d.). URL: https://biochartoday.com/news/science/sunflower-seed-husk-biochar-a-sustainable-solution-for-reducing-nitric-oxide-pollution/ (Last accessed: 11.08.2025).
10. Kubaczyński, A., Walkiewicz, A., Pytlak, A., Grządziel, J., Gałązka, A., Brzezińska, M. (2023). Application of nitrogen-rich sunflower husks biochar promotes methane oxidation and increases abundance of Methylobacter in nitrogen-poor soil. *Journal of Environmental Management*, 348, 119324. https://doi.org/10.1016/J.JENVMAN.2023.119324
11. Klimek-Kopyra, A., Sadowska, U., Kubó, M., Gliniak, M., Sikora, J., Di Bene, C., Francaviglia, R., Farina, R., Álvaro-Fuentes, J., Zornoza, R., Przemysl, I. (2021). Sunflower Husk Biochar as a Key Agrotechnical Factor Enhancing Sustainable Soybean Production. *Agriculture*, 11(4), 305. https://doi.org/10.3390/AGRICULTURE11040305
12. Zgureva-Filipova, D., Gosheva, L., Petrova, R., Filipov, K. (2023). Utilizing of sunflower seed husk through production of biochar and its application in systems for purification of water contaminated with cadmium. *IOP Conference Series: Earth and Environmental Science*, *1128*(1), 012025. https://doi.org/10.1088/1755-1315/1128/1/012025
13. Kilkenny, C., Browne, W., Cuthill, I. C., Emerson, M., Altman, D. G., NC3Rs Reporting Guidelines Working Group. (2010). Animal research: reporting in vivo experiments: the ARRIVE guidelines. *British Journal of Pharmacology*, *160*(7), 1577–1579. https://doi.org/10.1111/j.1476-5381.2010.00872.x
14. McGrath, J. C., Drummond, G. B., McLachlan, E. M., Kilkenny, C., Wainwright, C. L. (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. *British Journal of Pharmacology*, 160(7), 1573–1576. https://doi.org/10.1111/j.1476-5381.2010.00873.x
15. Cotman, C. W. (1974). Isolation of synaptosomal and synaptic plasma membrane fractions. *Methods in Enzymology*, 31, 445–452. http://www.ncbi.nlm.nih.gov/pubmed/4278474
16. Pozdnyakova, N., Krisanova, N., Pastukhov, A., Dudarenko, M., Tarasenko, A., Borysov, A., Kalynovska, L., Paliienko, K., Borisova, T. (2024). Multipollutant reciprocal neurological hazard from smoke particulate matter and heavy metals cadmium and lead in brain nerve terminals. *Food and Chemical Toxicology*, 185, 114449. https://doi.org/10.1016/j.fct.2024.114449
17. Borisova, T., Kucherenko, D., Soldatkin, O., Kucherenko, I., Pastukhov, A., Nazarova, A., Galkin, M., Borysov, A., Krisanova, N., Soldatkin, A., El'skaya, A. (2018). An amperometric glutamate biosensor for monitoring glutamate release from brain nerve terminals and in blood plasma. *Analytica Chimica Acta*, 1022, 113–123. https://doi.org/10.1016/j.aca.2018.03.015
18. Krisanova, N., Pastukhov, A., Dekaliuk, M., Dudarenko, M., Pozdnyakova, N., Driuk, M., Borisova, T. (2024). Mercury-induced excitotoxicity in presynaptic brain nerve terminals: modulatory effects of carbonaceous airborne particulate simulants. *Environmental Science and Pollution Research International*, 31(3), 3512–3525. https://doi.org/10.1007/S11356-023-31359-X/METRICS
19. Larson, E., Howlett, B., & Jagendorf, A. (1986). Artificial reductant enhancement of the Lowry method for protein determination. *Analytical Biochemistry*, 155(2), 243–248. https://doi.org/10.1016/0003-2697(86)90432-X
20. Krisanova, N., Pozdnyakova, N., Pastukhov, A., Dudarenko, M., Maksymchuk, O., Parkho-

mets, P., Sivko, R., Borisova, T. (2019). Vitamin D3 deficiency in puberty rats causes presynaptic malfunctioning through alterations in exocytotic release and uptake of glutamate/GABA and expression of EAAC-1/GAT-3 transporters. *Food and Chemical Toxicology*, 123, 142–150. https://doi.org/10.1016/j.fct.2018.10.054
21. Borisova, T., Borysov, A. (2016). Putative duality of presynaptic events. *Reviews in the Neurosciences*, 27, 377–383. https://doi.org/10.1515/revneuro-2015-0044
22. Borisova, T. (2016). Permanent dynamic transporter-mediated turnover of glutamate across the plasma membrane of presynaptic nerve terminals: arguments in favor and against. *Reviews in the Neurosciences*, 27(1), 71–81. https://doi.org/10.1515/revneuro-2015-0023

БІОВУГІЛЛЯ З ЛУШПИННЯ НАСІННЯ СОНЯШНИКУ: СИНТЕЗ ТА ОЦІНКА РИЗИКУ ТОКСИЧНОСТІ

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Мета. Пошук ефективних біосумісних сорбентів, які мають нульову нейротоксичність, є актуальним завданням. Біовугілля є дуже перспективним вуглецевим матеріалом для адсорбції важких металів у навколишньому середовищі, стічних водах, а також в організмі людини.

Методи. Біовугілля з лушпиння насіння соняшнику (БЛС) було синтезовано шляхом піролізу при 800 °С без спеціальної функціоналізації. Ризик нейротоксичності БЛС оцінювали на тваринній моделі з використанням ізольованих пресинаптичних нервових закінчень кори головного мозку щурів (синаптосом).

Результати. В експериментах з використанням радіоактивно мічених нейромедіаторів було показано, що БЛС не змінює позаклітинні рівні збуджувального нейромедіатора L-[^{3}H] глутамату та гальмівного нейромедіатора [^{3}H] ГАМК у препаратах синаптосом в діапазоні концентрацій 0,25–1,0 мг/мл. У флуориметричних експериментах з використанням барвника JC-1, БЛС у концентрації 1,0 мг/мл не змінював мітохондріальний мембранний потенціал нервових закінчень.

Висновки. БЛС продемонстрував відсутність ознак нейротоксичності та високу біосумісність, тому БЛС має перспективи для використання як адсорбенту в біотехнології та медицині.

Ключові слова: сільськогосподарські відходи, лушпиння насіння соняшнику, біовугілля, нефункціоналізовані вуглецеві матеріали, ризик нейротоксичності, глутамат, ГАМК, пресинаптичні закінчення, мозок.

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TRANSPLANTATION OF MESENCHYMAL STROMAL CELLS IN EXPERIMENTAL ACUTE REVERSIBLE CEREBRAL ISCHEMIA (COMPARATIVE ANALYSIS)

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The issue of treating cerebrovascular disorders is very important due to their wide occurrence in the human population, especially in the elderly. The resulting ischemia of brain tissues leads to mortality, violent behaviour, biochemical and morphological changes in the brain. Correlation analysis allows evaluating the statistical relationship between two random variables or two-dimensional data. In recent years, the neuroprotective properties of mesenchymal stromal cells (MSCs) have been actively studied. Stem cell transplantation for ischemic stroke is one of the ways of the modern regenerative strategy in the treatment of this pathology.

Aim. The study was to analyse the correlations between biochemical indicators determined in the somatosensory cortex and hippocampus, morphological manifestations of neuroapoptosis, and parameters of CNS functioning in acute cerebral ischemia in rats after MSCs transplantation.

Methods. A 20-minute bilateral cerebral ischemia-reperfusion in rats. Experimental animals were intravenously injected with mesenchymal stromal cells from human umbilical cord Wharton jelly (hWJ-MSCs) or adult adipose-derived stem cells (hAD-MSCs). Rats were evaluated for mortality dynamics, neurological deficits, and biochemical parameters 7 and 14 days after surgery.

Results. Mortality after transplantation of hWJ-MSCs was 10% versus 65% in the control group and 32% in the group of rats that received hAD-MSCs. On day 7, the mean McGraw scores were 7.1 ± 0.19/ 8.9 ± 0.23 /11.8 ± 0.48 points in rats injected with hWJ-MSCs/ hAD-MSCs/saline; on day 14, these were 4.9 ± 0.15/ 5.7 ± 0.23/ 9.1 ± 0.30 points, respectively. Transplantation of mesenchymal stromal cells eliminated energy deficiency in ischemic rat brain tissue, reduced metabolic acidosis and oxidative damage to neurons, and had a positive effect on nitric oxide metabolism, but hAD-MSCs were less effective.

Conclusions. Transplantation of hWJ-MSCs had a better therapeutic effect than transplantation of hAD-MSCs.

Key words: somatosensory cortex, ischemia-reperfusion, mesenchymal stromal cells, Wharton jelly, adipose stem cells, biochemical parameters.

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Ischemic stroke is one of the dangerous vascular diseases with a high level of disability and mortality among people having acute cerebrovascular accidents [1]. Within the framework of the regenerative strategy, a new impetus has been given to stem cell transplantation in ischemic stroke. It is posible to use embryonic and fetal stem cells (SCs), cells of Wharton's jelly (substantia gelatinea funiculi umbilicalis) and umbilical cord (UC) blood, as well as SCs from an adult organism for cell therapy [2]. Encouraging results regarding endogenous mechanisms of neuroregeneration in response to ischemic damage of brain structures have been demonstrated by cell therapy using mesenchymal stromal cells (MSCs) [3]. MSCs are pluripotent cells obtained from adult tissues, which makes them ethically preferable for both preclinical and clinical research [4]. Mechanisms underlying favourable outcomes in stromal cell transplantation include "bystander" effects, paracrine mechanisms, or restorative effects mediated by extracellular vesicles [5]. Among all MSC types, MSCs derived from umbilical cord (frequently called Wharton's jelly mesenchymal stem cells — WJ-MSCs) are of interest. MSCs derived from bone marrow or adipose tissue, unlike perinatal organs MSCs, have some limits, such as an invasive procedure to get them, a higher risk of transmitting infectious diseases, the donor's age, and the limited proliferative potential [6].

In contrast to the available published results of experimental studies, our work will analyse and compare the therapeutic effects of MSCs transplantation (isolated from human umbilical cord Wharton jelly, i.e., hWJ-MSCs and adult adipose-derived SCs (hAD-MSCs)) in conditions of cerebral ischemia-reperfusion with the aim to identify the most active culture with cerebroprotective effect.

Materials and Methods

The research was done on 99 Wistar male rats weighing 160–190 g. Eighty-five animals underwent transient bilateral cerebral ischemia-reperfusion (IR) by ligation of the internal carotid arteries (ICA) for 20 min with subsequent blood supply restoration. Fourteen animals (the group of sham-operated rats) were subjected to the following interventions (anaesthesia, skin incision, vascular dissection) except for ICA ligation. The Bioethics Committee in the National Pirogov Memorial Medical University (protocol No. 2, dated 01.31.2024) approved all animals taken for the research. Surgical interventions, traumatic manipulations were performed under propofol anaesthesia ("Propofol-novo", Novopharm-Biosyntez production, Ukraine, 60 mg/kg intraperitoneally). After modelling the pathology, 20 animals were injected with hWJ-MSCs (1 million cells/animal suspended in 0.2 ml of saline) into the femoral vein. Another group of experimental rats ($n = 25$) was transplanted with hAD-MSCs (1 million cells/animal suspended in 0,2 ml of saline) intravenously. Animals of the control group ($n = 40$) were intravenously injected with 0.2 ml of saline. The method for obtaining MSCs to transplant them into rats is described in our previous publications [8].

For biochemical studies, the brain was removed from rats (by decapitation), washed with a cold 1.15% KCl solution. The tissue of the somatosensory region was homogenized in a medium of 1.15% KCl (ratio 1:3) at 3000 rpm (Teflon-glass). Succinate dehydrogenase (SDH) content was determined by the rate of potassium hexacyanoferrate (III) reduction in order to assess the parameters of energy and carbohydrate metabolism in the tissue of the somatosensory region of a rat brain. For the same purpose, the content of glucose (by glucose oxidase method using standard kits from Filisit-Diagnostics, Ukraine), lactate, and pyruvate (by colorimetric method) was determined [9].

Oxidative stress was assessed by determining the content of malondialdehyde (MDA), which is its final product (by reaction with thiobarbituric acid) [10]. In addition, the activity of superoxide dismutase (SOD) was measured (by the percentage of inhibition in quercetin oxidation) [11] to analyse the state of antioxidant protection and total NO synthase activity (by the amount of formed nitrite anion (NO_2^-) after incubation of the postnuclear supernatant in NADPH Sigma medium, USA (1 ml of which contains 50 mM KH_2PO_4-NaOH buffer (pH 7.0), 1 mM $MgCl_2$, 2 mM $CaCl_2$, 1 mM NADPH, 2.2 mM L-arginine) for 60 min [12].

We determined the dynamics of mortality, neurological deficit (according to the C.P. McGraw stroke-index scale), biochemical indicators such as glucose, lactate, succinate dehydrogenase (SDH), malondialdehyde (MDA), superoxide dismutase (SOD), total NO synthase (NOS) activity in the somatosensory cortex of rats with cerebral IR in the subacute period of ischemia (day 7) and the recovery period (day 14 after pathology modelling).

Statistical processing of the results was carried out using the computer program Statistica 7.0 (StatSoft Inc., USA) using non-parametric (Mann-Whitney U-test) statistical methods.

Results

In the group of sham-operated rats, in which ICA preparation was performed under propofol anaesthesia. Ligatures were applied without further arterial ligation, no case of mortality was recorded during the entire observation period (96 hours) (Fig. 1). An injection of 0.9% NaCl solution into the femoral vein of the rats from the control group (in 20 minutes after bilateral ICA ligation followed by subsequent reperfusion) was accompanied with a progressive increase in the animal mortality rate. Most animals (45%) died 12 hours after cerebral ischemia modelling, which can be considered a critical period in the development of this pathology. In 24 hours, mortality in this group reached 65% and didn't change further during the entire observation period (see Fig. 1).

Experimental therapy of acute cerebral ischemia using intravenous hWJ-MSC transplantation in rats contributed to a reduction in mortality, when mortality was recorded at 10% level, in contrast to the control group having 65% level ($P < 0.05$) (Fig. 1). Intravenous transplantation of hAD-MSCs to rats with cerebral IR also contributed to their survival, in which the mortality rate was at the 32% level and was significantly lower than in the control group of rats ($P < 0.05$) (Fig. 1). Therefore, intravenous transplantation of hWJ-MSCs significantly improved the survival of experimental animals after IR brain damage compared to intravenous transplantation of hAD-MSCs.

An integral indicator that allows us to assess the magnitude of the protective effect on the ischemic brain for a cerebroprotector, along with a decrease in the mortality rate, is the positive dynamics of changes in the neurological status of experimental animals. Thus, intravenous transplantation of hWJ-MSCs and hAD-MSCs led to a significant regression in neurological deficit. On the day 7 after IR, the average score on the McGraw Stroke-index scale was 7.1 ± 0.19 points in the rats administered with hWJ-MSCs and 8.9 ± 0.23 points in the rats having hAD-MSCs treatment versus 11.8 ± 0.48 points in the control group. On the day 14: 4.9 ± 0.15 points and 5.7 ± 0.23 points versus 9.1 ± 0.30 points respectively ($P < 0.05$).

Transplantation of hWJ-MSC tended to have a better modulating effect on the neurological deficit dynamics in rats with IR brain damage than hAD-MSCs transplantation (Fig. 2).

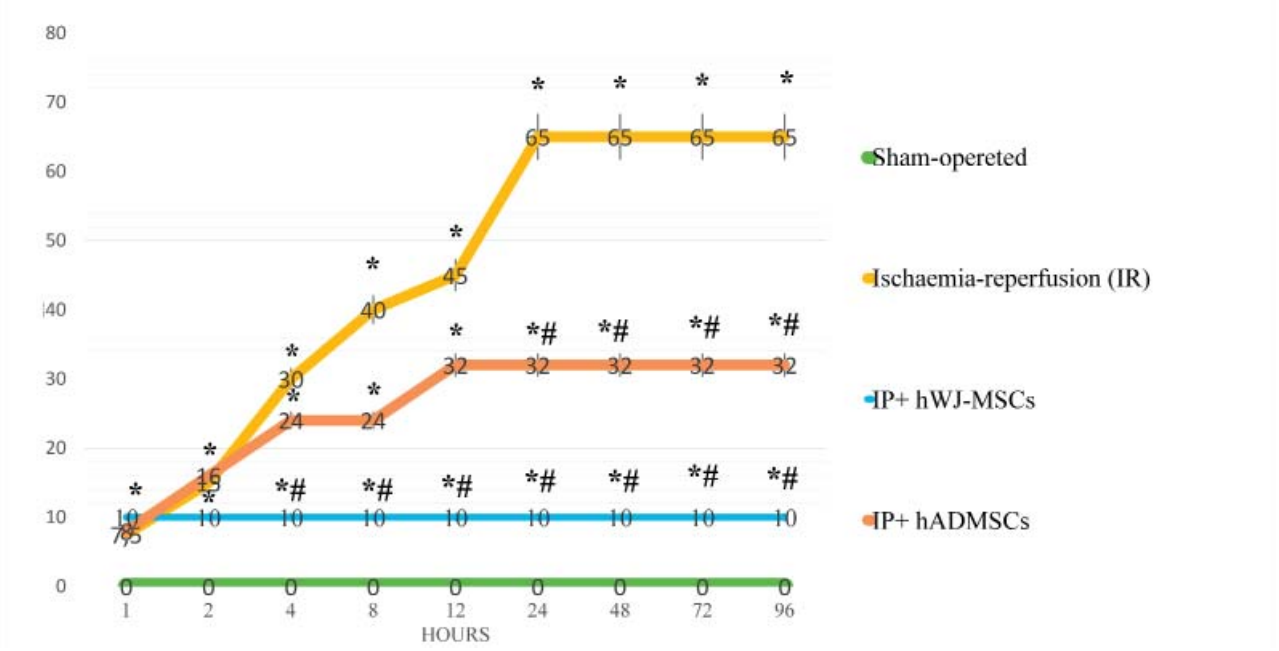

Fig. 1. **Rat mortality rates in the studied groups (%)**
* — $P < 0.05$ relative to the corresponding group of sham-operated animals;
\# — $P < 0.05$ relative to the control group of animals.

Taking into account the powerful cerebroprotective properties in MSCs, it is advisable to investigate the possible biochemical mechanisms in their influence on glucose metabolism, indicators of oxidative and nitrosative stress. The cerebroprotective effect of the studied MSCs is closely related to their sham-operated brain metabolism [13-15].

During our study (on days 7 and 14 after IR brain injury), a significant increase in glucose levels was observed in the somatosensory cortex of rats compared to sham-operated animals (Fig. 3), which averaged 3.2 ± 0.10 μmol/g of dry tissue and 2.9 ± 0.07 μmol/g of dry tissue, versus 2.1 ± 0.10 μmol/g of dry tissue and 2.2 ± 0.08 μmol/g of dry tissue ($P < 0.05$). hWJ-MSCs transplantation had a modeling effect on the increase in glucose levels in the somatosensory cortex of rats having IR lesion, which was manifested by a significant decrease in glucose content compared to the control group and was found to be on average 2.6 ± 0.07 μmol/g of dry tissue and 2.4 ± 0.05 μmol/g of dry tissue ($P < 0.05$). Simultaneously, intravenous transplantation of hAD-MSCs showed a tendency to normalize glucose levels in the somatosensory cortex in rats with IR (Fig. 3).

At the phase of energy shifts, it compensatory activates the anaerobic pathway in glucose metabolism. It improves the formation of lactate and hydrogen ions. causing metabolic acidosis development (Fig. 3). Thus, on days 7 and 14 after cerebral IR, a significant increase in lactate levels was observed in the somatosensory cortex of rats. Its level averaged 6.5 ± 0.14 μmol/g of dry tissue and 5.9 ± 0.13 μmol/g of dry tissue, respectively, compared to sham-operated animals — 1.6 ± 0.03 μmol/g of dry tissue and 1.5 ± 0.05 μmol/g of dry tissue ($P < 0.05$).

We found that hWJ-MSCs in conditions of brain IR significantly reduced metabolic acidosis development in the somatosensory cortex during the studied periods — the lactate level was 4.6 ± 0.08 μmol/g of dry tissue and 3.6 ± 0.12 μmol/g of dry tissue on days 7 and 14, respectively ($P < 0.05$). HAD-MSCs transplantation had no positive effect on lactate levels in the somatosensory cortex of rats with IR; hence, the average lactate concentration was 6.2 ± 0.11 μmol/g of dry tissue on day 7 after IR and 5.6 ± 0.14 μmol/g of dry tissue on day 14.

The leading cause of brain damage due to stroke is energy deficiency caused by changes in mitochondrial metabolism. Therefore, the next stage of our study was to assess the effect of MSC therapy on mitochondrial dysfunction through the investigation of a key enzyme of the Krebs cycle — SDH activity (Fig. 4). Thus, after cerebral IR in rats a sharp decrease in SDH activity was observed in the somatosensory cortex, both on day 7 (SDH activity was on average 3.1 ± 0.17 μmol/min • mg protein) and day 14 (4.0 ± 0.22 μmol/min • mg protein) in comparison with the sham-operated animals

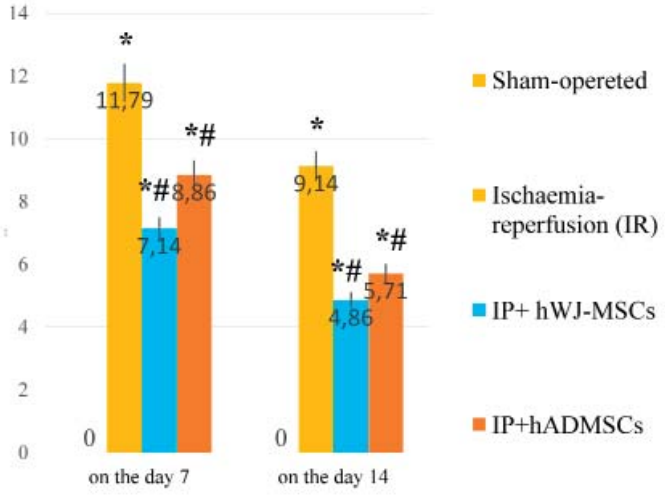

Fig. 2. **Dynamics of neurological deficit in rats of the observed groups in keeping with the McGraw Stroke-index scale**

* — $P < 0.05$ relative to the corresponding group of sham-operated animals;
# — $P < 0.05$ relative to the control group of animals.

in which SDH activity was on average 8.2 ± 0.21 μmol/min • mg protein and 8.4 ± 0.17 μmol/min • mg protein, respectively, (P <0.05) (see Fig. 4). A positive effect of hWJ-MSCs transplantation on SDH activity was noted in the studied periods, which significantly increased the level of SDH activity in comparison with the control animals (on average to 6.1 ± 0.36 μmol/min • mg protein and 7.7 ± 0.14 μmol/min • mg protein (P <0.05). Also, therapy with hWJ-MSCs transplantation was significantly better than hAD-MSCs transplantation, in which SDH activity averaged 3.5 ± 0.25 μmol/min • mg protein on day 7 and 4.3 ± 0.24 μmol/min • mg protein on day 14 (P < 0.05) (Fig. 4).

The implementation of oxidative stress processes occurred against the background of a significant lowering in the antioxidant enzymes activity, such as SOD (Fig. 5), and intensification of free radical oxidation, which induces lipid peroxidation processes. Thus, SOD activity in the somatosensory cortex of rats after IR during the subacute and recovery periods decreased in comparison to the indicators of the sham-operated

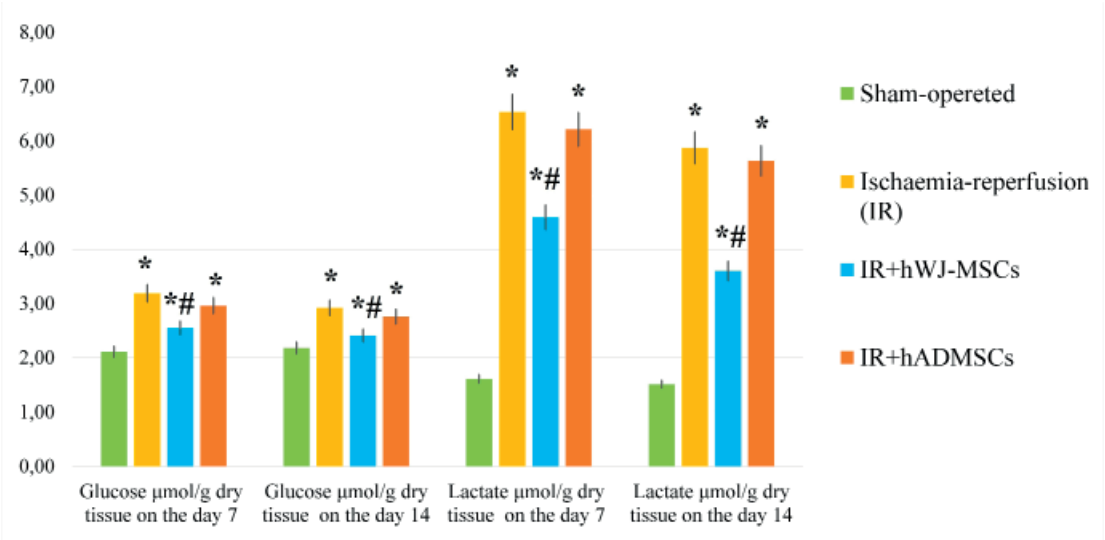

Fig. 3. **Glucose and lactate levels in the somatosensory cortex of the observed rat groups on days 7 and 14 after IR**
* — P < 0.05 relative to the corresponding group of sham-operated animals;
# — P < 0.05 relative to the control group of animals.

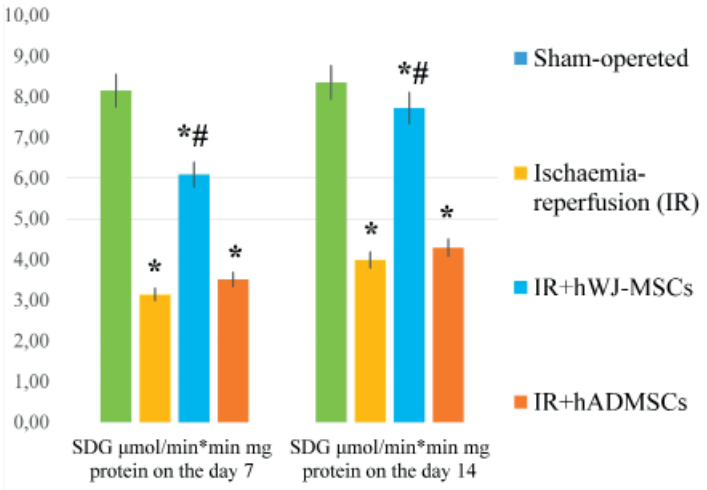

Fig. 4. **SDH activity in the somatosensory cortex of the observed rat groups on days 7 and 14 after IR**
* — P < 0.05 relative to the corresponding group of sham-operated animals;
# — P < 0.05 relative to the control group of animals.

animals and averaged 1.3 ± 0.26 standard units/mg protein on day 7 and 1.7 ± 0.15 standard units/mg protein on day 14, versus 2.7 ± 0.17 standard units/mg protein and 2.8 ± 0.10 standard units/mg protein respectively (P <0.05) (Fig. 5). Cure with hWJ-MSCs transplantation had an assertive effect on the antioxidant enzymes — SOD activity significantly exceeded the corresponding indicators in animals of the control group and amounted to an average of 2.2 ± 0.16 standard units/mg protein on the day 7 and 2.3 ± 0.16 standard units/mg protein on the day 14 (P <0.05). Additionally, hWJ-MSCs transplantation was credibly preferable than hAD-MSCs transplantation, after which SOD activity in the somatosensory cortex was on average 1.2 ± 0.20 standard units/mg protein (day 7) and 1.6 ± 0.08 standard units/mg protein (day 14) (P <0.05) (Fig. 5).

Therapeutic intravenous transplantation of MSCs to rats with cerebral IR reduced lipid peroxidation processes in the somatosensory cortex. Thus, MDA (Fig. 6) levels in rats having IR followed by intravenous hWJ-MSCs transplantation were on average 17.2 ± 1.02 μmol/g of dry tissue on the day 7 and 10.5 ± 0.58 μmol/g of dry tissue on the day 14, that was significantly lower than in the animals with IR receiving 0.9% NaCl solution intravenously — 31.1 ± 0.87 μmol/g of dry tissue and 24.9 ± 0.65 μmol/g of dry tissue respectively (p<0.05). Transplantation of hAD-MSCs was worse than hWJ-MSCs regarding lipid peroxidation reduction in the somatosensory cortex of rats with cerebral IR (Fig. 6).

One of the foremost mechanisms in the protective action of a modern cerebroprotective agent is its corrective effect on nitric oxide metabolism, in particular on the development of nitrosative stress in brain tissues. In the course of our studies it was found that IR in rats leads to growing in the total NOS (Fig. 7) activity in the somatosensory cortex on days 7 and 14 after IR which averages 223.6 ± 9.18 pmol/min · mg protein and 208.6 ± 8.70 pmol/min · mg protein, when in sham-operated animals the total NOS activity averaged 122.6 ± 4.66 pmol/min · mg protein and 121.3 ± 3.90 pmol/min · mg protein respectively, which may indicate hyperproduction of nitric oxide (P < 0.05) (Fig. 7). Treatment of rats using hWJ-MSCs in the subacute and recovery periods of cerebral ischemia had a positive modulating effect on the nitric oxide cycle and was superior to treatment with hAD-MSCs. Thus, during the indicated experimental days (7th and 14th), total NOS activity in the somatosensory cortex of rats transplanted with hWJ-MSCs decreased relative to the control group. It averaged 149.6 ± 5.11 pmol/min · mg protein and 145.0 ± 3.21 pmol/min · mg protein (P < 0.05) (Fig. 7). hWJ-MSCs were better than hAD-MSCs at restoring normal functioning of the nitric oxide system in acute cerebral ischemia in rats (both in the subacute and recovery periods of stroke), which may be one of the foremost mechanisms of its cerebroprotective action in post-reperfusion brain damage.

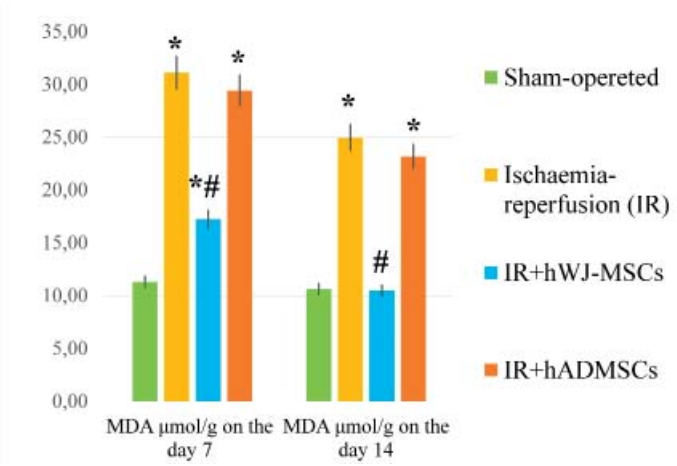

Fig. 5. **SOD activity in the somatosensory cortex of the observed rat groups on days 7 and 14 after IR**
* — P < 0.05 relative to the corresponding group of sham-operated animals;
# — P < 0.05 relative to the control group of animals.

Discussion

The pronounced cerebroprotective activity of hWJ-MSCs was demonstrated during their therapeutic application in conditions of IR modelling in rats. Thus, in the capability to reduce the mortality rate in the critical period of the research, hWJ-MSCs were superior to hAD-MSCs of rats with IR. An integrative indicator that allows for assessing the quality of the protective effect in cerebral ischemia is, along with a decline in mortality, the rapid disappearance of neurological deficit.

K.J. Wu et al. showed that intracerebral transplantation of hWJ-MSCs significantly reduced neurological deficit manifestations in rats on days 3 and 5 baft middle cerebral artery occlusion [16]. In another study made by H. Cao et al., it was found that intracerebral transplantation of human umbilical cord MSCs 24 hours after middle cerebral artery occlusion at a dose of 1×10^6 cells/animal credibly reduced the indexes of neurological deficit in rats [17].

This is consistent with the outcomes of our study in rats with acute cerebral IR, which, along with significant mortality, developed severe neurological deficits within the first 2 days. Experimental cell therapy in rats with the IR model contributed not only to a reduction in mortality, but also in

Fig. 6. **MDA levels in the somatosensory cortex of the observed rat groups on days 7 and 14 after IR**
* — $P < 0.05$ relative to the corresponding group of sham-operated animals;
\# — $P < 0.05$ relative to the control group of animals.

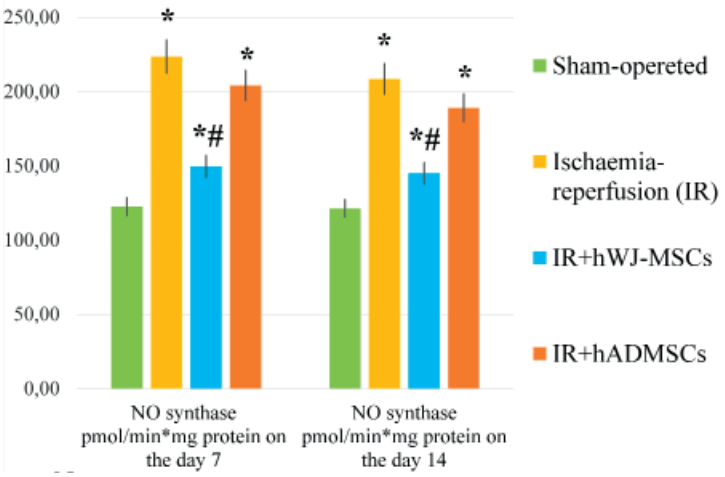

Fig. 7. **NOS activity in the somatosensory cortex of the observed rat groups on days 7 and 14 after IR**
* — $P < 0.05$ relative to the corresponding group of sham-operated animals;
\# — $P < 0.05$ relative to the control group of animals.

the manifestations of neurological disorders. HWJ-MSCs exerted the best modelling effect on neurological deficit.

Variations in paracrine factors of different MSC populations contribute to varying levels of regenerative activity. hWJ-MSCs or hAD-MSCs have different effects on CNS cell populations; thus, hWJ-MSCs have a better influence on the metabolic viability of somatosensory cortex neurons in rats. Therefore, during our study, an increment in the levels of glucose, lactate, MDA, total NOS activity, and a decrease in the activity of SDH and SOD were found in the somatosensory cortex of rats on days 7 and 14 after brain IR. A burst of free radicals and reactive oxygen species accompanies reperfusion. Free radicals' appearance next to blood vessels is one of the agents in reperfusion-induced damage by increasing blood-brain barrier permeability [18, 19]. At the same time, the liver, as the central metabolic organ, contributes not only to immunosuppression after stroke, but also to stress-induced hyperglycemia [20]. Systemic hyperglycemia associated with infarction may promote glucose entry into ischemic brain tissue due to injury to the blood-brain barrier [21].

It is known from literature that lipid peroxidation processes occur mainly in the membrane structures of neurons, which, with a sharp decrease in the function of the antioxidant defence system and appearance of uncompensated acidosis, inevitably leads to disruption of cytoarchitectonics, interneuronal connections, and death of a neuron as a structural unit of the CNS [22]. Therefore, intensive therapy of IR damage by hWJ-MSCs transplantation exhibited the most potent antioxidant effect, as evidenced by the ability to eliminate imbalance in the enzyme pro- and antioxidant systems and by slowing down of lipoperoxidation processes.

Conclusions

Twenty minutes of transient cerebral ischemia-reperfusion in rats, induced by ligation of the internal carotid arteries, was accompanied by substantive metabolic disorders in the somatosensory cortex in the mode of energy imbalance and appearance of lactic acidosis, nitrosative and oxidative stress, leading to severe neurological deficits and death of the experimental animals.

The complex mechanism of hWJ-MSCs' cerebroprotective action in acute cerebrovascular accident is associated with abolition of energy deficiency, metabolic acidosis, oxidative damage to neurons, affirmative effect on nitric oxide metabolism, which had a normalizing impact on the neurological status and increased survival of rats.

Intravenous transplantation of hWJ-MSCs to rats with cerebral ischemia-reperfusion contributed to better stabilization of neurological changes than hAD-MSCs, as well as increased survival of experimental animals, restored disturbed energy processes, and had a modulating effect on nitrosative and oxidative stress in the somatosensory cortex.

Prospects for further research

Results of the study substantiate the feasibility of creating a new medication for the therapy of ischemic stroke. The data obtained can be used to seek new ways to treat ischemic-reperfusion injury of the brain.

Compliance with ethical requirements

When executing the study, the authors adhered to the principles of the basic bioethical norms of the Helsinki Declaration adopted by the General Assembly of the World Medical Association, the Council of Europe Convention on Human Rights and Biomedicine (1977), the relevant provisions of the WHO, the International Council of Medical Societies, the International Code of Medical Ethics (1983), the Council of Europe Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of 18.03.1986.

Conflict of interest

The authors declare no conflict of interest.

Authors' contribution to the writing of the article

Konovalov S. V. — planning and conducting the research, analyzing the results, and writing the article; Moroz V. M. — development of the research concept; Yoltukhivskyi M. V. — conducting the research, writing certain sections of the article; Gusakova I. V. — final editing of the article; Stelmashchuk A. O. — design of graphic materials; Deryabina O. G. — writing certain sections of the manuscript; Kordium V. A. — contributed to the development of the research concept

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REFERENCES

1. Feske, S. K. (2021). Ischemic Stroke. *The American journal of medicine*, 134(12), 1457–1464. https://doi.org/10.1016/j.amjmed.2021.07.027
2. Derakhshankhah, H., Sajadimajd, S., Jafari, S., Izadi, Z., Sarvari, S., Sharifi, M., Falahati, M., …, Presley, J., F. (2020). Novel therapeutic strategies for Alzheimer's disease: Implications from cell-based therapy and nanotherapy. *Nanomedicine : nanotechnology, biology, and medicine*, 24, 102149. https://doi.org/10.1016/j.nano.2020.102149
3. Chung, J. W., Chang, W. H., Bang, O. Y., Moon, G. J., Kim, S. J., Kim, S. K., Lee, J. S., …, STARTING-2 Collaborators (2021). Efficacy and Safety of Intravenous Mesenchymal Stem Cells for Ischemic Stroke. *Neurology*, 96(7), e1012-e1023. https://doi.org/10.1212/WNL.0000000000011440
4. Guo, Y., Peng, Y., Zeng, H., Chen, G. (2021). Progress in Mesenchymal Stem Cell Therapy for Ischemic Stroke. *Stem cells international*, 2021, 9923566. https://doi.org/10.1155/2021/9923566
5. Brooks, B., Ebedes, D., Usmani, A., Gonzales-Portillo, J. V., Gonzales-Portillo, D., Borlongan, C. V. (2022). Mesenchymal Stromal Cells in Ischemic Brain Injury. *Cells*, 11(6), 1013. https://doi.org/10.3390/cells11061013
6. Semenova, E., Grudniak, M. P., Machaj, E. K., Bocian, K., Chroscinska-Krawczyk, M., Trochonowicz, M., Stepaniec, I. M., …, Rozwadowska, N. (2021). Mesenchymal Stromal Cells from Different Parts of Umbilical Cord: Approach to Comparison & Characteristics. *Stem cell reviews and reports*, 17(5), 1780–1795. https://doi.org/10.1007/s12015-021-10157-3
7. Patel, A. A., Mohamed, A. H., Rizaev, J., Mallick, A. K., Qasim, M. T., Abdulmonem, W. A., Jamal, A., …, Ahmad, F. (2024). Application of mesenchymal stem cells derived from the umbilical cord or Wharton's jelly and their extracellular vesicles in the treatment of various diseases. *Tissue & cell*, 89, 102415. https://doi.org/10.1016/j.tice.2024.102415
8. Konovalov, S., Moroz, V., Konovalova, N., Deryabina, O., Shuvalova, N., Toporova, O., Tochylovsky, A., Kordium, V. (2021). The effect of mesenchymal stromal cells of various origins on mortality and neurologic deficit in acute cerebral ischemia-reperfusion in rats. *Cell Organ Transpl.*, 9(2), 104–108. https://doi.org/10.22494/cot.v9i2.132
9. Chekman, I. S., Byelenichev, I. F., Nahorna, O. O., Horchakova, N.O., Luk'yanchuk, V. D., Bukhtiyarova, N. V., Horbachova, S. V., Syrova, H.O. (2016). Preclinical study of the specific activity of potential drugs of primary and secondary neuroprotection. Methodological recommendations. Kyiv.
10. Mihara, M., Uchiyama, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical biochemistry*, 86(1), 271–278. https://doi.org/10.1016/0003-2697(78)90342-1
11. Baylyak, M. M., Semchyshyn, H. M., Lushchak, V. I. (2006). The participation of catalase and superoxide dismutase in the response of Saccharomyces cerevisiae to the action of hydrogen peroxide in the exponential growth phase. *Ukr. biochem. journal*, 78(2), 79–85.
12. Gula, N. M., Kosyakova, G. V., Berdyshev, A. G. (2007). The effect of N-stearoylethanolamine on the NO-synthase pathway of nitric oxide generation in the aorta and heart of rats with streptozotocin-induced. *Ukr. biochem. journal*, 79(5), 153–158.
13. Han, M., Cao, Y., Xue, H., Chu, X., Li, T., Xin, D., Yuan, L., Ke, H., Li, G., Wang, Z. (2020). Neuroprotective Effect of Mesenchymal Stromal Cell-Derived Extracellular Vesicles Against Cerebral Ischemia-Reperfusion-Induced Neural Functional Injury: A Pivotal Role for AMPK and JAK2/STAT3/NF-κB Signaling Pathway Modulation. *Drug design, development and therapy*, 14, 2865-2876. https://doi.org/10.2147/DDDT.S248892
14. Tang, W., Lv, X., Huang, J., Wang, B., Lin, L., Shen, Y., Yao, Y. (2022). Neuroprotective Effect of Stroke Pretreated Mesenchymal Stem Cells Against Cerebral Ischemia/Reperfusion Injury in Rats. *World neurosurgery*, 165, e1-e11. https://doi.org/10.1016/j.wneu.2021.04.114
15. Pan, K., Peng, Q., Huang, Z., Dong, Z., Lin, W., Wang, Y. (2023). Temporal patterns and distribution of pyroptosis-related molecules and effects of human mesenchymal stem cells on pyroptosis following cerebral ischemia/reperfusion in rats. *Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association*, 32(8), 107199. https://doi.org/10.1016/j.jstrokecerebrovasdis.2023.107199
16. Wu, K. J., Yu, S. J., Chiang, C. W., Lee, Y. W., Yen, B. L., Tseng, P. C., Hsu, C. S., Kuo, L. W., Wang, Y. (2018). Neuroprotective Action of Human Wharton's Jelly-Derived Mesenchymal Stromal Cell Transplants in a Rodent Model of Stroke. *Cell transplantation*, 27(11), 1603–1612. https://doi.org/10.1177/0963689718802754
17. Cao, H., Cheng, Y., Zhang, J., Xu, M., Ge, L. (2020). The Effect of Umbilical Cord Mesenchymal Stem Cells Combined with Tetramethylpyrazine Therapy on Ischemic Brain Injury: A Histological Study. *Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association*, 29(12), 105298. https://doi.org/10.1016/j.jstrokecerebrovasdis.2020.105298

18. Ju, F., Ran, Y., Zhu, L., Cheng, X., Gao, H., Xi, X., Yang, Z., Zhang, S. (2018). Increased BBB Permeability Enhances Activation of Microglia and Exacerbates Loss of Dendritic Spines After Transient Global Cerebral Ischemia. *Frontiers in cellular neuroscience*, 12, 236. https://doi.org/10.3389/fncel.2018.00236
19. Liu, Q., Sorooshyari, S. K. (2021). Quantitative and Correlational Analysis of Brain and Spleen Immune Cellular Responses Following Cerebral Ischemia. *Frontiers in immunology*, 12, 617032. https://doi.org/10.3389/fimmu.2021.617032
20. Inderhees, J., Schwaninger, M. (2024). Liver Metabolism in Ischemic Stroke. *Neuroscience*, 550, 62–68. https://doi.org/10.1016/j.neuroscience.2023.12.013
21. Song, K., Li, Y., Zhang, H., An, N., Wei, Y., Wang, L., Tian, C., Yuan, M., Sun, Y., Xing, Y., Gao, Y. (2020). Oxidative stress-mediated blood-brain barrier (BBB) disruption in neurological diseases. *Oxidative Medicine and Cellular Longevity*, ID 4356386, 1–27. https://doi.org/10.1155/2020/4356386
22. Chavda, V., Lu, B. (2023). Reverse Electron Transport at Mitochondrial Complex I in Ischemic Stroke, Aging, and Age-Related Diseases. *Antioxidants (Basel, Switzerland)*, 12(4), 895. https://doi.org/10.3390/antiox120408

ТРАНСПЛАНТАЦІЯ МЕЗЕНХІМАЛЬНИХ СТРОМАЛЬНИХ КЛІТИН ПРИ ЕКСПЕРИМЕНТАЛЬНІЙ ГОСТРІЙ ОБОРОТНІЙ ЦЕРЕБРАЛЬНІЙ ІШЕМІЇ (ПОРІВНЯЛЬНИЙ АНАЛІЗ)

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Питання лікування цереброваскулярних порушень є дуже важливим через їх широку поширеність у людській популяції, особливо у людей похилого віку. Ішемія тканин мозку призводить до смертності, агресивної поведінки, біохімічних та морфологічних змін у мозку. Кореляційний аналіз дозволяє оцінити статистичний зв'язок між двома випадковими величинами або двовимірними даними. За останні роки набуло особливого значення дослідженнч нейропротекторних властивостей мезенхімальних стромальних клітин (МСК). Трансплантація стовбурових клітин при ішемічному інсульті є одним із шляхів сучасної регенеративної стратегії в лікуванні цієї патології.

Мета. Проаналізувати кореляцію між біохімічними показниками, визначеними в соматосенсорній корі та гіпокампі, морфологічними проявами нейроапоптозу та параметрами функціонування ЦНС при гострій ішемії головного мозку у щурів після трансплантації МСК.

Методи. 20-хвилинна двостороння церебральна ішемія-реперфузія у щурів. Експериментальним тваринам внутрішньовенно вводили мезенхімальні стромальні клітини з Вартонових драглів пуповини людини (hWJ-MSCs) або мезенхімальні стовбурові клітини жирової тканини дорослих людей (hAD-MSCs). Стан щурів оцінювали за динамікою смертності, неврологічним дефіцитом та біохімічними показниками через 7 та 14 днів після операції.

Результати. Смертність після трансплантації hWJ-MSCs становила 10% проти 65% у контрольній групі та 32% у групі щурів, які отримували hAD-MSCs. На 7-й день значення за шкалою McGraw становили 7,1 ± 0,19/8,9 ± 0,23/11,8 ± 0,48 балів у щурів, яким вводили hWJ-MSCs/hAD-MSCs/фізіологічний розчин; на 14-й день ці показники були 4,9 ± 0,15/ 5,7 ± 0,23/ 9,1 ± 0,30 балів, відповідно. Трансплантація мезенхімальних стромальних клітин усунула енергетичний дефіцит в ішемізованій тканині мозку щурів, зменшила метаболічний ацидоз та оксидативне пошкодження нейронів, а також позитивно вплинула на метаболізм оксиду азоту, але hAD-MSC були менш ефективними.

Висновки. Трансплантація hWJ-MSC мала кращий терапевтичний ефект, ніж трансплантація hAD-MSC.

Ключові слова: соматосенсорна кора, ішемія-реперфузія, мезенхімальні стромальні клітини, вартонівське желе, жирові стовбурові клітини, біохімічні параметри.

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INTEGRATIVE MORPHO-MOLECULAR ANALYSIS OF *Papilio polytes*, *Papilio polymnestor*, AND *Euploea core* FROM JHARKHAND (INDIA) USING ADVANCED BIOTECHNOLOGICAL AND BIOINFORMATIC APPROACHES

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Plants and butterflies have a coevolutionary relationship, and butterflies are essential to the ecosystem. They serve as ecosystem indicators as well since research on their populations and behaviour can reveal how healthy an environment is. They are efficient pollinators, and particular species have been known to migrate great distances to spread pollen, which causes genetic variety in plant species and increases their chances of surviving.

Aim. This work aimed to study the morpho-molecular features of *Papilio polytes, Papilio polymnestor,* and *Euploea core* from the Jharkhand state of India.

Methods. The study spans different areas from five districts, viz. Chatra, Koderma, Giridih, Godda, Ramgarh of the state. The specimens were collected, examined, and physical specimens of each species were submitted to the Insect Collection, Record and Identification, of the Department of Zoology, St. Xavier's College, Ranchi, and Voucher numbers were obtained. Modern biotechnological and bioinformatic tools were used in this work, five specimens of each species were sequenced for the mitochondrial cytochrome oxidase subunit 1 (CO1), on the basis of which the BLAST (Basic Local Alignment and Search Tool) search was performed for identification of the species on the basis of matching scores with sequences present in the nucleotide sequence databases.

Results. The latest biotechnologies were used. After identification, the sequences were submitted to GenBank, and accession IDs were obtained. The sequences were used to prepare a phylogenetic tree to ascertain the relationships among the collected specimens.

Conclusions. There is a paucity of knowledge related to the morphology and taxonomy of butterflies of the state; thus, this study is the first attempt of its kind. The study revealed significant intraspecific variation among specimens of *Euploea core*. The least variation was exhibited among specimens of *Papilio polymnestor*. The study contributes to the knowledge related to butterfly species of *Papilio polytes*, *Papilio polymnestor*, *and Euploea core*. It will be helpful in further studies related to the conservation and monitoring of the species.

Key words: morpho-molecular, Jharkhand, India, *Papilio polytes, Papilio polymnestor, Euploea core,* BLAST, phylogeny.

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Butterflies are keystone species that act as driver species [1] in the context of ecosystem services as pollinators. Mostly, some anthropogenic activity and, to some extent, also the natural result in habitat loss, the spread of invasive species, pathogens, and climate change. Pollinator populations, including the butterflies, have been declining in many parts of the world [2–4]. Consequently, the decline in pollinators has raised concerns about the consequences of the loss of one of the most critical ecosystem services, i.e., pollination [5]. Pollination, without doubt, is a vital ecosystem service that is accomplished through the involvement of multiple species of pollinators, among which insects like moths and butterflies share a notable contribution [6]. As per an estimate [7], the value of crop pollination on a global level in economic terms happens to be over €153 billion annually. More than 75% of crops, particularly fruits and vegetables, are dependent, to a considerable extent, on pollinators in order to provide optimized yield [5]. Having perceived that the pollinator species with a significant share of butterflies and moths, in the context of our global economy and food production, are essential ecosystem service providers [8], it is not only surprising but also alarming that there is a paucity of knowledge on the rapidly declining pollinator populations and vanishing of different species [8, 9].

The status of pollinators as a whole and butterflies in particular has attracted much attention from their identification and conservation viewpoint. More than 30 countries are members of Promote Pollinators, the Coalition of the Willing on Pollinators (initiated at the CBD CoP 13), committing themselves to take action to identify and conserve pollinators.

O'Connor et al. [10] reported the unavailability of data on declining population and community change for many taxonomic groups, including butterflies. This underlines the gap in knowledge pertaining to proper identification and subsequent efforts of conservation. The leading cause behind the gaps is that monitoring biodiversity is a task [4, 11, 12] that requires information from gene to species and ecosystem level. Biodiversity conservation demands, at its first step, the identification of the species with the genetic variation within the species. The situation is not different in this region. Butterflies of this region have not been adequately identified. Most of the information on butterflies from this region (Jharkhand) is either a survey or preliminary reports, lacking any morphological study and taxonomical observation [13–16]. For instance, in 2023, Kumar and Keshari prepared a checklist of the variety of butterflies encountered at Bhagwan Birsa Biological Park in Ormanjhi, Ranchi, Jharkhand. They enlisted *Papilio polytes, Papilio polymnestor,* and *Euploea core* among 86 butterfly species that belong to six families.

In contrast to their methods, which used a random encounter approach and involved taking pictures of butterflies, the whole paper lacked even a single photograph of the reported butterflies, including *Papilio polytes, Papilio polymnestor,* and *Euploea core*. Mahto et al. [17] studied the status of diversity and conservation of Rhopalocera in urban Ranchi. Osga et al. [18] studied the diversity of butterfly species in a work that was limited to the campus of St. Xavier's College, Ranchi.

Considering the paucity of authentic information on taxonomy and phylogeny of the region, we have initiated a project exploiting the latest biotechnological and bioinformatic tools for identification of the butterflies of this region based on morphological, anatomical, as well as DNA bar coding based morpho-molecular approach; so based on the latest biotechnologies.

The present communication is a part of the project stated above, and deals with morphological and molecular identification of *Papilio polytes* (*Papilio*nidae), *Papilio polymnestor* (*Papilio*nidae), and *Euploea core* (Nymphalidae) collected from different sites spread along the Jharkhand State of India.

Materials and Methods

Butterfly Sampling

Various parts of the Jharkhand state (Table 1) of India were surveyed for the collection of butterfly samples. Several butterflies were captured and released after preliminary identification based on study of traditional morphological characteristics of wings, locale, and other information as per the butterfly identification keys [19, 20]. In this work, the latest biotechnologies were used to rule out any error in the identification of morphologically similar (cryptic) species. For genetic level identification, the COI gene is preferred due to its high interspecific variation leading to species-level resolution, maternal inheritance, and universal primer compatibility. It also exhibits negligible intraspecific variation. This barcode gap makes it ideal for distinguishing species, thus for genome

Table 1

Details of sampling sites, date of survey, GPS coordinates of Sampling sites

Sp.	District	Site Code	Locality	GPS Co-ordinates (N/E)	Date	Voucher No. (SXCRAN-ENT-)
Papilio polytes	Chatra	A	Kathautia Talab	24.20585802, 84.85986758	04 Apr, 2024	0424-S17A
	Koderma	B	Raja Talab	24.46980312, 85.59268970	10 Apr, 2023	0423-S17B
	Giridih	C	S.S. U. Children Park	24.180694277, 86.30340222	26 Mar, 2023	0323-S17C
	Godda	D	Biodiversity Park	24.79002746, 87.220366	1 May, 2024	0524-S17D
	Ramgarh	E	Gadh Baba Mandir	23.63961972, 85.52425085	20 Apr, 2024	0424-S17E
Papilio polymnestor	Chatra	F	Nawi Talab	24.202032144, 84.87149307	4 May, 2023	0523-S18A
	Koderma	B	Raja Talab	24.469585704, 85.59092695	6 May, 2024	0524-S18B
	Giridih	G	Pampoo Talab	24.183487830, 86.31396327	9 June, 2023	0623-S18C
	Godda	H	Godda Park	24.839396892, 87.21427081	27 Apr, 2023	0423-S18D
	Ramgarh	I	Radha Rani Van	23.60687392, 85.5150253	4 June, 2024	0624-S18E
Euploea core	Chatra	J	Puraniya Talab	24.21051887, 84.87502824	10 Apr, 2024	0424-S19A
	Koderma	K	Koderma Town Station	24.465371166, 85.61008921	19 Apr, 2023	0423-S19B
	Giridih	L	Shashtri Nagar	24.1963680, 86.30239845	28 Mar, 2023	0323-S19C
	Godda	M	Biodiversity Park	24.7904938, 87.22146098	3 May, 2024	0524-S19D
	Ramgarh	N	Bijuliya Talab	23.620302331, 85.51636776	20 Apr, 2024	0424-S19E

(COI) sequence analysis. At least one specimen from each sampling site was preserved in 70% alcohol for DNA (deoxyribonucleic acid) extraction, PCR (Polymerase Chain Reaction) amplification, and sequencing [21]. Two to three specimens from every survey site, after being sacrificed under the ethyl acetate fumes, were pinned and spread for further reference. One representative of each specimen was submitted to the ICRI (Insect Collection, Record and Identification), Entomology Section, Department of Zoology, St. Xavier's College, Ranchi, and Voucher numbers were obtained (Table 1).

Morphological Investigations

Morphological cues were used to describe and record each sample's sex and colour pattern. Sex was investigated based on the wing markings [19, 20, 22].

DNA extraction, PCR amplification, and sequencing

A butterfly specimen's hind limb was used for extracting DNA. A 1.0% agarose gel was used to evaluate the quality. There was only one band of high-molecular-weight DNA visible. Using specific forward and reverse primers, fragments of the mitochondrial cytochrome c oxidase subunit I (COI) gene were amplified. There was only one identifiable PCR amplicon band visible when the sample was resolved on an agarose gel. The length of the sequences that were extracted from each sample varied. To get rid of contaminants, the PCR amplicon was subjected to further purification. The BDT v3.1 Cycle sequencing kit was used on an ABI 3730xl Genetic Analyser (ThermoFisher, 2024) to sequence the PCR amplicon using forward and reverse primers [23].

Molecular sequence analysis tools

The latest biotechnological tools were used, unique PCR amplicon was obtained by the Sanger sequencing method. The NCBI's (National Centre for Biotechnology Information) BLAST (Basic Local Alignment Search Tool) software was used to compare the resultant PCR amplicon with information found in nucleotide databases. The option of nucleotide blast (BLASTn) was used. The nucleotide query (our sequence) and the subject sequence (data from databases) are compared by BLASTn. The following parameters were used when running BLASTn: Program selection: highly similar sequences (megablast); search set: standard database. Sequences can be identified and compared within species using Megablast [24].

Submission of nucleotide sequence to GenBank

Based on the biotechnology of the BLASTn search results, the butterfly specimens were identified as *Papilio polytes, Papilio polymnestor, and Euploea core*. Additionally, accession IDs for nucleotide sequences were acquired after the COI nucleotide sequences were submitted to GenBank and the DNA Data Bank of Japan (DDBJ).

Phylogenetic Tree and Distance Matrix

Phylogenetic tree and Distance Matrix were obtained using other novel biotechnological and bioinformatic tools. The 15 sequences (5 from each species) derived from the COI nucleotide sequences of the three species collected from various survey sites restricted to the five districts (Chatra, Koderma, Giridih, Godda, and Ramgarh) of the state of Jharkhand were used to create the distance matrix and phylogenetic tree. The evolutionary distances (matrix), which are expressed in units of base substitutions per site (Tamura et al., 2004), were constructed using the Maximum Composite Likelihood approach [25]. In this study, ten nucleotide sequences were employed. All unclear sites were removed (pairwise deletion) for each pair of sequences.

Other biotechnological and bioinformatic tools were used for determining the evolutionary history. The evolutionary history was derived using the Maximum Likelihood method and the Kimura 2-parameter model [26]. The initial tree for the heuristic search was automatically created by applying the Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances determined using the Maximum Composite Likelihood approach. Next, we selected the topology with the highest log likelihood value. In this study, fifteen nucleotide sequences were employed. These analyses were performed using MEGA X (Molecular Evolutionary Genetics Analysis) software, version 10.2.6, build 10210527-x86_64 (Windows 11) [24, 27, 28].

Results and Discussion

Morphological Investigations

Papilionidae

The *Papilionidae* family of butterflies includes the swallowtail and Parnassian species. This is a family of big, vibrant butterflies. They vary in size from 60 to 180 mm. The genus Ornithoptera includes the largest butterflies in the world, the birdwing butterfly. From behind the hind wing, many swallowtail butterflies have long tails or extensions [29-31].

Papilio polytes

Commonly referred to as "Common Mormon," butterflies belonging to the *Papilio polytes* family come in three female forms: cyrus, stichius, and romulus, with only one male. Males are black with a white stripe across each hindwing and white specks along the outer margin of the forewing. The majority of the adult female *Papilio polytes*' polymorphism mimics that of other distasteful butterfly species. The cyrus form, which is present throughout the typical Mormon's entire range, resembles males but has paler colouring and more pronounced red crescents. Common Mormon populations from the Himalayas to Japan, Sulawesi, and Sri Lanka are home to the most prevalent stichius form, which resembles the typical rose (*Pachliopta aristolochiae*). The dull-coloured romulus form is similar to the Crimson rose (*Pachliopta hector*). This predator escape technique is an example of Batesian mimicry since *Papilio polytes* mimics unpleasant butterfly species and is palatable [21, 32, 33].

Wing venation of *Papilio polytes*

Forewing: *Papilio polytes* has a big, triangular forewing. Costa has a somewhat arched posture. Dc (discal cell), which is more than half the length of the wing, is closed. The Sc (subcostal) vein is located at the costal edge. R2 (radius 2) originates behind the Dc. It ends at the apex, while R1 (radius 1) emerges at one half of the costal edge near Sc. R3 (radius 3) forms an arch at the apex, emerging from the Dc's anterior corner.

Papilio polytes (female) *Papilio polytes (male)*

Fig. 1. **Photograph of Dorsal Side of specimen of *Papilio polytes***

Papilio polytes (female) *Papilio polytes (male)*

Fig. 2. **Photograph of Ventral Side of specimen of *Papilio polytes***

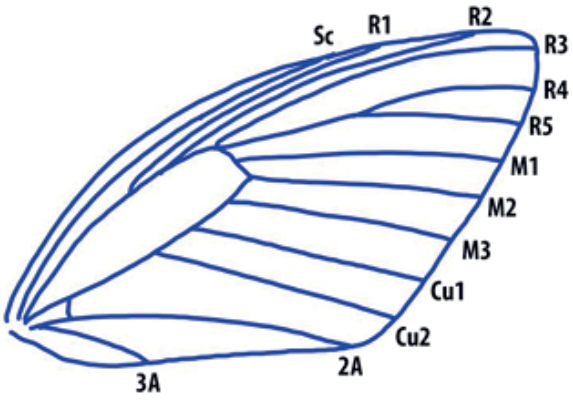

Fig. 3. **Venation in the forewing of *Papilio polytes***

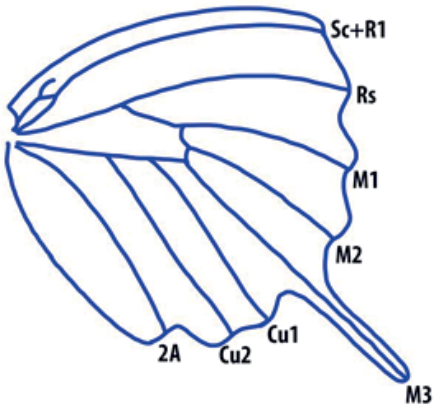

Fig. 4. **Venation in the hindwings of *Papilio polytes***

From a similar vein that emerges from the tip of Dc at the source of R3, R4 (radius 4), and R5 (radius 5) contact the wing's margin. The lower apex of Dc is where M1 (median 1), M2 (median 2), and M3 (median 3) originate, and each of the three median veins has a roughly equal length. Starting independently from the lower portion of Dc, the Cu1 (cubitus 1) and Cu2 (cubitus 2) reach the wing edge close to the tornus. Anal vein 2A (anal 2) emerges from the back and ends at the tornus' edge. 3A (anal 3) is extremely short, starting at the base and ending at the dorsum near the wing border.

Hindwing: The hindwing resembles something of a fan. DC is closed, and the coastal margin is somewhat arched. The humoral vein is located at the base's anterior-most point. Sc and R1 combine to form Sc+R1, which emerges from the base and travels parallel to the costa to the apex, or wing margin. The anterior sector of Dc is the origin of the radial sector vein (Rs), which ends at the anterior part of the termen at the wing's border. Both M1 and M2 emerge from Dc's terminal end and arrive at the margin at the term. The M3 vein lengthens along the wing margin to create a protrusion that resembles a tail. From the bottom portion of Dc, Cu1 and Cu2 emerge, reaching the wing

Fig. 5. **Photograph of Dorsal Side of specimen of *Papilio polymnestor***

Fig. 6. **Photograph of Ventral Side of specimen of *Papilio polymnestor***

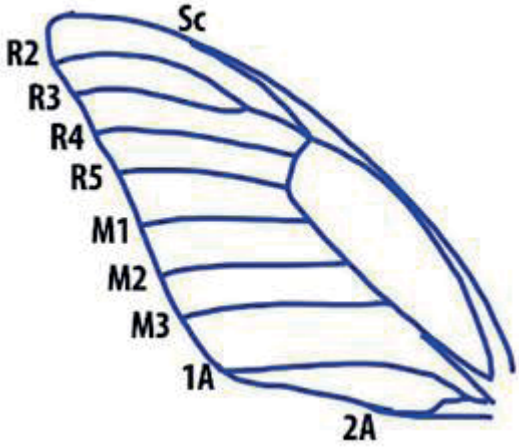

Fig. 7. **Venation in the forewings of *Papilio polymnestor***

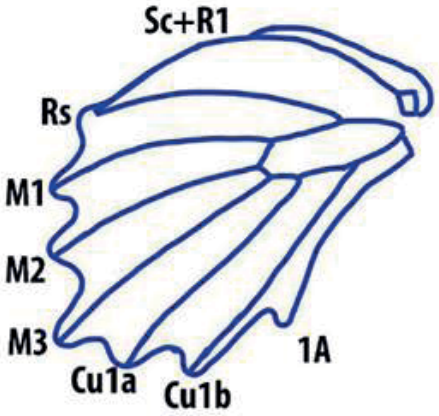

Fig. 8. **Venation in the hindwings of *Papilio polymnestor***

margin near the tornus. 2A rises from the wing's base to the tornus, which is the wing's edge.

Papilio polymnestor, often known as Blue Mormons, are giant swallowtail butterflies that are found in Sri Lanka, Southern India, and Northeastern India [34]. It is the state butterfly of Maharashtra, an Indian state. Between 120 and 150 mm is its wing span. It is the fourth-largest butterfly in India. The wings at the back have a glistening blue tint. In addition to the buff-coloured female of Sri Lanka's *Papilio polymnestor parinda* Moore, it has several characteristics with the latter. A few preliminary reports of it have been made from the state of Jharkhand [13-16], but none of them have included morphological, molecular, or documentation (photographs, sketches, sample preservation, etc.) research.

Wing venation of *Papilio polymnestor*

Forewing: The forewing contains twelve veins, as well as a vast, closed discal cell from which numerous veins radiate. The base is where the first and last of the 12 veins emerge, while the discal cell is where the others appear. Subcostal (Sc) joins the R5 vein on the forewing. There are five branches of the radial veins (R1–R5). The lower discal is the origin of the median veins (M1–M3). The last vein is the anal vein, which contains two branches, 1A and 2A, followed by the cubital veins, Cu1a and Cu1b.

Hindwing: Sc+R1 (fused) is the result of the first radial vein in the hind wing fusing with the subcostal. The term "radial sector" refers to the unbranched radius (Rs). There are three branches of the median vein (M) (M1-M3). There are cubital veins Cu1a and Cu1b. There is only one anal vein (1A). The Humeral vein is a little spur that extends towards the costa close to the base of the eighth vein.

Fig. 9. **Photograph of Dorsal Side of specimen of *Euploea core***

Fig. 10. **Photograph of Ventral Side of specimen of *Euploea core***

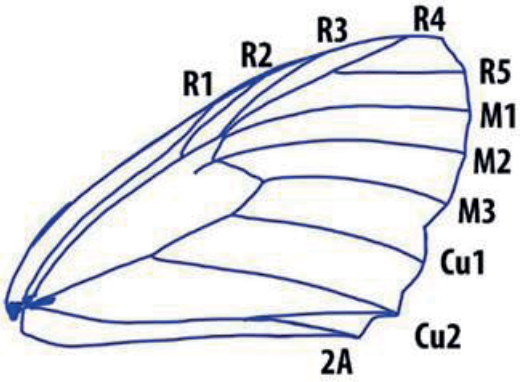

Fig. 11. **Venation in the forewings of *Euploea core***

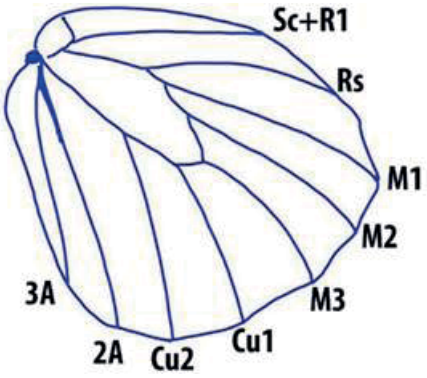

Fig. 12. **Venation in the hindwings of *Euploea core***

Nymphalidae

Two groups in this family are very different from one another in appearance. While the undersides of the fritillaries frequently have silver markings, the upper surfaces are orange and feature black markings. The other contains some of the brightest-coloured butterflies [19, 20, 28].

Euploea core

The medium-sized butterfly *Euploea core*, also called the "Common Crow," is a member of the Nymphalidae family. It has a glossy black appearance with white dots on the wing edges. The range of the wingspan is 85 to 95 mm. The female's forewing has a straight hind border, but the males have a bow-shaped one. Australia and South Asia are home to it. It is also occasionally called the "Common Indian Crow" in India and the "Australian Crow" in Australia.

Wing venation of *Euploea core*

Forewing: The *Euploea* core's forewing has a roughly triangular form. The Dc is closed. From the base of the wing, R1 arises to the edge in the centre of the costa. R2 emerges from Dc's anterior end and travels to the costa. The anterior terminal end of Dc is where R3 and R4 originate. R3 contacts the wing margin shortly before the apex, while R4 reaches the wing margin at the apex. R5 emerges from R4 as a branch and reaches the edge immediately below the wing's apex. The terminal end of Dc is where M1, M2, and M3 originate.

M2 enters the Dc for a very brief distance. The posterior portion of Dc gives rise to anterior Cu1 and posterior Cu2, to reach the wing's edge close to the tornus. As a shared vein, 2A and 1A emerge from the wing base and split apart near the wing's tornus, just short of the margin. At the margin, the 2A and Cu2 meet.

Hindwing: The rear wing of the *Euploea* core resembles a fan and achieves an oval-triangular form. The anterior-most vein, the humeral vein, begins at the base of the wing and ends close to the base at the wing's border. Sc and R1 combine to create Sc+R1, which emerges from the base of Dc and meets the wing edge close to the apex. The near-anterior tip of Dc is where Rs, M1, and M2 originate from. At the top, Rs satisfies the margin.

Table 2

Nucleotide frequencies and NT length of CO1 nucleotide region of the butterfly samples collected from Different parts of Jharkhand

Site Code	District	Butterfly species	Accession Ids	Nucleotide Frequencies, %				
				T(U)	**C**	**A**	**G**	**Total**
A	Chatra	*Papilio polytes*	PQ565876	40.1	15.6	29.8	14.5	704
B	Koderma		PQ565875	40.3	15.4	30.1	14.2	837
C	Giridih		PQ565873	40	16	29.8	14.3	658
D	Godda		PQ565874	40	16	29.8	14.3	658
E	Ramgarh		PQ565877	39.6	16.7	29.8	14	642
F	Chatra	*Papilio polymnestor*	PQ569085	39.7	16.2	30.3	13.8	630
B	Koderma		PQ569086	39.6	16.5	29.9	14	642
G	Giridih		PQ569087	39.7	16.3	30.1	13.9	638
H	Godda		PQ569088	38.9	16.3	30.7	14.1	596
I	Ramgarh		PQ569089	38.8	16.4	30.7	14.1	596
J	Chatra	*Euploea core*	LC849501	42.7	14	28.8	14.5	708
K	Koderma		LC849500	41.8	15.1	28.9	14.2	650
L	Giridih		LC849499	43.1	13	29.3	14.6	583
M	Godda		LC849498	42.9	14.4	28.9	13.9	613
N	Ramgarh		LC849497	42.9	14.3	28.9	14	630

The margin is met at the term by M1 and M2. M3 emerges from the Dc tip to reach the wing margin in the middle of the period. Cu1 emerges from the Dc's near-posterior end to meet the wing margin at the termen.

The 2A extends from two-thirds of the lower DC's length to the wing border at the termen above the tornus. The base of the wing is where anterior 2A and posterior 3A originate. Tornus is where 2A touches the wing's margin, whereas dorsum is where 3A touches the wing's margin.

Molecular analysis

DNA extraction, PCR amplification, and Nucleotide Sequencing

The process of verifying an organism's identity at the molecular level is known as molecular identification **[35].** One way for identifying an organism is DNA barcoding, which uses a brief DNA fragment from a particular gene to identify an organism. To determine the organism, the DNA sequence is compared to a reference library [36]. For example, the cytochrome c oxidase I (COI) gene is utilised for animals, the internal transcribed spacer (ITS) rRNA gene is utilised for fungi, and ribulose bisphosphate carboxylase/ oxygenase (RuBisCO) is utilised for plants **[37].** In this study, the COI gene sequence is used for DNA barcoding (identification of organisms at the molecular level).

Using a particular primer, a single distinct PCR amplicon of the COI region was obtained. Table 2 lists the specifics of the sequences that were extracted from the 15 specimens from the three species — *Papilio polytes, Papilio polymnestor, and Euploea core.* Using the NCBI (National Centre for Biotechnology Information)-based BLASTn program, all of the resulting nucleotide sequences (query) were compared with the nucleotide databases. The BLASTn search parameters were: program selection: highly comparable sequences (megablast); search set: standard database. The megablast is employed for intraspecies comparison and sequence identification **[38, 39].** The findings of the BLASTn similarity search were used for the identification of the specimens at the molecular level.

After identification on the basis of BLASTn search results, the 15 sequences obtained in this study were submitted to DDBJ (DNA Databank of Japan) and bankit to obtain accession IDs (Table 2). By looking up the accession IDs using the ENTREZ search engine, one can view the whole definition of CO1 sequences for each of the 15 sequences online. Additionally, each sequence's link has been provided with its QR (quick response) code, which is represented

as Fig. 13 (*Papilio polytes*), Fig. 14 *(Papilio polymnestor*), and Fig. 15 (*Euploea core*). The QR codes can be scanned to view the complete definition of the sequence online.

Distance Matrix and Phylogenetic Tree

Phylogenetic tree and Distance Matrix *were* obtained using other novel biotechnological and bioinformatic tools described below. The MEGAX software's ClustalW alignment function was used to align the 15 sequences, using a gap opening penalty of 15.00 and a gap extension penalty of 6.66. Following that, the alignment was exported in MEGA format. The distance matrix was subsequently created using this alignment (Table 3). There are many applications for distance matrices in bioinformatics, including phylogenetic tree construction, protein structure representation, protein structure comparison and alignment, inferring protein-protein interactions, and protein structure determination. A distance matrix is a two-dimensional array that contains the pairwise distances between a set of elements (in this case, nucleotide sequences) [40, 41]. The distance between two people, or the number of loci that separate them, is measured by pairwise distance. The more species separate from one another, the more unrelated they are, or the greater the distance matrix value, the more variety there is between any two species **[28].** In the distance matrix presented as Table 3, the value of pairwise distance ranged from 0.00 to 0.175.

Among the samples of *Papilio polytes*, the matrix score ranged from a minimum of 0.000 (close relationship) to a maximum of 0.017 (distant relationship). These ranging values indicated the intraspecific variation among the populations of *Papilio polytes* of the sampling regions. The specimen collected from Giridih was very closely related to the sample from Godda (0.00). The most distant relation was between *Papilio polytes* collected from Chatra and Godda; Chatra and Giridih, with a distance matrix score of 0.009 in each case.

Fig. 13. **Accession IDs and QR codes for accessing the full definition (online) of CO1 sequences of *Papilio polytes* submitted to GenBank**

Fig. 14. **Accession IDs and QR codes for accessing the full definition (online) of CO1 sequences of *Papilio polymnestor* submitted to GenBank**

Fig. 15. **Accession IDs and QR codes for accessing the full definition (online) of CO1 sequences of *Euploea core* submitted to DDBJ**

Table 3

Distance Matrix prepared using the 15 different sequences of *Papilio polytes, Euploea core* and *Papilio polymnestor* to estimate the evolutionary divergence between sequences

		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
Giridih_*P.polytes*_PQ565873	I		0.000	0.003	0.017	0.009	0.160	0.159	0.148	0.145	0.168	0.081	0.081	0.081	0.079	0.078
Godda_*P.polytes*_PQ565874	II	0.000		0.003	0.017	0.009	0.160	0.159	0.148	0.145	0.168	0.081	0.081	0.081	0.079	0.078
Koderma_*P.polytes*_PQ565875	III	0.003	0.003		0.016	0.007	0.164	0.166	0.162	0.144	0.175	0.074	0.076	0.074	0.073	0.070
Chatra_*P.polytes*_PQ565876	IV	0.017	0.017	0.016		0.001	0.155	0.155	0.162	0.137	0.161	0.080	0.082	0.080	0.079	0.077
Ramgarh_*P.polytes*_PQ565877	V	0.009	0.009	0.007	0.001		0.155	0.155	0.150	0.138	0.156	0.080	0.079	0.079	0.079	0.077
Ramgarh_*E.core*_LC849497	VI	0.160	0.160	0.164	0.155	0.155		0.000	0.000	0.144	0.000	0.158	0.158	0.158	0.163	0.165
Godda_*E.core*_LC849498	VII	0.159	0.159	0.166	0.155	0.155	0.000		0.000	0.140	0.000	0.159	0.159	0.159	0.162	0.164
Giridih_*E.core*_LC849499	VIII	0.148	0.148	0.162	0.162	0.150	0.000	0.000		0.141	0.010	0.160	0.160	0.160	0.160	0.163
Koderma_*E.core*_LC849500	IX	0.145	0.145	0.144	0.137	0.138	0.144	0.140	0.141		0.141	0.132	0.134	0.134	0.127	0.125
Chatra_*E.core*_LC849501	X	0.168	0.168	0.175	0.161	0.156	0.000	0.000	0.010	0.141		0.158	0.159	0.160	0.162	0.164
Chatra_*P.polymnestor*_PQ569085	XI	0.081	0.081	0.074	0.080	0.080	0.158	0.159	0.160	0.132	0.158		0.000	0.000	0.000	0.002
Koderma_*P.polymnestor*_PQ56908(	XII	0.081	0.081	0.076	0.082	0.079	0.158	0.159	0.160	0.134	0.159	0.000		0.000	0.000	0.002
Giridih_*P.polymnestor*_PQ569087	XIII	0.081	0.081	0.074	0.080	0.079	0.158	0.159	0.160	0.134	0.160	0.000	0.000		0.000	0.002
Godda_*P.polymnestor*_PQ569088	XIV	0.079	0.079	0.073	0.079	0.079	0.163	0.162	0.160	0.127	0.162	0.000	0.000	0.000		0.002
Ramgarh_*P.polymnestor*_PQ56908(	XV	0.078	0.078	0.070	0.077	0.077	0.165	0.164	0.163	0.125	0.164	0.002	0.002	0.002	0.002	

Among the samples of *Euploea core,* the distance matrix score ranged from a minimum of 0.000 to a maximum of 0.144. A very close relationship was exhibited between samples collected from Ramgarh & Godda; Ramgarh & Giridih; Ramgarh & Koderma; Godda & Giridih, Godda & Chatra, exhibiting a matrix score of 0.000. The distant relationship was shown among the specimens collected from Koderma and Ramgarh, with the highest matrix score of 0.144 among *Euploea core.*

Least variation was observed among samples of *Papilio polymnestor*, with a minimum of 0.000 to a maximum score of 0.002; this exhibits lower intraspecific variation among populations of *Papilio polymnestor* collected from the different sampling sites. The six pairs — Koderma & Chatra, Giridih & Chatra, Godda & Chatra, Giridih & Koderma, Godda & Koderma, Godda & Giridih exhibited very close relationships with a matrix score of 0.00.

Regarding interspecific variance, the matrix score varied between 0.070 and 0.175. The number of base substitutions between sequences is displayed for each location. The Maximum Composite Likelihood model was used for the analyses **[25].** For every pair of sequences, all ambiguous locations were eliminated (pairwise deletion). The final dataset contained 986 locations in total **[27].**

A figure that shows the evolutionary descent lines of various species, creatures, or genes from a common ancestor is called a phylogenetic tree, or phylogeny. Phylogenies are helpful for structuring classifications, organising knowledge of biological diversity, and shedding light on evolutionary events. Since Charles Darwin's time, tree diagrams have been employed in evolutionary biology**. [**42].

The Neighbor-Joining approach was used to estimate the evolutionary relationships of taxa [43]. The branches are accompanied by the proportion of duplicate trees where the related species clustered together in the bootstrap test (100 repetitions) [44]. The evolutionary distances are shown in terms of the number of base substitutions per site and were calculated using the Maximum Composite Likelihood approach [25]. Fifteen nucleotide sequences were analysed. In MEGA X, evolutionary studies were carried out [27]. Figure 16 displays the resultant phylogenetic tree.

As exhibited by the phylogenetic tree, the *Papilio polytes* from Giridih, Godda, and Koderma fell in a separate clade, and Chatra and Ramgarh fell in another clade. In the case of *Papilio polymnestor*, the sample from Koderma and Giridih fell in a separate clade as sister taxa. Interestingly, the samples of *Papilio polytes* and *Papilio polymnestor*, both species have a common genus under which fall the two species *polytes* and *polymnestor,* this is visible from the phylogenetic tree, which shows that both have originated from a common ancestor. The sample of *Euploea core* collected from Chatra showed most intraspecific variation, which is also exhibited by a high matrix score of 0.175.

Conclusions

Morpho-molecular studies on *Papilio polytes*, *Papilio polymnestor*, and *Euploea core* collected from various sampling sites from

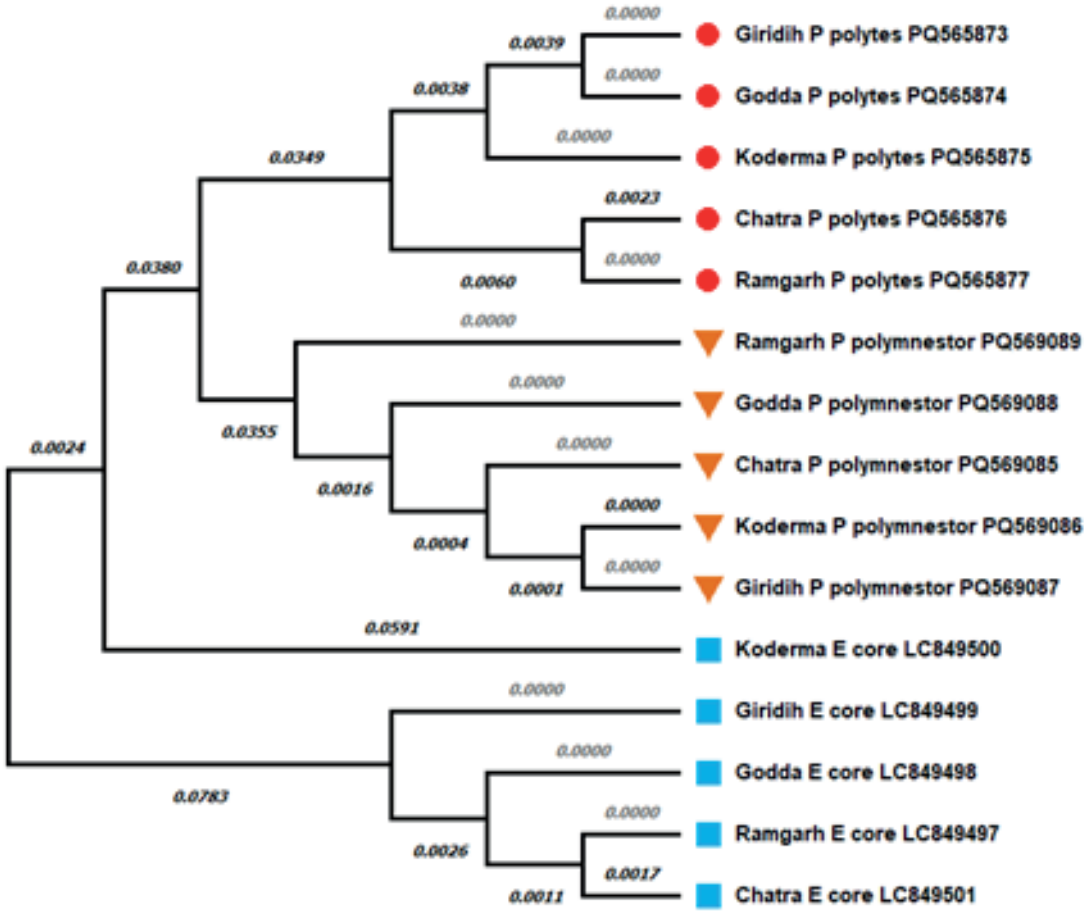

Fig. 16. **Phylogenetic tree prepared using 15 different CO1 sequences of the *Papilio polytes, Euploea core* and *Papilio polymnestor***

Koderma, Giridih, Godda, Ramgarh, and Chatra districts of the Jharkhand state of India were done. The study involved the use of the latest biotechnological and bioinformatic tools. There is a paucity of knowledge related to the morphology and taxonomy of butterflies of the state; thus, this study is the first attempt of its kind. The study revealed significant intraspecific variation among specimens of *Euploea core*. The least variation was exhibited among specimens of *Papilio polymnestor*. The study contributes to the knowledge related to butterfly species of *Papilio polytes*, *Papilio polymnestor*, *and Euploea core*. It will be helpful in further studies related to the conservation and monitoring of the species.

Authors' Contributions

Conceptualization: Kumar M, and Sinha MP; Data curation: Kumar M, and Ranjan R,; Formal analysis: Pratik R, Tirkey S, and Kumar A; Methodology: Kumar M, Hembrom T, Raipat BS; Project administration and Supervision: Sinha MP, and Raipat BS; Software: Kumar M; Writing original draft: Kumar M, Ranjan R, Sinha MP; Writing — review and editing: Raipat BS, Pratik R, Tirkey S, Kumar A, and Hembrom T. All authors read and approved the final manuscript.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

Ethical approval

Not Applicable.

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REFERENCES

1. Walker, B. H. (1995). Rangeland Ecology: Managing Change in Biodiversity. In: Perrings, C. A., Mäler, K. G., Folke, C., Holling, C. S., Jansson, B. O. (Eds.) *Biodiversity Conservation. Ecology, Economy & Environment*, v. 4. *Springer*, Dordrecht. https://doi.org/10.1007/978-94-011-0277-3_5
2. Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O, Kunin, W. E. (2010). Global pollinator declines: Trends, impacts and drivers. *Trends Ecol. Evol.*, 25, 345–353. https://doi.org/10.1016/j.tree.2010.01.007
3. Goulson, D., Nicholls, E., Botía, C., Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science,* 347, 1255957. https://doi.org/10.1126/science.1255957
4. Bergholz, K., Sittel, L. P., Ristow, M., Jeltsch, F., Weiss, L. (2022). Pollinator guilds respond contrastingly at different scales to landscape parameters of land-use intensity. *Ecol. Evol.,* 12, e8708. https://doi.org/10.1002/ece3.8708
5. Klein, A. M., Vaissiere, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. B Biol. Sci.*, 274, 303–313. https://doi.org/10.1098/ rspb.2006.3721. https://doi.org/10.1098/rspb.2006.3721
6. Rader, R., Bartomeus, I., Garibaldi, L .A., Garratt, M. P. D., Howlett, B. G., Winfree, R., ….Woyciechowski, M. (2016). Non-bee insects are important contributors to global crop pollination. *Proc. Natl. Acad. Sci.*, 113(1), 146–151. https://doi.org/10.1073/pnas.1517092112
7. Gallai, N., Salles, J. M., Settele, J., Vaissiere, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecol. Econ.*, 68, 810–821. https://doi.org/10.1016/j.ecolecon.2008.06.014
8. Sharma, S., Mansotra, D. K., Joshi, P. C. (2020) Role of butterflies in shaping an ecosystem: why to protect them?. *Ecology and Biodivdersity*, 39–44.
9. Segre, H., David, K., Ignasi, B., Michiel, F., Mark, J., Maarten, F.S., Jacinto, R., Thijs, P. M. F. (2023). Butterflies are not a robust bioindicator for assessing pollinator communities, but floral resources offer a promising way forward. *Ecological Indicators,* 154, 110842. https://doi.org/10.1016/j.ecolind.2023.110842
10. O'Connor, R. S., Kunin, W. E., Garratt, M. P. D., Potts, S. G., Roy, H. E., Andrews, …, Carvalheiro, L. (2019). Monitoring insect pollinators and flower visitation: The effectiveness and feasibility of different survey methods. *Methods Ecol. Evol.*, 10(12), 2129–2140. https://doi.org/10.1111/2041-210X.13292
11. Mandelik, Y., Roll, U., Fleischer, A. (2010). Cost-efficiencyof biodiversity indicators for Mediterranean ecosystems and the effects of socio-economic factors. *J. Appl. Ecol.*, 47, 1179–1188. https://doi.org/10.1111/j.1365-2664.2010.01864.x .
12. Breeze, T. D., Alison, P. B., Kelvin, G. B., Tom, B., …. Claire, C. (2020). Pollinator monitoring more than pays for itself. *J. Applied Ecology,* 58, 44–57. https://doi.org/10.1111/1365-2664.13755
13. Singh, N., Ahmad, J. (2017). A preliminary list of lepidopteran insects from Palkot Wildlife Sanctuary, Jharkhand. *Journal of Entomology and Zoology Studies*, 5(3), 654–661.
14. Kumari, K., Oraon, P. K., Kumari, K., Kumari, A., Banra, S., Thakur, A. K. (2021). Inventory report of butterflies (Lepidoptera: Rhopalocera) of family Nymphalidae, Pieridae and *Papilionidae* at Kanke, Ranchi. *Biospectra*, 16(2), 169–174.
15. Kumari, K., Thakur, A. K. (2022). Studies on the biodiversity of some families of butterflies (Lepidoptera: Rhopalocera) at Kanke, Ranchi, Jharkhand, India. *Biospectra*, 17(1), 93–98.
16. Kumar, M., Ranjan, R., Kumar, A., Pratik, R., Hembrom, T., Tirkey, S. (2025). Current studies on butterfly conservation in Jharkhand: *A Data-Oriented Analysis. Vat-Vriksha*. 1(1): OM03. https://doi.org/10.5281/zenodo.15834007 https://doi.org/10.5281/zenodo.12533671
17. Mahto, D., Osga, J., Khan, M. A., Kunkal, R., Herenj, S., Minz, P A., Pratap, R., Mahto, S., Masarat, F. (2023). Diversity and conservation of Rhopalocera in urban Ranchi. *In Jeune Chercheur* (Volume II): etudes en Entomologie. Ed. Manoj Kumar, Notion Press.
18. Osga, J., Kumar, M., Mahto, D., Minz, P. A., Herenj, S., Kunkal, R. L., Mahto, S., Khan, M., …., Raipat, B. S. (2025). Butterflies from the St. Xavier's College (Ranchi) campus: A Checklist. *Vat-Vriksha,* 1(1), OM06. https://doi.org/10.5281/zenodo.16572855
19. Kunte, K. (2006). *India-A lifescape: Butterflies of Peninsular India.* Universities Press. Hyderabad, India.
20. Smetacek, P. (2022). *A naturalist's guide to the Butterflies of India, Bangladesh, Nepal, Pakistan and Sri Lanka.* John Beaufoy Publishing Ltd.
21. Kumar, M., Ranjan, R., Sinha, M. P., Dhan, A., Naaz, F., Khanum, G., Rani, K.A., Sharma, S., Raipat, B.S. (2022). A review on insect

collection and preservation techniques. *European Journal of Pharmaceutical and Medical Research*, 9(7), 233–239. https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4171417
22. Mehra, D., Kirti, J. S., Sidhu, A. K. (2018). A taxonomic study of six species of the genus *Junonia* Hubner, [1819] (Insecta: Lepidoptera: Nymphalidae) from the northwestern Himalayan region in India. *Journal of Threatened Taxa*, 10(7), 11934–11947. https://doi.org/10.11609/jott.3731.10.7.11934-11947
23. ThermoFisher (2024): Applied Biosystems 3500 Dx Series Genetic Analyzer (CE-IVD, IVDR). Retrieved on 25th May, 2024; 08, 02 (IST). url: https://www.thermofisher.com/ie/en/home/life-science/sequencing/sanger-sequencing/sanger-sequencing-technology-accessories/applied-biosystems-sanger-sequencing-3500-series-genetic-analyzers/3500-series-genetic-analyzer/3500-dx-cs2-series-genetic-analyzer.html
24. Srivastava, R., Kumar, M., Ranjan, R., Dandapat, S., Sinha, M. P. (2024). Morpho-molecular taxonomy and bioinformatics based phylogeny of *Metaphire posthuma* (Sims and Easton, 1972). *The Bioscan*. 19(1), 45–52. https://dx.doi.org/10.2139/ssrn.4847732
25. Tamura, K., Nei, M., Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*), 101, 11030–11035. DOI: https://doi.org/10.1073/pnas.0404206101
26. Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120. https://doi.org/10.1007/BF01731581
27. Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution,* 35, 1547–1549. https://doi.org/10.1093/molbev/msy096
28. Manoj, K., Anshu, P., Rakesh, R., Manoranjan, P. S., Bharti, S. R. (2024). Morphological study and DNA barcoding of *Junonia atlites* collected from Jharkhand state of India. *International Journal of Entomology Research*, 9(11), 1–7. https://dx.doi.org/10.2139/ssrn.5096390
29. Rajeswari, N. B., Jeyabalan, D. (2017). Studies on biology and reproduction of butterflies (family: *Papilio*nidae) in Nilgiri Hills, Southern Western Ghats, India. International *Journal of Advanced Research in Biological Sciences*, 4(2), 1–11. http://dx.doi.org/10.22192/ijarbs.2017.04.02.001
30. Layberry, R.A., Hall, P.W., Lafontaine, J.D. (1998). *The butterflies of Canada.* University of Toronto Press, Toronto. https://doi.org/10.3138/9781442623163
31. Kioko, E., Musyoki, A. M., Kioko, M. D., Mwangi, E. W., Monda, L (2020). Swallowtail butterflies (Lepidoptera: *Papilio*nidae) species diversity and distribution in Africa: The *Papilio*nidae collection at the National Museums of Kenya, Nairobi, Kenya. *Biodiversity Data Journal,* 8, e50664. https://doi.org/10.3897/BDJ.8.e50664
32. Kunte, K. (2013). "Research: Molecular genetics of mimicry in the *Papilio polytes* butterfly» (On-line). *Biodiversity Lab. Accessed May* 28. URL: http://biodiversitylab.org/index/index.php/research/84-research/84-Papilio-polytes-mimicry-genetics.
33. Rangana, N. (2010). "Common Mormon" (On-line). *Butterflies of Sri Lanka.* Accessed February 10, 2012 URL: http://www.wildreach.com/butterflies/Papilio_polytes.php.
34. Kumar, M., Sahu, D., Kujur, A., Ehsan, H., Hembrom, L., Sinha, N., …. Raipat, B. S. (2024). First distributional record of the rare blue Mormon (*Papilio polymnestor*) from Gumla district (Jharkhand, India). *European Journal of Pharmaceutical and Medical Research*, 11(6), 195–196. https://doi.org/10.5281/zenodo.15528085
35. Antil, S., Abraham, J. S., Sripoorna, S., Maurya, S., Dagar, J., Makhija, S., Bhagat, P., …, Toteja, R. (2022). DNA barcoding, an effective tool for species identification: a review. *Wiley Oneline Library*, 50(1), 761–775. https://doi.org/10.1155/2014/406178
36. Kress, W. J., Erickson, D. L. (2008). DNA barcodes: Genes, genomics, and bioinformatics. *PNAS,* 105(8), 2761–2762. https://doi.org/10.1073/pnas.0800476105
37. Mampang, R. T., Auxtero, K. C. A., Caldito, C. J. C., Abanilla, J. M., Santos, G. A. G., Caipang, C. M. A. (2023). DNA barcoding and its applications: A Review. *Uttar Pradesh Journal of Zoology*, 44(20), 69–78. https://doi.org/10.56557/upjoz/2023/v44i203646
38. Zheng, Z., Scott, S., Lukas, W., Webb, M. (2000). A greedy algorithm for aligning DNA sequences. *J. Comput. Biol.*, 7(1–2), 203–214. https://doi.org/10.1089/10665270050081478
39. Aleksandr, M., George, C., Yan, R., Thomas, L. M., Richa, A., Schaffer, A. A. (2008). Database indexing for production MegaBLAST searches. *Bioinformatic*, 24, 1757–1764. https://doi.org/10.1093/bioinformatics/btn322
40. Kloczkowski, A., Jernigan, R. L., Wu, Z., Song, G., Yang, L., Kolinski, A., Pokarowski, P.

(2011). Distance matrix-based approach to protein structure prediction. *Journal of Struct. Funct. Genomics.* 10(01), 67–81. https://doi.org/10.1007/s10969-009-9062-2
41. Al-Neama, M., Reda, N. M., Ghaleb, F. F. M. (2014). An improved distance matrix computation algorithm for multicore clusters. *Biomed. Res. Int.*, 2014, 406178. https://doi.org/10.1155/2014/406178
42. Baum, D. (2008). Reading a Phylogenetic tree: Meaning of monophyletic groups. *Nature Education,* 1(1), 190. https://www.nature.com/scitable/topicpage/reading-a-phylogenetic-tree-the-meaning-of-41956/
43. Saitou, N., Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454
44. Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution,* 39, 783–791. https://doi.org/10.2307/2408678

МОРФО-МОЛЕКУЛЯРНІ ДОСЛІДЖЕННЯ ЗРАЗКІВ ОСЕРЕДКУ (?) *Papilio polytes, Papilio polymnestor* ТА *Euploea*, ЗІБРАНИХ У ШТАТІ ДЖАРКХАНД (ІНДІЯ) З ВИКОРИСТАННЯМ БІОТЕХНОЛОГІЧНИХ ТА БІОІНФОРМАТИЧНИХ ІНСТРУМЕНТІВ

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Рослини та метелики мають коеволюційний зв'язок, і метелики є важливими для екосистеми. Вони також служать екосистемними індикаторами, оскільки дослідження їхніх популяцій та поведінки можуть показати, наскільки здоровим є навколишнє середовище. Вони є ефективними запилювачами, і відомо, що деякі види мігрують на великі відстані для поширення пилку, що спричиняє генетичну різноманітність у видах рослин та збільшує їхні шанси на виживання.

Мета. Вивчити морфомолекулярні особливості осередку *Papilio polytes, Papilio polymnestor* та *Euploea* зі штату Джаркханд в Індії.

Методи. Дослідження охоплює різні ділянки п'яти районів, а саме штати Чатра, Кодерма, Гірідіх, Годда, Рамгарх. Зразки були зібрані, досліджені, а фізичні зразки кожного виду були передані до Відділу колекції, обліку та ідентифікації комах кафедри зоології коледжу Святого Ксаверія в Ранчі, та отримані номери ваучерів. У цій роботі були використані сучасні біотехнологічні та біоінформаційні інструменти, п'ять зразків кожного виду були секвеновані на предмет субодиниці 1 (CO1) мітохондріальної цитохромоксидази, на основі чого було виконано пошук за допомогою BLAST (Basic Local Alignment and Search Tool) для ідентифікації виду на основі збігів із послідовностями, присутніми в базах даних нуклеотидних послідовностей.

Результати. Були використані найновіші біотехнології. Після ідентифікації послідовності були передані до GenBank, і були отримані ідентифікатори доступу. Послідовності були використані для побудови філогенетичного дерева для встановлення взаємозв'язків між зібраними зразками.

Висновки. Існує недостатньо знань про морфологію та таксономію метеликів цього штата; тому це дослідження є першою спробою такого роду. Дослідження виявило значну внутрішньовидову варіацію серед екземплярів *Euploea core*. Найменша варіація спостерігалася серед екземплярів *Papilio polymnestor*. Дослідження сприяє розширенню знань про види метеликів *Papilio polytes, Papilio polymnestor* та *Euploea* і буде корисним у подальших дослідженнях, пов'язаних зі збереженням та моніторингом виду.

Ключові слова: морфомолекулярний, Джаркханд, Індія, *Papilio polytes, Papilio polymnestor, Euploea*, BLAST, філогенія.

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REINFORCING STARCH BIOPLASTICS WITH AGRICULTURAL WASTE

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Aim. The study was purposed to find alternative reinforcing fillers for the modification of starch-based bioplastics using agricultural waste.

Materials and Methods. The visual analysis method was used to compare the characteristics of materials with different types of fillers. The influence of various kinds of fillers on the mechanical properties and structure of starch-based bioplastics was evaluated. The study used corn starch according to the DSTU 3976-2000 standard and five different types of agricultural waste as fillers to modify biodegradable plastics. The method of manufacturing the bioplastics included preparing a 10% starch solution, mixing it with other fillers, heating the suspensions to 90 °C, and drying the resulting solutions at 60 °C, depending on the type of filler.

Results. The most successful options were those using technical cellulose fiber and sunflower seed husks compressed into granules. The obtained materials based on these fillers demonstrate better mechanical properties and better shape retention compared to starch-based materials without fillers. The optimal particle size was found to be in the range of 0.03-0.06 mm.

Conclusions. It can be concluded that agricultural vegetable waste has a high potential as an effective filler for starch-based bioplastics, which will significantly reduce the cost of biomaterials and expand the scope of their use, making them more accessible for a wide range of applications.

Key words: bioplastics, agricultural waste, reinforcing fillers, starch, properties.

The development of the bioplastics industry in Ukraine is an urgent issue, especially given the gradual abandonment of synthetic polymeric materials in the world. The lack of affordable foreign materials with reliable providers and national production stimulates further research in this area and the creation of local technologies [1].

The high cost is one of the main hurdles to the widespread use of bioplastics. Today, polylactic acid (PLA) and polyhydroxyalkanoates (PHA) are considered the most promising areas for the development of the biodegradable plastic market. However, the production of these polymers requires more sophisticated technologies than their synthetic counterparts, which significantly increases their cost [2].

Often, for reducing the cost and improving the mechanical properties of biodegradable materials, a certain amount of synthetic fillers is added to their composition. These are usually materials such as polyvinyl chloride or polyethylene. However, these components can negatively affect the biodegradability of polymers [3].

Therefore, the search for simplified technologies for the production of biodegradable materials using fillers of natural origin is relevant today [3].

Starch is one of the most readily available natural polysaccharides used to make biodegradable materials. An essential characteristic of starch-based bioplastics is their elasticity, which is provided by linear amylose, while amylopectin, which has a

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branched structure, controls tensile and elongation strength [4-5].

However, starch-based bioplastics are of limited use due to their insufficient strength. Therefore, modification using vegetable plasticizers, such as glycerin or sorbitol, as well as fillers that increase the mechanical strength and ductility of bioplastics, is necessary. In addition, the modification of hydrophilic OH-groups makes it resistant to moisture. Vegetable agricultural waste can be a promising alternative to synthetic fillers. Due to the high content of lignin and cellulose, these materials can be used as a reinforcing material to increase strength and stiffness for starch-based polymer metals [4].

The research aims to find reinforcing fillers for the modification of starch-based bioplastics using agricultural waste.

Materials and Methods

Materials

In the study of the process of obtaining biodegradable plastic based on starch, we used potato starch, which corresponds to DSTU 4286:2004 Potato starch. Technical conditions. Amended" [6].

To modify starch-based biodegradable plastics to improve their mechanical properties, the use of agricultural waste as a filler was investigated. For the study, five samples of fillers were selected:

– corn waste (dry stalks, leaves, cobs);
– waste peanut husks, crushed;
– sunflower husk pressed into pellets;
– technical fiber cellulose;
– crushed sunflower husk. The choice of specific types of plant waste for use as reinforcing fillers in starch plastic materials was based on their availability, physical properties, and economic advantages. The selected wastes include by-products of typical crops, so they are available in large quantities and have low costs. It is also important to note that obtaining fillers based on such materials can be an alternative method of their utilization and turn waste into a valuable resource [7]. All of these wastes have specific physical properties and chemical composition (high lignin and cellulose content), which can increase the strength and durability of bioplastics. The use of naturally occurring fillers helps to improve the biodegradation of polymeric material [7].

Corn waste (dry stalks, leaves, cobs). In terms of chemical composition, this type of raw material contains about 43% cellulose, 18% lignin, 26% pentosans, 4% inorganic substances, and 15–18% moisture [7, 8].

Peanut husks contain about 37–45% cellulose, 28-35% lignin, and 18–30% hemicellulose. Due to this composition, it is considered a promising raw material for biotechnological applications, in particular in the production of bioplastics, biofuels, and fermented products. To improve the fusion of this type of filler with starch-based biodegradable plastics, it is crushed.

Sunflower husk pellets (produced by Harveles OIL) are compressed cylindrical pellets 5 cm long and about 8 mm in diameter. The moisture content of the pellets does not exceed 8.6% [9].

Crushed sunflower seed husks. The chemical composition of the husk may vary depending on the sunflower variety, with high-oil species having a higher fat and ash content but lower fiber content. Sunflower husk composition: lipid content for different varieties ranges from 0.99 to 3.78%, fiber — 49–67%, of which cellulose — 31–42%, and 14 pentosans — 23–28%. The amount of lignin in the husk is 24–30%, and the proportion of inorganic components is 1.37–2.98% [10].

Fibrous technical pulp was also used as a filler. Cellulose has a fibrous structure and is insoluble in water. It was obtained from vegetable, mechanically processed raw materials by sulfite and alkaline cooking.

Methods

Methods of obtaining biodegradable materials based on starch

The technological properties of starch materials are still inferior to polyethylene and polypropylene, which they could replace. Materials made from starch are pretty fragile and not resistant to temperature or moistureю. However, they can provide the necessary flexibility to other biopolymers. Starch-based polymers can also be used as a separate type of material, but they must be reinforced with fillers to provide mechanical strength and stability [4].

The methodology for producing starch-based bioplastics consists of several steps. First, a 10% starch solution is prepared in a 100 cm^3 flask. 10 g of starch and 60 cm^3 of distilled cold water are mixed, stirred for 15 minutes to ensure uniform distribution of starch in the dispersion medium. Following starch dispersion, 5 cm^3 of glycerol is added, and the volume is brought up to 100 cm^3 with distilled water. The resulting mixture

is subsequently stirred for an additional 5 minutes [5].

The 100 cm^3 of starch was divided into five portions for mixing with the appropriate fillers. To 20 cm^3 of the prepared 10% suspension, 1 g of crushed fillers of various types was added [5].

Next, the resulting suspensions were heated to 90 °C with constant stirring for 25 minutes until a homogeneous solution with slight opalescence was obtained; the filler particles should be evenly distributed in the solution. [5].

The resulting solution was evenly distributed in a 5 mm thick layer on a flat surface and placed in a drying oven preheated to 60 °C. Drying was carried out with different durations for different types of filling: 3.5–6 h. [5]

To evaluate the biodegradation potential of polymeric materials, a vermicomposting method utilizing household organic waste and the earthworm species *Eisenia fetida* was employed. The vermicomposting conditions were maintained within a temperature range of 15–25 °C, a moisture content of 70–80%, and a pH between 7.0 and 7.6.

Starch-based polymeric materials were subjected to the vermicomposting process in order to determine their susceptibility to biodegradation. The results demonstrated a high degree of biodegradability, with complete decomposition of the materials observed within 60 days. The selected filler types demonstrated a high degree of compostability under the established vermicomposting conditions..

Results and Discussion

A polymeric material based on starch was obtained. A visual assessment of the material was carried out. According to which we can say that this material is hard, transparent, very elastic, not viscous, brittle, and easily crushed. The drying time of the material at a temperature of 60 °C is one hour. The main disadvantage observed in comparison with synthetic analogues is low strength and poor shape retention.

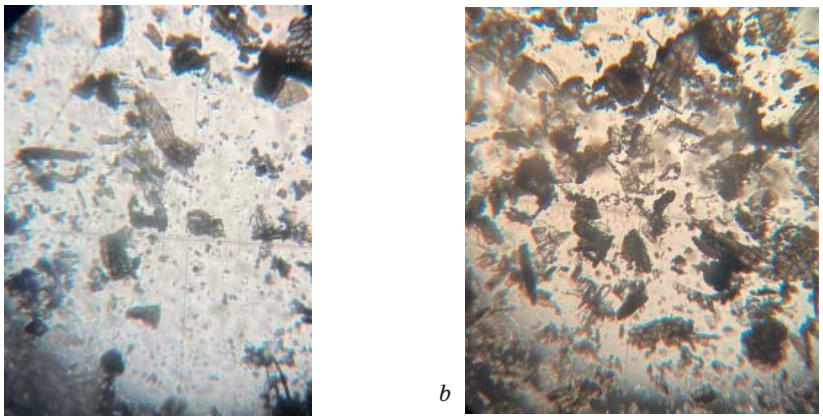
To improve the mechanical properties of the resulting material, crushed materials of plant origin were used. The following results were obtained by grinding different types of fillers.

The first type of raw material, based on corn stalks, leaves, and ears, is well crushed, and the resulting mixture is homogeneous. To determine the size of the ground particles, they were microscoped using a ULAB XSP-137 biological microscope; the results are shown in Fig. 1. The average particle size is 0.03–0.025 mm.

Another type of raw material — crushed peanut husks — is characterized by much better properties during mechanical processing. The degree of grinding is maximized, the resulting mixture is homogeneous, and the particles are easily ground to a powder. The average particle size is 0.05–0.08 mm, and the presence of larger fragments is not observed. To confirm the homogeneity and degree of grinding, the samples were microscoped using a ULAB XSP-137 biological microscope. The results of the study are shown in Fig. 2.

The raw material is based on sunflower husks, which are easily crushed, and a powder with a uniform distribution of particles was obtained.

To determine the size of the crushed particles, they were microscoped using a ULAB XSP-137 biological microscope. The results are shown in Figure 3. The average size of the crushed particles is 0.04–0.07 mm.



a *b*

Fig. 1. **Microscopy of crushed corn waste particles (ULAB XSP-137), ×10 objective and ×10 eyepiece, division price is 0.01 mm**

Sunflower husk pellets with a diameter of 8 mm and a length of 2–5 cm were crushed to improve the fusion of this type of filling with starch-based biodegradable plastic. As a result of grinding, a heterogeneous mixture was obtained. The smallest particles have a size of 0.03–0.05 mm; there are also fibers with a width of 0.002 mm, and the largest particles are within 2–5 mm. To determine the size of the crushed particles, they were microscoped using a ULAB XSP-137 biological microscope; the results are shown in Fig. 4.

The technical fiber pulp was not subjected to additional grinding, as the fiber size in its original form is sufficient for mixing with the polymer material. The fiber thickness was approximately 0.01 mm so that the length could vary significantly. To determine the size of the cellulose fibers, microscopy was performed using a ULAB XSP-137 biological microscope; the results are shown in Fig. 5.

Images of the materials obtained by mixing starch-containing polymer with various types of fillers are shown in Fig. 6.

A visual analysis of the differences in the characteristics of the materials was carried out, and the method of measuring Shore hardness (Shore hardness) was used using a durometer, which measures the ability of a material to resist the penetration of a steel pin into its surface. The following scales were used scale A — to measure the hardness of elastic materials such as rubber or soft plastics. Based on the data obtained, a conclusion was made about the uniformity of particle distribution in the polymer, as well as its hardness, strength, and ability to retain its shape.

Method in accordance with ISO 868-85 Plastics and ebonite. Determination of indentation hardness by durometer (Shore hardness).

Table 1 shows the measured hardness values for each of the samples.

The characteristics of the obtained samples of polymeric materials with different types of fillers are shown in Table 2.

A comparative analysis of starch-based polymeric materials with various plant-based additives revealed significant differences in their structural and mechanical properties, including surface texture, uniformity, and Shore A hardness.

The control sample, consisting of pure starch-based bioplastics without fillers, showed the lowest hardness value (13.87 A). Despite its high elasticity and smooth appearance, its mechanical stability and ability to retain its shape were significantly limited. This is in line with previously reported data on the inherent softness and flexibility of starch-based polymers in the absence of reinforcing agents.

Among the modified composites, the inclusion of sunflower seed husks compressed into pellets resulted in the highest hardness (47.00 A), indicating excellent mechanical strength. However, visual analysis revealed a coarse and granular structure, which, while effective in increasing stiffness, compromises material surface uniformity and elasticity. This filler has proven to be optimal in applications that require structural stability over flexibility.

The sample filled with crushed peanut husks also showed a significant improvement

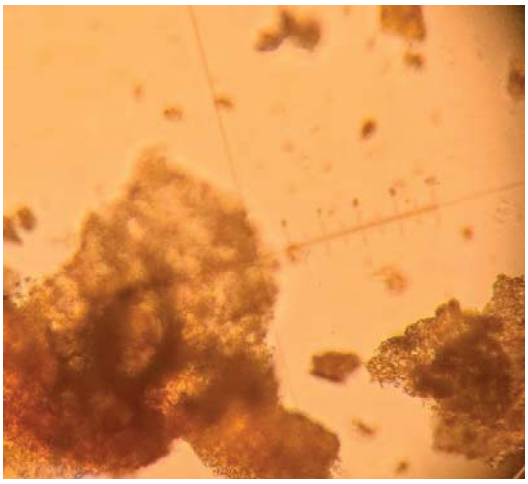


Fig. 2. **Microscopy of crushed peanut husks (ULAB XSP-137), ×10 objective and 10x micrometer eyepiece, division price is 0.01 mm**

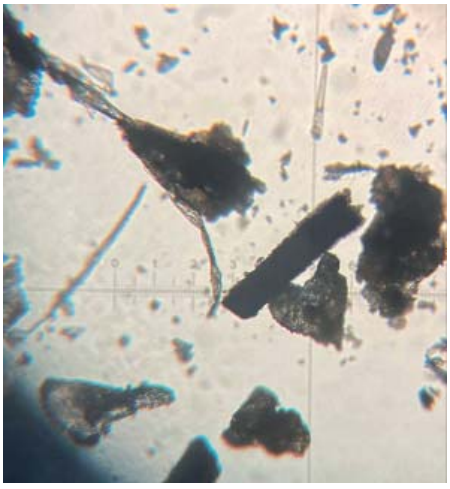


Fig. 3. **Microscopy of crushed sunflower seed husk particles (ULAB XSP-137), ×10 objective and ×10 micrometer eyepiece, division price is 0.01 mm**

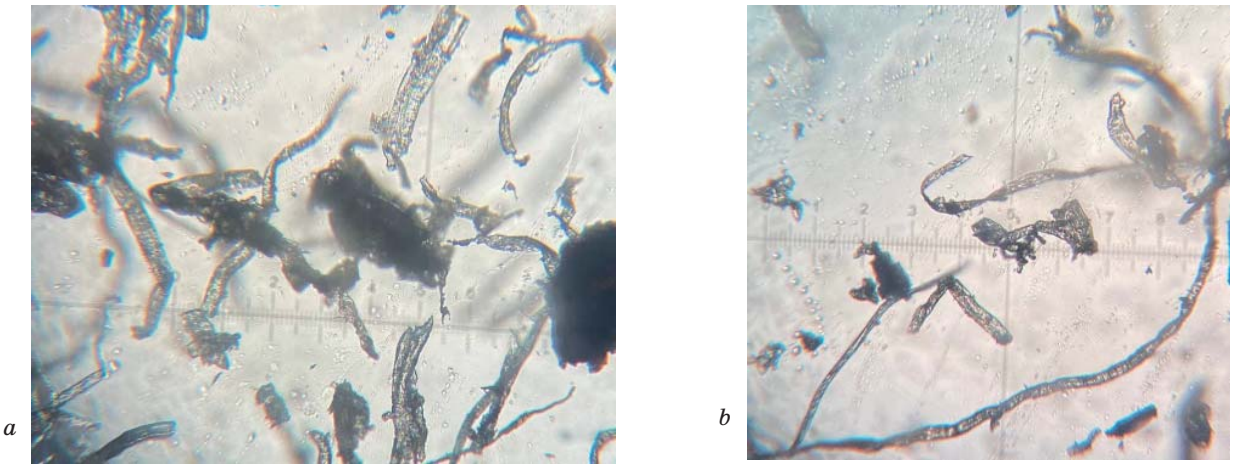


Fig. 4. **Microscopy of particles of crushed sunflower husk pellets (ULAB XSP-137), ×10 objective and ×10 micrometer eyepiece, division price is 0.01 mm**

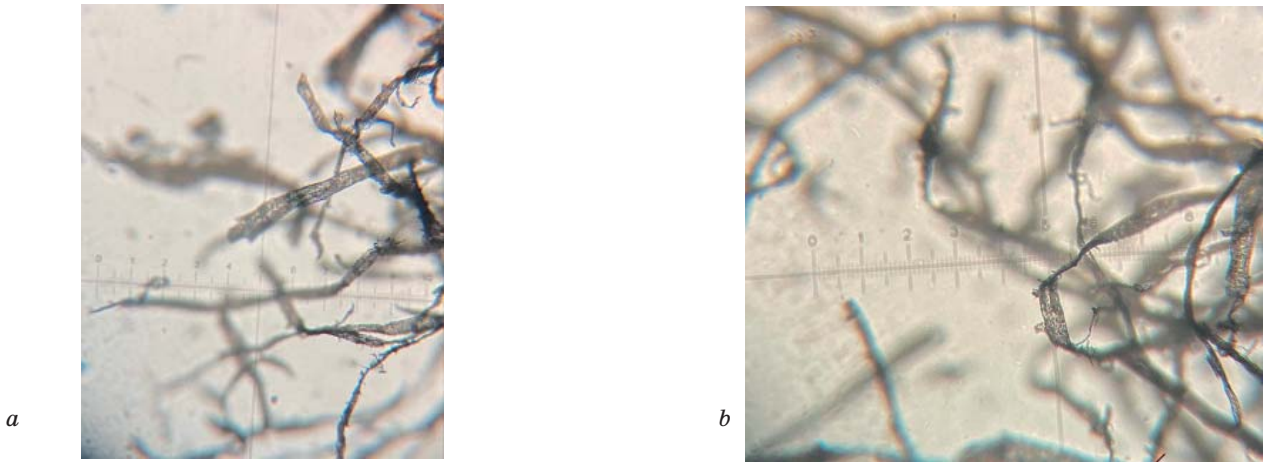


Fig. 5. **Fiber microscopy (ULAB XSP-137), 10× objective and 10× eyepiece, division price is 0.01 mm**

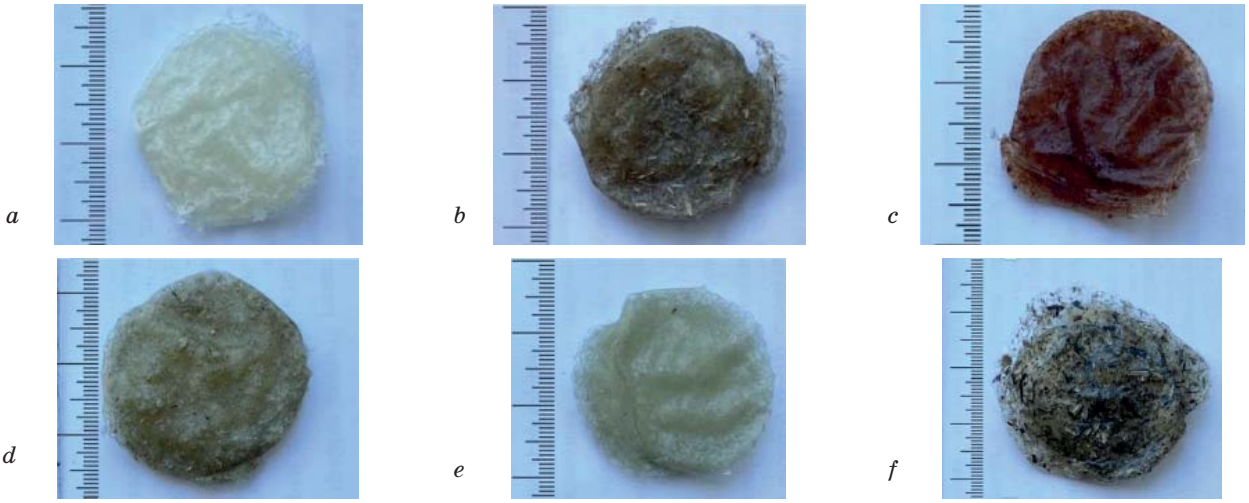


Fig. 6. **The obtained polymeric materials reinforced with different types of fillers based on crushed agricultural waste**

a — starch-based bioplastic without fillers; *b* — corn waste (dry stems, leaves, cobs); *c* — crushed peanut husks; *d* — sunflower husks compressed into granules; *e* — technical cellulose fiber; *f* — crushed sunflower husks

Table 1

Hardness values for each of the samples

Type of filler	Hardness indices in three repetitions. A	Average hardness value
starch-based bioplastic without fillers	16.1	13.87
	10	
	15.5	
corn waste (dry stems. leaves. cobs)	20.5	28.5
	24.5	
	40.5	
crushed peanut husks	35	32.83
	36	
	27.5	
sunflower husks compressed into granules	49.5	47.00
	46.5	
	45	
technical cellulose fiber	15.5	16.5
	18.5	
	15.5	
crushed sunflower husks	39.5	39.5
	42.5	
	36.5	

in hardness (32.83 A) compared to the control. Despite the somewhat uneven distribution of particles, the material retained a relatively balanced structure with acceptable shape retention and reduced brittleness. This indicates that finely ground peanut husks can serve as an effective reinforcing agent, especially in semi-solid biopolymer products.

The corn waste (dry stalks, leaves, and cobs) had a moderate hardness level (28.5 A), a more visually uniform structure, and a smoother surface texture. This filler resulted in a material that successfully combines stiffness and light flexibility, making it suitable for flexible packaging or biodegradable sheets.

In contrast, the samples filled with chopped sunflower husk (39.5 A) and technical cellulose fiber (16.5 A) showed mixed results. While the crushed sunflower husk contributed to the hardness increase, its poor distribution due to the coarse particle size resulted in localized brittleness and poor mold stability. On the other hand, industrial cellulose fiber improves material homogeneity and reduces brittleness, but does not significantly increase mechanical strength.

Conclusions

In the context of the ever-increasing awareness of environmental issues and changes in the global plastic materials market, the development of bioplastics production is becoming an urgent task, especially for countries like Ukraine. The research conducted in this paper aims to find alternative reinforcing fillers for the modification of starch-based bioplastics using agricultural waste.

The study results showed that the use of different fillers significantly affects the mechanical properties of starch bioplastics. The best results in terms of stiffness (47.00 A) and mechanical strength were obtained with sunflower husk granules. However, this filler has a coarse structure, which reduces the elasticity and homogeneity of the material, making it more suitable for applications where mold stability is essential.

Other materials, such as crushed peanut husks (32.83 A) and corn stover (28.5 A), showed moderate results, combining stiffness and elasticity. The crushed peanut husks provided good uniformity and reduced brittleness, while the corn stover was a material that combined flexibility and sufficient stiffness for biodegradable films and packaging.

Cellulose, although it does not provide a significant increase in mechanical strength (16.5 A), improves material uniformity and reduces brittleness. It is a good choice for products where a stable structure is important without high stiffness requirements.

Prospects for further research are to refine technological processes to improve the distribution of particles in the material and to study resistance to temperature, hydrolysis, and biodegradation. This will allow us to expand the use of bioplastics and ensure their stability in various conditions, in particular for the production of durable and environmentally friendly products. Based on our analysis, starch-based bioplastics reinforced with agricultural plant waste show promising potential in the following applications.

Short-term packaging, such as disposable food trays. The material demonstrates sufficient mechanical strength for food contact and is fully compostable, including through vermicomposting.

Table 2

Description of the properties of the obtained starch-based polymeric materials using different types of fillers

Type of filler	Properties of the resulting material	Time. h
Starch-based bioplastics without fillers	Semi-transparent material with a glossy surface, without visible inclusions. Demonstrates good elasticity, but has the lowest hardness among all samples (13.87 A), indicating low shape retention and mechanical instability under deformation.	3.5
Corn waste (dry stalks, leaves, ears);	Translucent yellowish sample with a homogeneous texture. The high degree of grinding ensured a uniform distribution of particles in the polymer. Still, there are particles of different shapes and sizes, which can cause insufficient resistance to deformation. It has a hardness of 28.5 A, which allows it to retain its shape better than bioplastics without fillers, while maintaining partial flexibility.	5.5
crushed peanut husks	The material is dark in color with noticeable particle inclusions. The sample has a hardness of 32.83 A, which indicates sufficient strength and shape retention, The husk particles are evenly distributed, which makes the material somewhat brittle at the edges, but generally holds its shape well.	3.5
Sunflower seed husks are pressed into pellets;	One of the most complex samples (47.00 A). The material is dark, with a rough texture and granular inclusions. The filler particles are embedded in the polymer matrix quite densely, but the insufficient degree of grinding reduces the overall resistance to damage. The material is inelastic but retains its shape very well.	5.5
Technical fiber pulp;	Translucent sample with a fibrous structure. The fibers are well distributed, which reduces brittleness. The material has medium hardness (16.5 A) and is stable in shape. Due to the good distribution of structural particles in the material, it has a good ability to retain its shape. It is suitable for the formation of rigid films that maintain their geometry under load.	5
Crushed sunflower seed husks;	A material with a high level of hardness (39.5 A) but an uneven distribution of particles. The surface is rough, with noticeable inclusions of husks. As a result, the sample does not retain its shape well under load and is prone to deformation. Low elasticity and increased brittleness reduce functionality in flexible applications.	4

Agricultural use, including biodegradable seedling pots and mulching films. After fulfilling their function, these materials can be left in the soil, where they naturally decompose without leaving harmful residues.

Authors' contribution

Kozar M.Y. — data collection and original draft preparation; Korneliuk O.A. — investigation, formal analysis. The authors have read and agreed to the published version of the paper.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Ranakoti, L., Gangil, B., Mishra, S.. K., Singh, T., Sharma, S., Ilyas, R.A. (2022). El-Khatib S. Critical Review on Polylactic Acid: Properties, Structure, Processing, Biocomposites, and Nanocomposites. *Mater.*, 15(12), 4312. https://doi.org/10.3390/ma15124312
2. ANALIZ RYNKU PLA (POLILAKTYD) TA PBAT (POLIMER) UKRAINY. (2021) [Electronic resource] Pro-Consulting — Resource ccess mode: https://pro-consulting.ua/ua/issledovanie-rynka/analiz-rynka-pla-polilaktid-i-pbat-polimer-ukrainy-2021-god
3. Stasiškienė, Ž., Barbir, J., Draudvilienė, L., Chong, Z., Kuchta, K., Voronova, V., Leal Filho, W. (2022). Challenges and Strategies for Bio-Based and Biodegradable Plastic Waste Management in Europe. *Sustainability*. 14(24), 16476. http://dx.doi.org/10.3390/su142416476
4. Costa, A., Encarnação, T., Tavares, R., Todo Bom, T., Mateus, A. (2023). Bioplastics: Innovation for Green Transition. *Polymers*,

15(3), 517. https://doi.org/10.3390/polym15030517
5. Holub, N. B., Shchurska, K. O., Kuzminskyi, Ye. V. (2021). Biomass Laboratory Workshop: a study guide. *Kyiv*, 76 p. (In Ukrainian).
6. DSTU 3976-2000 Corn Starch Dry. Specifications. With amendment No. 1 (IPS № 12-2013) and correction (IPS № 11-2000) (2013). *[Electronic resource]*. Resource access mode: https://online.budstandart.com/ua/catalog/doc-page.html?id_doc=70422. (In Ukrainian).
7. Safitri, A., Hidayati, H., Evi ,Y. (2022). The role of various plastisizers and fillers additions in improving tensile strength of starch-based bioplastics: A mini review. *IOP Conference Series: Earth and Environmental Science*. 1115(1), 012076. https://doi.org/10.1088/1755-1315/1115/1/012076
8. Mohammed, A. A. B. A., El-Bakry, A., Salama, H. E., Khalil, H. A. (2022). Corn: Its Structure, Polymer, Fiber, Composite, Properties, and Applications. *Polymers*. 14(20), 4396. https://doi.org/10.3390/polym14204396
9. Cui, X., Zhang, B., Li, Y., Wang, L. (2019). Pelletization of Sunflower Seed Husks: Evaluating and Optimizing Energy Consumption and Physical Properties by Response Surface Methodology (RSM). *Processes*, 7(9), 591. https://doi.org/10.3390/pr7090591
10. De'Nobili, M.D.M., Segatto, M. L., Lajolo, F.M. (2021). Sunflower (Helianthus annuus L.) Seed Hull Waste: Composition, Antioxidant Activity, and Filler Performance in Pectin-Based Film Composites. *Frontiers in Nutrition*. Vol. 8. https://doi.org/10.3389/fnut.2021.777214
11. Ali, A., Asif, M., Zhang, H., Chen, L. (2017). Preparation and characterization of starch-based composite films reinforced by corn and wheat hulls. *Journal of Applied Polymer Science*, 134(32), 45159. https://doi.org/10.1002/app.45159
12. Enawgaw, H., Edessa, D., Gebre-Mariam, T. (2023). Multiple Utilization Ways of Corn By-Products for Biomaterial Production with Bio-Refinery Concept; a Review. *Materials Circular Economy*, 5(1). https://doi.org/10.1007/s42824-023-00078-6
13. Omar, F. N., Hassan, E. S., El-Wakil, N. A. (2022). Starch-based composite film reinforcement with modified cellulose from bamboo for sustainable packaging application. *Materials Today Communications*. Vol. 33, 104392. https://doi.org/10.1016/j.mtcomm.2022.104392

АРМУВАННЯ БІОПЛАСТИКІВ НА ОСНОВІ КРОХМАЛЮ СІЛЬСЬКОГОСПОДАРСЬКИМИ ВІДХОДАМИ

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Мета. Пошук альтернативних армуючих наповнювачів для модифікації біопластиків на основі крохмалю з використанням відходів сільського господарства.

Матеріали й методи. Для порівняння характеристик матеріалів з різними типами сільськогосподарських відходів як наповнювачів використано метод візуальної оцінки та Метод вимірювання твердості за Шором. Оцінено вплив різних типів наповнювачів на механічні властивості та структуру біопластиків на основі крохмалю.

У дослідженні використовували кукурудзяний крохмаль за стандартом DSTU 3976-2000 та п'ять різних типів сільськогосподарських відходів як наповнювачі для модифікації біорозкладних пластиків. Метод виготовлення біопластиків включав підготовку 10% розчину крохмалю, змішування його з різними наповнювачами, підігрівання суспензій до 90 °C та сушіння отриманих розчинів при 60 °C залежно від типу наповнювача.

Результати. Найбільш вдалими виявилися варіанти з використанням технічного волокна целюлози та спресованого в гранули лушпиння соняшникового насіння. Отримані матеріали на основі цих наповнювачів демонструють кращі механічні властивості та краще збереження форми порівняно з матеріалами на основі крохмалю без наповнювачів. Оптимальний розмір частинок виявився в діапазоні 0,03–0,06 мм.

Висновок. Рослинні сільськогосподарські відходи є ефективними для біопластиків на основі крохмалю, що дозволить значно знизити собівартість біоматеріалів та розширити сферу їх використання, зробивши їх більш доступними для широкого спектру застосувань.

Ключові слова: біопластик, сільськогосподарські відходи, армуючі наповнювачі, крохмаль, властивості.

SHORT REPORTS

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ELECTROLYTE STATE OF CANINE RED BLOOD CELLS DURING HYPOTHERMIC STORAGE WITH THE ADDITION OF N-ACETYLCYSTEINE

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Aim. To evaluate the effect of adding N-acetylcysteine (NAC) to SAGM (adenine-glucose-mannitol saline) solution on the electrolyte composition and pH of canine erythrocytes during hypothermic storage.

Material and Methods. Red blood cells were stored in SAGM solution with or without NAC at 4–5 °C. The concentrations of sodium, potassium, chloride, and pH were determined on days 0, 7, 21, and 35 of storage.

Results. The sodium concentration increased in both groups, slightly less in the experimental group with NAC. The accumulation of potassium was less pronounced in the NAC-supplemented group. Chloride levels remained stable, and pH decreased, particularly in the experimental group.

Conclusions. NAC contributes to the stabilization of the electrolyte environment during storage, in particular to potassium retention and pH control.

Key words: erythrocytes, hypothermic storage, electrolyte balance, N-acetylcysteine, antioxidant protection.

Hypothermic storage of red blood cells is an essential practice in veterinary transfusion medicine, allowing for the long-term preservation of donor blood. Despite the effectiveness of this approach, storage at 4–5 °C leads to a gradual deterioration of red blood cell integrity due to metabolic changes and oxidative stress [1]. During storage, red blood cells undergo changes in volume, shape, membrane deformation, and metabolic profile, including progressive ATP depletion and lactate accumulation, which impair their functionality. When choosing red blood cell storage conditions, maintaining an optimal pH level and regulating electrolyte gradients play an essential role. These parameters affect the efficiency of gas exchange and provide the necessary conditions for the stability of red blood cell function [2].

Potassium (K^+) is the principal intracellular cation and plays a fundamental role in maintaining cell membrane potential, osmotic balance, and enzymatic activity. During storage, erythrocytes tend to lose K^+ to the extracellular environment due to changes in membrane permeability and energy depletion.

Sodium (Na^+), which is usually maintained at a low level within canine red blood cells, tends to accumulate during storage, altering the osmotic balance and indicating membrane instability. Chloride (Cl^-), a key anion, is also involved in regulating pH and fluid balance.

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A decrease in pH during storage leads to the accumulation of lactate and a reduction in buffer capacity, which negatively affects the enzymatic function and conformation of proteins in red blood cells [3]. Therefore, monitoring these parameters is essential for assessing the quality of storage.

N-acetylcysteine (NAC), an antioxidant and glutathione precursor, can help stabilize red blood cell membranes and buffer the intracellular environment. NAC acts as a thiol group donor, reducing oxidative stress and maintaining the structure of membrane proteins. This study evaluates its effect on the electrolyte profile and pH of canine erythrocytes stored for 35 days.

This study aimed to evaluate the effect of adding N-acetylcysteine (NAC) to SAGM (adenine-glucose-mannitol saline) resuspension medium on the electrolyte balance (Na^+, K^+, Cl^-) and pH of canine erythrocytes during 35-day hypothermic storage.

Material and Methods

Whole blood samples were collected from clinically healthy donor dogs and processed within 2 hours of collection. Red blood cells were obtained by centrifugation at 5000g for 7 minutes at 4 °C using blood bags equipped with satellite storage containers. The plasma was separated and frozen, and the erythrocyte mass was resuspended in a standard SAGM solution. The experimental group was supplemented with NAC at a specific concentration, while the control group was kept in standard SAGM without NAC.

All samples were stored at 4–5 °C in a blood storage refrigerator. Aliquots for analysis were taken on days 0, 7, 21, and 35 of storage.

Biochemical parameters, including the concentration of sodium (Na^+), potassium (K^+), chlorine (Cl^-), and pH, were evaluated using an EL-5 electrolyte analyzer (Quertimed, Ukraine).

The results were statistically processed using the Statgraphics software package (Manugistic Inc.; Statistical Graphics System, USA). The data were presented in the format M ± SE (mean ± standard error). Each series of experiments was performed at least five times.

Results and Discussion

Electrolyte analysis of the resuspension medium revealed dynamic changes during 35 days of hypothermic storage of erythrocytes. The sodium concentration increased progressively in both the experimental and control groups. On day 0, the Na^+ level was 159.6 ± 1.3 mmol/l in the experimental group and 157.6 ± 1.0 mmol/l in the control group. On day 35, these values increased to 165.4 ± 0.7 mmol/l and 167.75 ± 2.6 mmol/l, respectively. However, these differences were not statistically significant, indicating that NAC had no marked effect on sodium accumulation.

A more pronounced difference was observed in the concentration of potassium. In the experimental group, K^+ increased from 3.48 ± 0.3 mmol/l to 3.75 ± 0.21 mmol/l within 35 days, while in the control group, it increased from 3.51 ± 0.26 mmol/l to 4.59 ± 0.26 mmol/l. This statistically significant increase ($P < 0.05$) in the control group reflects a more substantial leakage of potassium, probably due to membrane instability. NAC supplementation seems to attenuate this effect, indicating improved membrane integrity.

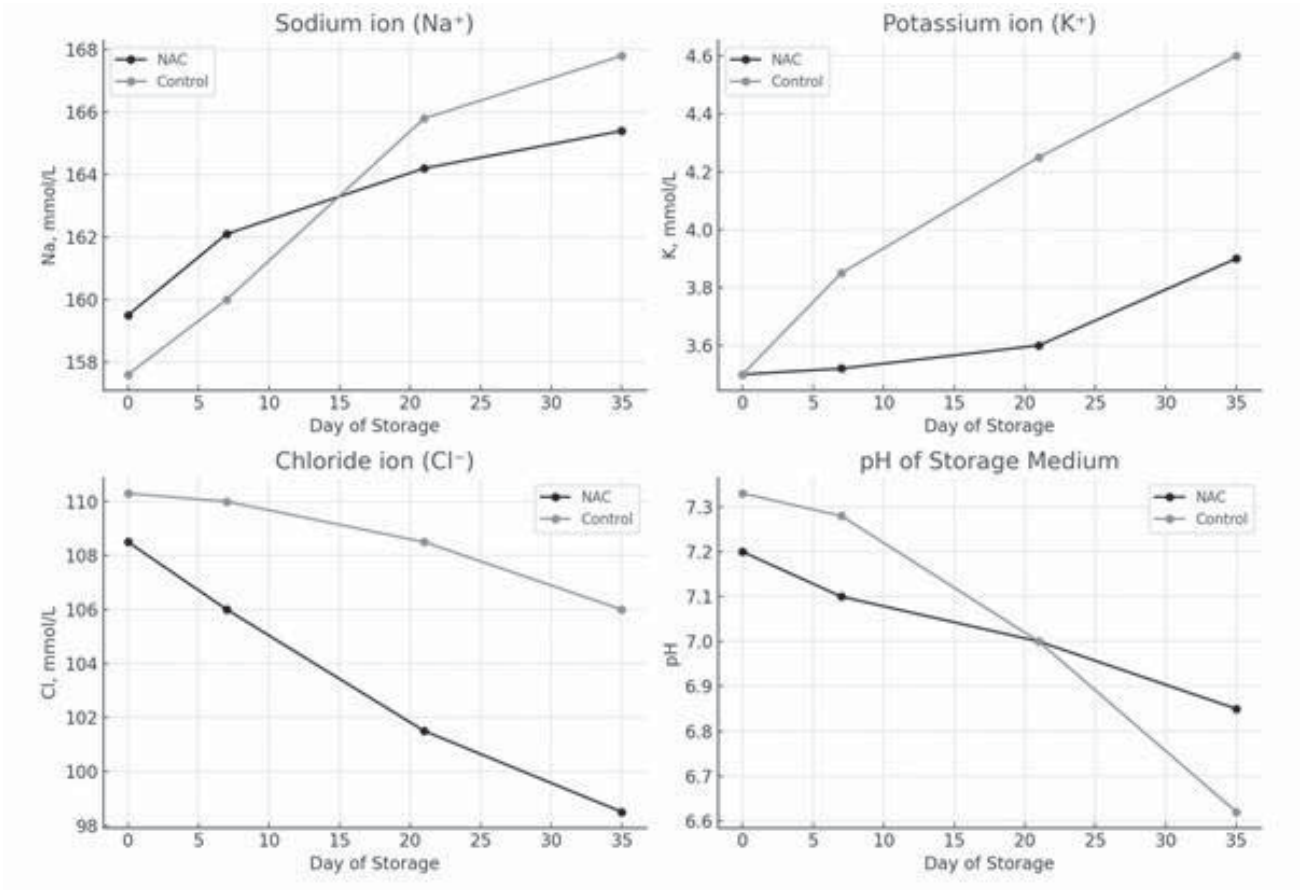
During the 35-day hypothermic storage, the chloride concentration in the resuspension medium gradually decreased in both NAC-treated and control samples. However, no statistically significant differences were found between the groups, indicating that the Cl^- dynamics remained relatively stable and within the physiological range during storage. The pH of the resuspending medium showed a steady downward trend in both groups, indicating acidification due to red blood cell metabolism. In particular, the pH value decreased from 7.21 ± 0.09 to 6.77 ± 0.24 in the NAC group and from 7.35 ± 0.07 to 6.62 ± 0.025 in the control group. Although acidification was observed in both groups, the presence of NAC helped to maintain a relatively higher pH level on day 35.

These results confirm that the addition of NAC can reduce the degree of electrolyte imbalance caused by storage, in particular by limiting potassium efflux and slowing the pH decline. This highlights its potential role in improving the quality of red blood cell storage for clinical use.

The data on changes in Na^+, K^+, Cl^-, and pH levels over time are shown in the Figure.

Conclusions

During 35 days of hypothermic storage, erythrocytes suspended in the SAGM medium showed progressive changes in electrolyte composition and pH. The addition of NAC helped to stabilize the level of Potassium,



Changes in the ionic composition of the SAGM resuspension medium with and without NAC on days 0, 7, 21 and 35

significantly reducing its leakage compared to the control group. Sodium levels increased in both groups, but the addition of NAC slowed the rate of accumulation, indicating improved membrane stability. Chloride concentrations remained within the physiological range, regardless of treatment.

The presence of NAC mitigated the acidification of the storage medium, with pH values decreasing less than in the control group.

These results support the potential use of NAC as a protective agent to improve electrolyte balance and maintain pH in stored red blood cells. The results highlight the potential of NAC as an effective additive to improve the quality and shelf life of red blood cells in veterinary transfusion practice.

Authors' contribution

KRH was responsible for the design of the experiment, preparation of red blood cell samples, data collection, and statistical analysis.

OMD supervised the study and participated in the interpretation of the results and critical revision of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Anastasiadi, A. T., Stamoulis, K., Papageorgiou, E. G., Lelli, V., Rinalducci, S., Papassideri, I. S., Kriebardis, A. G., Antonelou, M. H., Tzounakas, V. L. (2023). The time-course linkage between hemolysis, redox, and metabolic parameters during red blood cell storage with or without uric acid and ascorbic acid supplementation. *Front. Aging,* 4, 1161565. https://doi.org/10.3389/fragi.2023.1161565
2. William, N., Isiksacan, Z., Mykhailova, O., Olafson, C., Yarmush, M. L., Usta, O. B., Acker, J. P. (2023). Comparing two extracellular additives to facilitate extended storage of red blood cells in a supercooled state. *Front. Physiol.,* 14. 1165330. https://doi.org/10.3389/fphys.2023.1165330
3. Zhegunov, G., Denysova, O., Zhegunova, G. (2023). Blood Hypothermic Storage and Erythrocyte Cryopreservation in Dogs. *Probl. Cryobiol. Cryomed.,* 32(4), 245–255. https://doi.org/10.15407/cryo32.04.245

ЕЛЕКТРОЛІТНИЙ СТАН ЕРИТРОЦИТІВ СОБАКИ ПІД ЧАС ГІПОТЕРМІЧНОГО ЗБЕРІГАННЯ З ДОДАВАННЯМ *N*-АЦЕТИЛЦИСТЕЇНУ

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Мета. Оцінити вплив додавання N-ацетилцистеїну (NAC) до розчину SAGM (аденін-глюкозо-манітол-сольовий розчин) на склад електроліту та pH еритроцитів собак під час гіпотермічного зберігання.

Матеріали та методи. Еритроцити зберігали в розчині SAGM з NAC або без нього за температурою 4–5 °C. Концентрації натрію, калію, хлориду та pH визначали на 0, 7, 21 та 35 добу зберігання.

Результати. Концентрація натрію в обох групах була підвищена, дещо менше в експериментальній групі з NAC. Накопичення калію було менш вираженим у групі, яка отримувала NAC. Рівень хлориду залишався стабільним, а pH знизився, особливо в експериментальній групі.

Висновок. NAC сприяє стабілізації електролітного середовища під час зберігання, зокрема утриманню калію та контролю pH.

Ключові слова: еритроцити, гіпотермічне зберігання, електролітний баланс, N-ацетилцистеїн, антиоксидантний захист.