ANNOTATION

dissertation for the degree of Doctor of Philosophy (PhD) in
educational program "8D05101 – Biology"

Aitynova Arailym Eldoskyzy

Investigation of biological activity of a phytopreparation from felt burdock (*Arctium tomentosum* Mill.)

General characteristics of the work. This dissertation is devoted to studying the biological activity of a CO_2-extract from the root of *Arctium tomentosum* Mill. under *in vitro* and *vivo* conditions.

Relevance of the dissertation topic.

Medicinal plants remain a fundamental resource for pharmacotherapy. Of particular interest is *Arctium lappa* L. (Large burdock), a member of the *Asteraceae* family. Its root is traditionally used in extracts to treat dermatological, gastrointestinal, and hepatobiliary disorders. The therapeutic profile of burdock is due to its high concentration of biologically active compounds (BACs): phenolic acids (particularly chlorogenic and caffeic acids), flavonoids (quercetin, rutin, luteolin, and apigenin), and specific lignans (arctiin and arctigenin). This complex of compounds determines the plant's broad spectrum of pharmacological efficacy, including antioxidant, anti-inflammatory, hepatoprotective, antitumor, and antibacterial effects.

Despite the significant body of data on *A. lappa,* other species of the genus *Arctium* remain understudied, limiting our understanding of the chemosystematic and pharmacological diversity of this taxon. In this regard, *Arctium tomentosum* Mill. is of particular interest, being widespread in natural ecosystems and characterized by high ecological adaptability and the availability of raw materials. The fragmentary nature of data on the phytochemical profile and biological potential of this burdock species justifies its comprehensive study, particularly in relation to the treatment of hepatobiliary pathologies, the incidence of which is currently increasing for a number of reasons.

The rise in liver disease is due to a multifactorial etiology, ranging from toxic hepatopathies to metabolically associated fatty liver disease (MAFLD). Liver dysfunction, combined with carbohydrate and lipid metabolism disorders, initiates a cascade of systemic reactions: hyperglycemia, dyslipidemia, and insulin resistance, creating a vicious cycle of metabolic disorders. These processes are closely correlated with the risk of developing type 2 diabetes mellitus and cardiovascular complications. At the organ level, the accumulation of triglycerides and activation of lipid peroxidation lead to the transformation of steatosis into steatohepatitis. In the absence of adequate pharmacotherapy, chronic inflammation stimulates fibrogenesis, in which functional tissue is replaced by connective tissue, inevitably progressing to cirrhosis.

Despite the widespread use of classic herbal hepatoprotectors, particularly those based on silymarin, cynarin, glycyrrhizins, and curcumin, the problem of correcting complex metabolic liver disorders remains unresolved. The selective nature of existing agents, which often fails to address the full spectrum of systemic metabolic disorders, as well as the risk of developing adverse effects with long-term high-dose therapy – such as choleretic hyperstimulation (for cynarin and curcumin) or pseudohyperaldosteronism (for glycyrrhizins) – combined with the high resource dependence of production and the lack of a local raw material base for a number of introduced species, necessitate expanding the arsenal of drugs with polytropic action based on readily available domestic plant materials.

In this context, it is suggested that *A. tomentosum*, with its unique chemosystematic profile, can be considered a promising source of biologically active compounds with a complex mechanism of action. The rationale for studying this species is the hypothesis of the high pharmacological value of the lipophilic complex extracted by subcritical CO_2-extraction. It is assumed that the content of phenolic compounds (phenolic carboxylic acids and flavonoid glycosides) in the resulting extract will determine its biological potential. Literary data on the

phytochemical relationship of *Arctium* species suggest that the synergistic effect of polyphenolic components in the CO_2-extract of *A. tomentosum* will enable the integration of cytoprotection with the modulation of key components of pathological disorders.

Unlike classical pharmaceutical technologies based on the use of traditional extractants (water, ethanol and water-alcohol mixtures) in maceration, percolation, and Soxhlet extraction, this study utilized subcritical extraction using carbon dioxide (GOST 8050-85). This approach is considered a "green" technology because it eliminates the thermal evaporation step, ensuring the environmentally friendly process and the native preservation of heat-labile biologically active substances. Complete gas decompression at the final stage of extraction ensures the absence of trace amounts of extractant in the final product, ensuring its purity and ecological safety.

In summary, the choice of *A. tomentosum* as a research subject was dictated by its insufficient study, coupled with the high bioavailability of raw materials in the Republic of Kazakhstan. The synergistic nature of this species' secondary metabolites and the technological advantages of subcritical CO_2-extraction make it possible to create a unique, domestically produced, multi-targeted herbal product. Research into the biological activity of this herbal product, including its safety and efficacy in the pathogenesis of metabolic disorders and liver toxicity, is a pressing issue of strategic importance for the development of import-substituting pharmaceutical production.

Objective of the work: Study the biological activity of a phytopreparation from the root of *Arctium tomentosum* Mill. *in vitro* and *in vivo.*

Objectives of the study:

1. Determine the total flavonoid and phenolic content, identify the polyphenolic composition of a phytopreparation, obtained by CO_2-extraction from the root of *Arctium tomentosum* Mill. and evaluate its antioxidant potential in the test DPPH and FRAP;
2. Study the cytotoxic effect of the studied phytopreparation on the culture of mononuclear cells in the MTT assay;
3. Evaluate the antimicrobial activity of the phytopreparation on reference strains of gram-positive bacteria *(Staphylococcus aureus),* gram-negative bacteria *(Escherichia coli),* and yeast-like fungi *(Candida albicans);*
4. Examine body weight and internal organ function, hematological and biochemical blood parameters, and the histostructure of visceral organs in mice in an acute and subacute experiment;
5. Assess the morphofunctional state of mice, including body weight and organometric parameters, serum biochemical parameters, insulin resistance index, and liver histostructure in a study of the effectiveness of a phytopreparation in metabolic syndrome induced by a high-carbohydrate diet;
6. Study biochemical parameters of blood serum, lipid peroxidation markers and liver histostructure and morphometry in a CCl_4-induced hepatotoxicity model in mice.

Objects of the study: CO_2-extract from the root of *Arctium tomentosum* Mill. ("phytopreparation", "PT"), peripheral blood mononuclear cells, reference strains of gram-positive bacteria (*Staphylococcus aureus* ATCC 6538-P), gram-negative bacteria (Escherichia coli ATCC 11229) and yeast-like fungi (*Candida albicans* ATCC 10231), Swiss mice.

Subject of the study: total flavonoid and phenolic content, polyphenolic composition of the studied phytopreparation and its antiradical activity; The biological activity of the phytopreparation *in vitro* (antimicrobial activity against the above-mentioned reference microbial strains and cytotoxic influence in mononuclear cell culture); the biological activity of the phytopreparation *in vivo* (acute and subacute toxicity in mice; efficacy in metabolic syndrome induced by a high-carbohydrate diet in mice; antioxidant activity and hepatoprotective effect in CCl_4-induced hepatotoxicity in mice).

Research methods. In this dissertation the following methods were used: spectrophotometric methods for determining the total flavonoid and phenolic content; high-performance liquid chromatography with mass spectrometric detection for identifying the polyphenolic composition; DPPH and FRAP test methods for assessing the antioxidant potential;

MTT test method for assessing the viability of peripheral blood mononuclear cells; two-fold serial dilution method for assessing antimicrobial activity; hematological, biochemical, histological, morphometric and statistical methods.

Scientific novelty of the study:

1. For the first time, a phytopreparation was obtained from the root of *Arctium tomentosum* Mill. using the CO_2-extraction method, which ensures the extraction of the native complex of biologically active compounds; the total flavonoids and phenolic content were determined, the polyphenol composition of the phytopreparation was identified, and its antioxidant potential was established using DPPH and FRAP assays.
2. For the first time, based on the results of the MTT test by assessing the viability of blood mononuclear cells under in vitro conditions, the absence of a cytotoxic effect of the studied phytopreparation was revealed;
3. For the first time, the antimicrobial activity of the studied phytopreparation was established on reference strains of gram-positive bacteria *(Staphylococcus aureus),* gram-negative bacteria *(Escherichia coli),* and yeast-like fungi *(Candida albicans);*
4. For the first time, the safety of the studied phytopreparation was demonstrated in acute and subacute experiments in mice using a comprehensive assessment of body weight and organometric parameters, hematological and biochemical blood parameters, and morphological analysis of the histostructure of internal organs;
5. For the first time, the efficacy of the studied phytopreparation was demonstrated and its metabolic effect was established in a model of metabolic syndrome induced by a high-carbohydrate diet using a comprehensive approach, including assessment of body weight and organometric parameters, analysis of biochemical parameters of blood serum, and morphological examination of the histostructure of internal organs in mice;
6. For the first time, the antioxidant and hepatoprotective effect of the phytopreparation from the root *of Arctium tomentosum* Mill. was experimentally confirmed in a model of CCl_4-induced hepatotoxicity based on a comprehensive assessment of antioxidant activity, analysis of biochemical parameters of blood serum and liver enzyme activity, and morphological examination of the histostructure of the mouse liver.

The scientific and practical significance of this dissertation lies in its expansion and deepening of current understanding of the biological activity of a phytopreparation, obtained by CO_2-extraction from the root of *Arctium tomentosum* Mill. For the first time, the results of a comprehensive and *in vivo* safety assessment, along with data on antioxidant, antimicrobial, metabolic, and hepatoprotective activities, establish a scientifically grounded basis for further pharmacological research into this phytopreparation. The experimental findings obtained can be used to determine therapeutic dosages and administration regimens, as well as to plan clinical trials and evaluate the prospects for the practical application of the phytopreparation in managing metabolic and inflammatory disorders.

The main provisions submitted for defense:

1. A phytopreparation from the root of *Arctium tomentosum* Mill., obtained by CO_2-extraction, contains polyphenols, including flavonoids and phenolic compounds, which may contribute to its antioxidant potential;
2. The studied phytopreparation exhibits dose-dependent preservation of the viability of blood mononuclear cells in the MTT test *in vitro*;
3. The phytopreparation has been shown to have antimicrobial activity against gram-positive and gram-negative bacteria and yeast-like fungi;
4. The phytopreparation is safe and does not cause signs of toxicity when administered orally to mice in acute and subacute experiments.
5. In experimental metabolic syndrome induced by a high-carbohydrate diet, the phytopreparation exhibits an insulin-sensitizing effect, as evidenced by the normalization of glucose and insulin levels in the blood serum, and a lipid-correcting effect, as evidenced by a decrease in triglyceride, total cholesterol, and low-density lipoprotein levels, along with the

restoration of the high-density lipoprotein fraction in the blood serum of mice with metabolic syndrome.

6. In a CCl_4-hepatotoxicity model, the phytopreparation has a hepatoprotective effect, as evidenced by a decrease in the intensity of oxidative stress, normalization of the activity of liver marker enzymes, prevention of fatty infiltration, and preservation of the liver histostructure.

Applicant's personal contribution. The author analyzed the literature on the topic under study, performed all experimental studies, and statistically processed and analyzed the results. The dissertation manuscript was written and formatted according to a plan agreed upon with the academic advisors.

Research program affiliation. This dissertation was completed under the program-targeted grant funding: IRN AP23484003 "Development of new domestic and environmentally safe pharmacological substances for the treatment of skin diseases".

Results validation. The dissertation results were presented and discussed at the following international scientific conferences:

1. “Progress in Science” (Brussels, Belgium, December 15-16, 2022);
2. “Modern Trends in Sustainable Development of Biological Sciences” (Almaty, Kazakhstan, March 27-28, 2024).

Publications. The main results of this dissertation were published in 6 scientific papers: 3 articles in the Scopus database (Q3, SJR – 0.296, percentile – 52; Q3, SJR – 0.596, percentile – 45; Q1, SJR – 0.865, percentile – 87), one article in a journal recommended by CQASHE, and two abstracts in international conference proceedings (Q4, SJR – 0.205, percentile – 26).

Structure and scope of the work. The dissertation consists of an introduction, literature review, research materials and methods, research results, discussion of the results, conclusion, and a list of references. The work is presented on 100 pages of printed text, contains 15 tables, 21 figures, 224 references and 7 appendices.

Acknowledgments. The author expresses deep gratitude for their attentive and constructive guidance, valuable consultations, and advice during the course of this work to the domestic consultant, Doctor of Biological Sciences, Professor Tamara Minazhevna Shalakhmetova; the foreign consultant, Doctor of Veterinary Sciences, Professor Bogdan Sevastra; and the Head of the Pharmacology and Toxicology Laboratory, Candidate of Biological Sciences, Naila Akhtamovna Ibragimova.

Practical base. The study was conducted at the Pharmacology and Toxicology Laboratory, JSC Scientific Center for Anti-Infectious Drugs (Almaty, Kazakhstan); the Department of Biodiversity and Bioresources, Al-Farabi KazNU (Almaty, Kazakhstan); the Department of Patophysiology, University of Agricultural Science and Veterinary Medicine (Cluj-Napoca, Romania); and the Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy (Cluj-Napoca, Romania).