

ABSTRACT

for the dissertation for Doctor of Philosophy (PhD) degree on the educational
programm «8D05101 – Biology»

of **Yestemirova Gulfira Abdiquaymkyzy** on the theme

**«Antileukemic activity of novel cytotoxic and differentiation-inducing
combinations of bioactive natural compounds»**

General description of the research. The research presents a study of the antileukemic activity of novel cytotoxic and differentiation-inducing combinations of bioactive natural compounds in an *in vitro*, and their potential protective effect against the toxic effects of gasoline vapors in an *in vivo* experiment.

The relevance of the research: The rapid growth of the population, the increase in the number of motor vehicles and gas stations (GS), and of industrial plants are contributing to air pollution. Air pollution with gasoline vapors (GV) is not only an ecological threat to the planet but also a pressing issue that harms human health. Gasoline contains over 150 hydrocarbons, known carcinogens. Previous studies have shown that employees of oil refineries and GS, who are regularly exposed to GV, have a higher risk of developing lung, kidney, bladder, and hematological tumors, including leukemia. Leukemia, or leukosis, is a malignant disease of the hematopoietic system. According to WHO data, leukemia accounted for 2.4% of all cancer cases worldwide in 2022, ranking 13th, while in Kazakhstan, this figure was 1.8%, placing it 15th among the cancer diseases encountered in the country. Therefore, developing measures to protect against the toxic effects of GV and mitigate their consequences is a pressing issue. However, this issue is currently receiving little attention. One of the methods for preventing cancer is the search for natural compounds that enhance the body's resistance and compensatory-adaptive reactions.

Currently, several studies have demonstrated the protective effects of certain natural agents on leukemic cell models at the *in vitro* level. For example, it has been found that the use of active metabolites of vitamin D enhances differentiation in leukemic cell lines. Additionally, similar *in vitro* studies have shown that curcumin, a polyphenol found in the root of *Curcuma longa* L., significantly inhibits the proliferation of leukemic cells. However, despite the antileukemic properties of the aforementioned agents, their individual or combined effects in protecting against or mitigating the harmful effects of GV in animal models have not been studied *in vivo* to date. This research aims to investigate the prophylactic effects of vitamin D₃ and *Curcuma Longa* L. root powder against the toxicity of GV and to alleviate its negative consequences *in vivo*, based on the antileukemic activity of vitamin D₃ and curcumin observed *in vitro*.

The purpose of the research: Study the antileukemic activity of novel cytotoxic and differentiation-inducing combinations of bioactive natural compounds.

The main tasks of the research to accomplish purpose are as following:

1. To determine the differentiating and antiproliferative effects of 1,25D₃, curcumin, and their combination on leukemia cells;

2. Study of the phytochemical composition of *Curcuma Longa* L. root powder;

3. Investigating the effects of vitamin D₃, *Curcuma longa* L. root powder, and their combination on the dynamics of body weight in rats and the macroscopic and microscopic changes in their lungs, liver, kidneys, and spleen under the influence of gasoline vapor poisoning over periods of 30, 60, and 90 days.

4. Study of the effects of vitamin D₃, *Curcuma longa* L. root powder, and their combination on changes in the blood biochemistry of rats under gasoline vapor intoxication over periods of 30, 60, and 90 days.

5. Study of the effects of vitamin D₃, *Curcuma longa* L. root powder, and their combination on the hematological changes in rats under gasoline vapor intoxication over a period of 30, 60, and 90 days.

The research objects and materials: HL60 myeloblastic leukemia cell line; *Curcuma longa* L. root powder (Kevala International LLC); the body weight of three-month-old male albino rats was measured, and their lungs, liver, kidneys, spleen, and blood were studied.

Research methods: Cell culturing *in vitro*, flow cytometry method (CytoFLEX S, Kaluza 2.1, Beckman Coulter, California, USA), cell counting and viability determination using trypan blue staining (Vi-Cell'XR Cell Viability Analyzer, Beckman Coulter, California, USA), high-performance liquid chromatography (HPLC) method (Shimadzu LC-40, Shimadzu Corporation, Kyoto, Japan), body weight measurement (CAS SW-5, CAS Corporation, Seoul, South Korea), macroscopic, histological (MicroOptix MX 300 T, Micro-Optix, Wiener Neudorf, Austria), biochemical (HumaStar 100, Human Diagnostics Worldwide, Wiesbaden, Germany), hematological (Advia-2120i, Siemens, Munich, Germany), and statistical data analysis (GraphPad Prism 6.0, GraphPad, San Diego, California, USA).

The scientific novelty of the research:

1. For the first time, the effects of vitamin D and *Curcuma longa* L. root powder, both individually and in combination, on the body mass and lungs, liver, kidneys, and spleen of rats under gasoline vapor intoxication were studied over periods of 30, 60, and 90 days.

2. The effects of vitamin D₃ and *Curcuma longa* L. root powder, both individually and in combination, on the blood biochemistry and hematological parameters of rats were studied for the first time against the backdrop of gasoline vapor intoxication over periods of 30, 60, and 90 days.

3. For the first time, it has been proven that oral administration of vitamin D₃ protects against the reduction in body mass and harmful changes in the lungs, liver, kidneys, spleen, and blood caused by exposure to gasoline vapors in rats.

4. For the first time, it was determined that there is some antagonism between vitamin D₃ and *Curcuma longa* L. root powder, manifested either as a lack of interaction between them or as a weaker protective effect of their combination compared to the individual effects of each agent.

Scientific and practical significance of the research. The results obtained from preclinical *in vitro* and *in vivo* studies indicate that the identified antagonistic interactions between vitamin D₃ and curcumin/*Curcuma longa* L. root powder require careful consideration when developing combination therapy to prevent or mitigate the toxic effects of gasoline vapors. Furthermore, these findings can be used to develop preventive measures and therapeutic approaches aimed at reducing the harmful impact of gasoline vapors. The experimental data obtained are particularly important for individuals at high risk of gasoline vapor exposure, such as workers at oil refineries and gas stations.

The main provisions for the defense:

1. In the study of the effects of 1,25D₃ and curcumin on HL60 cells, the individual administration of vitamin 1,25D₃ demonstrated a significantly higher ($p < 0.0001$) differentiation property compared to the combination treatment of the two agents.

2. Phytochemical studies have identified a high concentration of the main curcuminoids in the root powder of *Curcuma longa* L.: curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Additionally, flavonoids, tannins, alkaloids, and saponins were also identified.

3. Oral administration of vitamin D₃ for 90 days significantly protected rats from the reduction in body weight and the macroscopic and microscopic damage to the lungs, liver, kidneys, and spleen caused by the toxicity of gasoline vapors.

4. An antagonistic interaction between vitamin D₃ and turmeric, indicating either an absence of synergistic effects or even a reduced protective effectiveness of their combination compared to the individual effects of each agent.

5. The effect of *Curcuma longa* L. root powder alone and in combination with vitamin D₃ against the toxicity of gasoline vapor in rats was similar, but it provided less effective protection compared to monotherapy with vitamin D₃.

Key research findings and conclusion:

1. It was found that the differentiation of leukemic HL60 cells was induced by 1,25D₃ at a concentration of 0.5 nM – 1.68%, and at 1 nM – 6.80%. Curcumin at a concentration of 2.5 μ M reduced HL60 cell viability by 77.12%, while at 5 μ M, it reduced viability by 62.87%. It was determined that vitamin 1,25D₃ alone exhibited a significantly higher ($p < 0.0001$) differentiation effect on HL60 cells compared to the combination treatment of 1,25D₃ and curcumin.

2. The phytochemical composition of *Curcuma longa* L. root powder was studied. The results revealed high concentrations of key curcuminoids, including curcumin (69.8 mg/g), demethoxycurcumin (31.6 mg/g), and bisdemethoxycurcumin (20.9 mg/g). Additionally, the amounts of flavonoids (2.8 mg/g), tannins (27.0 mg/g), alkaloids (15.8 mg/g), and saponins (41.8 mg/g) were identified.

3. It was proven that 90-day administration of vitamin D₃ significantly protected rats from weight loss induced by gasoline vapor toxicity, as well as from morphological damage to the lungs, liver, kidneys, and spleen, as observed in macroscopic and microscopic studies. However, *Curcuma longa* L. root powder

and its combination with vitamin D₃ provided weaker protective effects. When both agents were used together, *Curcuma longa* L. root powder exhibited antagonism against some of the protective effects of vitamin D₃. These findings suggest that the combined use of these two agents does not provide high beneficial activity against the harmful effects of gasoline vapor.

4. It was demonstrated that 90-day administration of vitamin D₃ protected rats from increases in liver (ALT – $p < 0.0001$ and AST – $p < 0.0001$) and kidney biomarkers (creatinine – $p < 0.001$ and urea – $p < 0.001$) caused by gasoline vapor toxicity in blood serum, as well as from decreases in protein ($p < 0.001$) and glucose ($p < 0.0001$) levels. However, *Curcuma longa* L. root powder and its combination with vitamin D₃ exhibited a weaker inhibition of these biochemical blood parameter fluctuations compared to vitamin D₃ alone.

5. It was demonstrated that 90-day administration of vitamin D₃ protected rats from a decrease in erythrocyte ($p < 0.01$), hematocrit ($p < 0.001$), hemoglobin ($p < 0.05$), leukocyte, lymphocyte ($p < 0.01$), granulocyte ($p < 0.05$), and platelet ($p < 0.01$) counts caused by gasoline vapor toxicity in the blood. However, *Curcuma longa* L. root powder and its combination with vitamin D₃ showed only a weak normalization of changes in these hematological parameters compared to vitamin D₃ alone.

The contribution of author for the results described in the dissertation.

The analysis of the literature related to the researched issue, the definition of the goal and objectives of the work, the conduction of experiments, data processing, statistical analysis of the obtained results, manuscript preparation for publication, interpretation and presentation of the results, and the presentation of the main results in the dissertation were carried out with the involvement of co-authors.

Relationship of the research with the scientific project.

The dissertation work was performed personally by the dissertation author and scientific supervisors. *in vitro* experiments were conducted in the laboratory of the Department of Clinical Biochemistry and Pharmacology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel. Phytochemical analyses were carried out at the Center for Medicinal Plant Research at Al-Farabi Kazakh National University, Almaty, Kazakhstan. *in vivo* experiments were conducted at the vivarium center of Al-Farabi Kazakh National University, in accordance with the protocol approved by the Ethics Committee of the RGP "Institute of Human and Animal Physiology," Ministry of Education and Science of the Republic of Kazakhstan (No. 12-28, February 3, 2023), Almaty, Kazakhstan.

Research approbation:

The results of the research and the main provisions of the dissertation were presented and discussed at the following international scientific conferences:

1. International scientific conference of students and young scientists "Farabi alemi", April 6-9, 2020, Almaty, Kazakhstan;
2. International scientific conference of young scientists, graduate students, students and schoolchildren "XX Satpayev Readings", April 10, 2020, Pavlodar, Kazakhstan;

3. International scientific conference of students and young scientists "Farabi alemi", April 6-8, 2021, Almaty, Kazakhstan.

4. The main results of the dissertation were presented every six months at the meetings of the Department of Biodiversity and Bioresources of the Faculty of Biology and Biotechnology at Al-Farabi Kazakh National University.

Publications:

The main results of the dissertation are presented in 10 published works: among them, one article in an international peer-reviewed journal with an impact factor included in the Scopus and Web of Science (Q1) databases; 6 articles in journals from the list of publications recommended by the Committee for Quality Assurance in Education and Science of the Republic of Kazakhstan for publishing the main results of scientific activity, 3 abstracts in collections of materials of international conferences.

Dissertation structure:

The dissertation consists of 124 pages of computer-typed text, symbols, and abbreviations, including the introduction, literature review, materials and methods, research results and discussion, conclusion, 220 references, 3 tables, 41 figures, and 1 appendix.