Review

by Prof. Ivanka Ilieva Kostadinova, MD, PhD,,

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REGARDING:

Dissertation on the topic: "Pharmacological studies with anethole in an experimental model of obesity"

Author: Elis Rafailova Gasanzadeeva, MD

Form of study - full-time doctoral student, Department of Pharmacology, Clinical Pharmacology and Therapy, MU "Paraskev Stoyanov" Varna,

enrolled in the doctoral program "Pharmacology (incl. Pharmacokinetics and Chemotherapy)" to obtain the PhD

Professional direction: 7.1 Medicine

Research supervisor: Prof. Stefka Vasileva Valcheva-Kuzmanova,MD, PhD, Dsc

Protection procedure:

Pursuant to Order No. P-109-168 / 22.05.2024 of the Rector of the MU - Varna, I have been appointed as a member of the Scientific Jury for awarding the educational and scientific degree "Doctor" in the scientific specialty "Pharmacology (incl. pharmacokinetics and chemotherapy)". of Elis Rafailova Gasanzadeeva,MD

Elis Rafailova was enrolled by Order R-109-450 / 28.10.2020 of the Rector of MU-Varna on the basis of a report entered No. 102 – 2384/07.10.2020 by Prof. Stefka

Kuzmanova, MD, PhD, Dsc., Head of the Department of Pharmacology, Clinical Pharmacology and Therapy, Medical Faculty, Medical University – Varna and decision according to protocol No. 30/12.10.2020 of the Faculty Council of Medical Faculty at University of Varna for a full-time doctoral student in the Ph.D. program "Pharmacology (incl. Pharmacokinetics and Chemotherapy)". The stages of the doctoral studies for admission to the public defense have been observed and the doctoral student has been dismissed on the proposal of a meeting of the Faculty Council of the Department of "Pharmacology, Clinical Pharmacology and Pharmacotherapy", according to Art. 61, para. 1 and para. 2 of the Regulations for the development of the academic staff at the MU - Varna. According to the defense procedure, Rafailova submitted all the necessary materials, according to the requirements of the Regulations for the Development of the Academic Staff of MU-Varna. This review was developed and presented in accordance with the requirements of the Law on the Development of the Academic Staff of the Republic of Bulgaria, the Rules for its Implementation and the Rules for the Development of the Academic Staff at the Medical University /MU/ - Varna. I declare that I have no conflict of interest with the author of the dissertation.

Brief biographical data and professional development

Elis Rafailova Gasanzadeeva, MD was born on March 15, 1993 in the city of Shumen. In 2012, he completed his secondary education at the "N.Y. Vaptsarov" Secondary School, Shumen. In May 2018, he graduated as a Master of Medicine at the "Prof. Paraskev Stoyanov" University of Varna. During her studies at the University of Varna, in the period March-July 2014, she conducted practical training at the "St. Anna" Medical Center, First Clinic of Surgery as an intern under the "Student Practices" project. From June to October 2017, Rafailova conducted practical training at St. Anna Medical Center, Endoscopy Office, about the "Student

Practices" project. She is a demonstrator at MU-Varna, Department of Pharmacology, Clinical Pharmacology and Pharmacotherapy for the period February 2018 to May 2018. From January 2019 to March 2019, she worked as a doctor in the Medical oncology department at the "Deva Maria" UMBAL, Burgas. From March 2019 to October 2019, Rafailova was a part-time assistant at the Medical University of Varna, Department of Pharmacology, Clinical Pharmacology and Pharmacotherapy. From October 2019 until now, she is an assistant in the same department.

Rafailova, MD conducted a short-term specialization at Ovidius university, 24.07-4.08.2023, in Constanta, Romania. Has completed a course in the protection and humane treatment of experimental animals used for scientific or educational purposes. In her CV, she listed a total of 19 scientific publications and 2 citations. He has a very good level of written and spoken English and a good level of Russian. He is a member of the Bulgarian Society of Pharmacology, Clinical Pharmacology and Therapeutics and the European Association of Clinical Pharmacology and Therapeutics (EACPT).

Structure of the dissertation

Rafailova's dissertation is presented in 157 pages, contains 40 figures and 27 tables. Includes: Table of Contents (7 pages), Abbreviations (1 page), Introduction (2 pages), literature review (34 pages), aim and tasks (2 pages), materials and methods (9 pages), own results and discussion (71 pages), conclusions (4 pages), contributions (1 page), list of the publications and participations related to the dissertation work (4 pages) bibliography (23 pages). 309 literary sources are noted in the bibliography, of which 2 are in Cyrillic and 307 are in Latin. From the last 5 years, 130 sources were used (41.80%), which is proof of the topicality of the topic of the dissertation work and the conclusions drawn and summaries of the obtained

results. The abstract of the dissertation work "Pharmacological studies with anethole in an experimental model of obesity" contains 100 pages and reflects the main content of the dissertation work and the obtained results. It is illustrated with 27 tables and 28 figures.

Relevance and significance of the dissertation work

The topic of the dissertation is relevant because it is related to the socially significant disease obesity. Obesity is one of the leading causes of preventable death, drastically reducing not only the overall quality of a person's life, but also their life expectancy. Obesity puts people at higher risk for diabetes, heart disease and some cancers. Additional health risks from diabetes include osteoarthritis, sleep apnea, kidney disease, strokes, and high blood pressure. Pregnant women who are overweight can suffer from complications that can lead to health problems for the mother or the child.

Obesity can be used as a basic general health indicator for the population because it leads to health problems. Worldwide, more than 1.9 billion adults are overweight, of which 650 million are obese. In Bulgaria, 61% of the population over the age of 18 is overweight, and about 20% is obese. The most significant changes in obesity statistics are seen in the period after 2016, with data showing obesity in up to 39% of the world's population and an increase in the number of people worldwide who are overweight. According to a 2015 global obesity survey of 195 countries, 604 million adults and 108 million children were overweight. Since 1980, the prevalence of obesity has doubled in 73 countries and increased in most others. The rate of increase is greater in childhood obesity. The incidence of metabolic syndrome is often comparable to the incidence of obesity and the incidence of type 2 diabetes. The greatest increase in the prevalence of obesity in young men (25–29 years) is seen in countries with a low socioeconomic index. Over the past three

decades, prevalence has increased from 1.1% in 1980 to 3.85 in 2015. Between 1990 and 2015, global mortality associated with high BMI increased by 28.3%. In preclinical and clinical studies related to obesity, conflicting and unclear data related to the influence of various factors and medicinal products on obesity worldwide have been registered. An advantage of natural products in the therapy of obesity is the unique chemical structures that are the basis for the creation of medicinal products. Only about 5 to 15% of the 2,500,000 species of plants have been studied chemically and pharmacologically. About 63% of medicinal products are from plant sources that can be investigated for new active ingredients. The search and research of combinations of plant sources has priority over the synthesis of new medicinal molecules because, according to the WHO, about 80% of the world's population uses medicinal products of plant origin for prevention and treatment. These products have an easy and convenient way of application, which ensures better participation of the patient in the prevention and treatment of various socially significant diseases.

In the introduction of the dissertation "Pharmacological studies with anethole in an experimental model of obesity", Rafailova points out the relevance of the problem. The PhD student notes a single study by Noreen et al., 2024 on the effects of the monoterpene anethole on obesity. This determined the need for a more in-depth study of anethole in an obesity model.

In the literature review, Rafailova presents the world's scientific achievements on the topic of the dissertation work. A definition of obesity and the factors leading to obesity are discussed. The Epidemiology of Obesity states that according to the WHO, obesity kills 4 million people each year. By 2035, the economic impact of obesity will exceed $800 billion annually. Risk factors for the development of obesity are discussed in detail. The influence of "endocrine disruptors", a sedentary lifestyle, genetic factors, the disturbance in energy homeostasis was examined.

Genetic defects in leptin or its hypothalamic receptor or melanocortin 4 receptor have been shown in the available literature to lead to obesity. The importance of central (visceral) obesity and peripheral obesity is discussed. Of interest is the existing positive correlation between BMI and the increase in systolic and diastolic pressure, vascular damage and the development of heart failure. The review indicates the changes in the function of the digestive, excretory and nervous systems in obesity and the risks to health and quality of life. The data of Grant et al are discussed. 2020 that obesity increases the likelihood of developing tumors, the link between markers of oxidative DNA damage and higher levels of body fat, faster aging of the genome and an increased risk of developing neoplasms. . Of interest are the data on the relationship between obesity, infectious diseases and COVID-19. Rafailova has examined in detail the methods for treating obesity. In the section "Pharmacological treatment" of obesity, the pharmacodynamic features of Phentermine, Phentermine/Topiramate, Orlistat, Lorcaserin, GLP-1 agonists, Naltrexone/bupropion, the benefit and risk of their use in the medical treatment of obesity are discussed in detail. In the section "Non-pharmacological treatment" of obesity, she examines the advantages and disadvantages of ziplosing diets, transcranial direct current stimulation, cognitive-behavioral interventions, increased physical activity, surgical treatment of obesity. Interesting facts are discussed by the PhD student in section 1.6 about the use of natural products such as monoterpenes and essential oils in combined obesity therapy such as limonene, thymol, citrol, eucalyptol, anethole, aurapten, carvacrol, paeoniflorin, iridoids, black pepper oil, essential oil from nutmeg, Mentha spicata. The physico-chemical characteristics of anethole, its pharmacokinetic features and pharmacological effects are described in detail. A synthetic derivative of anethole is the basis for the development of neuroprotective drugs for Parkinson's disease. Elis Rafailova describes in detail the experimental methods and models for the induction of obesity. The positive and

negative sides of the diets used as models for obesity are indicated, the reason for choosing high calorie diet (HCD|. Monogenic and polygenic models are considered, which present more accurate information about the biology of obesity.

They are the most widely used and more realistic models for studying obesity in humans. Surgical methods leading to hypothalamic obesity and the use of electromagnetic radiation to activate or inhibit ion channels are mentioned. From the comprehensive literature review, the manner of presentation, the competent and critical analysis of the data, the competent attitude of Rafailova in the researched area is evident. The main aspects of the dissertation are very well separated and addressed, making it easy for the reader to move to the resulting objective and tasks.

The aim of the dissertation was to investigate and generalize the pharmacological effects of anethole administered orally at increasing doses in rats in a model of high-calorie diet-induced obesity (HCD). The goal is precisely stated. Logically, the tasks resulting from the goal are well defined, which facilitates the presentation and discussion of the results. I believe that the set goal and tasks make it possible to carry out the planned studies in a complex manner. The main tasks include:

1. To induce obesity in healthy experimental animals by administering a high-calorie diet (HCD) for 10 weeks.

2. In an obesity model to study the effects of anethole on body weight, caloric and fluid intake, behavior, analyzing data on general motor activity, anxiety, depressive-like behavior, spatial memory, biochemical and clinical-laboratory indicators, indicators of antioxidant protection and oxidative stress - superoxide dismutase in serum, thiobarbituric acid-reactive substances in serum and tissue homogenates from brain, liver and heart, tissue indices: adipose tissue indices (of

retroperitoneal, mesenteric, perigonadal, paranephric and total adipose tissue), liver index, liver histology, retroperitoneal adipose tissue, myocardium and coronary vessels, immunohistochemical markers of apoptosis/anti-apoptosis and inflammation in liver and retroperitoneal adipose tissue, carrageenan-induced acute hind paw inflammation. In her dissertation work to fulfill the set tasks, Rafailova used modern, established research methods. The experimental animal groups and research methods are described in detail in material and methods. The animals were divided into 5 groups of 10 each: control, high calorie diet (HCD), HCD+62.5A, HCD+125A and HCD+250A. During 10 weeks, the animals of the control group (K) received standard laboratory pelleted food for rats and ordinary drinking water, and the a high-calorie diet groups (HCD| with an increased content of animal fat (17%) and simple sugars (17%), standard granules are enriched with lard and fructose. Drinking water was replaced with a 10% fructose solution in the animals of the HCD groups (Gancheva et al., 2015b).

Elis Rafailova has used the following behavioral methods: open field test, social interaction test, forced swimming test, object recognition test, biochemical and clinical-laboratory tests, determination of tissue indices, histological research, immunohistochemical methods, induction of acute hind paw inflammation. The data have been processed with appropriate statistical analyses, which ensures the reliability of the obtained results and the possibility of comparison with those of other authors.

In "Own results and discussion" the data are processed correctly. They are presented and described well. The PhD student has very good computer skills, which help her in presenting and publishing the obtained results. The body weight of the experimental animals was reduced by a dose of 250 mg/kg body weight of anethole, the dose of 125 mg/kg body weight. anethole significantly increased intake of

fructose solution per intake. Anethole in studied doses does not affect motor activity, prevents HCD-induced anxiety at a dose of 62.5 mg/kg body weight. A dose-dependent improvement of spatial memory was found, significant in the groups treated with anethole 125 mg/kg body weight. and 250 mg/kg.b.w. Anethole at the highest dose normalized serum levels of HCD -reduced superoxide dismutase. The three doses of anethole normalized HCD-increased lipid peroxidation in the brain. The results are interesting, which were registered in the tissue indices studied by the doctoral student. Anethole in a dose of 250 mg/kg body weight lowers the index of retroperitoneal adipose tissue reaching levels to those of the control, significantly reduces the size of adipocytes, which approach in size to those of the Control. The expression of apoptotic marker BAX was decreased by anethole 125 mg/kg body weight and increased by anethole in a dose of 250 mg/kg body weight. The highest dose also increased the BAX/BLC-2 ratio in the retroperitoneal adipose tissue compared to the data of the HCD- group of animals.

In the liver of the experimental animals, compared to the HCD - group, a decrease in the expression of BAX was recorded from the highest dose of anethole. BCL-2 expression was decreased by all three doses of anethole tested, with the decrease being most pronounced at the highest dose. The BAX/BCL-2 ratio was significantly increased by anethole at a dose of 250 mg/kg b. w, while the expression of MAC 387 was significantly decreased by anethole at a dose of 62.5 mg/kg b. w. In the discussion after the results, Rafailova compares the obtained results with those of other authors, discusses possible mechanisms that can explain the recorded data from the experiments. The dissertation is written in clear Bulgarian.

The conclusions drawn can be used to plan studies to elucidate the mechanisms of the established pharmacological effects of anethole. Studies in this direction, the establishment of dependence between effects and dose of anethole will

support the selection of a marker for a certain pharmacological effect related to behavior, biochemical and clinical-laboratory indicators, antioxidant protection of the body, changes in tissue indices, studied by Elis Rafailova.

The data from the conducted experiments are summarized in 13 conclusions with 36 sub-points. For the presentation, I recommend that the results be presented in summary tables, with an opinion expressed at what dose the research on the pharmacological effects of anethole in obesity can continue. The doctoral student knows very well the literature on the subject of the dissertation, expresses her position, analyzes the obtained results in a reasoned manner.

The dissertation is written and structured correctly. There are small inaccuracies that cannot affect my positive assessment of Rafailova's dissertation work. The goal and tasks are clearly set, the results and the discussion are connected logically. The conclusions are precisely formulated and correspond to the data obtained in the scientific experiment. Contributions are original. Data on effects of anethole on food consumption, fluid and caloric intake, behavior of anethole-treated rats, glucose tolerance, lipid peroxidation in brain, histological findings in retroperitoneal adipose tissue, data on effects of anethole on the programmed cell death in the retroperitoneal adipose tissue and liver, and data on the reduction of expression of the inflammatory marker MAC387 in the liver by anethole in an experimental model of diet-induced obesity in rats. I accept the scientific contributions that actually derive from the experimental research carried out and reflect important results obtained for the first time in our country. The scientific contributions have significant theoretical and real applied value.

The thesis abstract of the dissertation "Pharmacological studies with anethole in an experimental model of obesity" contains 100 pages. The results are illustrated with 28 figures and 27 tables. The thesis abstract reflects the main content of the

dissertation work and the obtained results. The requirements specified in the legal documents of MU-Varna have been met.

Elise Rafailova has presented a list of 5 publications and 14 participations in 3 international and 11 national forums with international participation related to the topic of the dissertation work. For her participation in scientific forums, Dr. Rafailova was awarded in Vienna in 2022, 35 ECNP, in 2023 36 ECNP in Barcelona and at the Ninth pharmaceutical forum and scientific-practical conference "Faculty of Pharmacy", city of Varna. With the presented publications, Dr. Rafailova meets the requirements of the Varna University of Medical Sciences for the ONS "Doctor". Dr. Rafailova possesses the qualities and skills to independently conduct scientific research and work in a team. It can be concluded that the presented publications and dissertation work are personal credit to the PhD student.

Elis Rafailova has theoretical knowledge and practical skills for scientific work. She can deal freely with the facts from her available literature, express and defend her own position with arguments, compare and contrast the data from the conducted research with those of other authors, looking for the reasons for the similarities and differences between them.

Conclusion

My comprehensive assessment of the presented dissertation work on the topic "Pharmacological study with anethole in an experimental model of obesity" by Dr. Elis Rafailova Gasanzadeeva is positive.

The topicality of the problem, the excellent knowledge of the issues under consideration, the possibility of interpretation and comparison of the obtained results with data from experimental studies of other authors, the expressed hypotheses, conclusions and contributions of theoretical and practical importance give me reason

to positively evaluate the conducted research presented by the reviewed above dissertation work, abstract, achieved results and contributions. Significant credit must be given to the scientific supervisor of the doctoral student Prof. Stefka Kuzmanova, MD, PhD, Dsc and to the head of the department Associate professor Silvia Gancheva Marinova, MD, PhD for the excellent opportunities created for scientific research in the department, for their professionalism and qualities of supervisors, for the team of young and enthusiastic teachers who want to develop, for the working environment and relations in the department that contribute to their success in science.

The presented dissertation work and the attached publications cover the requirements of the law on the development of the academic staff in the Republic of Bulgaria and the Regulations of the University of Varna in the field of higher education 7.0 Health care and sports, professional direction 7.1. Medicine, doctoral program "Pharmacology (incl. pharmacokinetics and chemotherapy)" for the educational and scientific degree "Doctor".

Based on the detailed positive aspects of the dissertation work, I strongly recommend to the honorable scientific jury to vote positively for awarding the educational-scientific degree "Doctor" to Elis Rafailova Gasanzadeeva, MD in the doctoral program "Pharmacology (incl. pharmacokinetics and chemotherapy) "

Reviewer: …

Prof. Ivanka Kostadinova, MD, PhD