

OPINION

of Assoc. Prof. Silvia Gancheva Marinova, MD, PhD

Head of the Department of Pharmacology and Clinical Pharmacology and Therapeutics at the Faculty of Medicine at Medical University "Prof. Dr. Paraskev Stoyanov"

regarding

a dissertation for acquisition of educational and scientific degree "**Doctor**" in the field of higher education 7. Healthcare and sport, professional direction 7.1. Medicine, scientific specialty „Pharmacology (incl. Pharmacokinetics and Chemotherapy)" of

Elis Rafailova Gasanzadeeva, MD – a doctoral student in full-time training at the Department of Pharmacology and Clinical Pharmacology and Therapeutics at Medical University of Varna

on the topic „**Pharmacological investigation with anethole in an experimental model of obesity**"

Scientific supervisor: Prof. Stefka Vasileva Valcheva-Kuzmanova, MD, PhD, DSc

On the basis of Order № P-109-168/ 22.05.2024 of the Rector of Medical University of Varna and decision of the Scientific jury (Protocol № 1/31.05.2024), I am appointed to prepare an opinion on the procedure for acquiring the educational and scientific degree "Doctor" with candidate Elis Rafailova Gasanzadeeva, MD.

The submitted materials for the procedure meet the requirements of the Law on Development of Academic Staff in Republic of Bulgaria and the Regulations for its application at Medical University of Varna.

Biographical data

Elis Rafailova was born in 1993. She completed her secondary education in 2012 at "N. Y. Vaptsarov" High School, Shumen with English and Russian language profile. She graduated as a "master" in medicine in 2018 at the Medical University of Varna. As a student, she conducted practical internships under the "Student Practices" project at St. Anna Hospital in 2014 and 2017. In 2018, she participated as a student-demonstrator in the Pharmacology seminars for 4th year medical students at Medical University of Varna. After graduation, she worked for a while in the Medical Oncology Department of Burgas University Hospital, after which she started working in the Department of Pharmacology and Clinical Pharmacology and Therapeutics at the Medical University of Varna as a part-time assistant professor. In October 2019, after winning a competition, she was appointed to the position of a full-time assistant professor at the department. Elis Rafailova hosts the disciplines of Pharmacology for medical and dental students and Clinical Pharmacology for medical students. She actively participates in the English language programs of

education. She speaks English and Russian. In 2023, she conducted a two-week specialization at Ovidus University in Constanta, Romania. She is a co-author of 19 scientific publications. Elis Rafailova is a member of Bulgarian Association of Pharmacology, Clinical Pharmacology and Therapeutics and European Association for Clinical Pharmacology and Therapeutics.

Relevance of the topic

Obesity is the most widespread disorder of energy metabolism. It is usually diagnosed by using the body mass index: values $\geq 25 \text{ kg/m}^2$ show overweight and values $\geq 30 \text{ kg/m}^2$ indicate obesity. The accumulation of visceral fat is associated with increased levels of oxidative stress and low-grade chronic inflammation. These abnormalities along with the insulin resistance play a leading role in pathogenesis of metabolic syndrome and associate with the development of type 2 diabetes, non-alcoholic fatty liver disease and other non-communicable diseases. The medical and social significance of obesity is enormous, as there is a persistent trend of increasing its rate worldwide not only among the adults, but also among the children and adolescents. The "Western" lifestyle, characterized by the consumption of high-calorie foods and drinks and reduced physical activity, contributes to this. Although obesity is recognized as a disease and a worldwide problem of a pandemic nature by the WHO, and in recent decades many efforts have been made to improve its early diagnosis and prevention, there is still no sufficiently effective and safe treatment. Therefore, efforts are directed to search for new approaches for prevention and therapy.

Plants are intensively studied as a potential source of biologically active substances with beneficial protective and/or therapeutic effects in a number of chronic disorders. The biologically active substances with natural origin evoke greater trust among the patients than the synthetic drugs and demonstrate a good safety profile. Anethole is a monoterpene found in the essential oil of various plants. Its beneficial effects have been established in *in vitro* and *in vivo* experimental studies. Its anti-inflammatory and antioxidant activity serves as a basis for the hypothesis that anethole would favorably affect energy metabolism disorders such as obesity.

Elis Rafailova's dissertation is dedicated to this relevant topic - obesity and its potential alleviation by biologically active substances of natural origin, in this case anethole.

Structure of the dissertation

Elis Rafailova's dissertation is presented by 157 standard pages. It is designed in accordance with the requirements for acquiring the educational and scientific degree "Doctor". The dissertation includes all obligatory sections, which are properly balanced, as follows: Introduction – 2 pages, Literature review – 33 pages, Objective and tasks – 2 pages, Materials and methods – 9 pages, Results and discussion – 72 pages, Conclusions – 4 pages, Contributions – 1 page, List of publications and congress participations related to the dissertation – 4 pages, References – 22 pages. The dissertation is illustrated with 40 figures and 27 tables. The bibliography includes 311 references.

The **literature review** is focused on obesity as it is the main problem concerned in the dissertation. The author reviews its epidemiology, the factors related to its development, the other chronic disturbances associated to it, and the current pharmacological and non-pharmacological strategies

for its treatment. The review includes the experimental data available to date on the beneficial impact of natural products, incl. monoterpenes, on this disorder of energy metabolism. The second part of the literature review is dedicated to anethole as it is the substance used for treatment of the animals in the experimental part of the dissertation. The author describes its physico-chemical properties, pharmacokinetic characteristics, and the data available so far on its pharmacological effects – anti-inflammatory, antioxidant, neuroprotective, antiplatelet, etc. The effects of anethole established so far suggest that this substance has a potential to improve the parameters of energy metabolism and influence the pathogenesis of obesity when consuming a high-calorie diet, which is the main hypothesis of the dissertation. The review is well illustrated with 2 figures.

The **objective and tasks** of the dissertation are logically related to the presented literature review. The objective is precisely and clearly formulated, and the tasks are adequate for its implementation.

The **materials and methods** used in the dissertation are suitable for the fulfillment of the tasks set. The author describes in detail the experimental model utilized for induction of obesity and the animal treatment. A wide range of research tools is used for the realization of the dissertation: behavioral tests for assessment of motor function, spatial memory, anxiety- and depressive-like behavior; biochemical tests for evaluation of energy homeostasis, liver function and oxidative stress; histological methods for detection of structural changes of liver, heart, coronary vessels and fat tissue; immunohistochemical methods for measurement of expression of pro-inflammatory, pro- and anti-apoptotic markers in liver and fat tissue; a model of carrageenan induced inflammation for detection of anti-inflammatory activity. It is noteworthy that dissertation is realized by using both classical and modern methods, which make possible not only to determine the changes in biochemical indicators, but also to reveal the pathogenetic mechanisms underlying them. All methods are described in detail. The statistical analysis is selected and performed adequately.

The **results and discussion** are combined in one section and follow the tasks. The author presents sequentially the effects of anethole in animals fed high-calorie diet on: body weight, consumption of food and fluids and total caloric intake; behavior and spatial memory; biochemical parameters of energy metabolism, antioxidant defense and oxidative stress; fat tissue; myocardium and coronary vessels; liver; carrageenan-induced paw edema. The results are presented clearly. The visualization with figures, tables and pictures facilitates their perception by the reader. After the presentation of each group of results, they are discussed in the context of the currently available data on the mechanism of action and effects of anethole. Many of the results confirmed the initial hypothesis of the doctoral student – anethole improved the spatial memory of experimental animals, prevented the development of anxiety in one of the experimental groups, showed a tendency for antidepressant-like activity, prevented the impairment of glucose tolerance and antioxidant defense and reduced visceral adiposity at the highest dose used. Another part of the results differed from what was originally expected – for example, the author did not find anti-dyslipidemic, antiplatelet or hepatoprotective effect, as well as an anti-inflammatory action in the carrageenan-induced paw edema model. Of interest are immunohistochemical studies, which showed that the reduction of visceral obesity in the experimental group receiving the highest dose of anethole was due to the activation of apoptosis of fat cells. A similar activation of apoptosis was also found in the liver. Another interesting finding is the effect of anethole on food and fluid

consumption and total caloric intake, indicating a possible impact on central appetite regulation. It is noteworthy that despite increased caloric intake, the group receiving the highest dose of anethole had reduced body weight gain and reduced visceral adiposity.

On the basis of the results, the author forms 2 main **conclusions**, which correspond to the set tasks. Each conclusion is further divided and described in subsections. I would recommend to Elis Rafailova to summarize the conclusions and to present them briefly without a detailed description.

The **contributions** of the dissertation are adequately formulated by the doctoral student. The contributions can be assessed as original, since this is the first study showing that anethole administered to rats with diet-induced obesity:

- Increases consumption of food and fluids and total caloric intake
- Improves spatial memory and shows anti-anxiogenic action and tendency for antidepressant-like activity
- Prevents impairment of glucose tolerance and suppresses brain lipid peroxidation
- Prevents the structural changes of retroperitoneal fat tissue
- Stimulates the pro-apoptotic phenotype in retroperitoneal fat tissue and liver
- Suppresses liver expression of the pro-inflammatory marker MAC387

Publications and participations in scientific events

Elis Rafailova has presented a list of 5 full-text scientific publications related to the dissertation. She is the first author of all of them. The high activity of the doctoral student is visible from the presented list of 14 participations at scientific forums, 3 of which were held abroad, with Elis Rafailova being the first and presenting author of 11 of them. At 3 of the scientific forums, Elis Rafailova has been awarded, she has won twice the Excellence award for young scientists from the European College of Neuropsychopharmacology, which is an indicator of the high quality of the presented results.

Summary of the dissertation

The summary of the dissertation is prepared in accordance with the requirements. The summary consists of 100 pages. It is appropriately structured and illustrated.

Conclusion

The dissertation of Elis Rafailova Gasanzadeeva MD entitled „Pharmacological investigation with anethole in an experimental model of obesity” is dedicated to one of the most relevant problems of modern society. A variety of research methods have been utilized for the implementation of dissertation tasks. Results of high significance have been obtained, representing an original scientific contribution. The dissertation of Elis Rafailova meets the requirements of the Law on Development of Academic Staff in Republic of Bulgaria and the Regulations for its application at Medical University of Varna.

The dissertation of Elis Rafailova shows that she possesses theoretical knowledge and professional skills, knows to use a wide range of experimental methods, analyzes and presents the obtained results, and demonstrates qualities and abilities for independent conduct of scientific research.

In conclusion, I give my **positive** assessment to Elis Rafailova Gasanzadeeva, MD and I offer the esteemed Members of the Scientific Jury to award her the educational and scientific degree “Doctor” in the field of higher education 7. „Healthcare and sport”, professional field 7.1. “Medicine”, scientific specialty „Pharmacology (incl. Pharmacokinetics and Chemotherapy)”.

25.06.2024
Varna, Bulgaria

Prepared by:

/Assoc. Prof. ~~Sil~~^Kvia Gancheva, MD, PhD/

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