

OPINION

By Assoc. Prof. Miroslav Tsonkov Eftimov, MPh, PhD

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

regarding

a dissertation for obtaining the educational and scientific degree "Doctor" in the field of higher
education 7. Healthcare and Sports, professional direction 7.1. Medicine, doctoral program
"Pharmacology (incl. pharmacokinetics and chemotherapy)"

of

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

on the topic "**Pharmacological studies with anethol in an experimental model of obesity**"

with scientific supervisor Prof. Stefka Vasileva Valcheva-Kuzmanova, MD, PhD, DSc

Information about the procedure

As a member of the Scientific Jury in accordance with Order No. P-109-168 of 22.05.2024 by the Rector of the Medical University – Varna, and based on Protocol No. 1 of 31.05.2024, I am appointed to prepare an **opinion** on the procedure for obtaining the educational and scientific degree "Doctor", with the candidate **Elis Rafailova Gasanzadeeva, MD**.

Dr. Gasanzadeeva has submitted all the necessary documents in accordance with the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria, the Rules for the Implementation of the Law for the Development of the Academic Staff, the Rules for the Development of the Academic Staff at the Medical University – Varna, and the Procedure for Obtaining the Educational and Scientific Degree "Doctor" at the Medical University – Varna.

Biographical Data

Elis Gasanzadeeva was born in 1993 in the city of Shumen. In 2018, she graduated from the Medical University – Varna and obtained a Master's degree in Medicine. From January to March 2019, she worked as a physician in the Medical Oncology Department at University Hospital "Deva Maria" in Burgas. In March 2019, she began as a part-time assistant, and since October of the same year, she has been appointed as a full-time assistant at the Department of Pharmacology and Clinical Pharmacology and Therapeutics at the Medical University – Varna. Dr. Gasanzadeeva leads classes in both Bulgarian and English language programs in the disciplines "Pharmacology" and "Clinical Pharmacology". She speaks Turkish fluently and has an intermediate level in English, and basic level in Russian. She has a short-term specialization at Ovidius University in Constanța, Romania, and has completed a course on "Protection and Humane Treatment of Experimental Animals Used for Scientific and Educational Purposes". She is a co-author of 19 full-text

publications and has 2 citations. Elis Gasanzadeeva is a member of the Bulgarian Society of Pharmacology, Clinical Pharmacology, and Therapeutics, as well as the European Association of Clinical Pharmacology and Therapeutics.

Relevance of the Dissertation

Obesity is a global health problem with increasing prevalence. Its causes are multiple and variable – genetic, socio-economic, endocrine-metabolic, disease, medication therapy, etc. It is associated with an increased risk of developing cardiovascular diseases, diabetes, infertility, neurodegenerative and cognitive diseases, cancer, etc. The therapy for obesity includes lifestyle changes, medication therapy, and even surgical methods. Phytochemical products have long been used in the prevention and as an additional treatment of numerous chronic diseases. According to WHO data, 80% of people receive their primary medical care from traditional medicine through the use of plant products and their active components. Against the backdrop of numerous studies on plant extracts and compounds contained in them, which are used in the fight against obesity, there is only one study on the monoterpene anethole in weight gain. In this context, the research in the present dissertation is focused on an important and current medical and social problem, and the results from it would have undeniable scientific and practical potential.

Structure of the Dissertation

Elis Gasanzadeeva's dissertation consists of 157 pages and is formatted according to the requirements for obtaining a PhD. It is structured as follows: Introduction – 2 pages, Literature Review – 33 pages, Aim and Objectives – 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 – 3 pages, Bibliography – 23 pages. The individual sections are properly arranged and balanced in volume. The work is illustrated with 40 figures and 27 tables. The bibliography includes 309 English-language sources and 2 in Cyrillic.

The **Literature Review** is divided into three parts. In the first part, Dr. Gasanzadeeva examines the data available in the literature related to the problem of obesity, emphasizing the risk factors for its development, diseases associated with obesity, and pharmacological and non-pharmacological methods of treatment. Attention is given to natural products as an alternative with fewer side effects compared to conventional drugs in the therapy of obesity. The focus logically falls on monoterpenes and the essential oils containing them, as the monoterpene anethole is central to the present dissertation.

The second part of the review focuses on the phenylpropanoid anethole. Its physicochemical characteristics, pharmacokinetics, and the pharmacological effects described in the literature are outlined.

The third part presents various experimental models for inducing obesity in laboratory animals, along with their advantages and disadvantages.

The structure of the literature review is sequential and logically leads to the necessity of studying the effects of anethole on obesity using experimental models in laboratory animals.

Aim and Objectives

The presented dissertation has a clearly and precisely formulated aim - to investigate and summarize the pharmacological effects of anethole, administered orally in increasing doses to rats in a model of obesity induced by a high-calorie diet.

The objectives are sequential and logically presented, clearly outlining the algorithm for achieving the stated aim.

Materials and Methods

The study design is described in detail and in a logical sequence. The methods used are clearly presented, and their selection is appropriate for achieving the stated objectives. The statistical methods are also correctly chosen, allowing for proper interpretation of the obtained results.

Results and Discussion

The results presented by Dr. Gasanzadeeva are based on original data obtained because of completing the tasks of the dissertation project. They can be divided into seven sections. The first section examines the effects of anethole on the change in body weight of the experimental animals. The data discussed in the literature show varying effects, whereas in the conducted study, a high dose of anethole leads to a significant reduction in weight gain. The second section discusses the effects of anethole on animal behavior. A dose-dependent effect on the improvement of spatial memory was found. Similar beneficial effects of anethole on memory in various other models of cognitive impairment are discussed. The third section focuses on biochemical and clinical laboratory indicators. It is established that anethole normalizes the increased lipid peroxidation in the brain caused by the high-calorie diet at all administered doses, which can be used to explain its beneficial effects on the central nervous system. The fourth section presents the effects on adipose tissue. At a high dose, anethole lowers the retroperitoneal fat index, significantly reduces adipocyte size, and increases its apoptotic potential, which can be used in the treatment of obesity. The fifth section discusses the effects on the myocardium and coronary vessels, revealing that anethole does not prevent myocardial damage and does not antagonize focal necrosis in the endothelium of the coronary vessels. In the sixth section, Dr. Gasanzadeeva describes the effects on the liver. Anethole does not demonstrate a protective effect against micro-vesicular steatosis but significantly increases apoptotic potential at a high dose and at a low dose reduces the expression of the inflammatory marker MAC387, indicating anti-inflammatory action. The final section presents the results in carrageenan-induced paw edema, where anethole does not lead to significant changes in the edema.

Conclusions and Contributions

The presented conclusions clearly reflect the results of the dissertation and fully correspond to the stated objectives.

Six contributions of original character are derived, which are well-formulated and substantiated. For the first time, the effects of anethole in an experimental model of diet-induced obesity in rats are demonstrated on:

- Food, fluid consumption, and caloric intake
- Behavior of experimental animals
- Glucose tolerance and lipid peroxidation in the brain
- Histology of adipose tissue
- Programmed cell death in adipose tissue and liver
- Expression of the inflammatory marker MAC387 in the liver

The obtained results provide the opportunity to discover new, beneficial effects of anethole in obesity, which is a socially significant problem growing at a rapid pace. Additionally, the dissertation addresses the question of the potential mechanisms underlying these effects.

Publications and participations in scientific events

A list of 5 publications related to the dissertation is presented, with Dr. Gasanzadeeva being the first author of all five. Participation in scientific forums totals 14, of which 3 are international, and 11 are in Bulgaria. It is important to note that Dr. Gasanzadeeva has also received three awards from participation in national and international scientific conferences.

Summary of the dissertation

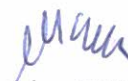
The summary of the dissertation is 100 pages in length, logically structured, and fully reflects the content of the dissertation.

Conclusion

The dissertation "Pharmacological studies with anethole in an experimental model of obesity" by Dr. Elis Gasanzadeeva represents an original scientific work on a relevant and socially significant topic. A variety of methods were used, enabling a detailed picture of the researched problem. The obtained results are of theoretical and practical significance and represent an original contribution to science. Dr. Gasanzadeeva possesses in-depth theoretical knowledge on the subject, has mastered a wide range of experimental methodologies, and is able to competently interpret the obtained results. Her dissertation fully meets the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the Application of the Law on the Development of the Academic Staff, the Regulations for the Development of the Academic Staff at the Medical University of Varna, and the Procedure for Obtaining the PhD Degree at the Medical University of Varna.

For the above reasons, I give my **positive assessment** and propose to the esteemed members of the Scientific Jury to award the educational and scientific degree "Doctor" in the field of higher education 7. Healthcare and Sports, professional direction 7.1. Medicine, doctoral program "Pharmacology (including pharmacokinetics and chemotherapy)" to Elis Rafailova Gasanzadeeva.

19.06.2024
Varna, Bulgaria

Prepared by: 
/Assoc. Prof. Miroslav Eftimov, MPh, PhD/

ДЕКЛАРАЦИЯ

Долуподписаният/та Мирослав Цонков Ефимов.....
в качеството си на член на научно жури в процедура за придобиване на ОНС „Доктор“ в
Медицински университет „Проф. д-р Параскев Ив. Стоянов“ – Варна от д-р Елис
Рафаилова Гасанзадеева, по област на висше образование 7. Здравеопазване и спорт,
професионално направление 7.1. Медицина, докторска програма „Фармакология (вкл.
фармакокинетика и химиотерапия)“.

ДЕКЛАРИРАМ, ЧЕ:

1. Участвах в заключителното заседание по процедурата за защита на дисертационния труд, проведено на **12.07.2024 г.** чрез технически средства;
2. На проведеното заседание потвърдих своята положителна..... (вписва се положителна/ отрицателна) оценка, за придобиване на ОНС „Доктор“ от д-р Елис Рафаилова Гасанзадеева.
3. Запознат/а съм с протокола от проведеното заседание на научното жури и съм съгласен/а със съдържанието му, в т.ч. и с взетото решение.

Дата: **12.07.2024 г.**

Декларатор: Мц.....

Заличено на основание чл. 5,
§1, б. „В“ от Регламент (ЕС)
2016/679

/име и подпис/

