

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

Mehmed Reyzov Abtulov, 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 of the effects of Aronia melanocarpa fruit juice in an experimental model of metabolic syndrome**"

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

On the basis of Order № P-109-282/ 08.07.2022 of the Rector of Medical University of Varna and decision of the Scientific jury (Protocol № 1/13.07.2022), I am appointed to prepare an opinion on the procedure for acquiring the educational and scientific degree "Doctor" with candidate Mehmed Reizov Abtulov, 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

Mehmed Reizov was born in 1992 in the village of Bisertsi, Razgrad region. He completed his secondary education in 2011 at "Hristo Botev" Secondary School, Kubrat. He graduated as a "master" in medicine in 2017 at the Medical University of Varna. From 2018 to the present, he works as an assistant professor at the Department of Pharmacology and Clinical Pharmacology and Therapeutics at Medical University of Varna. In 2022, he was appointed to the position of administrative assistant at the Department. Mehmed Reyzov participates in one scientific project. He speaks English and Turkish. He has excellent computer skills, incl. ability to work with graphic and statistical software. He works with specific analytical equipment for biochemical research.

Relevance of the topic

Metabolic syndrome is a widespread disorder of energy metabolism. It represents a set of clinical and biochemical deviations, incl. visceral obesity and insulin resistance. These metabolic abnormalities are associated with increased levels of oxidative stress and low-grade chronic inflammation and play a leading role in pathogenesis of the syndrome. The medical and social

significance of metabolic syndrome is enormous, as there is a persistent trend of increasing its rate worldwide, both among adults and children. The "Western" way of life, characterized by consumption of high-calorie diet and low physical activity, contributes to this. Metabolic syndrome is a well-known risk factor for development of cardiovascular diseases and type 2 diabetes. According to recent epidemiological studies, it is also associated with neuro-psychiatric diseases, such as anxiety and depressive disorders, as well as cognitive impairment. Despite progress in the study of etiology and pathogenesis of metabolic syndrome, there is still no effective treatment. Therefore, efforts are directed to search for alternatives in therapy.

Plants are intensively studied as a potential source of biologically active substances with beneficial protective and/or therapeutic effects in metabolic syndrome. Polyphenols, possessing anti-inflammatory, antioxidant and organoprotective properties, are some of the most widely studied biologically active substances of natural origin. An additional advantage of plant polyphenols is their safety. *Aronia melanocarpa* is a plant whose fruits are extremely rich in polyphenols. A number of experimental and clinical studies have confirmed that *Aronia melanocarpa* fruit juice possesses various beneficial effects, incl. anti-hyperglycemic, cholesterol-lowering and anti-obesity.

Mehmed Reizov's dissertation is dedicated to this relevant topic - metabolic syndrome and its potential alleviation by biologically active substances of natural origin, in this case *Aronia melanocarpa* fruit juice.

Structure of the dissertation

Mehmed Reizov's dissertation is presented by 159 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 – 43 pages, Objective and tasks – 1 page, Materials and Methods – 10 pages, Results and discussion – 50 pages, Conclusions – 4 pages, Contributions – 2 pages, List of publications and participations related to the dissertation – 2 pages, References – 34 pages. The dissertation is illustrated with 30 figures and 27 tables. The bibliography includes 410 references.

The **literature review** is focused on metabolic syndrome and potential beneficial effects of *Aronia melanocarpa* fruit juice. Metabolic syndrome is reviewed in details, paying attention to its etiopathogenesis and current therapeutic strategies. The social significance of the disorder is also emphasized, due to its high epidemiological rate and the association with number of other diseases. The author also describes the main diet-induced experimental models of metabolic syndrome. *Aronia melanocarpa* is considered by the author as a plant with expected beneficial effects on metabolic syndrome. He describes in details the content of the fruits, as well as the currently available data on their biological activity. Logically, this part of the review is focused on studies demonstrating *Aronia melanocarpa* beneficial effects on energy metabolism. The review is well illustrated with 5 tables and 4 figures. The review concludes with a brief summary of the available information on the topics covered to date, noting the lack of data on the effect of *Aronia melanocarpa* fruit juice on visceral adiposity, antioxidant defense system and animal behavior in a model of metabolic syndrome induced by high-fat high-fructose diet.

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 content of the fruit juice used in the experiments, as well as the models inducing metabolic syndrome and acute inflammation. The treatment of animals is also well explained. 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 and antioxidant defense system; histological and immunohistochemical methods. All methods are described in detail. The combination of classic research methods with the most modern ones is impressive. The statistical analysis is selected and performed adequately.

The **results and discussion** are combined in one section and follow the tasks. The author presents in different subsections the effects of *Aronia melanocarpa* fruit juice in animals with diet-induced metabolic syndrome on: behavior and spatial memory, biochemical parameters of energy metabolism, antioxidant enzymes, adipose tissue, myocardium and coronary arteries, liver, and carrageenan-induced paw edema. Results are clearly presented. The visualization with figures and tables facilitates their quick perception by the reader. Most of the results confirm the initial hypothesis of the doctoral student – *Aronia melanocarpa* fruit juice improves a significant part of the behavioral, biochemical, histopathological and immunohistochemical impairments associated with the metabolic syndrome. Each subsection ends with a discussion in which the author competently analyzes the obtained results.

The results described in Mehmed Reyzov's dissertation are of great theoretical and practical importance, as they could be used as a basis for designing nutritional supplements or medications for prevention and/or treatment of the clinical-biochemical abnormalities associated with the metabolic syndrome.

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

The **contributions** of the dissertation are well structured. They can be assessed as original, since data on the effects of *Aronia melanocarpa* fruit juice in an experimental model of diet-induced metabolic syndrome are reported for the first time, as follows:

- *Aronia melanocarpa* fruit juice produces an anxiolytic-like effect and improves spatial memory
- *Aronia melanocarpa* fruit juice produces a glucose-lowering and antihypertriglyceridemic effect
- *Aronia melanocarpa* fruit juice suppresses oxidative stress
- *Aronia melanocarpa* fruit juice reduces visceral obesity
- *Aronia melanocarpa* fruit juice produces a protective effects against the histological alterations in adipose tissue, myocardium, coronary vessels and liver

- *Aronia melanocarpa* fruit juice affects the programmed cell death in adipose tissue and liver:
 - The juice suppresses the programmed cell death in adipose tissue at doses of 2.5 and 5.0 ml/kg. and might induce adipocyte apoptosis at high dose (10 ml/kg)
 - The juice suppresses hepatocyte apoptosis at all doses
- *Aronia melanocarpa* fruit juice produces an anti-inflammatory effect after carrageenan-induced hind paw edema

Publications and participations in scientific events

Mehmed Reyzov has presented a list of 4 full-text scientific articles related to the dissertation. He is the first author of all of them. One of the articles is accepted for publication in a scientific journal with impact factor. The results of the dissertation have been presented at 4 scientific forums, including international.

Abstract of the dissertation

The abstract of the dissertation is prepared in accordance with 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 abstract consists of 78 pages. It is appropriately structured and illustrated.

Conclusion

Mehmed Reyzov's dissertation entitled „Pharmacological investigation of the effects of *Aronia melanocarpa* fruit juice in an experimental model of metabolic syndrome” is dedicated to one of the most relevant problems of modern society. A variety of classic and modern research methods have been utilized for the implementation of dissertation tasks. Results of high theoretical and practical significance have been obtained, representing an original scientific contribution. The dissertation of Mehmed Reyzov 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.

Mehmed Reyzov's dissertation shows his ability to use a variety of research methods and competently analyze and present the results obtained. Mehmed Reyzov demonstrates in-depth theoretical knowledge and professional skills in scientific field of pharmacology and is capable to conduct independently a scientific research.

In conclusion, I confidently give my **positive** assessment to Mehmed Reyzov Abtulov, MD for awarding 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)”.

05.09.2022
Varna, Bulgaria

Prepared by:


/Assoc. Prof. Silvia Gancheva, MD, PhD/