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

Klementina Moncheva Moneva-Marinova, 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 Chaenomeles maulei 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-109-46/ 14.02.2024 of the Rector of Medical University of Varna and decision of the Scientific jury (Protocol № 1/23.02.2024), I am appointed to prepare an opinion on the procedure for acquiring the educational and scientific degree "Doctor" with candidate Klementina Moncheva Moneva-Marinova, 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

Klementina Moneva was born in 1993 in Stara Zagora. She completed her secondary education in 2012 at "Romain Rolland" High School, Stara Zagora. She graduated as a "master" in medicine in 2018 at the Medical University of Varna. After graduation, she started working at the Department of Pharmacology and Clinical Pharmacology and Therapeutics at Medical University of Varna as a part-time assistant professor, and in April 2019, after winning a competition, she was appointed to the position of a full-time assistant professor at the department. Klementina Moneva teaches 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 fluently and has basic knowledge of German. She is a co-author of 9 full-text scientific publications and has participated in more than 15 national and international scientific forums. Her research articles have been cited 20 times. Klementina Moneva is a member of Bulgarian Association of Pharmacology, Clinical Pharmacology and Therapeutics and European Association for Clinical Pharmacology and Therapeutics.

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. A major concern is that the age of onset of metabolic syndrome is progressively falling, with about 3% of children and 5% of adolescents worldwide affected by it in 2020. The rate increases with age. In a population where average life expectancy increases, which is the tendency worldwide in the last decades, we can expect that the medical and social importance of metabolic syndrome will also rise in the future. 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 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, incl. metabolic syndrome. The biologically active substances with natural origin evoke greater trust among the patients than the synthetic drugs and demonstrate a good safety profile. The Japanese quince plant, belonging to genus *Chaenomeles,* family of Rosaceae, has been used for therapeutic purpose for thousands of years in the traditional Eastern medicine. Most of its biological effects, although observed empirically, still lack scientific proof.

Klementina Moneva's dissertation is dedicated to this relevant topic - metabolic syndrome ant its potential alleviation by biologically active substances of natural origin, in this case *Chaenomeles maulei* fruit juice.

Structure of the dissertation

Klementina Moneva's dissertation is presented by 123 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 – 29 pages, Objective and tasks – 2 pages, Materials and methods – 8 pages, Results and discussion – 54 pages, Conclusions – 3 pages, Contributions – 2 pages, List of publications and congress participations related to the dissertation – 2 pages, References – 14 pages. The dissertation is illustrated with 30 figures and 13 tables. The bibliography includes 187 references.

The **literature review** is focused on metabolic syndrome and potential alleviation of its clinical and biochemical characteristics by *Chaenomeles maulei* based on the high polyphenol content of its fruit juice. The pathogenesis of metabolic syndrome is reviewed in detail. The author pays attention to the well-known classical mechanisms for its development, as well as to those whose pathogenetic role has been recognized recently, such as circadian dysrhythmia and the role of

intestinal microbiota. The main models of metabolic syndrome utilized to study it in experimental settings are also described. *Chaenomeles maulei*, whose fruit juice is the main experimental substance in the dissertation, is characterized in terms of botanical data, chemical composition of the fruit and fruit juice, and biological activity known to date. It should be noted that this plant with potentially many beneficial effects is still poorly studied and data on biological activity, especially in *in vivo* experiments, are scarce. The author also reviews the currently known effects of polyphenols on metabolic syndrome and its components. The experimental studies in this field are substantial and represent the basis on which the hypothesis of the dissertation was built, that the *Chaenomeles maulei* fruit juice could favorably affect the clinical and biochemical characteristics of metabolic syndrome. The review is well illustrated with 6 figures. The literature review is comprehensive and presented clearly and logically. It demonstrates Klementina Moneva's in-depth theoretical knowledge of the issues covered in her dissertation.

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 experiment, the model inducing metabolic syndrome and the animal treatment. A wide range of research tools is used for the realization of the dissertation: biochemical tests for evaluation of energy homeostasis and oxidative stress, histological methods for detection of structural changes of liver, heart, coronary vessels and fat tissue, behavioral tests for assessment of motor function, spatial memory, anxiety- and depressive-like behavior. 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 *Chaenomeles maulei* fruit juice in animals with diet-induced metabolic syndrome on: consumption of food and fructose solution and total caloric intake; body weight and visceral obesity; biochemical parameters of energy metabolism, antioxidant defense and oxidative stress; histological changes in the structure of myocardium, coronary arteries, liver and adipose tissue; behavior and spatial memory. The results are presented clearly. The visualization with figures and tables facilitates their quick perception by the reader. Many of the results confirm the initial hypothesis of the doctoral student – *Chaenomeles maulei* fruit juice reduced visceral obesity and improved the antioxidant defense in experimental animals fed high-fat high-fructose diet. Although the biochemical parameters of lipid metabolism and glucose homeostasis did not show statistically significant improvement, the histological changes in the myocardium, coronary vessels, liver and adipose tissue were almost completely prevented by the fruit juice. Of interest is the unexpected effect of the fruit juice on food and fructose solution consumption and total caloric intake, indicating a possible impact on central appetite regulation. 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 polyphenols.The discussion is clear and logical and demonstrates the competence of Kementina Moneva to analyze the obtained results.

On the basis of the results, the author forms 3 main **conclusions**, each of which is further divided and described in subsections. The effect of the fruit juice on consumption of food and fructose

solution, and total caloric intake is presented as a separate conclusion. In this way, the author highlights its significance.

The results described in Klementina Monveva'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 metabolic syndrome.

The **contributions** of the dissertation can be assessed as original, since data on the effects of *Chaenomeles maulei* fruit juice in an experimental model of diet-induced metabolic syndrome are reported for the first time. For the first time, within this experimental model, are reported the effects of *Chaenomeles maulei* fruit juice on:

- Total caloric intake and consumption of food and fructose solution
- Visceral obesity
- Antioxidant defense and lipid peroxidation
- Histology of the myocardium, coronary vessels, liver and adipose tissue
- Locomotor activity, behavior and spatial memory

The conducted research on *Chaenomeles maulei* fruit juice contributes to the better understanding of the effects of the juice and of the polyphenols in its content in the context of metabolic syndrome.

Publications and participations in scientific events

Klementina Moneva has presented a list of 4 full-text scientific articles related to the dissertation. She 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 2 scientific forums, including an international one.

Summary of the dissertation

The summary of the dissertation is prepared in accordance with the requirements. The summary consists of 79 pages. It is appropriately structured and illustrated.

Conclusion

The dissertation of Klementina Moncheva Moneva-Marinova, MD entitled „Pharmacological investigation of the effects of *Chaenomeles maulei* fruit juice in an experimental model of metabolic syndrome" 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 theoretical and practical significance have been obtained, representing an original scientific contribution. The dissertation of Klementina Moneva 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 Klementina Moneva shows her ability to use a variety of research methods and competently analyze and present the results obtained. Klementina Moneva 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 Klementina Moncheva Moneva-Marinova, 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)".

26.03.2024
Varna, Bulgaria

Prepared by:
/Assoc. Prof. Silvia Gancheva, MD, PhD/