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 field 7.1. Medicine, scientific specialty „Pharmacology (incl. Pharmacokinetics and Chemotherapy)" of

Milena Todorova Salbashian, MD – a self-training doctoral student at the Department of Pharmacology and Clinical Pharmacology and Therapeutics at Medical University of Varna

on the topic **„Pharmacological study of behavioral effects of biologically active substances of plant origin in experimental animal models of depression"**

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

Consultant: Prof. Roman Emilov Tashev, MD, PhD, DSc

On the basis of Order № P-109-441/ 11.11.2022 of the Rector of Medical University of Varna and decision of the Scientific jury (Protocol № 1/15.11.2022), I am appointed to prepare an opinion on the procedure for acquiring the educational and scientific degree "Doctor" with candidate Milena Todorova Salbashian, 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

Milena Todorova was born in 1966 in the city of Varna. In 1987, she graduated with a degree in Nursing at Institute for Training of Health Personnel, and in 1999 she graduated as "master" in medicine at Medical University of Varna. In the period 1987-1991, she worked as a nurse in a Cardiology Department in the city of Varna, and subsequently from 2000 to 2005 as an epidemiologist and general practitioner in the town of Dobrich. From 2005 to 2013, she worked as a medical assistant, phlebotomist in the USA. From 2015 to the present, she works as an assistant professor at the Department of Pharmacology and Clinical Pharmacology and Therapeutics at Medical University of Varna, teaching the disciplines "Pharmacology" and "Clinical Pharmacology" to medical and dental students in the Bulgarian- and English-language form of training. Milena Todorova participates in three scientific projects. She speaks English and Russian. She has excellent computer skills, incl. ability to work with graphic and statistical software. She is

a member of the Bulgarian Medical Union and the Bulgarian Scientific Society of Pharmacology and Clinical Pharmacology and Therapeutics.

Relevance of the topic

Affective disorders represent an extremely serious problem of the modern society with great health, economic and social significance. According to WHO data from 2021, about 5% of the world's adult population suffers from depression. Statistics shows that 1 in 6 people will experience depression at some time in their life. Depression is a leading cause of disability in the world, especially in young adults. It is the second most common cause of suicide among young people between the ages of 15 and 29. The rate of affective disorders has increased dramatically in recent years as a result of the COVID-19 pandemic, which has greatly increased the relevance of this serious health problem.

Despite the availability of a wide range of antidepressants and their widespread use, a large proportion of patients do not respond satisfactorily to therapy. The effect of most antidepressants develops slowly, the therapy is long-term and often accompanied by various adverse drug reactions. Therefore, efforts are directed to search for new therapeutic approaches.

Medicinal plants are widely used for prevention and treatment of a number of chronic diseases, both alone and as an adjunct to conventional therapy. Polyphenols, possessing anti-inflammatory, antioxidant and organoprotective properties, are some of the most widely studied biologically active substances of natural origin. The discovery that they pass the blood-brain barrier serves as a basis for studying their potential neuroprotective and neuromodulatory properties and searching for beneficial effects in affective disorders and neurodegenerative diseases. An additional advantage of plant polyphenols is their safety. *Aronia melanocarpa* is a plant whose fruits are extremely rich in polyphenols – the fruit juice contains high concentrations of anthocyanins, procyanidins and phenolic acids, such as chlorogenic, ferulic and gallic acid.

Milena Todorova's dissertation is dedicated to this relevant topic – affective disorders and their potential alleviation by biologically active substances of natural origin.

Structure of the dissertation

Milena Todorova's dissertation is presented by 177 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 – 54 pages, Objective and tasks – 2 page, Materials and methods – 13 pages, Results and discussion – 50 pages, Conclusions – 4 pages, Contributions – 1 page, List of publications and participations related to the dissertation – 2 pages, References – 39 pages. The dissertation is illustrated with 41 figures and 12 tables. The bibliography includes 631 references.

The **literature review** is focused on the characteristics of polyphenols. The botanical data of *Aronia melanocarpa* are described in detail, as well as the chemical composition of the fruits, paying special attention to the polyphenolic compounds contained in them. The pharmacokinetics of polyphenols found in the fruit juice of *Aronia melanocarpa* are reviewed, highlighting their ability to pass the blood-brain barrier and accumulate in relatively high concentrations in the

hippocampus, cortex, striatum and cerebellum of experimental animals, especially in long-term administration. Experimental studies focusing on the effects of polyphenols on behavior, pain perception, memory and cognition, neuronal inflammation, neurogenesis, and central neurotransmission are described. The author reviews the data available to date on the modulation by polyphenols of neuronal and glial signaling pathways regulating synaptic plasticity and neurogenesis. Logically, this part of the review focuses on the symptoms and pathogenesis of depression, as well as on the influence of polyphenols on them. It would be appropriate in this part of the dissertation to review and compare the currently known experimental models for inducing depression. The literature review is well illustrated with 16 figures and 1 table. The review concludes with a brief summary of the information available to date on the topics covered, noting the lack of sufficient data on the effects of *Aronia melanocarpa* fruit juice and phenolic acids (chlorogenic, ferulic and gallic) on animal behavior in surgically induced depression by ovariectomy and bilateral olfactory bulbectomy.

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 experimental models of bilateral ovariectomy and bilateral olfactory bulbectomy aiming to induce depression. A wide range of behavioral methods is used for the realization of the dissertation: tests for assessment of motor function and exploratory behavior, learning and memory, anxiety- and depressive-like behavior, pain perception. The statistical analysis is selected and performed adequately.

The **results and discussion** are combined in one section and follow the tasks. The author first presents the effects of *Aronia melanocarpa* fruit juice and chlorogenic acid on the behavior of ovariectomized rats, evaluating locomotor activity (after 30 and 75 days of treatment), anxiety (after 31 and 76 days of treatment), depressive-like behavior (after 33 and 78 days of treatment) and pain sensitivity (after 79 days of treatment) of experimental animals. Then the author presents the effects of chlorogenic, ferulic and gallic acid on axiety, memory and learning in rats with bilateral olfactory bulbectomy after 14 days of treatment. Results are clearly presented. The visualization with figures and tables facilitates their quick perception by the reader. The author discusses each performed test immediately after the presentation of its results. In the discussion section, the author competently analyzes the possible mechanisms through which the obtained changes in behavior, pain perception, learning and memory are realized.

The results confirm the initial hypothesis of the doctoral student – *Aronia melanocarpa* fruit juice and phenolic acids contained in it improve a significant part of the behavioral changes, hyperalgesia, memory and learning impairments induced by ovariectomy and bulbectomy.

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 Milena Todorova 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 and phenolic acids contained in it in ovariectomized and bulbectomized rats are reported for the first time, as follows:

- In rats with a model of bilateral ovariectomy:
  - *Aronia melanocarpa* fruit juice suppresses motor activity, exhibits a certain anxiolytic effects, produces an antidepressant-like effects and increases threshold sensitivity for thermal pain;
  - Chlorogenic acid causes a certain decrease in general locomotor activity, prevents the development of anxiety, increases threshold sensitivity for thermal pain and has no effect on depressive symptoms.
- In rats with a model of bilateral olfactory bulbectomy chlorogenic, ferulic and gallic acids prevent hyperactivity, exhibit an anxiolytic-like effect and improve memory and learning processes.

The dissertation of Milena Todorova is of great theoretical and practical importance, as the results reveal future possibilities for improving the therapy of affective and neurodegenerative disorders.

Publications and participations in scientific events

Milena Todorova has presented a list of 4 full-text scientific articles related to the dissertation. She is the first author of 2 of them. Three of the articles are published in scientific journals that are referenced and indexed in world-renowned scientific information databases, and one of the articles has an impact factor. Milena Todorova's publication activity covers and exceeds the requirements for acquisition of the educational and scientific degree "Doctor". The results of the dissertation have been presented at 9 scientific forums, most of them international. Five of the abstracts of the reports are published in supplements of international scientific journals with an impact factor.

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 83 pages. It is appropriately structured and illustrated.

Conclusion

The dissertation of Milena Todorova Salbashian entitled „Pharmacological study of behavioral effects of biologically active substances of plant origin in experimental animal models of depression" is dedicated to a relevant problem of modern society. A variety of methods for assessment of animal behavior, pain perception, learning and memory 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 Milena Todorova 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 Milena Todorova shows her ability to use a variety of research methods and competently analyze and present the results obtained. Milena Todorova 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 **Milena Todorova Salbashian, 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)".

09.01.2023
Varna, Bulgaria

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