

TO

Assoc. Professor Silvia Gancheva, MD

Chairman of the Scientific Jury

under the procedure for the acquisition of ESD "Doctor"

PhD program "Pharmacology (incl. pharmacokinetics and chemotherapy)"

OPINION

by Assoc. Prof. Galya Tsvetanova Stavreva-Marinova, MD, PhD

Department of Pharmacology and Toxicology

Medical University - Pleven

According to the procedure for awarding the **educational and scientific degree "Doctor"** in

Field of higher education: 7. "Health and sports"

Professional direction: 7.1. "Medicine"

PhD program: Pharmacology (incl. pharmacokinetics and chemotherapy)

PhD student: Dr. Milena Todorova Salbashian

Form of doctoral studies: independent form of study

Dissertation topic: "PHARMACOLOGICAL STUDY OF BEHAVIORAL EFFECTS OF BIOLOGICALLY ACTIVE SUBSTANCES OF PLANT ORIGIN IN EXPERIMENTAL ANIMAL MODELS OF DEPRESSION"

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

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

I present the opinion in my capacity as a member of the Scientific Jury, determined by the order of the Rector of the University of Varna (No. R-109-441/11.11.2022) and the decision of the 1st absent meeting of the Scientific Jury held on 15.11.2022. I declare that I have no conflict of interest, including co-authorship with the PhD student Dr. Milena Todorova Salbashian.

General presentation of the procedure

The presented set of materials on an electronic medium is in accordance with the requirements of Art. 69 of the Regulations for the Development of the Academic Staff at the Medical University - Varna. On the basis of the set of materials and documents, I declare that the rules and conditions for the defense of a dissertation work for the acquisition of the ESD "Doctor" have been complied with, in accordance

with the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Rules for its application and the Regulations for the Development of the Academic Staff at the Medical University – Varna.

Education and qualification

Dr. Milena Todorova Salbashian is a Master of Medicine (diploma No. 093581/1999 from Medical University – Varna). Since September 2015, she is currently working as an assistant at the Department of Pharmacology and Clinical Pharmacology and Therapy of the University of Varna. By Order of the Rector of MU-Varna (No. R-109-127 of 01.04.2019) Dr. Milena Salbashian enrolled as a doctoral student with the topic of the dissertation work "Pharmacological study of behavioral effects of biologically active substances of plant origin in experimental animal models of depression" with scientific supervisor Prof. Stefka Valcheva-Kuzmanova and consultant Prof. Roman Tashev.

Dr. Salbashian finished PhD program with the right of defense by order of the Rector of MU-Varna No. R-109-441/11.11.2022.

She participated in 3 projects, funded by MU-Varna, related to the effects of biologically active substances of plant origin in experimental models. She has 28 full-text publications and 39 participations in scientific forums.

She speaks English and Russian, has the skills to work with a computer and experimental equipment.

The dissertation is written on 177 pages and contains 12 tables and 41 figures. All the main elements of the generally accepted in our country structure for presenting a dissertation are included: introduction – 3 pages; literature review – 52 pages, aim and tasks – 2 page; materials and methods – 12 pages; results and discussions – 50 pages; conclusions - 4 pages; contributions - 1 pages; references – 42 pages, including 628 sources in Latin and 3 in Cyrillic, with 97 articles (15.6%) published after 2017. A good impression is made by the good technical execution of the dissertation work and the scientific style of expression.

Relevance of the topic and appropriateness of the aim and tasks

I believe that the dissertation deals with current issues. Depression is one of the most common diseases worldwide. According to the WHO, 300 million people suffer from this disease [<https://www.who.int/en/news-room/fact-sheets/detail/depression>]. Approximately one in six people will experience at least one major depressive episode at least once in their lifetime, and 1 in 10 individuals will be absent from work due to depression.

On the other hand, after the undisputed achievements of synthetic chemistry in the last century, modern researchers and doctors are turning to the therapeutic possibilities of medicinal plants. *Aronia melanocarpa* is a light-loving shrub native to North America, but in our country there are favorable conditions for its cultivation. Fruit juice is rich in polyphenolic compounds - procyanidins, flavonoids, mainly from the subclass of anthocyanins and phenolic acids, with diverse biological activity; contains vitamins, microelements, sugars, amygdalin, etc. The team of the Department of "Pharmacology and Clinical Pharmacology and Therapy", MU-Varna has experience and significant achievements in the study of the pharmacological activity of *Aronia melanocarpa* fruit juice.

Based on the long-term experience of the department's team with *Aronia melanocarpa* and other biologically active substances of natural origin, as well as on the data from the scientific literature, the hypothesis is logically derived that their application has a place in optimizing the complex prevention and treatment of depression, affective and anxiety disorders upsets. **The goal and objectives** are adequate to investigate and generalize the pharmacological effects of biologically active substances of plant origin (*Aronia melanocarpa* fruit juice and phenolic acids) in experimental models of behavioral disorders induced by ovariectomy and bulbectomy in experimental animals.

Research methodology

The set of experimental methods makes a good impression; they are described briefly and precisely. Adequate experimental models of depression and other behavioral changes in rats induced by ovariectomy and bulbectomy were used. Methods for the investigation of motor activity, anxiety, depressed behavior, pain sensitivity, learning and memory are validated, reproducible and informative. Appropriate methods were used for statistical analysis of data. The selected and applied methodical approaches allowed Dr. Salbashyan to fulfill the tasks of the research and achieve the set goal.

The presented results of the dissertation show that the study was not only well planned, but also successfully executed. The research data are described briefly and clearly, well-illustrated with 12 tables and 41 figures. From the obtained results and the discussion presented after the results of each task, it can be concluded that Dr. Salbashian knows the methods used, skillfully and comprehensibly presents the obtained data. The discussion is done in a good scientific style, calmly handling the data of other authors and comparing them with one's own results. The discussion of the results by tasks introduces some disproportionality into the overall structure of the thesis, but makes the results easier to understand. An advantage of the work would be the presentation of a comprehensive, concise, concluding discussion.

The conclusions correspond to the tasks set and are a logical reflection of the results obtained. Twenty-four conclusions are presented, which can be expressed in a more general form.

Contributions and significance of the development for science and practice

The dissertation is an in-depth scientific study of effects of *Aronia melanocarpa* fruit juice and phenolic acids - chlorogenic, ferulic and gallic in experimental models of behavioral disorders induced by ovariectomy and bulbectomy in rats. The results of the study enrich the knowledge about the effects of polyphenols on the CNS, which could expand the possibilities for the prevention/therapy of anxiety-depressive diseases.

I accept the contributions formulated by the PhD student, which I qualify as scientific-theoretical and scientific-applied:

Abstract of the doctoral thesis

The abstract meets the requirements both in terms of content and layout. It accurately and correctly reflects the essence of the dissertation work. It is illustrated with highly informative figures and tables. It reflects the methodology, the main results with their discussion and the relevant conclusions, contributions and publications related to the work.

Assessment of publications

The results of the dissertation were published in 4 real articles. Two of them are published in journals, refereed and indexed in Scopus, have SJR and are in the fourth quartile (Q4). An article has been published in the journal *Farmacia*, indexed and referenced in WoS (IF 1.48 and Q4) and in Scopus (SJR 0.265 and Q2). In two published articles, Dr. Salbashian is the first author. The articles are related to the subject of her research work.

Critical remarks and recommendations

I take the liberty of presenting omissions and inaccuracies, some of which are technical, and do not detract from the value of the dissertation.

- A comprehensive, concluding discussion that synthesizes the discussions made on the individual tasks' results would significantly contribute to the paper.
- The presented 24 conclusions can be expressed in a more general form.
- There are discrepancies between the sources cited in the text and those in the too extensive bibliography. E.g. Bernaras et al., 2017 was not found in the references; articles numbered 28,

465, etc. are not mentioned in the text; article 25 matches article 241; there are incomplete descriptions, e.g. in 13 no journal name given (Mol Nutr Food Res).

- Some abbreviations listed are not found in the text, e.g. TH, CXT, ASTN, etc.

CONCLUSION

The dissertation intiled "**Pharmacological study of behavioral effects of biologicly active substances of plant origin in experimental animal models of depression**" contains scientific-theoretical and scientific-applied results that represent an original contribution. The work meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its Implementation and the relevant Regulations of the Medical University "Prof. Dr. P. Stoyanov" - Varna. The presented materials and dissertation results fully correspond to the specific requirements of the University.

The dissertation shows that the PhD student Dr. Milena Todorova Salbashian has bibliographic culture at a scientific level, has mastered experimental models and methods, is able to analyze, synthesize results and formulate conclusions; has in-depth theoretical knowledge and professional skills in Pharmacology. From the above, it follows that the two qualifying tasks of the doctorate have been fulfilled - educational and scientific, and the completed written work can receive a well-deserved positive evaluation.

The results of Dr. Salbashian's dissertation enrich the long-term research of biologically active substances of plant origin conducted in the department. There is convincing evidence that substances contained in aronia berries have a sedative, known anxiolytic effect, antidepressant-like effect, increase the threshold of sensitivity to thermal stimuli. Chlorogenic, ferulic and gallic acids affect experimentally induced hyperactivity, have an anxiolytic effect, improve memory and learning processes. These results and those of the team's previous research should lead to the creation/implementation of medications/supplements to prevent and affect anxiety-depressive conditions.

Due to the above, I confidently give my positive assessment and propose to the respected members of the Scientific Jury to award the **ESD "Doctor"** to **Dr. Milena Todorova Salbashian** in the PhD program in Pharmacology (incl. pharmacokinetics and chemotherapy), Professional direction 7.1. Medicine.

05.01.2023

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Signature:



/Assoc. Prof. Galya Stavreva-Marinova, MD, PhD/