OPINOIN

by Prof. Ivanka Ivieva Kostadinova, MD, PhD,

Department of Pharmacology and Clinical Pharmacology

of the Medical Faculty at MU-Plovdiv, determined by

Order № 408 / 16.07.2020 of the Director of

Institute of Neurobiology of BAS

for a member of the Scientific jury on the grounds of Art. 4 of the Law on the Development of the Academic Staff of the Republic of Bulgaria

about awarding the educational-scientific degree "Doctor" to part-time doctoral student Stela Toshkova Dragomanova
for her dissertation thesis "Pharmacological, toxicological and neurobiological studies of Myrtenal – bicyclic monoterpenoid of natural origin"
in the field of higher education 7.0 Healthcare and sports, professional field 7.1. Medicine, scientific specialty: "Pharmacology"

Professional Development

Stela Dragomanova graduated from the Medical College as an assistant pharmacist in 1998 in Varna. In 2004 she graduated from MU Sofia as a master of pharmacy. In 2014 she was enrolled as a part-time doctoral student at INB-BAS, headed by Prof. Tancheva, PhD. In 2017 she acquired a degree in clinical pharmacy. From 2000 to 2005 the doctoral student worked in an open pharmacy as a pharmacist, from 2005 to 2010 she was a pharmacy manager. Since 2010 she has been an assistant in the departments of Pharmacology and Clinical Pharmacology and Therapy at the Medical Faculty at MU Varna, the Department of Preclinical and Clinical Sciences, the Faculty of Pharmacy at MU Varna and the Department of Pharmacology, Toxicology and Pharmacotherapy at the Faculty of Medicine. Varna. From 2014-2020 she was an administrative assistant in the Department of Pharmacology, Toxicology and Pharmacotherapy at the Medical University of Varna. As an assistant Stela Dragomanova participates in the teaching of pharmacology and toxicology to pharmacy

students and pharmacology to medicine and dentistry students. She is a co-author of 4 published textbooks for the preparation of students in medicine, dentistry and pharmacy at MU Varna. In 2015, Stela Dragomanova, master of Pharmacy, participates in a course at the Thracian University of Stara Zagora on "Protection and welfare of experimental animals used for scientific and educational purposes."

Assessment of the preparation of Stella Dragomanova, master of Pharmacy, as a part-time doctoral student according to the BAS credit system:

1. Implementation of the educational program

   Master Pharmacist Stela Dragomanova has collected 130 points / Information on exams taken and credits received, issued by Eng. T. Todorova /.

2. Approbation of the implementation of the scientific program

   Scientific reports on the results on the topic of the dissertation

   Master of Pharmacy Dragomanova has participated in 17 scientific forums on the topic of the dissertation. In 9 of them she is the first author. Six of the scientific reports have been exported in Bulgaria, five - abroad. For her participation in a scientific forum in Amsterdam Stela Dragomanova has won a grant. The total number of points in this section is 312 with a mandatory minimum of 40 points.

3. Publications of scientific results on the topic of the dissertation

   There are three publications related to the topic of the dissertation. Two of them are in a magazine with an impact factor and 1 publication in a Bulgarian thematic magazine. Total number of points on this indicator is 168, with a mandatory minimum of 80 points.

   Stela Dragomanova, master of Pharmacy, has 2.4 times more points than the required minimum of 250 credits. The preparation of the doctoral student at the Doctoral School and in the elaboration and reporting of the results of the dissertation topic at scientific forums can be assessed as excellent. Stela Dragomanova presented data for 4 citations of articles on the topic of the dissertation.

   The doctoral student participated as a researcher in project DN03/8/2016 at the Research Fund with a project "Galantamine and 4-aminopyridine derivatives

containing a peptide motif with an expected effect on Alzheimer's disease and Multiple sclerosis."

These science-metric data confirm the relevance and significance of the problems considered in the dissertation of Stela Dragomanova and their scientific value. The doctoral student's knowledge of foreign languages and excellent computer skills help her to incorporate the latest trends in her teaching and research activities.

Structure of the dissertation

The dissertation of Master Pharmacist Dragomanova contains 255 standard typewritten pages. It is constructed according to the requirements of INB-BAS for dissertation work for awarding the Educational-Scientific Degree "Doctor". It consists of: introduction (3 pages), literature review (62 pages), purpose and tasks (2 pages), materials and methods (22 pages), results and discussion to each section (101 pages), conclusions (2 pages), reference for the contributions (1 page), list of literature sources. The dissertation is illustrated with 9 tables and 118 figures, in which the results of the experiments are presented in a very good way, with great informative value. The bibliography for the dissertation contains 534 sources, two in Cyrillic and 532 in English. 108 of them are from the last 5 years.

Relevance and significance of the dissertation

The significance of the study is related to:

- Increasing the number of people with Alzheimer's disease, vascular dementia, social phobias and anxiety due to prolongation of life. The treatment of these socially significant diseases with traditional medicines is long, with many established ADRs. Worldwide, nearly 44 million people have Alzheimer's disease or dementia associated with the disease. 5.8 million Americans live with Alzheimer's disease. In 2050, more than 16 million Americans will have AD. Alzheimer's disease is the 6 leading cause of death in the United States. One in three adults die of Alzheimer's disease or another type of dementia. Life expectancy after a diagnosis of Alzheimer's disease is 4 to 8 years. Mortality from Alzheimer's disease increased in the United States by 145% from 2000 to 2017. These facts determine the demand for drugs that can be used

prophylactically to preserve learning and memory and slow the development of the disease.

- The most common anxiety disorder is social anxiety disorder (social phobia). Social anxiety disorder in the United States occurs in 7% of people. It is a risk factor for subsequent depressive illness.
- According to the WHO, about 80% of the world's population uses herbal medicines for prophylaxis and primary health care.
- Herbal products that are used for therapeutic and prophylactic purposes have an easy and convenient way of application, which ensures better patient participation in the prevention and treatment of various diseases.
- Only about 5 to 15% of the 2,500,000 plant species have been chemically and pharmacologically studied.

Studies of the pharmacological, toxicological and neurobiological properties of various products of plant origin are needed to improve the quality of life of patients and reduce mortality from these socially significant diseases.

The topic of the dissertation is relevant because it is related to a socially significant problem. In the introduction to the dissertation, the doctoral student points out that so far no data have been found on the impact of Myrtenal on experimental animal models of neurodegeneration. The doctoral student substantiates the need to conduct pharmacological, toxicological and neurobiological studies of Myrtenal in healthy rodents and in an experimental model of Alzheimer's dementia, as well as for the discovery of new mechanisms of action of the monoterpenoid in neurodegenerative disorders. Stela Dragomanova points out the urgency of the problem and the motives for choosing the dissertation topic.

In the literature review the doctoral student presents the world scientific achievements on the topic of the dissertation. A general review of natural terpenes and terpenoids of plant origin has been made. The natural sources, structure, biological features and pharmacological effects of Myrtenal-containing essential oils and Myrtenal are discussed, as well as the affinity of Myrtenal for different metabolotropic receptors and ion channels, GABA receptor, glutamate receptor and the influence of terpenoids on the various enzymes activity. The hypothesis of linking the central action of Myrtenal with interaction with the GABA receptor

is presented. Issues on which there are conflicting opinions that require further study have been addressed. The literature review presents in detail the etiology, genetic predispositions, pathogenesis and patho-anatomical changes, the mechanisms of neurodegeneration, risk factors, the clinical picture, the main stages of Alzheimer's disease and therapeutic approaches and trends in its treatment. The doctoral student pays special attention to the strategies in the search for drugs for the treatment of neurodegenerative diseases. The main directions in the search for new therapeutic agents and the therapeutic benefits of currently used medicinal products are discussed. The main therapeutic goals in influencing the symptoms of Alzheimer's disease related to secretory modulators and optimizing choliergic neurotransmission are considered. The advantages of experimental animal models in the study of neurodegenerative disorders are considered. The model of scopolamine dementia, which was used in the dissertation, is described. The inclusion of this model in the design of the study is justified by the considered hypotheses about the damaging effect of scopolamine, the multifactorial etiology and pathogenesis of neurodegenerative diseases. At the end of the literature review, Stella Dragomanova discussed the benefits of natural products, as well as the choice of biologically active substances in the treatment of neurodegenerative diseases. There is limited information on the influence of Myrtenal on the levels of major neurotransmitters. There are no data in the available literature on the effect of Myrtenal on experimental animal models of neurodegeneration, which is the reason for conducting experimental studies with Myrtenal on healthy animals and those with neurodegenerative disabilities. The data presented in the literature review prove that Dragomanova knows very well the literature on the topic of the dissertation, analytically and competently discusses the unsolved problems.

The purpose is formulated accurately and correctly. The tasks set in the dissertation work support the fulfillment of the purpose. The tasks are divided into four sections. The design of the experimental study takes into account the effects of Myrtenal in healthy rodents and those with dementia caused by scopolamine and comparing the results. The doctoral student's tasks include: determination of mean lethal dose (LD_{50} i.p.) in mice and prolonged toxicity after treatment with toxic doses for mice, study of pharmacological effects of Myrtenal on the CNS of mice and rats, anxiolytic and analgesic effects of various pain models, neurobiological effects of Myrtenal in mice and rats, biochemical effects of Myrtenal in the brain of experimental rodents, verification of a scopolamine-induced dementia model in laboratory mice and rats.

The purpose and tasks have both scientific and applied clinical significance. In her dissertation, Dragomanova used modern, established models and methods for reporting pain sensitivity, training, memory, anxiolytic action, neuroprotective, antioxidant action of Myrtenal in healthy mice and rats and those with scopolamine-induced dementia. The "Materials and Methods" section describes in detail the experimental models and methods used. Tests were performed after single and repeated administration of Myrtenal to mice and rats. Also of interest are docking studies on the ability of Myrtenal to affect AChE activity by comparison with galantamine effects as a standard for anticholinesterase activity. The results are processed correctly. They are well presented and described. Appropriate statistical tests have been used to process the data from the experimental studies, which ensure the reliability and comparability of the obtained results with data from other authors. The PhD student found that Myrtenal in healthy experimental rodents showed no detrimental effects on memory and learning, neuromuscular coordination and spatial orientation. She discusses the possibility that the depressant effects of Myrtenal on the CNS may be related to affecting GABA-ergic neurotransmission. Neuromodulatory properties of the test compound have also been reported, manifested by increased levels of the major brain mediator acetylcholine. The obtained results are a prerequisite for expanding the study in the direction of studying the effects of monoterpenoid on models of impaired memory functions and experimental dementia. The doctoral student has very good computer skills, which help her in presenting and publishing the results. The design of the experiment also included the verification of a scopolamine-induced model of dementia in two species of animals, mice and rats, after repeated administration of Myrtenal. Scopolamine at the administered dose, after repeated treatment, caused memory impairment in rats. Dragomanova connects the lack of effect on memory abilities of mice with their more intensive metabolism. In the discussion, the doctoral student compares the results of the present study with data from other authors. The probable reasons for these differences are also discussed. The data from the conducted experiments are summarized in 10 conclusions. Four of the conclusions were for the results obtained with Myrtenal experiments in healthy mice and rats and six conclusions about the protective effects of Myrtenal in dementia rodents as a model of neurodegeneration. The doctoral student knows very well the literature on the topic of the dissertation, expresses her position, analyzes the results obtained and the conclusions made.

The dissertation is written and structured correctly. The goal and tasks are clearly set. The results and the discussion are logically connected. The conclusions are precisely

formulated and correspond to the data obtained in the scientific experiment. The dissertation of Stela Dragomanova has contributions of scientific and applied significance. Contributions were divided into original – 6 and 2 with confirmatory character, but with dose modification of scopolamine-induced dementia and with determination of mean lethal dose of Myrtenal in intraperitoneal administration to mice. I accept and evaluate as significant the contributions indicated in the dissertation of Stela Dragomanova, master of Pharmacy.

The dissertation "Pharmacological, toxicological and neurobiological studies of Myrtenal - bicyclic monoterpenoid of natural origin" presented for an opinion meets the requirements set out in the regulations of the Institute of Neurobiology at BAS. The dissertation is written in accurate and clear Bulgarian. Stela Dragomanova, master of Pharmacy, has theoretical knowledge, practical skills and competencies of a scientist. She can freely handle the facts of the available literature, express and defend her own position, compare and contrast the obtained results with those of other authors, looking for the reasons for the differences and similarities between them. The advantage of the dissertation is the study of the pharmacological effects, biochemical parameters and neuroprotective effect of two species of animals, mice and rats, as well as of healthy and damaged by scopolamine rodents after single and repeated administration. The cooperation with leading specialists from different scientific units, the management of the doctoral student by an established lecturer and a researcher are a guarantee for the great scientific value of the dissertation presented for an opinion. The doctoral student has the qualities and skills to conduct research independently. Based on the presented publications, participation in scientific forums and dissertation, it can be concluded that they are her personal merit. Stela Dragomanova manages under the guidance of her supervisor to master a number of modern methodologies, to work in a team and to maintain her research interests and contacts on issues that she develops consistently and in collaboration with other researchers.

The autoreferat "Pharmacological, toxicological and neurobiological studies of Myrtenal – bicyclic monoterpenoid of natural origin" contains 92 pages and reflects the main content of the dissertation and the results obtained. The abstract of the thesis is constructed correctly, according to the requirements specified in the normative documents of the Institute of Neurobiology at BAS. It is illustrated with 1 table and 44 figures.

Conclusion: My assessment of the presented dissertation "Pharmacological, toxicological and neurobiological studies of Myrtenal – bicyclic monoterpenoid of natural origin" by Stella Dragomanova, master of Pharmacy, is positive. The dissertation is relevant with scientific and

applied significance. The dissertation topic has a significant contribution to science and practice. The doctoral student knows the well-presented problem, she has mastered many modern methods, which she uses in the experimental part of the doctoral thesis.

What is different in the dissertation of Stela Dragomanova is the interdisciplinary consideration of the problem. The topic of the dissertation is relevant and socially significant. A long and complex experiment was performed with a large number of animals of two animal species, healthy and damaged by scopolamine rodents. Modern research methods have been used. The original idea, the urgency of the problem, the excellent knowledge of the issues, the ability to interpret and compare the results with data from experimental studies of other authors, the hypotheses, conclusions and contributions of theoretical and practical significance give me reason to evaluate the study positively, presented by the peer-reviewed dissertation, abstract, achieved results and contributions. **I propose to the esteemed members of the Scientific Jury to award the educational and scientific degree "Doctor"** to Stela Dragomanova, master of Pharmacy, in the field of higher education 7.0 Health and sports, professional field 7.1 Medicine and doctoral program in Pharmacology.

Opinion drown by:

Prof. I. Kostadinova, MD, PhD