

REVIEW

by Prof. Krassimira Simeonova Yakimova, MD, PhD, DMS

In reference with dissertation thesis for award of a scientific and educational degree “Doctor” in Scientific specialty "Pharmacology", Professional field 7.1. Medicine, field of higher education 7. Healthcare and sports.

Author: Stela Toshkova Dragomanova

Form of doctoral studies: part-time

Scientific unit: Institute of neurobiology, Bulgarian Academy of Sciences

Topic of the dissertation thesis: *"Pharmacological, toxicological and neurobiological studies of Myrtenal – a bicyclic monoterpenoid of natural origin"*

Scientific supervisor of the doctoral student: Prof. Dr. Lyubka Pavlova Tancheva

I present this review as a member of the Scientific Jury, approved by a decision of the Scientific Council of the Institute of Neurobiology – Bulgarian Academy of Sciences (Protocol № 03/09.07.2020) and according to Order № 408/16.07.2020 of the Director of the INB – BAS.

Stella Toshkova Dragomanova, Master of Pharmacy, **has presented all the necessary documents** in accordance with the Law on the Development of Academic Staff in the Republic of Bulgaria, the Regulations for application of the Law for the development of the academic staff in the Republic of Bulgaria for acquiring the educational-scientific degree "Doctor" and in accordance with Art. 24 (1) of the Regulations for training of doctoral students at INB – BAS.

Stela Toshkova Dragomanova completed her master's degree in 2004 at the Medical University – Sofia, majoring in Pharmacy. She worked as a pharmacist and pharmacy manager until 2010, when after winning a competition she started working as an assistant in the Department of Pharmacology and Clinical Pharmacology and Therapy at the Medical University – Varna, Faculty of Medicine. Since 2015 he has been an assistant at the Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy at MU-Varna. Since 2014, after a competition,

she has been enrolled as a part-time doctoral student at INB – BAS. She has conducted training in accordance with the Regulations for training of doctoral students at INB - BAS, having received the required number of credits (Reference Ex. №331 / 08.07.2020). According to an order of the Director of Institute of Neurobiology – BAS (№ 265 / 01.06.2018), she was expelled from a doctoral program with the right to defense. The internal defense (approbation) was held on 30.06.2020 at an extended scientific seminar of scientific directions "Behavioral Neurobiology" and "Synaptic Signaling and Communications" at INB - BAS, as a result of which the Scientific Council of INB has opened a procedure for defence of the dissertation work under Art. 9 of the Regulations for training of doctoral students at INB - BAS.

Stela Toshkova Dragomanova has conducted postgraduate training (2013 - 2017) at MU-Sofia and has acquired a specialty in "Clinical Pharmacy".

The presented dissertation is properly structured and contains 255 pages, including the main parts: Introduction (3 pages); Literature review (63 pages); Purpose and tasks (2 pages); Materials and methods (21 pages); Results and discussion (112 pages); Conclusions (2 pages); Contributions (1 page); References used (36 pages); List of publications and participations in scientific forums related to the dissertation (4 pages).

The dissertation is illustrated with 118 figures and 9 tables.

The topic of the dissertation thesis is very relevant. Neuropharmacology is undoubtedly one of the most dynamically developing fields of pharmacology. Despite the advances of modern medicine, neurodegenerative diseases (multiple sclerosis, Alzheimer's disease, etc.) lead to permanent and irreversible disability, which defines them as socially significant diseases. A number of established drugs are used, but clinical practice remains significantly limited in terms of its real possibilities and the need to develop new pharmacologically active substances and search for new approaches is obvious, which is of great interest worldwide. A significant number of scientific researches and publications in this direction appear annually in the world scientific literature. The doctoral student shows **a very good knowledge** of scientific literature, creatively evaluating the literary material. A significant volume of literature was made, covering 534 sources.

The literature review is constructed in four main parts: *the first main part* examines the pharmacological effects of natural monoterpenes and terpenoids of plant origin and in particular - the bicyclic monoterpene myrtenal; *the second main part* deals in principle with the general mechanisms of neurodegeneration in neurodegenerative diseases; *the third main part* focuses specifically on Alzheimer's disease – etiology, pathogenesis, risk factors, clinical picture, pharmacotherapy; *the fourth main part* deals with modern strategies in the search for drugs against neurodegenerative diseases – a multitarget approach to the treatment and prevention of neurodegenerative diseases, highlighting the benefits of natural products.

From the literary review naturally follows **the correctly motivated purposes**, as well as **the clearly and concretely formulated tasks** of the dissertation.

In her dissertation, the doctoral student uses a variety of modern experimental approaches to achieve the goal of her research.

From a methodological point of view, the use of a wide range of adequately selected **experimental methods** is impressive, allowing to perform the tasks and achieve the goal – an experimental model of chemically induced dementia in experienced rodents, a wide range of behavioral tests (memory and learning, spatial orientation and neuromuscular coordination), biochemical, histopathological and software docking research.

Appropriate methods for statistical processing are used, which contributes to the objectification of the obtained results.

The conducted research and the obtained results are divided into three main directions: 1. Pharmacological and toxicological effects of Myrtenal in healthy rodents; 2. Effects of Myrtenal in a model of neurodegenerative damage in rodents; 3. Comparison of the effects of Myrtenal in healthy rodents with those in rodents with an experimental model of dementia.

As a result of the rich experimental material, the doctoral student drew **4 conclusions** regarding the pharmacological and toxicological effects of Myrtenal in healthy rodents and **6 conclusions** regarding the protective effects of Myrtenal in rodents with a model of neurodegeneration.

After getting acquainted with **the results** of the research and their discussion, I consider that **with her dissertation the doctoral student Stela Dragomanova makes the following more important scientific contributions:**

▲ *Scientific-theoretical and scientific-applied contributions:*

- The pharmacological and toxicological effects of Myrtenal on healthy rodents were studied for the first time, and the mean lethal dose (LD50) in mice after intraperitoneal administration was determined.

- Original data have been established on the pharmacological effects of Myrtenal in intact rodents – analgesic properties, anxiolytic potential and effects on memory, coordination and exploratory behavior.

- Important drug interactions have been identified – potentiation of the action of drugs affecting GABA-ergic neurotransmission, such as barbiturates and benzodiazepines, by the monoterpenoid Myrtenal.

- **The neuroprotective effect of Myrtenal was first established in rodents with an experimental model of dementia associated with its antioxidant and neuromodulatory properties.**

- **For the first time, a comparative analysis of the effects of Myrtenal in healthy rodents and rodents with an experimental model of dementia was performed. The analysis showed a significantly more pronounced effect of Myrtenal in animals, with an experimental model of dementia.**

▲ *Contributions of methodological significance:*

- A new dose modification of scopolamine-induced dementia has been proposed in order to reproduce the non-linear progression of the lesion, which has been behaviorally, biochemically and histopathologically verified.

Excerpts from the dissertation have been published in English in three Bulgarian scientific journals. Two of these publications are cited by foreign authors in journals with an impact factor, which meets the criteria for obtaining the educational and scientific degree "Doctor" at the Institute of Neurobiology – BAS. The doctoral student is the first author of two of the printed publications.

In connection with the dissertation, the doctoral student presented 17 scientific reports (in 12 of them she is the first author) at scientific forums in Bulgaria (12) and

abroad (5). She was the coordinator of a scientific project under the Science Fund at MU-Varna, on the topic "Preventive effects of natural monoterpenes on memory disorders of experimental rodents."

The evaluation of the credit system of the preparation of the doctoral student at BAS Stela Dragomanova shows coverage (and exceeding according to some criteria) of the necessary credits in the three main directions: I. Implementation of the educational program; II. Approbation of the implementation of the scientific program; III. Publications of scientific results on the topic of the dissertation.

The autoreferat project is made according to the requirements and corresponds to the dissertation, reflecting the main results achieved in the dissertation.

In conclusion, I consider that the dissertation work of Stela Toshkova Dragomanova, Master of Pharmacy, proposed for the award of the educational and scientific degree "Doctor", is a competent, modern level of scientific work, which successfully solves current scientific problems. A large number of labor-intensive experiments have been performed, the results of which have been interpreted objectively and competently. Most of the data are published in scientific journals and are presented at scientific forums at home and abroad. Original scientific contributions have been formulated.

I give a positive assessment of the dissertation of Stela Toshkova Dragomanova, Master of Pharmacy, part-time doctoral student at the Institute of Neurobiology – BAS, with scientific supervisor Prof. Dr. Lyubka Pavlova Tancheva, on "*Pharmacological, toxicological and neurobiological studies of Myrtenal – a bicyclic monoterpene of natural origin*" **and I propose to the esteemed Scientific Jury to vote positively for the award of the scientific and educational degree “DOCTOR”.**

18. 08. 2020

Sofia

Review drawn by:

(Prof. Krassimira Simeonova Yakimova, MD, PhD, DMS)