

OPINION

by Prof. NIKOLAY ELENKOV LAZAROV, MD, PhD, DSc

ON DISSERTATION WORK FOR ACQUISITION OF THE EDUCATIONAL AND SCIENTIFIC DEGREE "DOCTOR" IN SCIENTIFIC SPECIALTY "PHARMACOLOGY"

PHD THESIS AUTHOR : STELA TOSHKOVA DRAGOMANOVA

PHD TITLE: „*PHARMACOLOGICAL, TOXICOLOGICAL AND NEUROBIOLOGICAL STUDIES OF MYRTENAL – A BICICLIC MONOTERPENOID OF NATURAL ORIGIN*”

Stela Toshkova Dragomanova is a master pharmacist, assistant in the Department of Pharmacology, Toxicology and Pharmacotherapy at the Medical University (MU) -Varna, and since 2014 a part-time doctoral student in the Department of Behavioral Neurobiology at the Institute of Neurobiology (BAS), where she conducted the main part of the experimental work on the present dissertation. Small parts of the research were performed at the Medical University-Varna, Medical University-Sofia and the Military Medical Academy-Sofia. After the implementation of the educational program in accordance with the Rules of the Center of Education at the Bulgarian Academy of Sciences, including raising the required minimum number of credits, successfully passing exams in pharmacology, English and computer skills, she was expelled by the Scientific Council of the Institute of Neurobiology with the right to defend her thesis.

The dissertation on "Pharmacological, toxicological and neurobiological studies of myrtenal - bicyclic monoterpene of natural origin" is written on 255 pages and is illustrated with 9 tables and 118 figures, representing schemes from scientific publications or freely available in Internet publications and graphs from own results. The bibliographic reference includes 534 literature sources, a large part of them from the last decade, which is an indirect indicator of the relevance of the investigated problem.

The topic of the dissertation is well chosen. It is devoted to an interesting and contemporary problem of experimental pharmacotherapy and in particular on elucidating the neurobiological effects of the myrthenal monoterpene myrtenal and its neuroprotective effect on the cerebral cortex of rodents with an experimental model of dementia. It is known that this natural product, along with its numerous (bronchodilator, anti-inflammatory, antiplatelet, antihemolytic, antibacterial, antioxidant and antitumor) effects, also has a cellular membrane stabilizing action with a potential effect on neurotransmitter systems in the brain. In this regard, a good knowledge of the mechanisms of these systems response could serve as a reliable basis for developing an effective strategy for prevention and treatment of neurodegenerative diseases.

In vivo studies were performed on sexually mature healthy mice and rats, as well as on animals with chemically-induced with scopolamine dementia of Alzheimer's type. Biological, biochemical and behavioral parameters in their brains were monitored after intraperitoneal administration of myrtenal. Its toxicity and neuropharmacological activity on the CNS in healthy animals have been specifically studied. Prolonged

administration of myrtrenal has been shown to have an anorexigenic effect and to induce analgesia in treated animals, even in acute administration. At the central level, it potentiates the action of CNS depressants, probably by binding to GABA_A receptors, as shown by doctoral studies conducted by the PhD student, and when applied alone demonstrates anxiolytic activity. Its effect on the memory of intact animals is expressed in shortened latency with a single application, while the 5-day treatment with mirtrenal does not affect their training skills. No appreciable effects on the neuromuscular coordination and exploratory activity were reported during the study in healthy rodents.

On the other hand, after conducting these experiments in dement rodents, convincing data were obtained for a significant improvement in their memory and learning abilities. Myrtrenal has been shown to restore the scopolamine-damaged exploratory activity of these animals without affecting their neuromuscular coordination, and in addition, it has antioxidant properties. It was also demonstrated that myrtrenal does not have anticholinergic activity, but to increase acetylcholine levels in the dement brain. The histopathological finding in the brains of animals treated with scopolamine visualized obvious signs of enhanced neuronal degeneration and cell loss in the hippocampus and cerebral cortex. In animals treated with the scopolamine-myrtrenal combination, a reduced number of damaged neurons and concomitant microgliosis in the cerebral cortex and hippocampus were observed. The performed quantitative analysis of the data verifies a statistically significant neuroprotective effect of myrtrenal in the studied brain structures.

In my opinion, the dissertation would acquire a complete form with a final section summarizing the main results of the study and outlining directions of future research on the problem. Analyzing the obtained results, Dragomanova has drawn 10 main conclusions that accurately and reliably reflect the author's findings, their interpretation and conclusions. The significant scientific achievements of the dissertation, most of which have an original character, are presented in a separate section. Some of them (№ 1, 4, 6) can be formulated more tightly as findings, without unnecessary and detailed description of their nature and, in particular, of the causal relationship between them. There is no doubt that the present dissertation is the personal work of Stela Dragomanova. In this regard, undisputed proof are the scientific publications and communications presented by her on the topic, most of them with her leading participation. The author's findings are published in three scientific articles in peer-review Bulgarian journals, in two of which she is the first author. Two of these articles have been cited a total of four times in scientific journals, but two of the citations are self-citations. Attached is a list of 17 reports presented at various scientific forums at home and abroad, in 12 of which Dragomanova is a leading co-author. Резюмета на три научни съобщения са публикувани в реномирани научни списания с импакт фактор.

The PhD thesis autoreferate adequately and sufficiently reflects the state of the studied problem, the goal, the methods used for its implementation, the obtained own results, their analytical description and interpretation, as well as the author's conclusions and contributions.

In conclusion, I consider that the dissertation of Stela Dragomanova is a complex, time-consuming and extensive study on an extremely contemporary issue of phytopharmacology and phytotherapy, namely the study of the effects of myrtenal on the CNS and its potential application in neurodegenerative brain damage. It is well thought out and precisely methodically justified, conducted very accurately and illustrated perfectly. The results of the study make an original theoretical and applied contribution to clarify the role of biologically active substances for the prevention and treatment of neurodegenerative diseases. All this gives me the inner conviction to give a positive assessment of the dissertation thesis developed by the PhD student and as a member of the Scientific Jury on the procedure to support with a positive vote the award of the educational and scientific degree "Doctor" in "Pharmacology" of Stela Toshkova Dragomanova.

Opinion drawn by:

(Prof. Nikolay Lazarov, DSc)

28.07.2020

Sofia