

## OPINION

On a dissertation with the topic: **"Pharmacological, toxicological and neurobiological studies of Myrtenal – bicyclic monoterpenoid of natural origin"**

*For awarding a general education and scientific degree "DOCTOR"*

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The dissertation submitted to me for an opinion is written on 255 standard typewritten pages. Contains as follows: 3 pages introduction, 62 pages literature review, purpose and tasks 2 pages, materials and methods 21 pages, results, discussion and summary of results 109 pages, conclusions 2 pages and contributions 2 pages, bibliography – 36 pages. It is illustrated with 118 figures and 9 tables. The bibliographic reference covers 534 sources.

The thesis is devoted to the pharmacological, toxicological and neurobiological study of the bicyclic monoterpenoid of natural origin Myrthenal in healthy rodents and in animals with an experimental model of Alzheimer's dementia. In recent decades, there has been an increased interest in medicinal products from natural sources. Only for the period 1981 – 2006, thanks to the efforts to develop natural medicines, new natural biologically active products were introduced in the medical treatment practice, which make up 63% of all registered pharmacotherapeutic agents.

On the other hand, after the identification of Alzheimer's disease, for a hundred years, a number of scientific teams have tried to develop effective and harmless therapeutic agents for this specific neurodegenerative disease, but so far their efforts have not been successful.

The disease is characterized by a protracted onset, multifactorial etiology and pathogenesis and a diverse clinical picture in individual patients, which makes it difficult to find effective therapeutic products. At this stage, the available drugs are aimed only at the symptomatic response to neurodegenerative changes and do not significantly affect the course of the disease process. Therefore, the detection of biologically active substances with potential effects for the prevention and treatment of neurodegenerative disorders, incl. Alzheimer's disease, is an important field in modern neurobiological science. On the other hand, in recent decades there has been a huge scientific interest in essential oils in our country and worldwide due to their indisputable valuable pharmacological properties. And in this sense, the topic of the dissertation is extremely relevant. This is well presented in the introduction, with a skillful emphasis on the healing effects of essential oils, including the group of terpenoids, which includes the secondary metabolite of alpha-pinene – Myrtenal. The pharmacological spectrum of action of Myrtenal is outlined on the basis of previous experimental studies and the fact is emphasized that in the field of neuropharmacology there are no significant data on its biological activity. The doctoral student describes very well the need to conduct various types of research to study the effects of Myrtenal in healthy rodents and in an experimental model of Alzheimer's dementia, which can contribute to verification and supplementation of existing scientific data and the discovery of new mechanisms of action. of the monoterpenoid in neurodegenerative disorders.

The structuring and summarizing of all systematized scientific facts within the framework of the comprehensive literary review, which in itself is worthy of a separate publication in the renowned journals for scientific reviews, makes an extremely good impression. The visualization (including color figures - see between pages 23 and 29) in this first main part of the dissertation, contributes significantly to the overall impression of Dragomanova's work and speaks clearly about the erudition and precision of the dissertation. The opportunity for acquainting the reader with the diverse facts is also greatly facilitated.

The comprehensive literature review is the basis for adequate formulation of the scientific purpose and the resulting tasks for complex and targeted research and comparative characterization of the effects of Myrtenal in healthy rodents and rodents with an experimental model of scopolamine amnesia, which is considered a verified model of Alzheimer's dementia. All used experimental materials and methods are considered in detail.

At the end of the literature review, the exact basis for the planned research is formulated, the specific experimental goals and tasks are clearly described and all used experimental materials and methods are considered in detail. In the chapter "Materials and methods" the experimental material is very well described and classified, all types of experimental protocols, chemicals and reagents are given. The 9 main experimental directions with the included in each of them multicomponent researches, proving the wide scale and multidisciplinary of the present work, are correctly selected and accurately characterized.

Chapter V presents in detail, precisely and with original handwriting the various groups of scientific data obtained as a result of pharmacological, toxicological and neurobiological experiments with Myrthenal on healthy rodents and rodents with scopolamine amnesia. And in this part of the dissertation the

doctoral student with skill and love has made every effort to perfectly illustrate all the results. All tables, figures and diagrams are accurate and easy for the reader to perceive, due to the good arrangement and shaping of the legends below them. This is especially important when comparing the many different simultaneously monitored parameters. The discussion follows the course of the obtained experimental results. The style is clear, concrete and based on the facts of the literary review. This speaks of good awareness of the doctoral student and her ability to properly navigate the many results obtained, to extract the essence and to formulate correctly the conclusions and contributions arising from the discussion. From the precisely presented numerous results follow the logically formed and well-formulated conclusions and contributions. The skilful formation of the overall picture of the obtained data in two main groups of originally presented conclusions with undoubted scientific and scientific-practical value is impressive:

Myrtenal was found to be non-toxic after repeated administration to mice and rats, and anorexigenic effect with improved lipid profile was observed with prolonged administration. The data obtained on the central anxiolytic properties of Myrtenal and potentiation of the action of barbiturates and benzodiazepines confirm the results of docking studies showing the affinity of the substance for GABA A receptors. Undoubtedly, the greatest scientific and practical significance is given to the data on the protective effects of Myrtenal in rodents with a model of cerebral neurodegeneration. Myrtenal improves memory and learning abilities in dement rodents without affecting the neuromuscular coordination of the animals. Activity towards exploratory behavior was found, manifested by recovery of the scopolamine-damaged habituation. Myrtenal also exhibits distinct antioxidant properties, as in dementia mice it lowers LPO and dose-dependently increases tGSH levels, significantly reducing the increased SOD activity of scopolamine to

levels close to those of the reference Lipoic acid. The latter conclusion regarding the absence of anticholinesterase properties of Myrtenal is important in light of the existing hypothesis of acetylcholine neurotransmitter pathogenesis of memory disorders in Alzheimer's dementia.

Based on the obtained results and the conclusions made, 8 main contributions are made in the dissertation and all of them are of original character:

1. The effects of Myrtenal on healthy rodents (mice and rats) were studied for the first time.

2. New data have been obtained enriching the toxicological characteristics of Myrtenal in experimental rodents.

3. Central nervous effects of potentiation of the action of barbiturates have been established, which are superior to those of diazepam and are related to the affinity of Myrtenal for GABA A receptors.

4. New data have been obtained on the pharmacological effects (analgesic properties) of Myrtenal in healthy rodents. The first studies of the central nervous properties of the monoterpenoid did not reveal a detrimental effect on the memory, research behavior, and coordination of experimental animals.

5. For the first time, studies on the anticholinesterase properties of Myrtenal in *in vivo* model rule out the possibility of blocking enzyme activity, in contrast to the available *in vitro* data.

6. For the first time, the protective potential of the substance in rodents with a pattern of dementia associated with its antioxidant and neuromodulatory properties was established.

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

8. For the first time, a comparative analysis of the effects of Myrtenal in healthy and dementia rodents was performed, which showed a more pronounced effect in animals with induced impairment.

Giving an extremely high assessment of the topic, the selected experimental methods, the conducted comparative research, the efforts and work, the overall scientific and practical contribution of the work of Stela Dragomanova, master of Pharmacy, and her scientific supervisor, I would like to emphasize that I do not find a reason for any recommendations and negative comments.

**ASSESSMENT OF THE CRITERIA IN ACCORDANCE WITH THE LAW FOR DEVELOPMENT OF THE ACADEMIC STAFF OF THE REPUBLIC OF BULGARIA AND RULES FOR TRAINING OF DOCTORAL STUDENTS OF INB – BAS**

In connection with the dissertation, the doctoral student presents 3 scientific publications, two of which are in journals with impact factor, of which she is the first author. On the presented publications 4 quotations have been noticed so far, which once again confirms the relevance of the topic and the high level of this work.

From the documents attached to the procedure, I understand that the required number of credits has been collected, in accordance with the Law for Development of the Academic staff of the Republic of Bulgaria and the Rules of Procedure for its implementation.

**CONCLUSION:** The dissertation presented for my opinion is a multidisciplinary, relevant, interesting and original study of the pharmacological, toxicological and neurobiological properties of the natural monoterpenoid Myrtenal in healthy and rodents with scopolamine-induced amnesia. I believe that the new results obtained for the antioxidant protective ability of Myrtenal in

dementia rodents not only expand the pharmacobiological passport of this natural terpenoid, but represent a valuable find and basis for future research to develop effective natural drugs for the prevention and treatment of dementia.

All this gives me reason to convincingly suggest to all members of the Scientific jury to vote positively for the award of Stella Dragomanova, master of Pharmacy, to the general education and scientific degree "DOCTOR".

24.08.2020

Sofia

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