



OPINION

from

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Head of the Department "Pharmacology, Toxicology and Pharmacotherapy"

at the Faculty of Pharmacy of the Medical University of Plovdiv

for

- dissertation work for awarding the **educational and scientific degree "DOCTOR"**

- professional direction: 7.3. "**Pharmacy**"

- doctoral program "**Pharmacology (incl. pharmacokinetics and chemotherapy)**"

Author: **mag. pharm. STELLA TOSHKOVA DRAGOMANOVA**

Form of doctoral study: **Doctoral student of an independent form of study**

Department: **Pharmacology, toxicology and pharmacotherapy, Faculty of Pharmacy at
Medical University "Prof. Dr. Paraskev Stoyanov" Varna.**

Subject:

**"NEUROPHARMACOLOGY STUDY OF MYRTENOL
AMINOADAMANTANE CONJUGATES"**

Scientific supervisor: **Assoc. prof. VELICHKA ANDONOVA, DF**

Scientific consultant: **PROF. DR. LYUBKA TANCHEVA, MD, PHD**

1. General presentation of the procedure and the PhD student

The presented set of materials on paper / electronic media is in accordance with the Regulations for the terms and conditions for acquiring scientific degrees and holding scientific positions at the Varna University of Medical Sciences and includes the following documents:

- Application to the Rector for the disclosure of a protection procedure;
- European format curriculum vitae with the doctoral student's signature;
- Copy of diploma for educational qualification degree "master" (2016)
- Enrollment order: R.O.-109-253/23.06.2022
- Minutes of exams held for the doctoral minimum: by specialty and by foreign language;
- Minutes from the Dept. C. 01/12/01/2024 with a positive decision on readiness for protection;
- Deduction order with right of defense: R.O.-109-18/23.01.2024;
- Declaration of originality;
- List of publications related to the topic of the dissertation work with the doctoral student's signature;
- Copy of publications related to the topic of the dissertation;
- Declaration of authenticity of the submitted documents;
- Declaration for registration of profiles in scientific databases;
- Similarity report from a similarity checker program from the Publishing Department
- Certificate of availability of an up-to-date profile with a complete list of scientific publications in Google Scholar and ORCID issued by the library of the MU-Varna.
- Dissertation work;
- Abstract, formatted according to the requirements;
- Documents for participation in scientific forums (congresses, conferences, etc.);

2. Brief biographical data for the doctoral student

Stella Dragomanova graduated from the semi-higher medical institute (PMI) in the city of Varna with a specialty of assistant pharmacist in 1998. In her quest to master new knowledge, a few years later in 2004 she graduated as a master pharmacist at the Faculty of Pharmacy of the MU Sofia. In 2010, he started working as an assistant at the department of pharmacology, clinical pharmacology and therapy of the Faculty of Medicine, at the Medical University of Varna. For a short period from 2014 to 2015, he was an assistant in the "Preclinical and Clinical Sciences" Department, "Pharmacology and Toxicology" Faculty of Pharmacy of the Medical University of Varna. From 2015 to the present M.Sc. Ph.D. Drahomanova is successively assistant and chief assistant in the Department of "Pharmacology, Toxicology and Pharmacotherapy" of the same faculty. In 2017, he acquired a specialty in Clinical Pharmacy. She speaks and uses Bulgarian and

English in her practice. Possesses very good personal computer skills with basic office programs, statistics and electronic student learning platforms. Her membership in a number of professional and scientific organizations shows high civic and social responsibility. In support of this are: membership in the Bulgarian Pharmaceutical Union (2004); Chairman of the Committee on Ethics and Quality of RPC Varna of the BPU; Organizer of the continuing education of master pharmacists in the city of Varna and the region in the sense of the Law on Class Organizations; member of the Board of Directors of BSPCPT (Bulgarian Society of Pharmacology, Clinical Pharmacology and Therapy); member of scientific organizations such as EUTOX and BSPCPT, and in 2018 she was awarded the "Pharmacist of the Year" Prize of RPC Varna. The PhD student has 4 participations in scientific projects, a number of participations in scientific conferences and congresses, and co-authorship in 20 scientific publications. Publications and participation in scientific forums have significant scientific value. The editions in which they are published ensure publicity of scientific achievements among a wide range of specialists from various fields of pharmacy and medicine.

3. Relevance of the topic and appropriateness of the set goals and tasks

The dissertation outlines new opportunities for experimental research of the pharmacological activity of natural monoterpenes and proof of their potential medicinal properties for the needs of various fields of medicine and pharmacy. The use of compounds from this group for the treatment of various diseases is a promising and progressive direction in experimental pharmacology. Moreover, the problem is particularly relevant because information in the scientific literature is scarce regarding the therapeutic effects of monoterpenes in experimental models of damaged CNS. In the available literature, there are too few studies on the neuroprotective potential of myrtenal conjugates in experimental models of dementia. The two compounds of myrtenal with aminoadamantane were designed and synthesized to avoid the shortcomings of the natural monoterpene with a view to increasing its biological efficacy. Conducting neuropharmacological studies to study the effects of both compounds in intact and demented experimental animals contributes to uncovering specific mechanisms of action in neurodegenerative disorders. The sequence of the set tasks logically follows the main goal.

4. Knowledge of the problem

Monoterpenes as natural products have been the subject of particular attention in recent years in the search for new molecules with medicinal properties. Therefore, the inclusion of available monoterpenes as building blocks in the design and synthesis of new and effective therapeutic agents in various diseases is an innovative endeavor in experimental pharmacology. A promising direction in scientific research is the use of monoterpenoids in the design of new drugs, due to their ability to minimize adverse drug reactions and toxicity of the resulting compound. The overview of the

dissertation reveals the possibilities of the mag. pharm. Stella Dragomanova to get into the essence of a problem important for medical practice and to show a scientific approach in its experimental modeling and study. The analysis of research on the topic from 482 literary sources allows her to summarize data from other authors and suggest ideas for new design solutions.

5. Research methodology

The dissertation is the result of using a number of modern methods, united in an appropriate methodology to achieve the final goal - in vivo research of the potential neuropharmacological effects of synthetic myrtenal conjugates with aminoadamantane and revealing their main neuroprotective mechanisms on experimental animals with an experimental model of dementia from Alzheimer type. Extensive software and docking research has been conducted. Physicochemical characteristics of myrtenal conjugates with aminoadamantane for calculation of molecular descriptors were determined using the program QikProp 6.2 (2009, NY, USA). With the help of In silico analysis, the prediction of the biological effect was carried out, and with the web-based SwissADME (Swiss Institute of Bioinformatics) software, a theoretical assessment was made for the binding of the myrtenal conjugates to different biological targets. The affinity of myrtenal conjugates to the enzyme acetylcholinesterase was verified with the Scigress Program version FJ 2.6 (Scigress Ultra V. FJ 2.6. 2016, Krakow, Poland). The results of this assay were used to construct inhibitors of this enzyme. Using the CONFLEX method and the MM3 force field, the global minimum was identified, followed by structural optimization (Go \bar{t} o and Osawa, 1993). With the help of a number of physical and physicochemical methods, the stability of the myrtenal emulsion and the solutions of the investigated compounds with the code names MAC-197 and MAC-198, respectively, was determined. To study the potential neuropharmacological effects of the studied synthetic myrtenal conjugates, the doctoral student conducted appropriate behavioral tests with 90 male white Wistar rats, namely the Step through/Passive avoidance test and a test for research behavior (Hold board test). In another set of experiments, an experimental Alzheimer's type dementia model in rats was used to reveal the underlying neuroprotective mechanisms of myrtenal conjugates. The experiments ended with a wide range of biochemical studies in rat brain: for the level of MDA and tGSH; for activity of CAT, SOD, GPx, AchE and measuring the level of mediators NA and 5-HT. Moreover, the oxidative status, bile content, lipid peroxidation product levels and total glutathione content were determined in the cerebral cortex and hippocampus of experimental animals. In addition, the activity of the enzymes superoxide dismutase, catalase, glutathione peroxidase, acetylcholinesterase and the content of monoamines were determined. The experiments with experimental animals were carried out in compliance with the rights of animals according to the International Guiding Principles for Animal Research and the ethical principles in

planning and conducting experiments according to the requirements of the BAFS and the Committee on Ethics of Scientific Research at the University of Varna.

GraphPad Prism 7.0 (San Diego, CA 92108, USA) and Microsoft Excel 2003 programs for statistical data processing and graphical presentation of results were used to process the results.

6. Characterization and evaluation of the dissertation work

The dissertation is written on 220 standard typewritten pages and illustrated with 41 figures, 7 tables and 5 appendices. The bibliography includes an enviable number of literary sources: 480 in Latin and 2 in Cyrillic. Over 75% of the literature sources are from the last 5 years.

The main part of the dissertation was carried out at the Faculty of Pharmacy of the Medical University "Prof. Dr. P.Stoyanov" - Varna, and another part of the research and experiments were carried out with specialists from other scientific centers: Institute of Neurobiology at BAS and Keele University in Great Britain.

Mag. Ph.D. Drahomanova has successfully structured the *literature review* in two topics, which give an idea of adamantane as a pharmacophore for the development of new drugs and a second topic characterizing myrtenal derivatives as potential sources of biologically active substances. The two parts of the review are logically connected with the set goal and provide arguments for the logic of the research conducted in the dissertation work.

In the material and methods section, the PhD student presents in detail the *in vivo* methods used for working with experimental animals in learning and memory tests, exploratory behavior tests, biochemical studies on oxidative status and brain enzyme activity. The substances, solutions and emulsions used are described in detail.

The most important part of the dissertation is the *results section*, which covers 61 pages. The research was conducted under the precise guidance of Assoc. Prof. Velichka Andonova, Ph.D. and the competent consulting activity of Prof. Dr. Lyubka Tancheva, MD, Ph.D. from the Institute of Neurobiology at the BAS. The results are graphically illustrated using the Microsoft Excel program after statistical processing with GraphPad Prism 7.0 as follows:

- Investigation of the presumed ability of the two derivatives of myrtenal to pass through the blood-brain barrier and their ability to influence the CNS based on their physicochemical relationships;
- prediction of potential targets of myrtenal conjugates with aminoadamantane using SwissADME software in rat and human.

- Physical stability of myrtenal emulsion and MAC-197 and MAC-198 solutions for intraperitoneal administration

Experiments on intact rats reveal the influence of myrtenal-aminoadamantane derivatives MAC-197 and MAC-198 on the memory abilities of laboratory rodents and the changes that occur in the enzyme activity of brain acetylcholinesterase and the content of some monoamines in the cerebral cortex and hippocampus.

To establish the neuroprotective activity of the two myrtenal conjugates - MAC-197 and MAC-198, the doctoral student used a suitable experimental model of induced dementia with scopolamine in rats. The changes in the levels of the two target monoamines - serotonin and norepinephrine - were recorded.

The results chapter concludes with a special section comparing pharmacological effects and changes in biochemical parameters of intact experimental animals and those of rats in a state of experimental Alzheimer-type neurodegeneration. In the short but meaningful discussion to this section, a general interpretation of the obtained results is made. Natural myrtenal and its two synthetic derivatives were found to exhibit specific neuroprotective effects, which warrants future development as neuroprotective agents in memory disorders.

7. Contributions and significance of the dissertation work

The established neuroprotective properties of the newly synthesized conjugates of myrtenal with aminoadamantane on experimental animals are a serious basis for extended preclinical studies. For the first time, the potential of both compounds (MAC-197 and MAC-198) to improve memory has been demonstrated in in vivo experiments, without damaging effects on exploratory behavior. A complex mechanism of neuroprotection was revealed for the first time - anticholinesterase, antioxidant and neuromodulating. The potential of the newly synthesized compounds to affect diseases with an imbalance of noradrenergic and serotonergic neurotransmission in the cortex and hippocampus has been experimentally proven. The synthetic conjugates were found to possess specific neuroprotective properties that were more pronounced in memory-impaired rodents than healthy ones. The contributions in the dissertation work are summarized in 5 separate topics, which have a fundamental nature and are a prerequisite for future application in therapeutic practice in neurodegenerative conditions.

8. Assessment of publications related to the dissertation work

Master of Pharmacy Drahomanova has attached evidentiary material for participation in 2 publications in journals with an impact factor and 4 scientific announcements, in which the most important results of the dissertation work are reflected. The publication activity of the doctoral student in our and international scientific forums is a prerequisite for popularizing the obtained

results among research teams in the field of pharmacy and medicine. They are of interest to dosage form technology specialists, pharmacologists, biopharmacists, neurologists, psychiatrists, geriatricians, pediatricians, and many others.

9. Personal participation of the doctoral student

I believe that the dissertation work is the personal work of M.Sc. Dr. Drahomanova. This is supported by the fact that she is first lead author on all publications and scientific communications. The obtained results and formulated contributions are a personal merit under the skillful guidance of the scientific supervisor of the doctoral student – Assoc. Professor, Master of Pharmacy Vili Andonova, MD and scientific consultant Prof. Dr. Lyubka Tancheva, MD, PhD.

10. Abstract

The abstract to the dissertation work is comprehensive, excellently illustrated and completely sufficient as a volume of 68 pages to present the essence of the work. It has been prepared according to the requirements of the Regulations for the terms and conditions for acquiring scientific degrees and occupying scientific positions at the Varna University of Medical Sciences.

11. Critical remarks and recommendations

I have no critical remarks, and my recommendation to the doctoral student is to increase her publication activity in refereed international publications. This will promote the interesting results of the dissertation work and lead to a high citation rate.

CONCLUSION

The dissertation contains *scientific and applied results* that represent an original contribution to science and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB) and the Regulations for its implementation. The presented materials and results correspond to the requirements of the Regulations on the terms and conditions for acquiring scientific degrees and occupying scientific positions at the Varna University of Medical Sciences.

The dissertation shows that the Master of Pharm. Dragomanova possesses in-depth theoretical knowledge and professional skills in the scientific specialty of Pharmacology (including pharmacokinetics and chemotherapy), demonstrating qualities and skills for independent conduct of scientific research.

Due to the above, I confidently give my positive assessment of the conducted research, presented in the above-reviewed dissertation work, abstract, achieved results and contributions. *I propose to the honorable scientific jury to award the educational and scientific degree 'Doctor' to*

Master of Pharmacy Stella Toshkova Dragomanova in a doctoral program in "Pharmacology (incl. pharmacokinetics and chemotherapy)".

11.03.2024

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