

REVIEW

By Prof. Dr. Rumen Pavlov Nikolov, MD
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Member of a scientific jury (order of the Rector of the University of Medicine - Varna No. R-109-441/11.11.2022 in connection with the procedure for the defense of a dissertation on the topic "Pharmacological study of the behavioral effects of biologically active substances of plant origin in models of depression in experimental animals" by Dr. Milena Todorova Salbashyan, doctoral student in independent training preparation in a doctoral program in pharmacology (incl. pharmacokinetics and chemotherapy) at the Department of Pharmacology and Clinical Pharmacology and Therapy at the Faculty of Medicine, Medical University - Varna, professional direction "7.1. Medicine" and field of higher education 7. Health and sports". Scientific supervisor of the doctoral student: Prof. Dr. Stefka Valcheva-Kuzmanova, DSci. Scientific consultant: Prof. Dr. Roman Tashev, DSci.

All documents in accordance with the requirements of the Regulations for the conditions and procedures for acquiring scientific degrees and holding academic positions at the Medical University - Varna were provided to me.

Dr. Milena Salbashyan has successfully passed the required exams: doctoral minimum (pharmacology) and foreign language.

Biographical data

Dr. Milena Todorova Salbashyan completed her secondary education in 1966 in the city of Varna. Acquired secondary special education - nurse in 1987 at IPZK - city of Varna and a master's degree in medicine at Medical University

"Prof. Dr. Paraskev Stoyanov" - Varna in 1999. From September 2015 to the present, he is an assistant at the Department of Pharmacology and Clinical Pharmacology and Therapy at the Medical University "Prof. Dr. Paraskev Stoyanov".

Dr. Salbashyan is fluent in written and spoken English. She has very good computer skills with basic office programs.

Assessment of the submitted dissertation work

Structure of the dissertation

Presented dissertation contains 177 pages and is illustrated with 41 figures and 12 tables. 631 literary sources are cited, of which 3 are in Cyrillic.

The scientific work is properly structured in the following sections: introduction – 2 pages, literature review – 53 pages, aim and tasks – 2 pages, materials, and methods – 12 pages, results – 44 pages, discussion – 5 pages, conclusions - 4 pages, contributions - 1 page, appendices - 1 page, list of publications and participation in scientific forums - 3 pages, bibliography - 42 pages.

Relevance of the dissertation topic

The dissertation is written thoroughly, competently, and comprehensively. The topic of the dissertation is dedicated to a current problem related to the study of the behavioral effects of biologically active substances of plant origin on experimental models of depression.

Anxiety disorders are the most common mental disorders, with studies showing that up to 33.7% of the population is affected by an anxiety disorder in their lifetime. According to WHO data, as of 2017, 300 million people worldwide suffer from depression. Untreated depression increases the risk of suicide (one of the 10 leading causes of death). Despite the availability of many

antidepressants (TCAs, SSRIs, SNRIs, NRIs, NDRI, selective and non-selective MAO-inhibitors and other heterocyclic antidepressants), in about 30-35% of patients with depression, no therapeutic effect is observed. This necessitates the search for new therapeutic approaches, including substances of plant origin.

Aronia melanocarpa (called the black chokeberry) fruits are a rich source of polyphenols. Polyphenols are antioxidants and their effects may contribute to the anti-inflammatory, anti-diabetic and cardioprotective effects of chokeberry plant extracts. In recent years, the anti-depressant and anxiolytic activity of polyphenols contained in aronia berries has been studied in experimental rat models.

Literature review

The literature review is competently written and includes contemporary sources related to the topic of this dissertation.

The review contains 4 parts: *Aronia melanocarpa* - botanical data and biologically active substances in the fruit, pharmacokinetics of polyphenolic compounds, effects of polyphenols on the central nervous system and a summary of literature data.

The literature review shows that the doctoral student is thoroughly familiar with the issues related to the dissertation work, incl. the latest studies in this direction.

Purpose and tasks of the study

The purpose of the study is precisely and clearly formulated. The tasks for its implementation are well defined, specific and correspond to the set goal.

The aim is to study the effects of biologically active substances in *Aronia melanocarpa* fruit juice in experimental models of behavioral disorders in experimental animals induced by ovariectomy and bulbectomy.

To achieve this goal, Dr. Salbashyan has set himself 4 tasks, which are well formulated and specified and precisely correspond to the set goal.

Materials and methods

To the dissertation, the effects of fruit juice from *Aronia melanocarpa* and phenolic acids - chlorogenic, ferulic and gallic acid on two experimental models of depressive behavior in fluff were investigated. The content of polyphenolic compounds in chokeberry fruit juice was standardized by an analytical method according to Valcheva-Kuzmanova et al., 2014, using spectrophotometric analysis and HPLC.

Two experimental models were used in the study: a rat bilateral ovariectomy model and a bilateral olfactory bulbectomy model. A few pharmacological methods have been used (examining locomotor activity and exploratory behavior through the open field test, active two-way avoidance training methods, passive avoidance training, elevated plus maze test, social interaction test, forced swim test, hot plate test "). Appropriate software programs were used to process the survey results.

The methodical approach is modern and sufficient to fulfill the set goals and tasks.

Results

The obtained results are very detailed and competently analyzed.

With long-term administration of *Aronia melanocarpa* fruit juice, a beneficial effect on anxiety- and depression-like behavior was observed: reduction of locomotor activity of ovariectomized rats, prolongation of the time of active social contacts and shortening of immobility time.

Chlorogenic, ferulic and gallic acids prevent the development of hyperactive behavior and exhibit an anxiolytic-like effect, improve memory and learning processes in bulbectomized rats.

After long-term administration of Aronia melanocarpa fruit juice and chlorogenic acid, an increase in pain threshold sensitivity was observed in ovariectomized rats.

Conclusions and scientific contributions

The main conclusions reached by the doctoral student are presented in 2 groups: 16 conclusions were formulated from the results in an experimental model of bilateral ovariectomy in rats and 8 conclusions were formulated from the results in an experimental model of bilateral olfactory bulbectomy.

There are 4 contributions to the dissertation, 3 of which are original. The formulated contributions are mainly scientific in nature with potential importance in the treatment of depressive disorders.

Scientometric indicators related to the dissertation work

In connection with her dissertation work, Dr. Milena Salbashyan has submitted a list of 4 publications, 1 of which is in a journal with IF. In two of the presented publications, she is the first author.

Dr. Milena Salbashyan presented a list of 9 participations in congresses and conferences. She participated as a researcher in 1 scientific project related to the topic of the dissertation work.

Doctoral Thesis

The abstract of the dissertation contains 83 pages, 18 figures and 11 tables. The attached doctoral thesis adequately reflects the main content of the dissertation and the results obtained.

Recommendations and critical remarks: I recommend the doctoral student to continue her publication activity.


Conclusion

The dissertation contains scientific and, scientific applied, which 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 (ZRASRB), the Regulations for the Implementation of ZRASRB and the Regulations of the Medical University of Varna. The presented materials and dissertation results fully correspond to the specific requirements adopted in connection with the Regulations of the Medical University - Varna.

I believe that the presented dissertation work is well-formed, as it uses modern experimental methods, the obtained results are processed and presented excellently and confirm the set goals and tasks, the conclusions drawn summarize the experimental results.

Based on the detailed positive aspects of the dissertation submitted to me for review, I strongly recommend to the respected members of the scientific jury to vote positively for awarding the educational and scientific degree "Doctor" in the doctoral program in pharmacology (incl. pharmacokinetics and chemotherapy) to Dr. Milena Todorova Salbashyan.

12/17/2022

Prepared the review: 
/Prof. Dr. Rumen Nikolov, MD/