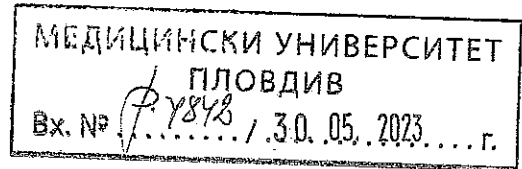


STATEMENT



by Assoc. Prof. Iliya Dimitrov Kostadinov, MD, PhD
Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Medical
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On PhD thesis for awarding the educational and scientific degree 'Doctor'

Professional direction: 7.1 "Medicine"

Field of higher education: 7. "Healthcare and sports"

Doctoral program: "Pharmacology (including Pharmacokinetics and Chemotherapy)"

Author of PhD thesis: Master Pharmacist Nikolay Borisov Yanchev- full-time PhD student

Department: "Pharmacology and Clinical Pharmacology"

Topic: "Experimental Pharmacological Study of Extract from *Sideritis scardica*, Lamiaceae".

Scientific supervisor: Assoc. prof. Delyan Delev, MD, PhD; Head of Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Medical University of Plovdiv

1. General presentation of the procedure and the PhD student

Based on the Decision of the meeting of the Faculty Council at the Faculty of Medicine, Medical University of Plovdiv (Protocol № 8/28.09.2022) and by Order of the Rector of the Medical University of Plovdiv № P-983/19.04.2023, I have been appointed as a member of the scientific jury in the procedure for awarding the educational and scientific degree "Doctor" to Nikolay Borisov Yanchev. On the basis of Protocol from 24.04.2023, I am appointed to prepare a statement on the above-mentioned procedure.

The presented set of materials on paper and electronic media is in accordance with the requirements specified in Art. 70 (1) of Section I. Acquisition of educational and scientific degree "Doctor" at MU-Plovdiv; Regulations of MU-Plovdiv from 28.01.2021 and includes the following documents:

- application to the Rector of MU-Plovdiv for disclosure of the procedure for the defense of PhD thesis;
- curriculum vitae in European format with the doctoral student's signature;
- a notary certified copy of certificate for recognition of professional qualification master-pharmacist;
- an order of the Rector for enrollment of Nikolay Borisov Yanchev as a full-time doctoral student № P-331/28.02.2019; order of the Rector for deduction with the right of public defence for up to one year № P-2411/07.10.2022;
- an order for conducting an exam from the individual plan ((№ P-1354/07.06.2022) and a corresponding protocol for passing the doctoral minimum in the specialty;
- protocol of the Extended Departmental Council for preliminary discussion of the PhD thesis and the decisions taken for the disclosure of the procedure and for the composition of the scientific jury № 97/31.08.2022;
- PhD thesis - 151 pages;
- author's abstract - 54 pages;
- list of scientific publications on the subject of the dissertation;
- copies of scientific publications;
- list of participations in scientific forums;
- certificate of received credits from the group study plan;
- declaration of originality and authenticity of the attached documents;
- other documents related to the course of the procedure - order for defining the topic of the PhD thesis, order No. P-2433/23.12.2021 for extending the period of doctoral studies.

Nikolay Borisov Yanchev has submitted 6 full-text articles. Two of them are published in scientific journals refereed in international databases (Scopus). I have no objections to the documents.

Nikolay Borisov Yanchev was born on August 22, 1988 in the city of Plovdiv. He graduated from secondary education in 2007. Nikolay Yanchev studied pharmacy at the Free University of Berlin in the period 2007-2014. During his studies in pharmacy, he conducted practical training on the validation of analytical methods for the determination of alcohols in various matrices at the Institute of Legal Medicine and Forensic Sciences Charité, Berlin. Nikolay Borisov Yanchev graduated and acquired the professional qualification "Master of Pharmacy" and European certificate for a licensed pharmacist in 2014. In the following years he worked in pharmacies in Berlin. He was enrolled as a full-time doctoral student in the

doctoral program "Pharmacology (including Pharmacokinetics and Chemotherapy)", after successfully passing the competitive exam (order № P-331/28.02.2019 of the Rector of MU-Plovdiv). Nikolay Yanchev was deducted with the right of public defense for up to one year by order No. P-2411/07.10.2022. The doctoral student studied at the Doctoral School of MU-Plovdiv and has acquired 136 credits from the mandatory minimum of 128. He is fluent in German, English and Russian language. Nikolay Yanchev has a certificate for the "SPSS statistical software package" qualification course and skills in working with Windows, MS Office. The PhD student has undergone additional training on the protection and ethical treatment of experimental animals used for scientific or educational purposes. He completed a course, organized by the vitamin manufacturer Orthomol, for acquiring competence in the field of orthomolecular medicine.

2. Relevance of the topic

The PhD thesis of Nikolay Yanchev is about a relevant problem related to the study of the chemical composition, toxicity, antioxidant, anxiolytic, antidepressant, procognitive, immunomodulatory and anti-inflammatory effect of *Sideritis scardica* dry extract. Medicinal plants are an important source of biologically active substances that have a variety of therapeutic effects. This is of scientific interest because medicinal plants produce beneficial effects with minimal risk of adverse reactions. A number of plant-derived compounds are currently employed in modern therapy or have played a role in the synthesis of more complex biologically active molecules. Therefore, studies on the pharmacological effects of medicinal plants are important for theoretical and applied science.

Sideritis scardica, which is endemic to the Balkan Peninsula, is one of the promising medicinal plants. It is a rich source of polyphenols, flavonoids, terpenoids, etc. The rich phytochemical composition suggests a pronounced biological activity of the extract with a wide variety of biological effects. *Sideritis scardica* has been used in folk medicine for centuries but scientific studies on its pharmacological effects are quite insufficient. The plant is cultivated in our country and the systematic pharmacological research of the extract is important for further investigations on its use in the adjuvant therapy of a large number of diseases.

Stress is characterized by low-grade systemic inflammation and increased production of free oxygen radicals. Therefore, it plays an important role in the onset and progression of a large number of socially significant diseases - neurodegenerative, cardiovascular, etc. Pharmacological research on the effects of *Sideritis scardica* dry extract in acute and chronic stress model will provide the scientific basis for further study regarding its clinical application in the prevention and treatment of stress-induced diseases. The study on the levels of pro- and anti-inflammatory molecules under conditions of acute and chronic stress will expand the knowledge about their probable use as biomarkers of body response to stress. *Sideritis scardica*-induced changes in serum concentrations of these molecules are essential to reveal the role of the immunomodulatory effect in the mechanism of its anti-stress action.

The pharmacological study on the anxiolytic and antidepressant effect of *Sideritis scardica* extract under conditions of acute and chronic stress is the basis for further research on its possible application in the complex prevention and therapy of anxiety and depressive disorders. The relevance of this problem is related to the high prevalence of these diseases, significant adverse drug reactions and interactions associated with their contemporary pharmacotherapy. Research on the pro-cognitive effect of *Sideritis scardica* extract will provide

the scientific base for further studies on its use in the therapy of dementia, another significant health problem. Dementia affects nearly 50 million people worldwide and lead to disability, low quality of life and increased mortality.

3. Knowledge of the problem

Nikolay Yanchev demonstrates excellent knowledge of the problem that is subject of the PhD thesis. The review of the dissertation work is based on an extensive literature reference. It is laid out on 31 pages and is based on above 150 sources. The review is illustrated with 11 figures and 3 tables, which greatly facilitates the perception of the presented information. The analysis of literature shows that the doctoral student knows the problem that is subject of the dissertation and is able to focus on unresolved issues, summarize and analyze the data from a large number of scientific sources.

The literature review is competently and comprehensively written. The individual parts follow in a logical sequence. At the beginning of the review, the doctoral student presented detailed information on the biology and chemical composition of *Sideritis scardica*, the mechanisms of free oxygen radicals production, natural antioxidants and the role of free radicals in the pathogenesis of neurodegenerative diseases. The doctoral student focuses on the biological activity of compounds found in *Sideritis scardica*. Based on the data about the neuroprotective effect of these compounds the doctoral student developed the hypothesis for investigating the neuropharmacological effects of *Sideritis scardica* extract - anxiolytic, antidepressant and pro-cognitive.

A special section in the literature review is dedicated to the available experimental data about the effects of *Sideritis scardica* on the CNS. The mechanisms of its neuropharmacological effects are discussed. The available data regarding these effects of *Sideritis scardica*, the involved mechanisms and experimental models are summarized in a table. Data on the anti-inflammatory activity of plants of the genus *Sideritis* and in particular of *Sideritis scardica* are also summarized in tables. This manner of presenting the information demonstrates the doctoral student's ability to summarize and systematize literature data on the problem.

In the next part of the review, the doctoral student examines the relationship between stress, the immune system and diseases of the nervous system. Data for experimental stress models are presented. The role of certain pro- and anti-inflammatory cytokines in the pathogenesis of stress, systemic and neuroinflammation is discussed. Based on this information, the PhD student focused on the study of certain cytokines in models of stress and lipopolysaccharide-induced systemic and neuroinflammation. In the last part of the review, the doctoral student discusses the essence, advantages and disadvantages of the cognitive deficit models used in the dissertation.

The summary of the literature review is short, but comprehensive and systematized. It justifies the choice of *Sideritis scardica* as a subject of the research in the PhD thesis and outlines the main directions of the study - neuroprotective, anti-inflammatory and immunomodulatory.

4. Research methodology

The study design is described in-depth. The chosen methods are contemporary, reliable and provide the achievement of the aim of the study. They are described in detail and precisely, which guarantees reproducibility of the obtained results. A wide range of *in vivo* and *in vitro* methods have been used. This is indicative for the good practical training of the doctoral

student. Successful collaboration has been realized with two other departments of MU-Plovdiv - Department of Medical Microbiology and Immunology "Prof. Dr. Elisey Yanev" and Department of Anatomy, Histology and Embryology. The phytochemical composition and antioxidant activity of *Sideritis scardica* extract were studied at the Department of Organic Chemistry and Inorganic Chemistry, University of Food Technology, Plovdiv.

Four different methods (DPPH, ABTS, FRAP and CUPRAC) are used to determine the antioxidant activity of the extract. This allows more complete characterization and corresponds with the requirements for the use of at least two methods for evaluation of antioxidant activity. Carbohydrates and total content of polyphenols and flavonoids are quantified by using a spectrophotometric method.

Histological examination of liver, kidney, stomach and brain with hematoxylin-eosin staining are used to investigate subchronic toxicity of *Sideritis scardica* extract. The PhD student acquired skills for using methods of hematological and biochemical analysis with an automatic hematological (Rt-7600Vet) and biochemical (Chemray 120vet) analyzer. The serum cytokines levels are measured with ELISA methodology.

The PhD student has used a large number of behavioral and other methods for evaluating anti-inflammatory (plethysmometer), anxiolytic (elevated plus maze, social interaction test) and antidepressant (forced swim test) effect, locomotor activity, learning and memory (activity cage, two-way active avoidance, step-through, step-down, novel object recognition test, T- and Y-maze). Models of acute cold stress and chronic unpredictable stress; three models of impaired memory (with diazepam, scopolamine and sodium nitrite) and two models of inflammation (carrageenan-induced paw edema and systemic inflammation with lipopolysaccharide) are used.

The obtained data are analyzed with the appropriate statistical methods using the IBM SPSS 19.0 software product.

5. Characterization and evaluation of the PhD thesis and contributions

The PhD thesis is structured according to the requirements for the educational and scientific degree "Doctor". It is laid out on 151 standard typewritten pages and is well balanced between its individual parts - introduction (2 pages), literature review (31 pages), aim and objectives (1 page), scientific idea and working hypothesis (1 page), material and methods (11 pages), results (28 pages), discussion (20 pages), conclusion (1 page), main research findings (1 page), scientific contributions (1 page), appendix (15 pages). The bibliographic reference is written on 28 pages and includes 303 authors. The dissertation is illustrated with 39 tables and 46 figures.

The aim is clearly and precisely formulated. The doctoral student planned 7 objectives for the achievement of the aim of the study.

The results are correctly processed statistically. They are described accurately and in-depth and follow the objectives logically. The results are illustrated with 36 tables and 1 figure. 30 additional figures (in the appendix) also illustrate the obtained results. At the beginning of the "Results" section, the PhD student presents data on phytochemical composition and antioxidant activity of *Sideritis scardica* extract. Study on acute and subchronic toxicity (after 12 weeks of administration) of the extract is performed. This is a necessary prerequisite for further studies on its pharmacological effects and biological activity. Further in the section, the results of the conducted research on anxiolytic, antidepressant, immunomodulatory, anti-

inflammatory and memory-improving effects are presented. Pharmacological effects are studied after 14-day, 30-day or 8-week treatment of the experimental animals. The results of all conducted experiments are summarized in three tables, which greatly facilitates the perception of information from this long and extensive research.

The "Discussion" section of the dissertation demonstrates the doctoral student's ability to analyze and interpret the results obtained. At the beginning of the section, the obtained results about the toxicity of *Sideritis scardica* extract are compared with the available data in the literature. The choice of doses in the conducted study is justified. The discussion of the results is presented in four main directions - anti-inflammatory (including neuroinflammation), immunomodulatory, memory-improving and anti-stress effect. Nikolay Yanchev competently and intelligently discusses the possible mechanisms of the registered pharmacological effects. The doctoral student proficiently uses the available data about the biological activity of the compounds found in the extract to explain the obtained results. The discussion of the results is summarized in 4 figures which illustrate the complex mechanisms of the observed pharmacological effects of *Sideritis scardica* extract.

In the conclusion, the main results of the systematic pharmacological study of *Sideritis scardica* extract are presented.

The doctoral student has formulated 7 conclusions that correspond to the obtained experimental results and fully meet the set objectives.

The contributions of the dissertation work are divided into 2 groups: scientific-theoretical and scientific-practical. Contributions with theoretical significance are: systematic analysis of acute and chronic stress experiments was made to establish the anxiolytic effect of substances of plant and synthetic origin; systematic analysis of learning and memory experiments in different cognitive impairment models was conducted to establish a neuroprotective effect of substances of plant and synthetic origin; relationship between the development of anxiety and increased levels of proinflammatory cytokines has been established in preclinical studies. Contributions with practical application have high scientific value. Systematic pharmacological study of the anti-inflammatory, anxiolytic and neuroprotective effect of *Sideritis scardica* was conducted for the first time in Bulgaria; a dose-effect relationship of the extract was established; markers for the study of anxiolytic and anti-inflammatory effects were documented. I accept the contributions of the PhD thesis.

6. Evaluation of the publications and personal contribution of the PhD student

Nikolay Yanchev has submitted 6 full-text articles and 6 participations in scientific forums on the topic of the dissertation. Two of the full-text articles are in scientific journals refereed in international scientific databases - one publication (in print) in a Bulgarian journal refereed in Scopus (Folia Medica) and one in a foreign journal refereed in Scopus (Tropical Journal of Natural Product Research). The doctoral student participates in one scientific forum abroad and five in Bulgaria. In all publications the doctoral student is the first author.

The PhD student's publication activity indicates his ability to analyze, summarize and present the data from experimental studies in publications and scientific forums. The number and type of publications meet the requirements for the acquisition of the educational and scientific degree "Doctor" according to the Act on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its Implementation and the Regulations of MU-Plovdiv.

I have known Nikolay Yanchev since his participation in the competition for full-time PhD student at the department. He has high theoretical and practical training. Nikolay Yanchev is responsible, organized and has analytical thinking. My personal impressions, the PhD thesis, the author's abstract, the presentation at the time of deduction, as well as the scientific publications and participation in scientific forums in which Nikolay Yanchev is the first author convinced me that the conducted research, obtained results and formulated conclusions and contributions are the personal merit of the doctoral student.

I have no critical remarks and recommendations.

7. Author's abstract

The author's abstract of the dissertation is structured according to the requirements and contains 54 pages. It is illustrated with 35 figures and 6 tables, and sufficiently reflects the main content of the dissertation - the methods used, the results obtained, discussion, formulated conclusions and scientific contributions.

Conclusion

The PhD thesis of Nikolay Borisov Yanchev is dedicated to an important and relevant topic of contemporary pharmacotherapy. Researched problems are important not only for theoretical but also for practical knowledge. The doctoral student shows an exceptional knowledge of the researched problems and uses a wide range of contemporary *in vitro* and *in vivo* scientific methods for achieving the formulated objectives of the study. The obtained results are a consequence of extensive and long scientific research that is precisely planned and conducted. They are documented precisely and in-depth. The contributions of the PhD thesis are important for the development of the science in the researched field and its practical application.

Nikolay Borisov Yanchev is an established specialist in pharmacology, who possesses in-depth theoretical knowledge and practical training, and demonstrates qualities for independent planning and conducting scientific research.

The presented dissertation fully meets the requirements of the Act on Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for its implementation and the Regulations of the Medical University of Plovdiv. The presented materials and dissertation results fully correspond to the specific requirements regarding the Regulations of the Medical University of Plovdiv for implementation of the ADASRB.

I confidently give **my positive assessment** of the research, presented by the above peer-reviewed dissertation, author's abstract, obtained results and scientific contributions, and would recommend to the honorable members of the scientific jury to award the educational and scientific degree "Doctor" to Nikolay Borisov Yanchev in a doctoral program " Pharmacology (including Pharmacokinetics and Chemotherapy)".

29.05.2023

Заличено на основание
Чл. 51, б. "В" Регламент (ЕС) 2016/679

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(Assoc. Prof. Iliya Kostadinov, MD, PhD)