

**REVIEW**

**by Prof. Dr. Stefka Vasileva Valcheva-Kuzmanova, PhD, DSc**  
**Department of Pharmacology and Clinical Pharmacology and Therapeutics,**  
**Faculty of Medicine, Medical University – Varna**

of a dissertation for awarding the educational and scientific degree 'doctor'

professional direction **7.1. Medicine**

doctoral program **Pharmacology (including pharmacokinetics and chemotherapy)**

**Author: Natalia Borisova Vilmosh**

**Form of doctoral studies:** independent training

**Department:** Pharmacology and Clinical Pharmacology

**Topic:** Investigation of pharmacological effects of *Satureja montana*

**Research supervisor:** Prof. Ivanka Ilieva Kostadinova, MD, PhD, MU – Plovdiv

By Order of the Rector of the MU – Plovdiv P-2782/04.11.2022, I was approved as a member of the Scientific Jury, and by a decision of the meeting of the Scientific Jury (Protocol No. 1/07.11.2022) I was chosen to prepare a review on the procedure for awarding the educational and scientific degree "doctor" to Natalia Borisova Vilmosh, MD.

### **1. General presentation of the procedure and the doctoral student**

The presented set of documents in an electronic format is in accordance with Act on Development of Academic Staff in the Republic of Bulgaria, the Regulations for the implementation of the Academic Staff Development Act and the Regulations of MU-Plovdiv dated 28.01.2021 – Art. 70 (1) of Section I. Acquisition of educational and scientific degree "DOCTOR" at MU-Plovdiv

Dr. Natalia Vilmosh has presented 3 publications, 2 are of which referenced in Scopus and 1 is in a non-refereed journal. In this way, the minimum requirements of the Regulations for Academic Development at MU-Plovdiv (2021) have been fulfilled.

### **2. Brief biographical data for the doctoral student**

Dr. Natalia Vilmosh was born on 04. 10. 1993. She has a Master's degree in Medicine - she graduated from the Medical University of Plovdiv in 2018. Since 2019, she has been working as an Assistant Professor in the Department of Pharmacology and Clinical Pharmacology, Medical Faculty

at Medical University of Plovdiv. She was enrolled as a doctoral student on independent training with Order of the Rector of MU – Plovdiv P-1275/31.05.2022.

She is fluent in German (level C1) and English (level C1). She is a member of the Bulgarian Society of Clinical Pharmacology since 2021 and of YSA “Asclepius” since 2020. She has completed courses and trainings: Statistical software package SPSS, Protection and welfare of experimental animals used for scientific or educational purposes, School of personalized medicine, History of Medicine, Sports Medicine and Homeopathy.

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

In recent years, plants have been intensively studied as a source of molecules with potential application as drugs. The study of the composition of medicinal plants aims to lay the scientific foundations of their traditional use in folk medicine. An advantage of biologically active substances of plant origin is their good safety profile. Despite many years of clinical and research experience, it is believed that only a small part of plants have been studied for their composition and medicinal effects.

In this sense, the study of the plant *Satureja montana* is very up-to-date and has important contributions of scientific and scientific-applied value. The review of the scientific literature shows that so far some pharmacological effects of *Satureja montana* – antioxidant, antibacterial, antiviral – have been established.

The dissertation is devoted to hitherto unexplored pharmacological effects of *Satureja montana*, with the aim to investigate a standardized dry extract of the plant for antioxidant activity in vitro, as well as for toxicity, and for analgesic, anti-inflammatory, anxiolytic and antidepressant effects after oral administration to male white rats.

To achieve this goal, 9 very voluminous tasks were set – starting with standardizing the extract, recording the effects listed above and investigating markers and biochemical indicators that could be used to explain the observed effects.

### **4. Knowledge of the problem**

Dr. Natalia Vilmosh has done a very comprehensive review of the literature in several aspects: stress, correlation between stress and oxidative stress, the role of oxidative stress and chronic low-grade inflammation in the pathogenesis of socially important diseases and psychiatric diseases, correlation between stress and inflammation, and between oxidative stress and inflammation. From

the extensive literature review, it is clear that Dr. Vilmosh is well aware of the role of proinflammatory cytokines in inflammatory processes. She discusses the modern therapeutic options for oxidative stress and chronic low-grade inflammation.

The detailed knowledge of the pathogenetic mechanisms of stress, inflammation and pain has guided Dr. Vilmosh to select appropriate indices to evaluate the presence of an effect of *Satureja montana* extract on pain and inflammation, as well as to investigate psychopharmacological effects in stress models.

An important section in the literature review is devoted to the characterization of the plant *Satureja montana*, as well as the effects of extracts from it that are known up to now – antioxidant, antibacterial and antiviral. A good knowledge of the effects known so far is a prerequisite for the research carried out by Dr. Vilmosh in search of activities and actions that have not been established until now, with the aim of possible future practical application of the extract.

The pharmacological effects of rosmarinic acid and carvacrol are described. They were used to standardize the dry extract of *Satureja montana* applied in the experiments. The characterization of these substances is also very important due to the fact that they were also used in the experiments – the effects of a dry extract of *Satureja montana* were compared with their effects.

From the literature review, it is obvious that Dr. Vilmosh has studied the established experimental research models and methods for investigation of inflammation and pain, models of stress, and methods for the evaluation of anxiety and depression. This enables her to make the right choice of models and methods for her research work carried out with the *Satureja montana* extract.

## **5. Research methodology**

The research related to the dissertation includes an extremely diverse methodology. There are several groups of methods: I. Extraction, followed by standardization of the extract by HPLC, and spectrophotometric determination of its polyphenol composition; II. In vitro determination of the antioxidant activity of the extract by Oxygen Radical Absorbance Capacity, Hydroxyl Radical Averting Capacity and electrochemical method; III. Studies in experimental animals. The last group of methods includes: 1. Determination of acute and chronic toxicity, verified by hematological parameters, biochemical analysis and histological examination of the liver, kidney and brain; 2. Investigation of analgesic effect for 2 periods of treatment – 14 and 30 days, using 2 tests: tail withdrawal test and plantar test; 3. Investigation of the analgesic effect of the two doses of *Satureja montana* extract at 2 treatment periods – 14 and 30 days, in a model of carrageenan-induced inflammation of the paw; 4. Studies in 2 models of stress – acute cold and chronic. In these 2 stress

models, a very extensive research work has been carried out, evaluating the effects of the two doses of *Satureja montana* extract (250 mg/kg and 500 mg/kg) on the levels of cytokines IL-1 $\beta$ , IL-6 and TNF- $\alpha$  by using by ELISA method; the effects on behavioral responses and recognition memory were established, and in the chronic stress model, the effects on biochemical parameters (cholesterol, glucose, triglycerides and uric acid) as well as on the weight of the animals were traced. The behavioral tests performed in both stress models were: the elevated plus maze test, the social interaction test, the Vogel conflict test and activity cage locomotor activity, which measured anxiety levels; the novel object recognition test, which measured the recognition memory; the forced swim test, which measured depressive behavior.

In all experiments, the effects of 2 doses of *Satureja montana* dry extract (250 mg/kg and 500 mg/kg) were determined and compared with the effects of carvacrol (500 mg/kg) and rosmarinic acid (15 mg/kg).

Very adequate methods and models were chosen to establish the pharmacological effects of *Satureja montana* extract. They are widely used in pharmacological research practice and enable adequate conclusions to be drawn, providing an answer to the research tasks.

I highly appreciate the fact that an extremely large number of pharmacological methods were used, the mastery of which is a prerequisite for a very successful future research work of Dr. Natalia Vilmosh.

## **6. Characterization and evaluation of the dissertation**

The dissertation is well structured and includes the following sections: Introduction – 4 pages, Literature review – 38 pages, Aim and tasks – 1 page, Material and methods – 19 pages, Results – 80 pages, Discussion – 36 pages, Summary – 2 pages, Conclusions – 1 page, Contributions – 1 page, Bibliography – 31 pages, including 443 sources in English.

With the comprehensive and very targeted literature review, Dr. Natalia Vilmosh directs the reader to the aspects of the research work carried out. The pathogenetic mechanisms of inflammation, pain, oxidative stress, stress, as well as the correlation between them, elucidated in the review, are skillfully used further as an explanation for the observed effects of *Satureja montana* extract.

Knowledge of the literature on already published research with the *Satureja montana* plant enables Dr. Vilmosh, competently guided by her scientific supervisor, Prof. Dr. I. Kostadinova, to direct her work to hitherto unexplored effects, which contributes to originality of the results obtained.

The goal is clearly stated. In order to achieve the goal, nine tasks were set, which required extremely diverse and very voluminous research work.

The materials and methods are very detailed and accurately described, which shows a very good knowledge of research methods and testifies to the personal commitment of Dr. Vilmosh.

The experimental groups are presented very accurately, and the corresponding treatments are indicated in a tables. Treatments for comparison were correctly selected – carvacrol and rosmarinic acid, which are components of *Satureja montana* extract, were used in all experiments, while in the study for analgesic effect there was an additional comparison group – treated with metamizole, and in the study for anti-inflammatory effect – treated with diclofenac. In each experimental group there is an optimal number of experimental animals – eight.

The research results are are presented in detail and are illustrated with 97 tables and 4 figures. In the tables, values are presented as mean  $\pm$  SEM. Statistical significance is indicated, which was determined with IBM SPSS 19.0 software by One Way ANOVA test and Independent sample T test. Correctly performed statistical processing of the experimental data enables correct and reliable conclusions from the studies.

Important results of an original character, with scientific and scientific-practical value, were obtained. The effects registered in the studies are skillfully discussed, being supported by the data so far known in the scientific literature. Since the main carriers of the biological activity of *Satureja montana* extract are rosmarinic acid and carvacrol, Dr. Natlia Vilmosh refers to the established activities of these biologically active substances to explain the effects of the extract.

The results are very clearly summarized in the Summary of the dissertation work, and the effects of the dry extract of *Satureja montana* are highlighted in a comparative aspect against the comparators rosmarinic acid and carvacrol, as well as metamizole (for analgesic effect) and diclofenac (for anti-inflammatory effect).

Based on the precisely conducted and described experiments, 10 conclusions were drawn, which correctly reflect the results of the research.

## **7. Contributions and significance of the research for science and practice**

As a result of the fulfillment of the tasks set in the dissertation, important contributions of scientific theoretical importance were obtained, namely, for the first time in Bulgaria, a systematic pharmacological study of a dry extract of *Satureja montana* was conducted, and important effects were established: anxiolytic, antidepressant, analgesic, antioxidant and anti-inflammatory. The effective dose for the manifestation of these effects has been established. The anxiolytic and

antidepressant activities of *Satureja montana* dry extract have been shown to correlate with its antioxidant and anti-inflammatory effects.

Contributions of applied value were also obtained, with the standardization of a dry extract of *Satureja montana* and determination of its acute and chronic toxicity, which is an important prerequisite for its future safe use. The dissertation work would help future researchers in two aspects: through the developed protocol for the Vogel test, as well as with the result obtained in the experiments that the serum concentrations of the pro-inflammatory cytokines IL-6, IL-1b and TNF-a are not a reliable marker for registering a systemic anti-inflammatory effect in models of acute and chronic stress.

The experimental studies conducted with *Satureja montana* extract are a prerequisite for conducting clinical trials and its future therapeutic use in humans.

#### **8. Assessment publications related to the dissertation**

Dr. Natalia Vilmosh has applied 3 publications in relation to the dissertation work, 2 of which are in refereed journals, one of which has an impact factor. Both publications are in English. One of the publications has a review character and was published in Bulgarian in a non-refereed journal.

Dr. Vilmosh popularized the results of the research included in the dissertation through 2 participations in a prestigious international forum, as well as 4 participations in national forums.

#### **9. Personal participation of the doctoral student**

Dr. Vilmosh is the first author of all publications and abstracts of participation in scientific forums, which shows her great personal commitment and personal credit for the dissertation research, as well as for the results obtained and the contributions formulated.

#### **10. Abstract**

The abstract is written in 73 pages, made according to the requirements and reflects the main results achieved in the dissertation. The results are well illustrated with figures that replace the tabular presentation of the same data in the dissertation.

#### **11. Critical remarks and recommendations**

I have no critical remarks. The materials submitted for review are very well organized and timely presented.

I would recommend Dr. Natalia Vilmosh to continue to apply the rich set of acquired pharmacological research methods that will ensure her successful future development as a researcher and scientist.

## **12. Personal impressions**

I have no prior personal impressions of Dr. Vilmos, but the conductance of the studies and the presentation of the results thereof, as well as their interpretation, show precision, exactingness, organization and logic.

## **13. Recommendations for future use of dissertation contributions and results**

I recommend Dr. Vilmosh to conduct future clinical trials with *Satureja montana* extract.

## **CONCLUSION**

The dissertation contains scientific and scientific-applied results, which represent an original contribution to science and meet all the requirements of Act on Development of Academic Staff in the Republic of Bulgaria, the Regulations for the implementation of the Academic Staff Development Act and the Regulations for Academic Development at MU – Plovdiv (2021). The presented materials and dissertation results fully correspond to the specific requirements of the MU – Plovdiv.

The dissertation shows that Dr. Nataliya Vilmosh possesses in-depth theoretical knowledge and professional skills in the scientific specialty of Pharmacology (including pharmacokinetics and chemotherapy). She shows great personal commitment in the work on the dissertation, demonstrating qualities and skills for independent conduction of scientific research. The presentation of the results and their interpretation show precision, exactingness, organization and logic. It is commendable that Dr. Vilmosh was able to draw as much as possible from the experience of her scientific supervisor - Prof. Dr. Ivanka Kostadinova, as well as the other colleagues with whom she carried out the scientific research. She has mastered an exceptionally large number of pharmacological methods, which is a prerequisite for very successful future research work.

Due to the above, I confidently give my **positive assessment** of the conducted research, presented by the above-reviewed dissertation work, abstract, achieved results and contributions, and I propose to the honorable scientific jury to award a scientific degree of "doctor" to **Natalia Borisova**

Vilmosh in the doctoral program **Pharmacology (including pharmacokinetics and chemotherapy)**.

Заличено на основание  
Чл.5 §1, б. "В" Регламент (ЕС)2016/679

18.11. 2022

Reviewer:.....

Prof. Stefka Valcheva-Kuzmanova, MD, PhD, DSc