

## STATEMENT REVIEW

By: Prof. Dr Ivanka Ilieva Kostadinova, PhD

Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Medical University Plovdiv

In reference to: procedure for award of a Educational and scientific degree` „Doctor“ to **Mag. Pharm. Stanislav Yordanov Marchev, independent PhD student**

**Dissertation title:** “Pharmacological studies of steroid hormones, natural products and newly synthesised 2H-substituted hydrasid hydrasones in experimental models of epilepsy, pain and osteoporosis”, **Field of Higher Education:** 7. Healthcare and sport, **Professional field:** 7.3 Pharmacology, **Scientific specialty:** Pharmacology

Scientific supervisors: *Prof. Dr Stefka Valcheva - Kuzmanova, DSc.*  
*Ass. Prof. Polina Gateva, PhD*

Scientific consultant: *Prof. Mila Vlaskovska, DSc.*

### **Professional development:**

Stanislav Yordanov Marchev graduated with a master's degree in pharmacy in 2017 in Germany, Freie Universitat Berlin. He currently lives and works as a Master Pharmacist in Berlin. Mag. Pharm. S. Marchev is enrolled as a doctoral student in an independent form of study at the Department of Pharmacology, Toxicology and Pharmacotherapy of MU Varna "Prof. Dr. P. Stoyanov".

### **Structure of the dissertation:**

The dissertation of Mag. Pharm. Marchev consists of 129 pages. The research presented in the dissertation is funded by research projects NSF DN 13/16 21. 12. 2017 and MUS D-74 / 2017. Stanislav Marchev's dissertation is constructed according to the requirements of MU Varna for the award of Educational and scientific degree "Doctor". It is illustrated with 24 tables and 11 figures, in which the results of the experiments are presented in a very good way, with great informative value. The bibliography of the dissertation contains 155

sources written by foreign authors and 15 publications by Bulgarian authors. Twenty six titles are published in the 5 years.

### **Relevance and significance of the dissertation**

Epilepsy is one of the most common chronic neurological diseases, affecting up to 60 million people worldwide without age, race, social class, national or geographical differences. According to the definition of the International League Against Epilepsy, the disease affects almost 1% of the population. Epilepsy causes increased physical and psychosocial morbidity and imposes a great economic burden on health care systems. Drug-resistant epilepsy is reported in up to 30% of patients with epilepsy. Patients with uncontrolled seizures have been diagnosed with psychosocial disabilities among which unemployment, impaired socialization, more injuries, lack of education and psychiatric disorders. Seizure control is especially important in preventing injury, morbidity and mortality in people with drug-resistant epilepsy. There are evidences in the literature that sex hormones are involved in the pathogenesis of epileptic seizures.

The role of steroid hormones, accumulated in the structures involved in controlling seizures by influencing their mechanisms, determines their follow-up in the study design. In this aspect, new directions are being sought for more effective treatment of seizures.

The ageing of the European and world population underlines the importance of prevention and treatment of osteoporosis. According to Compendium of Osteoporosis (2017), there were 22 million women and 5.5 million men diagnosed with "osteoporosis" in the EU. The total number of newly diagnosed fractures in osteoporosis were 3.5 million. Herbal products used for therapy and prevention are of convenient and easy use, which might augment patient participation in the prevention and treatment of diseases. According to the WHO, about 80% of the world's population uses herbal medicines for prevention and in primary health care.

The dissertation combines research on the role of steroid and sex hormones on epileptogenesis with the treatment of osteoporosis with products of plant origin and study of the pharmacological effects of newly synthesised compounds. The topic of the dissertation is relevant and related to socially significant diseases such as epilepsy and osteoporosis.

The introduction of the dissertation resumes the relevance of the research problem and motivates the choice of the dissertation topic.

In the literature review Mag. Pharm. Marchev reviews the scientific achievements in the field. Various study results on the influence of steroid adrenal and sex hormones on epileptogenesis are discussed as well as scientific gaps and need for further research. The literature review describes in detail the areas for research of new AELs. The main directions in the search for new therapeutic agents and the therapeutic benefits of medicinal products used for the treatment of epileptic seizures are discussed. One of the directions is to study new drug molecules with a selective mode of action on important parts of epileptogenesis. In recent years, there has been increased interest in the synthesis of new compounds, coumarin derivatives with anticonvulsant effect. In the review the author points out the main chemical characteristics, pharmacological and toxic effects of newly synthesised coumarin and 2H-chromene-substituted hydrazide-hydrazones and justifies the inclusion of 4a, 4b, 4c, 8a, 8b in the study design. The increased risk of osteoporosis and fractures with long-term AEL treatment has been discussed. Previously published data on the pathogenetic mechanisms for the osteoporosis development epileptic patients are divergent which led to discussion. Risk factors are the female sex, the duration of therapy, AEL, diet, combination drug therapy in severe forms of epilepsy. Gene mutation, the action of AELs as enzyme inducers, and those that do not affect the metabolism of other drugs are discussed. Epidemiological and clinical data on osteoporosis, pathogenetic mechanisms for its development are considered as well as the main fields of research for pharmacological treatment. The types of pain, the mechanisms and pharmacotherapy of the pain syndromes are discussed in detail. At the end of the literature review, Mag. Pharm. Stanislav Marchev discussed the benefits of natural products, the composition of *Aronia* fruit juice and celery extract and their pharmacological effects. In the review, Marchev underlines the reasons to include a study of the effects of *Aronia* fruit juice in an experimental model of estrogen-deficient (post-menopausal) osteoporosis in the design of the PhD thesis. Systematic multilayer *in vivo* and *in vitro* studies of Vlaskovska et al. (Tsakova, A., et al., 2015, Tsakova, A., 2016) showed evidences for the beneficial effect of clinical and paraclinical symptoms in chronic administration of extract of celery (*Apium nodiflorum*) in experimental models of postmenopausal osteoporosis. Marchev points out the need for additional experiments in order to clarify the mechanism of action of celery extract. The data presented in the literature review proved that Mag. Pharm. Marchev is familiar with the recent publications on the topic of the dissertation and is able to analyse and discuss in competent way the unsolved scientific issues.

The aim of the dissertation is divided into two areas:

1. To study the effect of steroid adrenal and sex hormones on the intensity, dynamics and latency of Kainate-evoked seizures and lethality in an experimental model of epileptogenesis in rats. To study the effect of a series of newly synthesised hydrazide-hydrazone compounds on nociception;
2. The second direction is to study the effect of phytoproducts of *Aronia melanocarpa* and *Apium nodiflorum* on bone mineral density (BMD) and bone mineral content (BMC) in animals with experimental oestrogen-deficient osteoporosis. The tasks set in the dissertation work support the fulfilment of the set aims. For the implementation of the first one 5 tasks are indicated, and for the second - 6 tasks.

The aims and tasks have both scientific and applied significance. Mag. Pharm. Marchev used modern, well-established models and methods for reporting pain sensitivity, behavioural reactions, anticonvulsant activity. Through surgery, the doctoral student removed the gonads and adrenal glands of male rats and performed bilateral ovariectomy of female rats. The assessment of the intensity of the epileptiform syndrome was according to the initially introduced by Prof. Dr. M. Vlaskovska and associates new original six-degree cumulative scale. The analgesic study included 5 newly synthesised compounds administered at a mean effective dose and tested by Hot plate test and formalin test.

The material and methods described in detail the experimental models and methods used. The results are presented correctly and in detail. Appropriate statistical tests have been used to process the data from the experimental studies, which ensure the reliability and reproducibility of the obtained results with data from other authors. The doctoral student has very good computer skills that help him in presenting and publishing the results. In the discussion Mag. Pharm. Marchev compared the results of his studies with data from other authors. The probable reasons for observed differences were also discussed. The data from the conducted experiments are summarised in 10 conclusions. The doctoral student knows very well the literature on the topic of the dissertation, expresses his position, analyzes the results obtained and set the proper conclusions.

The dissertation is written and structured correctly. The aims and tasks are clearly set. The results and the discussion are logically connected. The conclusions are precisely formulated, correspond to the data obtained in the scientific experiments. The dissertation of mag.pharmacist Marchev has contributions of scientific and applied significance. The five newly synthesised hydrazide-hydrazone derivatives were found to have analgesic

activity. It was found that long-term treatment with *Aronia* extract could affect osteoporotic bone changes in an experimental model of osteoporosis. A quantitative cumulative scale for analysis of somatic/convulsive and cognitive manifestations in experimental epilepsy has been validated. I do accept and evaluate as significant the contributions indicated in the dissertation of Mag. Pharm. Stanislav Marchev.

The dissertation “Pharmacological studies of steroid hormones, natural products and newly synthesised 2H-substituted hydrasid hydrasones in experimental models of epilepsy, pain and osteoporosis” submitted for an opinion meets the requirements specified in the regulations of MU Varna. The dissertation is written in accurate and clear language. Mag. pharmacist Stanislav Marchev has proven theoretical knowledge and practical skills. He can handle freely the facts from the available literature, express and defend his position, discuss and compare his results with those of other authors, comment differences and similarities between them. The cooperation with leading specialists from different scientific units, the management of the doctoral student by established lecturers and researchers are a guarantee for the scientific value of the dissertation presented for opinion.

Mag. Pharm. Stanislav Marchev has the qualities and skills to conduct research independently. Based on the presented publications, participation in scientific forums and dissertation, a conclusion for personal contribution can be made. Stanislav Marchev succeeded under the guidance of his supervisors and the scientific consultant to master a number of modern methodologies, to work in a team and to maintain his research interests and contacts on issues, which he develops consistently and in collaboration with other researchers.

The abstract of the dissertation “Pharmacological studies of steroid hormones, natural products and newly synthesised 2H-substituted hydrasid hydrasones in experimental models of epilepsy, pain and osteoporosis” contains 62 pages and reflects the main content of the dissertation and the results obtained. The abstract is constructed correctly, according to the requirements specified in the regulatory documents of MU Varna. It is illustrated with 20 tables and 5 figures.

**Publications and conference participations of mag. pharmacist Marchev in scientific forums on the topic of the dissertation:**

Mag. Pharm. Stanislav Marchev presented a list of 5 publications. Two of the articles have been published in journals with an impact factor, in two of the scientific articles Marchev is the first

author. The doctoral student has 9 participations in scientific forums on the topic of the dissertation, two of the participations are in Bulgaria and 7 in international scientific forums abroad. These scientometric data confirm the relevance and significance of the problems discussed in the dissertation of the mag. pharm. Stanislav Marchev and their scientific value. Stanislav Marchev's knowledge of foreign languages and excellent computer skills help him to incorporate the latest trends in world science into his scientific work.

**Summary:**

My evaluation of the presented dissertation "Pharmacological studies of steroid hormones, natural products and newly synthesised 2H-substituted hydrazid hydrasones in experimental models of epilepsy, pain and osteoporosis" by Stanislav Marchev is strongly positive. The dissertation is relevant with scientific and applied significance. The doctoral student knows in dept the presented problem, he has mastered many modern methods, which he uses in the experimental part of the doctoral thesis.

I do evaluate as an advantage of the dissertation the interdisciplinary approach to the problem. Modern research methods have been used. The original idea, the topicality of the problem, the excellent knowledge of the issues, the ability to interpret and compare the results with data from experimental studies of other authors, the hypotheses, conclusions and contributions of theoretical and practical significance give me reason to evaluate the study positively, presented by the peer-reviewed dissertation, abstract, achieved results and contributions. I would propose to the esteemed members of the Scientific Jury to award the educational and scientific degree "Doctor" to Mag. pharm. Stanislav Marchev in the field of higher education 7.0 Health and sports, professional field 7.3 Pharmacology, Scientific specialty: Pharmacology and doctoral program in Pharmacology.

**Signed By**

Prof. Dr. I. Konstadinova, PhD