C R I T I C A L R E V I E W

Written By:

Prof. D-r Nikolai Danchev, PhD, Head of the Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Sofia. Member of a scientific jury, Order No P-109-248 / 15.07.2020` of the Rector of MU-Varna.

In reference to:
Dissertation thesis of ***Stanislav Jordanov Marchev,*** independent doctoral student in the Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Varna;
For award of a scientific and educational degree` **„**Doctor**“** in a field of higher education 7.Healthcare and sport, professional field 7.3 Pharmacology, following accredited doctoral programme in Pharmacology, including pharmacokinetics and chemotherapy.
Title: **Pharmacological studies of steroid hormones, natural products and newly synthetised 2H-substituted hydrasid hydrasones in experimental models of epilepsy, pain and osteoporosis**

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

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

I have not found missing parts in the documentation attached by Stanislav Martchev related on the procedure. I declare that I do not have studies and publications in co-authorship with the candidate.

Summary of the candidate’s CV

Stanislav Jordanov Marchev was born in 1989 in Plovdiv. He studied pharmacy in Berlin up until 2017. After his graduation he currently works in a company with three pharmacies, a hospital pharmacy and a warehouse fo medical devices.

Relevance and content of the dissertation

The main topic of the dissertation is dedicated to a current issue, namely experimental pharmacology study on steroid hormones like corticosterone, oestrogen, progesterone and others in kainic acid induced convulsive syndrome. The author also introduced an experimental model of oestrogen deficit osteoporosis after bilateral ovariectomy in rats. He studied the influence of *Aronia* extract on oestrogen deficient osteoporosis and also on the nociception of this widely studied plant from the Varna Pharmacological School.

The dissertation consists of 129 pages as follows: title 1 page, content 2 pages, list of abbreviations 2 pages, introduction 3 pages, review of the literature 46 pages, materials and methods 25 pages, results 20 pages, discussion 10 pages, conclusions 2 pages, contributions 1 pages, references 23 pages.

The **review of the literature** is dedicated on the influence of the suprarenal and sex steroids on the epileptogenesis including the third generation anti-epileptic drugs. The author underlined the risk of developing osteoporosis during treatment with current anti epileptic drugs, which logically motivated the tasks of the dissertation. This fact is explained by lowering of vitamin D levels.
The candidate thoroughly described the mechanism of the pain as the main pathophysiological phenomenon in the osteoporosis as well as the effect of opioid and non-opioid agents on the pain. The scientific literature on the pharmacodynamics and effects of *Aronia melanocarpa* is very well described. Unfortunately, there is no summary of the scientific studies to background the large-scale experimental works performed by the PhD-student. The reference list includes 170 titles, twenty seven of them published in the last five years, which confirms the relevance of the studied subject. There are some technical

inaccuracies and incompletenesses: in 61 references there are only authors cited, and in 70 lacks the year of publication.

There are three **contributions** in the author's abstract and in the thesis with scientific and applied character. The most significant and original are the discovered protective effect of *Aronia* for osteoporosis as well as the analgesic effect of the original studied hydrasid-hydrasone molecules.

The chapter **"Materials and methods"** is thoroughly described, as by my opinion, some of the protocols might be presented in slightly shorter manner or annexed. On the other hand, the methodological set covers in practice a wide range of methods for experimental study of anti-epileptic action and pain *in vivo*. Estrogen-dependent osteoporosis of the rat was induced by bilateral ovariectomy and bone density was densitometrically tested in various controls and treated animals. The selected set of experimental and statistical methods is adequate for the implementation of the tasks set out in the research program of the dissertation.

The **"Results"** chapter presents the most important part of the author's own research. The chapter is subdivided to smaller subsections each presenting the experimental studies on the main aims and tasks of the work. The methods are described in detail and allow for reproducibility. Especially advanced is the densitometric bone examination of rats, conducted with modern equipment, which provides a great opportunity to study the processes of osteoporosis. This approach allows for gradual penetration and understanding of the scientific novelties, achieved and discussed by the doctoral student.

The **"Discussion"** chapter is concisely written given the many areas of research. The author discusses adequately the experimental data obtained including in comparison and in reference with those already published in the literature. The results could be summarised as follows:

1. A scale for quantitative assessment of symptoms in an experimental model of epileptic seizure has been developed and validated, which allows an adequate study of somatic and cognitive manifestations in an experimental model of epilepsy.
2. It was found that the original hydrasid hydrasone molecules showed a profile of anticonvulsants *in silico* and have moderate analgesic activity when using the classic models of hot plate, as well as in formalin-induced Randall-Selito edema of the paw of a rat.
3. Very interesting is the fact that the extract of *Aronia melanocarpa* at a dose of 10 mg per kg could significantly slow down osteoporosis and at the same time reduce the sensation of pain in postmenopausal osteoporosis. This is of great importance given the wide availability and safety of *Aronia melanocarpa* on the one hand and the epidemiological significance of osteoporosis on the other. I would suggest these very promising and interesting results to be published as soon as possible.

In general, the dissertation is written clearly, with proper handling of terminology, and the candidate has mastered a number of key pharmacology methods.
The diverse and in-depth experimental results combined with the ability to interpret them make the personal contribution of the candidate undoubted.

The presented **author's abstract** reflects his own research and discussions, summarizes them and logically leads to the conclusions and contributions, although there are some inconsistencies in the ranking and the last contribution could be the first.

The publications list, related to the dissertation includes five articles in full text, two of them, published in 2017 and 2019 respectively are in the journal Biotechnology and Biotechnology equipment, which has an impact factor of 1,277. Other publications are mostly reviews.
Nine participations in conferences have been announced, six of which are интернатионал congresses and conferences abroad.

There are three contributions оф scientific-theoretical and applied character. I do accept and agree with their wording, of note one of them is of a methodical nature.

Conclusion

In conclusion, the dissertation of Stanislav Yordanov Marchev exceeds the quantitative and qualitative criteria of the Regulations and rules of MU Varna and the Law for Development of the Academic Staff in Republic of Bulgaria for awarding the Educational and Scientific degree of Doctor of Accredited Doctoral Research Program in Pharmacology. All of the above supports and reasons my decision to vote positively on the award of educational and scientific degree Doctor to Master pharmacist Stanislav Yordanov Marchev.

Sofia, 29.08.2020

Signed by
Prof. Dr Nikolay Damianov Danchev, PhD