STATEMENT REVIEW

By: Ass. Prof. Dr Maria D. Zhelyazkova - Savova, PhD
Department of Pharmacology and Clinical pharmacology
Medical University Varna

In reference to: dissertation for award of an educational and scientific degree` **„Doctor“**
Professional field: 7.3 Pharmacology
Doctoral programme in scientific specialty: Pharmacology
Author: **Mag.pharm. Stanislav Yordanov Marchev**

Doctoral form: independent
Department: Pharmacology, Toxicology and pharmacotherapy, Faculty of Pharmacy, Medical University Varna

T**itle**: 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.*

The presentet statement review is done according order N Р-109-248/15.07.2020 of the Rector of the Medical University Varna.

1. Presentation of the procedure and the author

The presented set of documents on paper and electronic media includes:

- Application to the rector of MU-Varna for opening a dissertation defence procedure
- CV European format, signed by the PhD student
- Orders for enrolment in doctoral studies and for final right to defence
- Dissertation in electronic media
- Author’s summary in paper and in electronic media
- List of publications related to the dissertation
- Reprints of the scientific papers (five)
- Declaration of originality and authenticity of the attached documents

CV of the student

Stanislav Marchev was born in Plovdiv. He has graduated Freie Univeristat Berlin in 2017. After his graduation he currently works in healthcare sector in Berlin as a pharmacist in Apotheke Rosa-Luxemburg-Platz. Apart from the dissertation, he presented 3 more publications and two congress participations on various topics.

2. Relevance of the topic

Three problems are developed in the dissertation, united by the topic of epilepsy as serious widespread neurological disease. They include: 1. study of the role of steroids (sex and glucocorticoids) in the pathogenesis and development of the kainic acid induced epileptiformic seizures; 2. study of the potential analgesic activity of synthetic compounds previously shown anticonvulsive activity in experimental model of epilepsy; 3. study of osteoporosis activity of natural products based on their known antioxidant and anti-inflammatory properties. Regarding “epilepsy” it is reasonable to look for solutions and clarify known but in need of further study aspects such as prevalence among males, osteoporosis risk in patients on long term antiepileptic therapy, pain as common comorbidity. In this sense, the issues raised in the dissertation are relevant and of important research interest.

3. Knowledge on the topic

The topics covered in the dissertation are multifaceted. The in-depth literature review should provide a good orientation in the scientific knowledge in the field and allow the scientist to freely handle the data and use them to analyse his own results. In the proposed dissertation the review of the literature occupies a disproportionately large part of the total volume of the work. It follows structurally the main studied topics. The chapters “Osteoporosis” and “Pain” are are unnecessarily detailed, which distances the author from the specific direction he should follow in clarifying the issues under discussion. In these section there is a disturbingly unregulated borrowing from another author. Apart from moral side, this questions the author's independent work with literature, his ability to go into details, to analyse information and to use it adequately.

The aims of the dissertations follow the review of the literature. In brief they include the study of the role of steroids in epileptogenesis, of the newly synthesised compounds for analgesic activity and of natural products for antiosteoporotic action. Specific tasks for their achievement are formulated.

4. Study methods

One of the strengths of the dissertation is the methodology support. A variety of modern methods and experimental sets have been used, many of which are time-consuming and labor-intensive due to the long-term treatment of animals. Among used methods are surgical (gonadectomy, adrenalectomy, ovariectomy), use of established (three different tests for analgesic activity) or modified models (kainic acid induced epileptiformic syndrome), osteodensitometry of small animals. It is not clear if bone density and mineral content are studied *in vivo* as described in the chapter “Materials and methods” or *ex vivo* as reported in “Results”. A merit of the work is the validation of a new scale for quantitative assessment of the intensity of the somatic and cognitive manifestations of the experimental kainate induced epileptoform syndrome, but it is not specified what exactly are cognitive manifestations and how they are observed.
The experimental work is of large volume. Laboratory Wistar rats (70 male rats in antiepileptic tests and 94 female rats in osteoporotic models) and 96 male albino mouses in experiments on analgesic activity are used according ethic and human norms for animal experiments. All procedures and studies have been approved by Bioethics Committee of the scientific studies of The Sofia Medical University.

5. Main characteristics and evaluation of the dissertation

The dissertation consists of 129 pages, 11 figures and 24 tables. The structure is standard and includes: introduction (3 pages), review of the literature (46 pages), Aims and tasks (3 pages), materials and methods (18 pages), own results (18 pages), analyses and discussion (10 pages), conclusions, contributions, publications and congress participations (6 pages), list of references (20 pages). The reference list consists of 170 titles, of which four are in cyrillic, the left are in english; 26 titles are published in the last 5 years. f
The following original results could be pointed out:

- Analgesic activity of hydrazid hydrasone newly synthesised compounds with antiepileptic effect has been shown. The dual pharmacology activity hypothetically could be useful in epilepsy with concomitant pain. As far as the studied compounds has different antinociceptive activity during the two tests, it would be fine should the author discuss the results in clinical point of view, knowing the specific informative value of the tests.

There are some differences between the results shown in the thesis and those published in the paper. The cytokine studies in the brain tissue are also not shown in the dissertation, which by my opinion deprives the thesis of additional value.

- A protective effect of *Aronia melanocarpa* on the bone mineral density was revealed in long term treatment of oestrogen-deficient osteoporotic female rat model. The observed hyperalgesia in this model is of interest. The analgesic activity of the extract in adition to a beneficial effect on the bones might be of important clinical value. In the context of the common “epilepsy” line a question why an osteoporosis, induced by antiepileptic drugs, was not used rises, as far as the last might be more adequate to the whole concept of the thesis.

Other results have partially or entirely supportive character:

- In regard of the role of steroids in the epileptogenesis it was concluded that the hormonal imbalance was an important pathogenetic factor. The conclusion was based on the found pro-convulsive activity of androgens and oestrogens, neutral to protective effects of gestagens and protective role of corticosterone. The analyses of the observed effects was good. It wold be good to discuss how the doses were chosen as far as they might direct the effect. The reasons for choosing the kainate-induced epileptoform model under drastically non-physiological conditions of simultaneous removal of the gonads and adrenal glands are not completely clear. Were there any advantages of this modification in clarifying the sexual predisposition of epilepsy compared to intact animals? What was the working hypothesis. Was the model previously described in the literature or is original idea? Could the results therefore be compared with already published data? By my opinion separate experiments with gonadectomy and adrenalectomy might be more informative regarding the role of respective hormones in the seizures control.
- In the studies with *Apium* extract, the author explained the uncertain effect on BMD with shorter duration, despite it was not quite different in the cited referent work. Other reasons could be discusses as lack of effect of ovariectomy, different age of the rats during treatment, mode of application.

6. Publications and self contribution of the author

The author presented five full text publications, being the first author in two of them. Two of the papers are published in peer-reviewed journal with impact factor and reflect the dissertation results. The rest are reviews in the field of epilepsy, published in Bulgarian scientific journals.

Nine congress (Bulgarian and international) abstracts are presented, related to the dissertation. In two of them the candidate is first author; four of the abstracts are published in the supplemental editions of IF international journals.

7. Author's abstract

The authors abstract consists of 63 pages, includes figures and tables from the dissertation, as well as the discussion. The abstract meets the requirements. There is a bit of difficulty in reading due to lack of content.

Summary:

The dissertation submitted to me for an opinion contains practical and applied results of original and confirmatory nature and meets the requirements of the Law for Development of the Academic Staff in Republic of Bulgaria and the Regulations for the Development of the Academic Staff in The Medical University Varna.
The presented work shows that the doctoral student mag.pharm. S. Marchev has theoretical training and demonstrates professional qualities and skills for independent scientific research. The critical remarks made are well-intentioned and constructive in nature. I hope they would support the author in his preparation for the presentation of the work as I would recommend to include an appropriate comments and answers.
In conclusion I suppose that the dissertation titled "Pharmacological studies of steroid hormones, natural products and newly synthesised 2H-substituted hydrasid hydrasones in experimental models of epilepsy, pain and osteoporosis" meets the criteria for scientific work necessary for the award of educational and scientific degree "Doctor" to its author mag.pharm. Stanislav Marchev and I give my ***positive assessment.***

27.08.2020
Varna

Signed By
Ass. Prof. Dr. Maria Zhelyazkova-Savova, PhD