R E V I E W

By Prof. Georgi Tsvetanov Momekov, DSc

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External member of the Scientific Jury, in compliance to Order № P109-248/15.07.2020 of the Rector of MU-Varna

Regarding a dissertation titled "Pharmacological studies of steroid hormones, natural products and newly synthesized 2H-substituted hydrazide-hydrazones in experimental models of epilepsy, pain and osteoporosis" in fulfillment of the requirements for acquiring the educational and scientific degree "Doctor (PhD)"

Area of higher education "Healthcare and Sports", professional field 7.3. Pharmacy under the doctoral program "Pharmacology, incl. pharmacokinetics and chemotherapy"

By Stanislav Yordanov Marchev, MSc(Pharm)

PhD student in independent form of education at the Department of Pharmacology, Toxicology and Pharmacotherapy, "Prof. Dr. Paraskev Stoyanov“ Medical University of Varna.

Brief biographical data

Stanislav Marchev was born on September 4, 1989, in the city of Plovdiv. In 2017 he obtained his MSc degree in pharmacy from the Freie Universität Berlin, Germany. The PhD student has been enrolled in 2019 in an independent form of training in the doctoral program "Pharmacology, incl. Pharmacokinetics and Chemotherapy " at the Department of Pharmacology, Toxicology and Pharmacotherapy, "Prof. Dr. Paraskev Stoyanov” Medical University of Varna with scientific supervisors Prof. Dr. Stefka Valcheva Kuzmanova, DSc and Assoc. Prof. Pavlina Gateva, PhD, and scientific adviser BAS Corresponding Member Prof. Dr. Mila Vlaskovska, DSc. Evident from the submitted documents, the thesis of Mr. Stanislav Marchev has been found to be compliant with the requirements for launching of the defense procedure in execution of order № P-109-248 / 15.07.2020.

Structure and chapters of the dissertation

The dissertation of Mr. Stanislav Marchev, MSc(Pharm) is based on a research program focused on two largely autonomous fields: on the one hand, a study of the effects

of gonadal and adrenal steroid hormones, as well as a series of newly synthesized hydrazide-hydrazones on the intensity, dynamics and latency of kainate-evoked seizures and lethality in an experimental model of epileptogenesis in rats; the other field is focused on evaluating the effects of natural products from *Aronia melanocarpa* and *Apium nodiflorum* on osteodensitometric and algometric endpoints in an experimental model of estrogen-deficient "post-menopausal" osteoporosis

Length and chapters. The dissertation of Mr. Marchev, MSc(Pharm) is written on 129 pages, and is illustrated with 11 figures and 24 tables. The dissertation is structured in accordance with the standards adopted in our country. It contains the following main sections: Introduction - 3 pages; Literature review - 46 pages; Goals and objectives - 3 pages; Materials and methods - 15 pages; Own results - 19 pages; Summary and discussion of the results - 10 pages; Conclusions - 2 pages; Scientific contributions - 2 pages; Literature - 21 pages. The bibliography comprises 155 publications by foreign authors and 15 publications by Bulgarian authors. Lists of publications, participation in scientific conferences are also attached.

Literature review. The doctoral student examines in sufficient depth the theoretical foundations in the two autonomous fields - the influence of steroid hormones on epileptogenesis, a review of existing therapeutic strategies for pharmacotherapy of epilepsy, and the potential risk of osteoporosis and fractures with antiepileptic drugs. In this context, a newly reviewed newly synthesized hydrazide-hydrazones containing coumarin or 2H-chromene pharmacophore moiety were made with the formulation of a theoretical hypothesis for their study in the context of this paper. In the other scientific direction, an in-depth evaluation of the clinical significance, pathogenetic basis and existing approaches for hormonal and non-hormonal pharmacotherapy of osteoporosis has been made. The products of chokeberry and celery are briefly reviewed in terms of the secondary metabolites contained, ethnopharmacology and therapeutic use thereof. The review also contains a significant section on pain, nociceptive and neuropathic mechanisms, relevant afferent and efferent pathways, and the relevant strategies for pharmacological modulation.

Research methods. The armamentarium of research methods that have been acquainted and employed is impressive. A study was performed in an experimental model of kainate-induced epileptiform syndrome in rats and in addition to the approbation of the methodological setting, an original algorithm for cumulative quantitative assessment of cognitive and somatic symptoms was applied and validated. The analgesic effect of the newly synthesized chromene and coumarin hydrazide-hydrazones was studied using the formalin and hot-plate assays.

An experimental estrogen-deficient osteoporosis was employed, using a model of bilateral ovariectomy, and the pharmacological assessment was based on osteodensitometric measurement of bone mineral density and bone mineral content as

quantitative endpoints. Regarding the effects of chokeberry juice, a study of nociception in animals with experimental estrogen-deficient osteoporosis was performed using two alternative methods. The methodological section is described in an exemplary manner, as the presented experimental protocols are complete enough to allow the reproduction of the employed methods. The tested compounds and phytoproducts with the respective analytical and standardization parameters are described in detail as well.

Original results. The data, well-illustrated with 11 tables and 4 figures, are included in 2 sections and 4 subsections. In the first research field, the author presents the validation of a newly created quantitative scale for assessing the intensity of somatic and cognitive manifestations of experimental kainate-induced epileptiform syndrome. The pharmacological study showed that treatment with 17-β-estradiol worsened the clinical and pathophysiological characteristics of experimental kainic acid-induced epileptiform syndrome. Administration of the androgen 5-α-dihydrotestosterone is associated with potentiation of the pro-convulsive action. At the same time, the administration of glucocorticoids attenuates the manifestations of kainate-induced epileptiform syndrome, incl. of relevant life-threatening reactions. This gives the author reason to conclude that hormonal imbalance comprises a major pathogenetic factor for the development of epileptiform/convulsant activity.

The newly synthesized hydrazide-hydrazone hybrids containing a chromene or a coumarin pharmacophore fragment **4a, 4b, 4c, 8a** and **8b**, which have demonstrated an anticonvulsant profile in a preceding virtual *in silico* screening, showed moderate analgesic activity.

In the other research direction, the approbation and validation of the estrogen-deficient model of experimental "post-menopausal" osteoporosis is described with the corresponding pharmacological tests of the studied plant products. The presented data show that the long-term treatment with *Aronia melanocarpa* juice has a protective effect on bone mineral density. The performed nociceptive modulation assays show that chokeberry juice attenuates the pain sensation in the osteoporosis model. The use of *Apium nodiflorum* extract showed marginal activity in the experimental model of experimental post-menopausal osteoporosis.

In the A**nalysis and discussion,** the author explores his own results and interprets them in the light of the reviewed literature data**.**

The **nine conclusions** are a reasonable result of the conducted research. The formulated three contributions come as a logical interpretation of the research findings.

Critical remarks and questions

Although there are preliminary *in silico* data on the anticonvulsant properties and published antiepileptic effects in maximal electroshock and pentylenetetrazole models for the newly synthesized hydrazine-hydrazone series, the present work would have benefited from the inclusion of these compounds in the kainate-induced epilepsy studies. Moreover, the clinically used hydrazide isoniazide causes vitamin B6 depletion, neurotoxicity and seizures that are at least in part due to the formation of hydrazone adducts with the aldehyde function of pyridoxal phosphate, which justifies the consideration of the hydrazide functional group as a toxicophore. In this sense, the characterization of any hydrazide compound as a potential drug should include a study of direct and surrogate biomarkers of vitamin B6 status, which could of course be the subject of a separate research project.

As the tested agents are a series of original, newly synthesized compounds, it is appropriate to mention explicitly the team that synthesized, characterized and provided them for pharmacological evaluation.

There is a certain imbalance in some of the individual chapters of the literature review - e.g. the nociceptive pathways and approaches to pain pharmacotherapy, as well as the subsection for osteoporosis have been developed in great detail, whereas the existing antiepileptic drugs are presented quite concisely.

Some stylistic remarks can be made as well: the work is full of foreign words (*voltage-dependent* Na^+ *and/or* Ca^{2+} *channels; тридименсионален; деривати; трипликат*, etc.), in addition the names of hormones, endogenous substances, plant biologically active substances and IUPAC chemical names could easily be written in Cyrillic. These purely editorial remarks are minimal and in no way diminish the quality and contributions of the work.

Scientific production

In connection with the dissertation Mr. Marchev has published 3 full-text articles in Bulgarian and 2 in English. Both English-language publications are in an impact factor (Thomson-Reuters/Clarivate analytics) journal. In two of the publications the PhD student is the first author. There are also 9 participations in international congresses, two in Bulgaria, and seven abroad (in Poland, France, Czech Republic, Sweden, Japan).

Concise overview (autoreferate)

The autoreferate presented by the doctoral student is prepared in compliance to the requirements of the relevant legislation, has an adequate length and summarizes the data from the dissertation.

Funding

The fact that the research this dissertation is based on has received financial support from the NSF and the MSC is very impressive.

Conclusions:

The dissertation of Mr. Stanislav Yordanov Marchev, MSc (Pharm) is state-of-the-art, original and is based on an adequate methodological armamentarium. The work presented in its entirety, as well as the scientific publications make the personal contribution of the PhD student indisputable. The doctoral student shows excellent theoretical expertise on the scientific field and has been successful in mastering and applying an enviable number of research methods and was able to present his original results, to interpret them and to formulate the relevant conclusions and contributions. Taken together the aforementioned considerations give me reason to conclude a confident positive assessment and to recommend the esteemed members of the scientific jury to vote for awarding Mr. Stanislav Yordanov Marchev, MSc(Pharm), the educational and scientific degree "Doctor (PhD)".

Sofia, 01.09.2020 Prof. Georgi Momekov, DSc