STATEMENT REVIEW

By: Prof. Dr Petko Penkov Marinov, PhD
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In reference to: procedure for award of a scientific and educational degree` **„**Doctor**“ to Stanislav Yordanov Marchev**

By the order N Р-109-248/15.07.2020 of the Rector of the Medical University Varna on the basis of a decision of the Faculty Council of the Faculty of Pharmacy under protocol N2/02.07.2020 I was elected a member of the Scientific Jury. Under the Protocol N 1/24.07.2020 of the first meeting of the Jury I was appointed to prepare a statement review on the procedure of the award of a scientific and educational degree` „Doctor“ to Stanislav Yordanov Marchev

PhD title: Pharmacological studies of steroid hormones, natural products and newly synthetised 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 (incl. pharmacokinetics and chemotherapy)
Scientific supervisors: *Prof. Dr Stefka Valcheva - Kuzmanova, DSc.*
Ass. Prof. Polina Gateva, PhD
Doctoral form: independent
Department: Pharmacology, Toxicology and pharmacotherapy, Faculty of Pharmacy, Medical University Varna

The procedure is followed in compliance with Law for Development of the Academic Staff in Republic of Bulgaria, The regulation of its application and according to the Regulation of the Development of the Academic Staff in The Medical University “Prof. Dr Paraskev Stoyanov” Varna.

Summary of the candidate’s CV and professional qualification:

Stanislav Jordanov Marchev was born in 1989 in Plovdiv. He studied magister programme in pharmacy in Berlin up until 2017. After his graduation he currently works in a Berlin pharmaceutical company. For his relatively short professional career he has already participated in 11 international congresses and conferences as well as is among authors of eight full text publications.

Relevance of the thesis: Epilepsy, a disease known since ancient times is one of the most serious and common neurological diseases. It is characterised by the appearance of generalised and partial (tonic/clonic) seizures. Epileptic patients on long-term anticonvulsant treatment has increased risk of osteoporotic bone changes. These changes lead to significant decrease of bone strength, and pathological fractures with minimal trauma. The osteoporotic pain is another important clinical feature in these patients. Osteoporosis prevention especially in postmenopausal women often consists of oestrogen replacement treatment. The last could be related with serious adverse effects like increased risk of uterine and breast cancer, thromboembolic complications and menorrhagia.

Alternatively, a phytotherapy could be used in the osteoporosis prevention. Plant medicines do not cause the above mentioned complications of hormone replacement therapy. Different phytoproducts known for their culinary and medicinal properties may be suitable for phytochemical and pharmacological studies in experimental models of oestrogen-deficient osteoporosis.

Aronia Melanocarpa fruits are popular for their organoleptic properties. Recently, the interest in the plant has been significant due to the possible pharmaco-therapeutic application of *Aronia* phytoproducts. Studies on other medicinal plants should be discussed in the same way. There are experimental and clinical data of multiple health effects of *Apium Nodiflorum* products. Phytochemical analyses of celery parts of products reveal multiple chemicals acting as secondary metabolites. Chronic treatment with *Apium Nodiflorum* extract have beneficial effects on clinical and laboratory symptoms in experimental models of postmenopausal osteoporosis. The prarmacology studies on *Aronia Melanocarpa* and *Apium Nodiflorum,* steroid hormones and newly synthesised 2H-substituted hydrazid hydrasones in experimental models of epilepsy, pain and osteoporosis are important for assessing their therapeutic potential in women with postmenopausal osteoporosis.

Structure of the dissertation

The dissertation consists of 129 pages, 11 figures and 24 tables. The reference list consists of 170 titles, of which fifteen are from Bulgarian authors and 155 are from abroad. The dissertation has classic structure including: introduction 3 pages, review of the literature 45 pages, Aims and tasks 3 pages, materials and methods 15 pages, results 19 pages, analyses and discussion 10 pages, conclusions 2 pages, contributions 1 pages, list of references 23 pages, publications related to the thesis 1 page, participation in local and international scientific forums, related to the thesis 2 pages, one thanksgiving page.

I. Introduction

The author presents in short medical and social significance of epilepsy and osteoporosis and back up the opportunity of using phytoproducts and newly synthesised 2H-substituted hydrazid hydrasones in experimental models of the above mentioned diseases.

II. Review of the Literature

The author covers several main topics as follows:

1. Characteristics of the epilepsy as a disease, influence of suprarenal and sex steroids on the epileptogenesis, epileptic and anticonvulsant drugs currently used in medical practice, opportunities to influence the natural history of the disease with newly synthesised 2H-substituted hydrazid hydrasones. The increased risk of osteoporosis and bone fractures in case of antiepileptic medicines is also discussed.
2. Characteristics of the osteoporosis, epiedemiology, mechanism of the disease, clinical features, pharmacotherapy.
3. Characteristics of the pain and treatment opportunities
4. Characteristics of *Aronia Melanocarpa* and *Apium Nodiflorum* phytoproducts.

Based on the review of the literature, the author concluded:

1. Treatment of the epilepsy is symptomatic, currently without effective mechanism based therapy or effective prophylaxis. Steroid treatment of the epilepsy currently is understudied, which suggests additional studies on the effect of suprarenal and gonad steroids on the intensity, dynamics and latency of the kainic acid induced seizures and lethality in an experimental models of epileptogenesis in rats.
2. There is an increased interest in new coumarin derivatives with pronounced anticonvulsant effect. It is shown that the molecules with code 4b, 4c and 8b have real pharmacotherapeutic potential as anticonvulsants. There is however a need for further studies on their analgesic activity and on the mode of action.

3. Prevention of the osteoporosis could induce serious adverse effects. In tis context, phytoproducts from popular plants, known for their culinary and medicinal properties may be suitable for further phytochemical and pharmacological studies in experimental models of oestrogen-deficient osteoporosis. *Aronia Melanocarpa* and *Apium Nodiflorum* might be good examples of such plants.

III. Aim and tasks

The firs aim of the dissertation is to study the effect of the suprarenal and gonadal steroids on the intensity, dynamics and latency of the kainic acid induced seizures and lethality in an experimental models of epileptogenesis in rats as well as the effect of a series of newly synthesised hydrazid hydrasones compounds on the nociception.

The second aim is to study the effect of the phytoproducts from *Aronia Melanocarpa* and *Apium Nodiflorum* on bone mineral density (BMD) and the bone mineral content in animals with experimental oestrogen-deficient “postmenopausal” osteoporosis.

There are eleven tasks set, with experimental character, subdivided in two groups. The aims and tasks are specific and correctly formulated.

IV. Materials and methods

The laboratory 260 animals used in the experimental models (male rats, male albino mouses and female rats), are treated after approval by the Bioethics Committee of the scientific studies of The Sofia Medical Univeristy and the Bioethics Committee of the Institute of Neurobiology of the BAS. There are several main models:

1. Experimental model of kainic acid - induced epileptogenesis; methods for experimental pharmacological and cognitive studies
2. Pharmacological methods for study of the analgesic effect of the 2H-substituted hydrazid hydrasones.
3. Experimental model of oestrogen-deficient osteoporosis. Osteodensitometric and algometric studies.

The experimental, instrumental, pharmacological, cognitive and statistic methods used in the dissertation allow solving the tasks and achieving the aim.

V. Candidate’s own results

The removal of the suprarenal glands and gonads (bilateral orchiectomy) in male rats significantly worsens the clinical and pathological parameters of the kainic acid induced

experimental epileptoform syndrome. On this background, the administration of oestrogens or androgens has a pronounced unfavourable effect on the main studied parameters. Among the original newly synthesised 2H-substituted hydrazid hydrasones there are compounds with pronounced antinociceptic effect. Using the “hot plate” test the author reveals an antinociceptic effect in three of the newly synthesised 2H-substituted hydrazid hydrasones - 4c, 8a and 8b with analgesic power decrease as follows: 8b>8a>4c. The ‘formalin’ tests showed moderate analgesic effect of the compounds 4a and 4c, verified as 4amore pronounced than 4c (4a > 4c). The newly synthesised 2H-substituted hydrazid hydrasones have therapeutic potential and could be of interest in the development of new anti-epileptic drugs.

It is shown that experimental oestrogen-deficient osteoporosis in rats could be used as a reliable model of post menopausal osteoporosis in women. Long term treatment with *Aronia* extract in dose 10 ml/kg might delay the development of osteoporotic bone changes. The obtained data for changes in pain sensitivity are in line with with the results on studies on bone density. The nociceptive thresholds are significantly decreased in osteoporosis in hot plate model. Treatment with *Aronia* extract in dose 10 ml/kg might have beneficial effect on pain relief in overt osteoporosis. The proposed regimen of pharmacology treatment with *Aronia* extract in dose 10 ml/kg and 5 ml/kg respectively does not cause significant changes in bone mineral content. There is a trend to decrease of the BMC of the femur, which might be delayed after *Aronia* 10 ml/kg. Pharmacotherapy with *Apium* as antiosteoporotic agent has an uncertain effect, probably attributed to relatively short course of treatment.

VI. Analyses and discussion

The analysis of the results shows that corticosterone has more pronounced antiepileptic effect as compared with progesterone. Testosterone exhibits significant pro-convulsive activity. This requires careful dosing regimen in subjects receiving anabolic steroids. More, the hormonal imbalance might influence the manifestations of epileptoform convulsive activity in epileptic patients. The developed quantitative cumulative allows detailed and adequate analysis of the somatic and cognitive symptoms in an experimental kainic acid induced epileptogenesis. Some of the newly synthesised 2H-substituted hydrazid hydrasones - 4a, 4c, 8a, 8b, might decrease the nociception. This feature could be used further as an additional useful feature in development of new anticonvulsants. The results showed that treatment with *Aronia* extract in dose 10 ml/kg delays osteoporosis combined with less bone destruction. It was verified with significantly

improved BMD and BMC after treatment with *Aronia* 10 ml/kg. The results with *Aronia* 5 ml/kg are not that convincing. Short-duration pharmacotherapy with *Apium* extract for prevention of osteoporotic changes had weak and uncertain effect.

VII. Conclusions

The author's nine conclusions from the experimental studies are logical reasoning of the dissertation.

VIII. Contributions

Based on the dissertation, Stanislav Marchev brings out and formulates three contributions which I completely approve. It is revealed that newly synthesised 2H-substituted hydrazid hydrasone compounds 4a, 4b, 4c, 8a, 8b, previously shown *in silico* anticonvulsant profile, have moderate analgesic activity. It was proven that long term treatment with *Aronia melanocarpa* 10 ml/kg could significantly delay the osteoporotic changes as well as decreases the pain sensitivity in experimental post-menopausal osteoporosis. The newly developed quantitative scale was validated to qualify the clinical symptoms in experimental epileptoform syndrome. The quantitative cumulative scale allows detailed in-dept analysis of the somatic/convulsive and cognitive manifestations of experimental epilepsy. The above described contributions enrich pharmacology science in the country and have scientific and applied character.

Publication activity

The author presents five publications related to the thesis, being a leading author in two of them and second in other two. This prove the own contribution of the PhD student in development of the thesis. Two of the papers were published in "Biotechnology and Biotechnological Equipment" journal, with IF of 1.277 and 1.327 respectively, an evidence for high scientific standards. The results were shown in nine scientific forums, six of them international, four of them with published abstracts abroad in IF journals (3.83, 3.83, 3.83, 2.997 respectively).

I would like to note that Stanislav's scientific works have significant scientific merits and are original contribution.

The author's abstract is prepared according the requirements and fully reflects the aim, tasks, and results in the dissertation.

Summary:

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” is up-to-date and covers problems of common diseases in clinical practice. The thesis fully meets the requirements of the Law for Development of the Academic Staff in Republic of Bulgaria, The regulation of its application and according to the Regulation of the Development of the Academic Staff in The Medical University “Prof. Dr Paraskev Stoyanov” Varna and criteria for educational and scientific degree Doctor. I therefore give my positive assessment and strongly recommend the esteemed members of the Scientific Jury to vote positively for the defence of the dissertation of Stanislav Yardanov Marchev and to award him with Educational and Scientific degree Doctor in Field of Higher Education: 7. Healthcare and sport, Professional field: 7.3 Pharmacology, Scientific specialty: Pharmacology (incl. pharmacokinetics and chemotherapy).

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18.08.2020

Varna

Signed By

Prof. Dr. Petko Penkow Marinov, PhD