BULGARIAN ACADEMY OF SCIENCES

INSTITUTE OF ORGANIC CHEMISTRY WITH CENTRE OF PHYTOCHEMISTRY

R E V I E W

of

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Institute of Organic Chemistry with Centre of Phytochemistry - BAS

Competition: *to occupy the academic position of "Professor" in area 7. "Health and Sport", Professional Field 7.1. "Medicine" for the scientific specialty "Pharmacology" for the needs of the Department "Behavioral Neurobiology" at the Institute of Neurobiology at the Bulgarian Academy of Sciences and decision of the National Council of the Institute of Neurobiology, Protocol N 4 / 28.03.2019, published in State Gazette, No. 10 / 01.02.2019.*

By Decision of the Scientific Jury Protocol No. 1 / 24.04.2019. and Order of the Director of Institute of Neurobiology-BAS No 274 / 24.04.2019 I have been elected as a member of the scientific jury and for preparing a review.

1. Personal and professional details of the applicant

Assoc. Prof. Lyubka Pavlova Tancheva, PhD from the Institute of Neurobiology - Bulgarian Academy of Sciences, Sofia is the only candidate in this competition. She was born on 26.05.1952 and graduated in 1975 from the Faculty of Pharmacy, Medical Academy, Sofia with a very good score. In 1982, she received the educational and scientific degree "PhD" after successfully defended dissertation on "Influence of hydrocortisone and deoxycorticosterone on the activity of some drug-metabolizing enzyme systems". Since 1987 she has worked at the Institute of Neurobiology, Bulgarian Academy of Sciences as a research associate (Assistant, Assistant Professor) and Associate Professor since 2007. During the period of her

academic development Assoc. Prof. Lyubka Tancheva has continuously increased her qualification. She specialized in the Institute of Pharmacology of the Slovak Academy of Sciences, Bratislava, Slovakia, 1988 (1 month); Friedrich Schiller University, Jena, Germany, 1989 (1 month); Faraday Institute of Cambridge University, Cambridge, United Kingdom, 2009 (1 month) and has worked as a visiting professor at Weizman Institute for Science, Rehoboth, Israel in 2015 - 2016.

Assoc. Prof. Lyubka Tancheva has extensive experience in project development, management and realization. Bulgaria's coordinator has been in 3 contracts and has participated in 1 contract under the EBR for bilateral co-operation with Israel (for the periods 2004 - 2010, 2018 - 2020). Coordinator from Institute of Neurobiology-BAS she was in 2 projects with the Scientific Research Fund - DN 03/13/2016 and DN 03/08/2016 and Work Package 4, KD 01-217 / 30.11.2018, Ministry of education, 2018 - 2021; participant from Institute of Neurobiology -BAS in contract FFNNIPO_12_01230 2014 – 2017. She has also participated in 3 projects with Medicinal University – Sofia, Medicinal University - Varna and Sofia University "St. Kliment Ohridski "in 9 projects with University of Chemical Technology and Metalurgy - Sofia and Southwest University" Neofit Rilski "- Blagoevgrad.

She is a member of the Union of Scientists in Bulgaria, the Bulgarian Toxicology Society, the European Society of Toxicology, the International Toxicology Society, the Bulgarian Peptide Society and the World Christian Doctors Network.

2. A general description of the scientific metrics in the presented materials

Assoc. Prof. Lyubka Tancheva presents a total of 90 publications with a general impact factor of 55.3, cited 130 times and h-index 5 (Web of Science).
In the "Professor" competition she participates with 36 publications, in 9 of which he is the first author. The 25 articles submitted for the competition are published in Impact Journals by Web of Science-Thomson Reuters with an aggregate
IF = 21.1, which do not repeat the "Associate Professor" publications.

Research results have been published in reputable international journals with a high impact factor such as: BBA - Molecular Basis of Disease, (IF = 5.725); Molecules (IF = 3.098); Journal of Molecular Neuroscience, (IF = 2.454); Phytotherapy Research (IF = 2.397); Termochimica Acta, (IF = 2.236); J Therm Anal Calorim,(IF = 1.781).

A total of 120 participations are presented in international and national scientific forums.

The summarized table of Assoc. Prof. Lyubka Tancheva's all scientific data shows that it meets the criteria for group A (PhD thesis - 50 pts.), group C (10 referenced articles - 100 pts.) and repeatedly exceeds the criteria for Group D (Publications in the "Professor" competition - 354 points at the required 220pts.), Group E (Quotations – 540pts. at the required 120pts.) and Group F (Participation / management of scientific projects and defense of PhD students – 605pts (at the required 150pts.).

From the submitted documents and references it is evident that in the announced contest Assoc. Prof. Lyubka Tancheva participated with a scientific asset, which fully meets the requirements for occupying the academic position "professor" according to SRARPB and the Rules of Institute of neurobiology-BAS.

3. Assessment of the applicant's teaching activity

I highly praise the teaching activities of Assoc. Prof. Lyubka Tancheva. Impressive is her desire to pass on her experience to younger colleagues. She has 5 PhD students all under Pharmacology at Institute of Neurobiology - BAS, one of which has been successfully defended in 2015. She has trained four masters at Sofia University "St. Kliment Ohridski", who defended their diploma theses, as well as four students from Medicinal University - Sofia and Sofia University "St. Kl. Ohridski". She also participated in the Mentor Program in 2016, where 3 students from Medicinal University - Sofia were trained. Assoc. Prof. Lyubka Tancheva has held lectures and exercises on "Pharmaceutical Toxicology" for Pharmacology students, lectures on postgraduate qualification of Master-Pharmacists on "Side and Toxic Effects of Drugs" at the Pharmaceutical Faculty of MA - Sofia in the period 11.05.1976. -

01.04.1987 and in the Faculty of Mathematics and Physics of the South-West University "Neofit Rilski", Blagoevgrad 2018 – 2019.

Assoc. Prof. L. Tancheva published books, which are used by students of the Medical University - Sofia, Sofia University, "Kl. Ohridski "and Southwest University" Neofit Rilski "- Blagoevgrad.

4. Valuation of the applicant's research activity

The research work of Assoc. Prof. Lyubka Tancheva makes an impression that it was conducted on a scientific topic in an extremely topical field, which is why I consider it important for the medical science and practice.

In recent years, special attention has been paid to socially significant and difficult-to-treat diseases caused by free-radical processes in the body and the application of antioxidant therapy, which involves strengthening the antioxidant protection of the body against the harmful effects of free radicals.

In the same aspect, the role of oxidative stress is also discussed.

The results obtained by Prof. Lyubka Tancheva can be used to enrich the knowledge of oxygen and drug metabolism in biological environments. In a scientifically applied aspect, they can facilitate the development of effective preventive regimes against the initiation and development of diseases related to the harmful effects of free radicals.

The scientific and scientific contributions from the Assoc. Prof. L. Tancheva can be summarized in the following main directions:

1. Directed synthesis and drug design for selecting biologically active substances - in vitro and in vivo (articles: 2, 3, 5, 6, 7, 8, 9, 10, 12, 13, 14, 25);

2. Neurobiological and psychopharmacological studies of newly synthesized analogues on experimental models of socially significant diseases (articles: 6, 7, 9, 14, 21);

3. Experimental approaches to protection and therapy of neurodegenerative diseases with newly synthesized substances (articles 11, 14, 15, 18, 21);

4. Multitarget strategies in treatment of neurogenic diseases with substances of

natural origin (articles: 1, 4, 16, 17, 19, 20, 22, 23);

5. New mechanisms of neurodegenerative processes and social exclusion (articles: 6, 7, 9, 11, 15, 16, 17, 18, 19, 21, 23).

It is noteworthy the largest number of papers (12) of the first strand, in which there is presented a directed synthesis and drug design of L-valine peptidomimetics (P-6 and M-6), cananavan and neurotensin analogs, as well as novel derivatives of galanthamine and amantadine. Significant better effects of the newly synthesized P-6 and M-6 were obtained on memory function in rodents, and related levels of neurotransmitters serotonin and acetylcholine in the brain were discussed.

Original data showing significant analgesic effects and memory and learning of cannaban and neurotensin analogues on experimental rodents are presented, as well as their ability to better pass the cell-brain barrier relative to the reference neurotransmitter. With molecular dockin application, new results are obtained for binding to three types of neurotensin receptors. For the first time, toxic and cognitive effects of new derivatives of galantamine and amantadine have been identified in rodent tests. Changes in neuromuscular coordination and metabolic behavior of model substrates have been identified.

The authors have been able to detect the diffuse effects of amantadine and galanthamine on their antioxidant and anticholinergic-non-steroid activity in vitro and in vivo in the brain. The new data revealed that galanthamine derivatives improve memory in rats, which is associated with a significant inhibitory effect on brain anticholinesterase activity, whereas amantadine derivatives exhibit moderate antioxidant activity.

I highly praise the research and the results obtained in Strand 2, where new valine and neurotensin derivatives have been studied through experimental models of socially significant diseases (aggression, social isolation and autism). On a model of aggression induced by social isolation, it has been shown for the first time that the two newly synthesized L-valine peptidomimetics M-6 and P-6 modulate the altered memory functions in aggressive rodents, their effects depending on gender

and social isolation of the animals.

It was shown for the first time that M-6 increases cerebral serotonin levels and P-6 has the opposite effect. It was demonstrated for the first time that the neuromodulatory effects of P-6 correlate with its effects on the memory of aggressive rats. Differences in the psychopharmacological and neuromodulatory effects of M-6 and P-6 have been identified and these effects are more pronounced on animals with a model of aggressive behavior.

Together with colleagues at the Institute of Neurobiology in Arizona at the Weizmann Institute of Science in Israel, the presence of significant effects of a newly synthesized HT4 analogue on the social activity of two mice (C57BL / 6 and BTBRT) was first established through tests of social behavior and social innovation. In heading 3 new protective effects of two newly synthesized HT2 and HT4 analogues on impaired motor coordination and memory in an experimental model of Parkinson's disease in rats are presented.

Their stronger neuroprotective and neuromodulatory effect of the reference neurotransmitter as well as increased dopamine content in the brain of rats with an experimental Parkinson's disease model were observed. The effect of HT4 is stronger than that of HT2.

The novel amantadine analogue (Amantir) has been shown to exhibit a neuroprotective and therapeutic effect with improved neurobiological and biochemical effects on a toxin-induced Parkinson's disease model in rats. It has been discussed that the effects of the new molecule are commensurate with those of amantadine but at significantly lower concentrations (about 2.5 times lower than the reference), which also exhibits lower amantadine toxicity.

The anti-parkinsonian effect of the new molecule is associated with the authors in part with its antioxidant properties. I highly praise these studies and the results obtained. It should be noted that the results for the new amantadine analogue have been summarized and a joint patent proposal was submitted by the Institute of Neurobiology - BAS and the Southwest University in Blagoevgrad.

Strand 4 presents interesting new results for the treatment of neurodegenerative

diseases with substances of natural origin.

New data have been obtained about the protective effects of the monoterpene Mirtenal on the memory functions of rodents with an Alzheimer type dementia model and its ability to slow the progression of the disease. Mirtenal's antioxidant and anticholinesterase activities are comparable to those used in the reference lipoic acid and galantamine, however, its mechanism of action being complex.

For the first time, an improvement in ellagic acid memory on experimental dementia, as well as its inhibitory effect on acetylcholinesterase activity in brain demented rodents, has been identified. It has been shown that the protective effect of ellagic acid is enhanced under conditions of scopolamine-induced oxidative stress in the brain.

Antioxidant mechanisms have also been identified in neuroprotective action of lipoic acid in rats in experimental dementia induced by scopolamine. Lipoic acid reduces oxidative lesions, restores glutathione levels and activity of catalase and superoxide dismutase in three brain structures linked to memory (rectal cortex, hippocampus and striatum).

I highly praise the comparison for the first time of the role of 3 natural bio-antioxidants with a different chemical structure on an experimental model of 6-OHDA-induced Parkinson's disease in rats. It has been demonstrated that elagic acid, lipoic acid and myrtenal significantly reduce oxidative disorders, with myrtenal showing the strongest protective effect. Regarding the increased levels of dopamine in the brain from the three bio-antioxidants the strongest is the effect of myrtenal. All three bio-antioxidants have been found to have a more potent memory-enhancing effect and motor coordination in Parkinson's disease rats than healthy animals.

I very much appreciate the research done in heading 5 on the mechanisms of neurodegenerative processes and social exclusion. For the first time, the dynamics of brain plasticity ability was recovered spontaneously over time, with early and late changes in the memory function of animals induced by scopolamine.

New relationships between the studied changes in oxidative status,

anticholinesterase activity and neurotransmitter levels in brain structures associated with memory, spatial orientation and motor coordination in neurodegenerative processes have been identified.

A change of the proteomic map of a hippocampus in healthy and dementia animals has been established jointly with colleagues from Institute of organic chemistry with Centre of Phytochemistry - BAS (Professor Pavlina Dolashka) as the correlation between behavioral, biochemical and proteomic parameters allows new conclusions to be drawn regarding the restoration of brain plasticity, the reversibility of disabilities and the mechanisms of the neurodegenerative process.

It is extremely important to study aggression and early social exclusion. A contributing factor is the evidence that social exclusion in combination with maternal deprivation in adolescent rats of both sexes leads to worsening of behavioral parameters.

It has been demonstrated for the first time that the modulation of memory functions in aggressive animals is gender-dependent and socially depopulated, demonstrating the complex interaction between biological, chemical and social factors.

In the materials of the competition is presented monographic work on the topic: "Drug metabolism and oxidative stress in influenza viral infection. Experimental Approaches and Antioxidant Protection ", publishing house of the Bulgarian Academy of Sciences, Sofia, 2019, 112 pp.

The monographic work examines the contemporary state of the problem and the following points can be made:

- Drug metabolism and free-radical processes
- Biological role and importance of drug metabolism
- Changes in drug metabolism in oxidative stress
- Influenza virus infection and free-radical processes
- Changes in drug metabolism in influenza viral infection

After an in-depth analysis of the state of the problem, the author draws attention to

the fact that influenza infection such as acute respiratory disease is important for human pathology, and more recent studies attribute it to the "Free radical" diseases. The unwavering interest of researchers in this mass disease is explained by the still unclear theoretical aspects / mechanisms of action and the relationship between the processes and their purely practical meaning for the treatment of this disease.

Assoc. Prof. Tancheva is right to study the effect of endogenous antioxidants (natural and synthetic) as they are known to be effective in overcoming oxidative stress. The author's studies show significant preventive effect of vitamin E, butylated hydroxy-toluene (BHT) and flavonoids during experimental influenza infection. And while their mechanism of action is associated with their antioxidant activity, the author rightly concludes that this does not explain all aspects of their diverse biological activity.

The conclusion of the author is that the metabolic drug metabolism changed by influenza infection is one of the major causes of increased drug toxicity in an infected organism, both in clinical and experimental conditions.

The author has two main objectives:

A. To predict, avoid and correct influenza-altered metabolism, resp. pharmaco-logical and toxic effects of drugs commonly used in influenza infection;

B. To investigate the effectiveness of certain natural antioxidants (vitamins, flavonoids and polyphenol products) and their combinations in influenza infection as well as possible mechanisms of antioxidant protection in oxidative stress.

It was found that during the course of influenza infection the concentration of both primary and secondary products of peroxidic lipid oxidation (POL) increased. The strongest is the pro-oxidant effect in the liver, but the fastest and most dynamic effect is in the lung. The author also found changes in antioxidant activity (AOA) during the course of influenza infection. A well-defined mathematical correlation was found between the virological parameters and the POL and AOA products between the liver and lung processes.

The author concludes that the reported potent oxidative stress associated with influenza infection is clearly a basic and key mechanism of damaging effects on the structure and function of liver cells, including their drug-metabolizing capacity.

Although the damage caused by membranes and membrane membrane-bound enzyme-mediated membranes and related enzymes is only one (not the only) cause, it is considered to be an important underlying mechanism responsible for the suppressed metabolism of the drug observed under influenza infection time.

I highly praise the work of the vitamin E and vitamin C individually and in combinations. A strong vitamin E restorative effect has been identified, which is strongly expressed in influenza-infected animals and is lacking in healthy animals. The author correctly concludes that their lower viral titre in the lungs of the vitreas treated by them animals can not be explained by its antioxidant properties. It is hypothesized that it is also possible to act to a large extent as an immunomodulator, reducing the production of anti-inflammatory cytokines and lipid mediators.

A stronger protective effect of lipophilic vitamin E has been observed compared to that of the hydrophilic vit. C, which is in agreement with other studies.

The combination of vit E and vit. C has shown a stronger protective effect on POL and drug metabolism compared to their self-administration. The observed effects are due to the highly proven synergy between the two antioxidants.

The effects of flavonoids-quercetin and rutin have also been investigated.

C-reductase is inhibited nearly 3 times by quercetin and 4 times by routine. In any case, quercetin is less potent than routine. The difference in the effects of the studied flavonoids in healthy and diseased animals according to the author should be sought in the modified pharmacokinetics and pharmacodynamics of the mono-oxygenase substrates tested in the influenza virus infected body.

A polyphenol complex isolated from Geranium sanguineum L (PFC) was also applied. A pronounced protective effect on infected animals in both the white and the liver was established. The author concludes that the protective effect may be due to the combination of more than one biological activity of the preparation - selective and specific antiviral activity, non-specific viral activating activity, non-selective immunomodulatory action, and certain pharmacological and biological properties known to natural polyphenols such as protein- binding and antioxidant activity.

Regardless of the complex and incomplete clarification mechanisms under which the PFC exerts its convincing protective action against influenza virus infection, the

author considers that PFC may be useful in the prophylaxis and treatment of influenza viral infection.

Comments and recommendations:

I would like to make some remarks and recommendations on the presented text in monographic work. Maybe they come from translating Bulgarian into English.
On page 29 - "... vitamin E / a-, b-, g- and d-tocopherol /" which is not correct - should be "α-, β-, γ-, δ- tocopherol ".
On page 30 - for singlet oxygen and hydroperoxide "102, H2O2" - should be "$^1O_2$, H_2O_2".
On page 36, "Because of its polyphenolic nature, flavonoids often exhibit strong antioxidant properties similar to the natural antioxidant - tocopherol they are structurally similar and can replace it with its antioxidant action in some model systems." Probably the author is referring alpha-tocopherol. Flavonoids are not structurally similar to alpha-tocopherol. The only resemblance is that they are phenolic antioxidants, but alpha-tocopherol has only one phenolic nucleus, a chroman ring and a long sidechain of 16 carbon atoms, while flavonoids have three nuclei, several phenolic groups and have no side-chain length. The mechanism of action is also different, so this judgment is not right in my view. Flavonoids can replace tocopherol but are not structurally similar.
On page 39, "Some researchers are using the synthetic analogue of alpha-tocopherol butylated hydroxytoluene (BHT) in experimental influenza infections." Butylhydroxytoluene is a synthetic antioxidant but is not an analogue of alpha-tocopherol. The synthetic alpha-tocopherol analogue is Chroman C1, synthesized by colleagues from the Russian Academy of Sciences, where the long alpha-tocopherol side chain is replaced by a methyl group.
On page 58 is written "... the accumulation of free radical intermedians ..." In my opinion, it must be: "the accumulation of intermediates from free-radical processes".
On page 68 - "... the ability of a vitamin C) located in the aqueous phase (recycle vitamin E) located in the membranes ... ". It is more correct to say that it is

"regenerated" rather than "recycled" because it is meant to recover the tocopherol molecule.

I recommend that Assoc. Prof. Tancheva uses these terms in the training of students and PhD students.

Conclusion:

Having in mind everything that has been said so far, my overall impression of the papers presented in the competition as well as my personal impressions, I am convinced that Assoc. Prof. Lyubka Tancheva meets the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the specific requirements of the Institute of Neurobiology - BAS for occupying the academic position " Professor ". She is also an authoritative scientist with a clearly expressed own scientific appearance, with sufficient scientific, applied and teaching activities, and the results of her research are significant for the medical science and practice. On the basis of all this, I strongly recommend to the honorable members of the scientific jury to vote positively for the award of the academic title "Professor" in the scientific specialty "Pharmacology" of Assoc. Prof. Lyubka Pavlova Tancheva at Institute of Neurobiology-BAS.

27.05.2019 Reviewer: 

(Prof. Vessela Kancheva, PhD)