

REVIEW

of dissertation for the EDUCATIONAL AND SCIENTIFIC DEGREE "Ph.D."

On the topic:

"ISOLATION AND ANALYSIS OF METHYLXANTHINE FRACTION, CATECHIN FRACTION AND WHOLE EXTRACT, ISOLATED FROM GREEN TEA BANCHA"

Field of higher education: 7. Health and sports

Professional field: 7.3. Pharmacy

PhD program: Pharmacology (incl. Pharmacokinetics and chemotherapy)

Author: Assistant Professor of Pharmacy **Maya Petrova Radeva-Ilieva** - full-time Ph.D. student

Department of Pharmacology, Toxicology and Pharmacotherapy,

Faculty of Pharmacy, Medical University "Prof. Dr. Paraskev Stoyanov" - Varna

Scientific adviser: Assoc. Prof. Kaloyan Dobrinov Georgiev, D.Sc.

Reviewer: Assoc. Prof. **Rumyana Lyubomirova Simeonova**, Ph.D.,

Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University of Sofia,

The review was prepared on the basis of order № P-109-92 / 24.02.2022 of the Rector of MU-Varna.

All documents required by the Regulations on the terms and conditions for obtaining scientific degrees and holding academic positions at the Medical University - Varna, were provided to me in electronic and paper form.

I declare, that I have no conflict of interest and common scientific papers with the candidate Maya Petrova Radeva-Ilieva

Biographical data and career development

In 2015, **Maya Radeva-Ilieva** graduated as a Master of Pharmacy with full honors and the Golden Galen Award from the Faculty of Pharmacy, Medical University "Prof. Dr. Paraskev Stoyanov" - Varna. She received an award as a student in 2014 from the Bulgarian Pharmaceutical Union. After

graduating, she worked as a stock manager and deputy head of a Pharmacy in Varna, and since 2016 she has been elected an assistant in the Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy, MU-Varna, where she still works today.

Fluent in written and spoken English.

Maya Ilieva participates in two research projects funded by MU-Varna and in three scientific forums related to the dissertation.

She is a specialist in "Pharmacology and Pharmacotherapy" under the POSTGRADUATE TRAINING.

She is a member of the Bulgarian Pharmaceutical Union.

EVALUATION OF THE DISSERTATION WORK

Relevance of the problem

The present work addresses current issues in medicine and clinical practice related to possible risks of clinically significant drug interactions. The potential risks of combining phytoproducts and plant extracts with conventional medicines are a growing problem. Adverse and little-known interactions are possible, which may occur with the daily use of some plant extracts and infusions, especially in the elderly, where the risk of side effects and interactions is higher due to physiological changes, comorbidities and the inevitable polypragmasia. The clinical significance of these interactions is greatly underestimated, moreover, when taking a medical history, patients often do not mention the chronic use of medicinal plants or teas that they take in their daily lives, as they consider them safe.

Structure of the dissertation

The dissertation is written on 139 pages, of which 112 pages of dissertation text and 27 pages of bibliography from 392 literature sources, of which 7 in Cyrillic and 385 in Latin.

The structure of the dissertation includes: Introduction - 3 pages, Literary review - 38 pages; Purpose and tasks - 2 pages; Materials and methods - 17 pages; Results - 17 pages; Discussion - 22 pages; Conclusions - 2 pages; Contributions - 1 p.

The literature review is comprehensive and purposeful, with deep knowledge of the phytochemical composition of green tea, its pharmacological and potentially toxic effects. From the thorough literature review on the mechanisms of drug interactions and methods for their prediction and study, manner of presentation, competent and critical analysis of literature data, shows the erudition and complex thinking of Assistant Ilieva. The main aspects of PD/ PK drug-drug, drug-plant interactions are very clearly and precisely summarized. Relevant literature data on the

interactions of green tea extracts and sildenafil, objects of the dissertation, which facilitates the reader to move to the resulting goal and objectives, are also thoroughly and analytically examined.

The aim of the dissertation reflects the author's intentions: "To evaluate the influence of methylxanthine fraction, catechin fraction and total extract of Japanese green tea Bancha on the pharmacokinetics of sildenafil" To fulfill this detailed goal four main tasks are specified:

1. Obtaining plant extracts of Japanese green tea Bancha, qualitative and quantitative analysis of the extracts obtained.
2. Qualitative and quantitative determination of sildenafil in the composition of plasma samples from experimental animals.
3. Experimental animal studies to evaluate the effect of the extracts obtained on the pharmacokinetics of sildenafil.
4. Development of a physiologically based pharmacokinetic (PBPK) model for predicting potential interactions between sildenafil and methylxanthine fraction, catechin fraction or whole green tea extract in humans.

I believe that the set goal and tasks are clearly formulated and allow for a comprehensive study and extraction of logical conclusions.

Materials and methods

The methods used are adequate to evaluate the planned study and are well described. In my opinion, the chromatographic conditions, the methods for sample preparation and sampling, the preparation of the working solutions, etc. are described in too much detail. In connection with the presented dissertation, reliable HPLC-UV methods for the analysis of biologically active substances contained in the various extracts of the studied green tea have been developed and validated. Given that the "PhD" is an educational and scientific degree, the methods used in the study show that the PhD student has mastered an extremely wide range of analytical, pharmacological, *in vitro*, *in vivo* and *in silico* methods. The confidence and detail with which the methods are described show the exceptionally good theoretical training and the rich literary analysis with which the candidate approached in developing the dissertation.

Results and discussion

The results obtained are described concisely, clearly and presented in 14 tables and 27 figures. I admire the readiness and patience of Assistant Ilieva to work with experimental animals, which requires great dedication. It is pleasant to note that all chromatographic methods of analysis have been validated and skillfully interpreted. The ability and ease with which the PhD student analyzes the established or calculated pharmacokinetic parameters gives me reason to believe that she is an established researcher with an analytical approach to the results obtained.

The discussion of the obtained results is done with an understanding of the matter, very analytically with logically derived conclusions and contributions to the dissertation.

The significant part of the results is original, the scientific contributions have both significant theoretical and real applied value and the conclusions sound convincing. In my opinion, the most significant contributions are clearly defined and have a real impact and priority theoretical and applied value.

Conclusions and scientific contributions

Conclusions and contributions are very well formulated based on calculated and well analyzed pharmacokinetic parameters and other numerical data. The conclusions reached by the PhD student are a logical consequence of the competent data analysis. The most significant conclusions and contributions of the dissertation are:

1. Isolation, qualitative and quantitative determination of methylxanthine fraction (MF), catechin fraction (CF) and whole extract (TE) of Japanese green tea Bancha
2. Bancha green tea TE contains less epigallocatechin-3-gallate (EGCG) and caffeine than other types of green tea.
3. Through the applied methods for extraction of CF and MF, a higher percentage of catechins and methylxanthines in the obtained fractions is achieved, compared to TE from Bancha green tea.
4. A liquid chromatographic method for analysis with high analytical reliability and sensitivity for qualitative and quantitative determination of sildenafil in biological samples has been introduced and tested.
5. Bancha Green Tea Extract has a significant effect on sildenafil PK in rats, despite the lower content of catechins and caffeine compared to other types of green tea.
6. Depending on the duration of administration of MF isolated from Bancha green tea, different changes in the pharmacokinetic parameters of sildenafil were observed in rats - after single administration, decrease in plasma concentrations of the drug was observed, and an increase in plasma levels was observed with repeated administration.
7. A similar increase in plasma concentrations of sildenafil has been observed with repeated administration of TE or CF of Bancha green tea.
8. Physiologically-based pharmacokinetic models (PBPk) have been developed. They have shown that there is a potential risk of pharmacokinetic interactions in humans when sildenafil is co-administered with green tea extract, EGCG or caffeine.

The contributions are divided into Original, Scientific-applied and confirmatory

ORIGINAL CONTRIBUTIONS:

- ❖ For the first time in Bulgaria TE, CF and MF of Japanese green tea Bancha were isolated and analyzed.
- ❖ For the first time, a pharmacokinetic study was performed and the effect of single and repeated administration of TE, CF and MF from Japanese Bancha green tea on sildenafil plasma concentrations in experimental animals was demonstrated to assess potential interactions.
- ❖ For the first time, static and dynamic PBPK models have been developed and implemented to predict potential interactions between sildenafil and CF or MF of green tea, both alone and in combination in humans.

CONTRIBUTIONS OF SCIENTIFIC AND APPLIED NATURE:

- ❖ A selective and reliable HPLC-UV method for qualitative and quantitative determination of EGCG, (+) - catechin, gallic acid and caffeine in samples of Japanese green tea Bancha has been developed and validated. The proposed analytical approach can be used in the analysis of other types of tea, as well as other plant extracts that are believed to contain these compounds.
- ❖ A sensitive and reliable HPLC-UV method for qualitative and quantitative determination of sildenafil in plasma samples from experimental animals has been developed and validated. The proposed analytical method can be used in the analysis of sildenafil in healthcare facilities or other institutions where analysis of drugs in biological samples is performed.

CONFIRMATIVE CONTRIBUTIONS:

- ❖ The lower quantitative content of ECG and caffeine in Bancha green tea has been confirmed.
- ❖ The efficacy of protein precipitation in the preparation of plasma samples from experimental animals treated with sildenafil has been confirmed.

Publications related to the dissertation

There are 3 publications, 2 of which have an impact factor according to *Thomson Reuters*. One citation was also noted.

The typographic layout of the dissertation and the author's abstract are excellent. The presented abstract fully reflects the content of the dissertation, although the book body of the abstract is

missing pages 38-40, which of course is not the fault of the PhD student. In the electronic version of the abstract, these pages exist, which is probably a typographical error.

Critical remarks and recommendations

It would be good to explain it at the first appearance of an abbreviation in the text. In my opinion, in a scientific work, it is not necessary to have photographic material from the work process. Probably this is the desire of each PhD student to prove their efforts in developing the dissertation, which of course does not reduce the value of the work done.

Keep in mind the term phytoproducts, not phytopreparations!

Questions

1. Do you have a certificate of origin and identity of the green tea you bought from the herbal pharmacy?

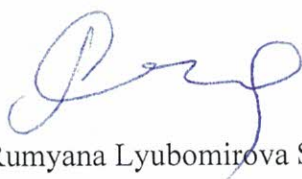
Conclusion

My complex assessment of the presented dissertation is POSITIVE.

The work is shaped by emphasized intelligence, professionalism and visible satisfaction with the successful career of the PhD student. The presented dissertation and attached publications meet the requirements of the Bulgarian law in the field of scientific education. 7. Health and sports, professional field 7.3. "Pharmacy", scientific specialty "Pharmacology (including pharmacokinetics and chemotherapy)" for EDUCATIONAL AND SCIENTIFIC DEGREE "PhD"

Based on the detailed positive aspects of the dissertation, I strongly recommend the esteemed members of the "Scientific Jury" to vote positively for the award of EDUCATIONAL AND SCIENTIFIC DEGREE "PhD" in the scientific specialty "Pharmacology (including pharmacokinetics and chemotherapy)" of Master Pharm. Maya Petrova Radeva-Ilieva

Reviewer



Assoc. Prof. Rumyana Lyubomirova Simeonova, Ph.D.

11. 04. 2022

Sofia