

Statement

By Prof. Zlatka Dimitrova Dimitrova, DSci, Head of TRL on Social Pharmacy, Department of Physical Chemistry, Faculty of Chemistry and Pharmacy at Sofia University "St. Kl. Ohridski" - Sofia, appointed a member of the Scientific Jury by order №P-109-92/24.02.2022r of Prof. Valentin Ignatov, MD, Rector of MU "Prof. Paraskev Stoyanov, MD" Varna.

Subject: procedure for the defense of a dissertation on the topic: "Isolation and analysis of methylxanthine fraction, catechin fraction and whole extract of green tea Bancha and study of their effect on the pharmacokinetics of sildenafil in rats" for the acquisition of ESD "doctor" of assist. prof. Maya Petrova Radeva-Ilieva, MPharm, PhD student in full-time education in the field of higher education 7. Health and Sports, Professional field 7.3 Pharmacy, in the doctoral program "Pharmacology" (including pharmacokinetics and chemotherapy) with supervisor: Assoc. Prof. Kaloyan Dobrinov Georgiev, M.Pharm, DSci.

This statement has been prepared in accordance with the requirements of Law on the Development of the Academic Staff in the Republic of Bulgaria /LDASRB/, Regulations for application of the Law for the development of the academic staff in the Republic of Bulgaria/RALDASRB/ and the Regulations for the development of the academic staff at MU "Prof. P. Stoyanov, MD" - Varna in force since 15.09.2020 (protocol №8 / 15.09.2020), Chapter II. Acquisition of scientific degrees, Section IV. Conditions and procedure for acquiring ESD "Doctor"

Details of the procedure. With Order With Order № P-109-57 / 31.01.2020r Ass.prof. ,MPharm Maya Petrova Radeva-Ilieva is enrolled as a PhD student in full-time education in the field of higher education 7. Health and Sports, Professional field 7.3 Pharmacy, in the doctoral program "Pharmacology" (including pharmacokinetics and chemotherapy) in the Department of Pharmacology, Toxicology and Pharmacotherapy at the Faculty of Pharmacy, MU-Varna. Two protocols from 2021 are presented for successfully passed examinations for covering the doctoral minimum in the specialty of pharmacology and for mastering a foreign language by the doctoral student before commissions appointed by orders of the Rector of MU-Varna, as well as Order № -P-109-92 / 24.02.2022 of prof. Valentin Ignatov, MD - Rector of MU "Prof. Paraskev Stoyanov, MD" Varna for expulsion of the doctoral student with the right to defend the dissertation after its discussion by the department council and a decision on its readiness for referral for public defense. The PhD student's file folder contains all the required documents.

Biographical data and career development of the candidate: Maya Petrova Radeva graduated in pharmacy and obtained a master's degree in pharmacy in November 2015 at the Faculty of Pharmacy at MU-Varna with excellent results and was awarded the Golden Galen Award. She is the winner of the annual award of the Bulgarian Union of Pharmacists for students of pharmacy in 2014. She is currently a specialist in Pharmacology and Pharmacotherapy. After graduation she worked as a stockman in Galen 2000 Ltd. and as vice manager of the pharmacy "Medunipharm EOOD" at the Faculty of Pharmacy, MU-Varna, and since January 2016 she has been an ass. prof. in the Department of Pharmacology, Toxicology and Pharmacotherapy of the Faculty of Pharmacy at MU-Varna.

Relevance and significance of the dissertation. The stories of the discovery of green tea are more myth than reality, like the incredible legend of the discovery of green tea in China by

accident. One day Shen Nü, the leader of China, was boiling water under a tea tree when several leaves accidentally fell into the boiling water, making the first green tea. After drinking the decoction of brewed tea leaves, Shen Nü, who was also an expert in medicine at the time, discovered the properties and beneficial effects of tea and immediately included green tea leaves in the list of herbs used in medicine. Fact or fiction, green tea has been used in China for over 4,000 years as a medicine. Green tea was imported from China to Japan by Buddhist monks in the early ninth century. Initially, this drink was only available to the upper class and was used as medicine. At the end of the 12th century, the famous priest Enshō began to use matcha tea for religious purposes in Japan. He wrote the first book on tea, focusing on the health benefits and health benefits of the drink, such as limiting the effects of alcohol on the body, stimulating its effects, quenching thirst, helping with indigestion, improving brain function, etc. .n. This gives green tea widespread popularity among the people. Between the 9th and 15th centuries, the production of green tea by brewing, roasting and frying in a pan began in different parts of Japan, and the production of traditional Japanese sencha and gyokuro teas became more widespread. Green tea was soon recognized as the nation's most popular beverage. Between the 9th and 15th centuries, the production of green tea by brewing, roasting and frying in a pan began in different parts of Japan, and the production of traditional Japanese sencha and gyokuro teas became more widespread. Green tea was soon recognized as the nation's most popular beverage.

The following 8 beneficial effects of green tea have been identified in the scientific and popular literature: it helps reduce weight, increases aerobic activity, boosts immunity and has anti-inflammatory effects, can reduce the risk of type 2 diabetes, and has a preventive effect against heart disease. Vascular diseases can help you live to a ripe old age, serve as a means of prevention against certain cancers of the breast, colon and prostate and supports learning and memory.

However, green tea has been shown to be used in combination with certain medications and substances to cause serious health problems. It should not be combined with stimulants such as amphetamines or ephedrine, with addictive substances such as cocaine and nicotine. Care should be taken when taking green tea together with drugs and substances such as: adenosine, antibiotics, contraceptives, estrogen, drugs for depression, liver, heart, stomach, circulatory system.

The composition of green tea can vary widely, depending on a number of factors such as the conditions under which the tea plants are grown, the time of leaf collection, the post-harvest processing processes and the storage conditions. Differences in the content of BAS are also related to different organoleptic properties of tea leaves and tea drink after preparation. In addition, different types of green tea are available, such as Chinese, Japanese, Korean and others, depending on the geographical conditions in which tea plants have been grown, and it has been found that this also affects the qualitative and quantitative content of BAS. For example, one of the most commonly consumed types of green tea in Japan is called Bancha. It is obtained from tea leaves, which are collected in late summer or early autumn, the so-called. late seasonal picking, while Sencha tea is obtained from the first and second harvest leaves. Sencha tea contains the highest levels of catechin EGCG (epigallocatechin gallate) compared to any other tea. These natural polyphenols are strong antibacterial and antiviral agents, strengthen the walls of capillaries, help rid the body of toxic compounds. Many scientists

believe that EGCG plays a positive role in the body's fight against cancer. Polyphenols act as a barrier to the absorption of cholesterol in the body and help eliminate excess cholesterol. Regular intake of Sencha protects the body from heart disease and atherosclerosis. The antioxidant properties of this drink help to preserve the youth of the skin, reduce wrinkles and hydrate. It has a beneficial effect on inflamed and damaged skin. Gargling with shadows leads to great relief from sore throat or cough. Tea is high in vitamin C, which makes it useful at all times. There is evidence that Bancha green tea contains less BAV than other popular Japanese green teas, such as Sencha and Matcha, but information in the scientific literature on the content of active substances, potential therapeutic and toxic effects of this type of Japanese tea. green tea, as well as the potential to participate in drug interactions is quite scarce, although Bancha is one of the most widely used teas in Japan. This is the reason why the PhD student chose this type of Japanese Bancha tea to conduct research in this dissertation.

In addition, taking into account the currently reported interactions between green tea extract and / or ECG with drugs, CYP3A4 substrates require further studies to identify potential interactions. Sildenafil is a selective inhibitor of the enzyme phosphodiesterase type 5 (PDE5) used in the treatment of erectile dysfunction and pulmonary arterial hypertension. In addition, there is evidence that sildenafil is a substrate of P-gp. It is high-permeability drugs that undergo significant metabolism, such as sildenafil, that are often involved in drug interactions. Moreover, due to the significant contribution of CYP3A4 to sildenafil metabolism, co-administration with drugs, foods, beverages or herbal extracts that affect the activity of this enzyme may lead to pharmacokinetic interactions. These, in turn, may lead to increased sildenafil toxicity or treatment failure. In addition, given the observed in vitro inhibition of CYP3A4 by methylxanthine fractions isolated from Bancha and Puer tea and pure caffeine, the doctoral student suggested that an in vivo pharmacokinetic interaction may occur after co-administration of sildenafil and methylxanthines, in particular caffeine. The dissertation aims to test the hypothesis. This determines the relevance and significance of this dissertation.

General characteristics of the candidate's research and applied research activity.

The PhD thesis of Maya Petrova Radeva –Ilieva on "Isolation and analysis of methylxanthine fraction, catechin fraction and total extract of Bancha green tea and study of their effect on the pharmacokinetics of sildenafil in rats" is 109 pages long and includes the following main parts: Introduction - 3 pages, Literary Review - 46 pages, Purpose and tasks - 2 pages, Materials and methods - 17 pages, Results - 18 pages, Discussion - 21 pages, and Conclusions - 2 pages. The bibliographic list includes 392 titles, of which 299 are in Latin and 3 in Cyrillic. The predominant number of used publications is from the last 10 years.

The literature review shows the good awareness of the doctoral student on the issues discussed: 1. Content of biologically active substances in green tea, / Methods for analysis of green tea extracts, Potential toxic effects of green tea extract, / 2. Drug interactions and interactions between drugs and phytopreparations / food / beverages, / Regulation of medicinal products and phytopreparations, Types of drug interactions, Interactions between conventional drugs, Interactions between conventional drugs and phytopreparations, Interactions between conventional drugs and green tea extract and / or its components, Interactions between sildenafil and phytopreparations / food / beverages, /3. Methods for research of drug interactions, / Physiologically based pharmacokinetic (FBPC) model, Construction of FBPC model, Software for preparation of FBFC model, Verification of FBPC

model, Application of FBPC modeling. / I am very impressed by the professionally responsible and critical attitude of the dissertation to the lack of strict regulation regarding food supplements containing plant substances, most often in the form of an extract in our country. Phytopreparations registered as food additives are not subject to mandatory pre-clinical and clinical trials before being placed on the market, so their pharmacokinetics, pharmacological activity and toxicological profile may not be fully studied and elucidated. . In addition, there are even dietary supplements on the market that contain plant extracts that are not standardized. The overview is illustrated with 5 tables and many structural formulas. Thus, the research in the dissertation is aimed at a more in-depth study of the potential interactions that could occur with the simultaneous use of different types of green tea and conventional drugs.

The goal and the five tasks are formulated very precisely and clearly, appropriate modern methods for their implementation are selected, the individual stages of the research are presented very precisely and consistently and the obtained results are discussed. Statistical analysis was performed using GraphPad Prism, version 9.2.0. Results are expressed as mean \pm standard deviation (SD). Statistical comparisons between the calculated pharmacokinetic parameters for the different groups in the two experiments were performed using analysis of variance (ANOVA), followed by Dunnett's test. The p-value ≤ 0.05 (two-sided test) is considered statistically significant. The results are presented textually and are illustrated with many photos, 27 figures and 14 tables.

As a result of the research conducted in the dissertation, appropriate conclusions are drawn, which logically follow from the results of the research.

Main scientific and applied scientific contributions. I accept the doctoral student's report on the scientific contributions of her dissertation. The main scientific and scientific-applied contributions in the dissertation are new or confirmed scientific facts, which are a result of the conducted by assoc. prof. Maya Petrova Radeva-Ilieva research and can be summarized in 3 groups as follows:

ORIGINAL CONTRIBUTIONS:

- ❖ For the first time in Bulgaria TE, CF and MF from Japanese green tea Bancha were isolated and analyzed and pharmacokinetic studies were conducted, which prove the influence of repeated use of TE, CF and MF and single use of MF from Japanese green tea Bancha on plasma concentrations of sildenafil in experimental animals to assess potential interactions.
- ❖ Static and dynamic FBPC models have been developed and implemented to predict potential interactions between sildenafil and CF or MF of green tea, both alone and in combination with humans.

CONTRIBUTIONS OF SCIENTIFIC AND APPLIED NATURE:

- ❖ Selective and reliable HPLC-UV method for qualitative and quantitative determination of ECG, (+) - catechin, gallic acid and caffeine in samples of Japanese green tea Bancha, which can be used in the analysis of other types of tea, has been developed and validated. as well as other plant extracts containing said 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, which can be used in the analysis of sildenafil in health facilities or other institutions analyzing drugs in biological samples.

CONFIRMATIVE CONTRIBUTIONS:

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

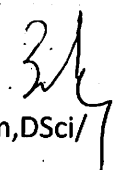
Reflection of the candidate's scientific publications in the literature. On the topic of the dissertation are presented 3 published scientific articles, two of which have been published in foreign journals with IF and 3 participations with posters at scientific forums in our country. He has documented participation in two research projects led by Assoc. Prof. Kaloyan Georgiev, D.Sci. in 2019 and 2020, funded by the Science Fund at the Medical University "Prof. Paraskev Stoyanov,MD ", Varna.

Conclusion. The dissertation of the assistant mag. farm. Maya Petrova Radeva-Ilieva contains new scientific facts with special relevance in the field of pharmacology and pharmacokinetics and social significance. The results of the research in the dissertation and their interpretation are at a high scientific level, are published in the specialized medical literature and are reported at scientific forums in our country with foreign participation.

The scientific work fulfills all the requirements of LDASRB and of the Regulations for its application / RALDASRB / and of the Regulations for development of the academic staff in MU "Prof. P. Stoyanov, MD" - Varna for acquiring ESD "Doctor". I give a positive assessment of this dissertation and will fully support the award of ESD "Doctor" of Pharmacy to Assistant Professor Maya Petrova Radeva-Ilieva, MPharm in the doctoral program "Pharmacology" / including pharmacokinetics and chemotherapy / by voting "Yes".

March 28, 2022,
Sofia

Prepared the opinion:



/Prof.Zl.Dimitrova,MParm,DSci/