

**To the Chairman of the scientific jury
formed with order № R-109-92/24.02.2022
of the Rector of the Medical University-Varna**

REVIEW

**From prof. Magdalena Spasova Kondeva-Burdina, PhD,
Department “Pharmacology, Pharmacotherapy and Toxicology”
Faculty of Pharmacy, MU-Sofia**

Included in the composition of the scientific jury, according order № R-109-92/24.02.2022 of the Rector of MU-Varna and elected as a reviewer on the first meeting of the scientific jury, which took place on 09.03.2022 (protocol № 1).

Concerning:

Procedure for the award of educational and scientific degree “Doctor” in the area of high education 7. “**Healthcare and sport**”, professional direction 7.3. “**Pharmacy**” and PhD program „**Pharmacology /including pharmacokinetics and chemotherapy/**”, with candidate assistant **Maya Petrova Radeva-Ilieva**.

Topic of the PhD thesis: **Isolation and analysis of the methylxantine fraction, catechin fraction and total extract from green tea Bancha and evaluation of the influence on the pharmacokinetics of sildenafil on rats.**

Scientific supervisor: assoc. prof. Kaloyan Dobrinov Georgiev, Dsci

Career development of the candidate

1. Education

January 2020 – until now – PhD student of the scientific specialty „Pharmacology /including pharmacokinetics and chemotherapy“;

August 2016 – until now – specialist in „Pharmacology and pharmacotherapy“;
September 2010 – November 2015 – Master of Pharmacy, specialty “Pharmacy” to
MU-Varna.

2. Professional activity

January 2016 – Until now – assistant, MU-Varna;

November 2016 – March 2021 – master of pharmacy, deputy head of pharmacy
“MEDUNIFARM” EOOD;

March 2015 – January 2016 – stockiest „GALEN 2000“ EOOD;

August 2014 – December 2014 – technical assistant „MANOLOVA“ EOOD.

Awards and prizes

Award „Golden Galen“ for finishing her Pharmacy education with FULL
EXCELLENT MARKS.

Prize from the Bulgarian Pharmaceutical Union for Pharmacy students, 2014.

Evaluation of the topic relevance

The green tea is one of the most consuming drinks in the world. It is produced from the leaves of tea plant *Camellia sinensis* (L.) Kuntze. The green tea is known with its antioxidant, anti-inflammatory and anti-microbial activity, as well as its anti-cancer effect. The tea leaves content lots of biologically active compounds (BAC): polyphenolic compounds, polysaccharides, alkaloids, saponins, free fatty acids, vitamins, minerals etc.

The most of the literature data showed that these effects of the green tea are connected with the effects of polyphenols in particular catechins. In high amount are found the follow catechins: (-)-epigallocatechin-3galat (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3galat (ECG) и (-)-epicatechin (EC). EGCG is in a high amount and consists from 50 to 70 % from the catechins. In addition, EGCG is shown to be the most active compound from the group of catechins, which is responsible for the most of the biological effects.

The usage of catechins as a potential therapeutic compounds is limited, because of their low stability, as well as their low oral bioviability, which leads to very low plasma concentrations.

Other important group BAC, which are included in the green tea are methylxantins. The natural methylxantins are: caffeine, theophylline and theobromine. In the tea leaves at highest concentrations is caffeine, theobromine is in very low concentrations and theophylline is not found.

The content of green tea may be different because of different factors like: conditions for breeding tea plants, the time for gathering the leaves, the processes for treatment and the storage conditions. The differences in the BAC's composition have been connected with different taste of the tea, prepared from the leaves.

There are many literature data about the potential interactions between green tea and some drugs, used for cardiovascular diseases and cancer, when they are taken at the same time. Although the green tea extract contains lots of active compounds, which can interact with the drug molecules, it is accepted that catechins are the main compounds, which are responsible for these interactions. Many pre-clinical and clinical studies evaluate the potential of the green tea extract or the catechins to modulate the activity of the drug-metabolizing enzymes, as well as some transmembrane transporters.

The results from the *in vitro* studies revealed that green tea extract or EGCG inhibit the activity of different isoforms of the cytochrome P450 like: CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2C8, CYP2C9, CYP2D6, CYP2E1, CYP3A4, as well as some transmembrane transporters as P-glycoprotein. On experimental animals was found that green tea extract, as well as EGCG, administered alone, increased the plasma concentrations of the drugs, which are substrates of CYP3A4 and P-glycoprotein but on clinical studies the results are contradictory.

On the other hand, the caffeine, as main compound from the methylxantins, also can interact with some drug groups. The interactions are on absorption's and distribution's levels, mainly with drugs, substrates of CYP1A2. In her previous study, the PhD student found that methylxantins' fractions, isolated from Bancha and Puer tea leaves, as well as clean caffeine inhibited the activity of CYP3A4 *in vitro*. There are also literature data about CYP3A4 and CYP2D6 inhibition from caffeine. Although there is a few information about such potential interactions between caffeine and drugs, substrates of other isoforms of cytochrome P450 except CYP1A2.

In her PhD thesis, assistant Radeva-Ilieva, investigates the effects of different BACs on the sildenafil's pharmacokinetics, substrate of CYP3A4. She chooses sildenafil, because of the fact, that the drug is used from healthy men. They use the drug for

increasing the sexual endurance. This fact can lead to possible increasing risk for occurring the unwanted drug reactions between sildenafil and other phytoproducts.

The structure of the PhD thesis

The dissertation includes 139 pages, 27 figures and 14 tables. The bibliography covers 392 titles.

The Literary review in details revealed the problems, connected with the possible drug interactions between some BACs, isolated from the extract of green tea Bancha, and the pharmacokinetics of some main drug's groups.

The Purpose and tasks are correctly formulated.

The Methods are presented in details. Their diversity showed the high methodological preparation of the candidate. These methods provide receiving depth information, which helps to reach the scientific aims and solving the formulated tasks.

The Results are presented systematically and followed a logical order. They are exhaustively illustrated with tables and graphics, which are enough clear and informative.

The Discussion of the results is very informative and presented in depth. The candidate managed to summarize the data and to compare them correctly with the literature information.

The interactions between drug and plant extract can be potentially dangerous and can lead to serious adverse drug reactions. The frequent usage of drugs in combination with plant products imposes more informative pre-clinical and clinical

investigations. These investigations can evaluate the possibility for clinically significant interactions between some main drug's groups and plant extract or their active compounds. These can decrease the possibility for interactions and increase the safety for the patients, when taking together plant products and drugs.

This is the reason for evaluating the influence of total extract, cathechins' and methylxantins' fractions from green tea Bancha on the pharmacokinetics of sildenafil, substrate of CYP3A4.

On rats, the PhD student found changes in the pharmacokinetics' parameters of sildenafil after chronic administration of total extract, cathechins' and methylxantins' fractions, isolated from Japanese green tea Bancha.

These changes in the pharmacokinetics' parameters of sildenafil might be due to possible activity of EGCG, which is found in the total extract and in cathechins' fraction, as well as caffeine, which is the main compound in the methylxantins' fraction.

In conclusion, on the basis of the made experiments, it is necessary to avoid the combination between sildenafil and drinks like green tea and coffee, as well as plant products, which contain high amounts green tea extract, EGCG and caffeine.

I receive *the Conclusions*, which the candidate made at her PhD thesis. They are presented clearly and correctly revealed the achieved results, connected with the aim and tasks of the work.

I agree with *the Contributions*, which are summarized as follow:

CONTRIBUTIONS WITH ORIGINAL CHARACTER:

- For the first time in Bulgaria were isolated and analyzed total extract, cathechins' and methylxantins' fractions from the Japanese green tea Bancha.
- For the first time was made pharmacokinetics' study and was proven the influence of multiple administration of total extract, cathechins' and methylxantins' fractions from Japanese green tea Bancha, on plasma concentrations of sildenafil in experimental animals. The aim is evaluating the possible interactions.
- For the first time was made pharmacokinetics' study and was proven the influence of single administration of methylxantins' fraction from Japanese

green tea Bancha, on plasma concentrations of sildenafil in experimental animals. The aim is evaluating the possible interactions.

- For the first time are developed static and dynamic physiologically based pharmacokinetics models for prediction the potential interactions between sildenafil and catehins' and methylxantins' fractions from green tea, administered alone, as well as in combination, on humans.

CONTRIBUTIONS WITH SCIENTIFIC-APPLIED CHARACTER:

- It was developed and validated selective HPLC-UV method for qualitatively and quantitatively determination of EGCG, (+)-catehin, galic acid and caffeine in samples from Japanese green tea Bancha. This analytic protocol can be used for analysis of others species tea, as well as other plant extracts, for which is supposed the content of these compounds.
- It was developed and validated selective HPLC-UV method for qualitatively and quantitatively determination of sildenafil in plasma samples from experimental animals. This analytic protocol can be used for analysis of sildenafil in hospitals or other laboratories for drug analysis in biologically samples.

CONTRIBUTIONS WITH CONFIRMATORY CHARACTER:

- It was confirmed the lower quantitatively content of EGCG and caffeine in green tea Bancha.
- It was confirmed the efficacy of protein precipitation in the preparation of the plasma samples, received from experimental animals, treated with sildenafil.

The Abstract of the PhD thesis is very comprehensive, created according the requirements and revealed the main results, receiving from the scientific development.

CONCLUSION

The PhD thesis of assistant Maya Petrova Radeva-Ilieva is with current character and contribution to the scientific data, which are connected with

evaluation the possible interactions between some BACs (methylxantins and cathehins), isolated from the green tea Bancha, and the sildenafil's pharmacokinetics.

On the base of the made analysis of her PhD thesis: "Isolation and analysis of the methylxantine fraction, cathelin fraction and total extract from green tea Bancha and evaluation of the influence on the pharmacokinetics of sildenafil on rats", I can give my POSITIVE EVALUATION and recommend to the Scientific jury to award to assistant Maya Petrova Radeva-Ilieva the educational and scientific degree "Doctor" to the PhD program "Pharmacology /including pharmacokinetics and chemotherapy/", professional direction 7.3. "Pharmacy" in the area of high education 7. "Healthcare and sport".

11.04.2022

Sofia

Reviewer:



/prof. Magdalena Spasova Kondeva-Burdina, PhD/