



Fund “Nauka” Project № 20019 Resume – Competition-Based Session 2020:

“Pharmacological study of methylxanthine fraction isolated from green tea”

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Green tea has been widely studied because of its numerous beneficial effects on the human body such as antioxidant, anti-inflammatory, diuretic, neuroprotective, antitumor, antiobesity, etc. It is believed that these effects are mainly due to the catechins content in green tea extract. However, the possibility that the biological activity of green tea may be related to the presence of other substances in the extract or due to synergism between catechins and other compounds should not be ruled out. Another group of substances present in green tea is the group of methylxanthines that have been reported to exert pharmacological effects. The best studied natural methylxanthines are caffeine, theophylline and theobromine. Interestingly, the percentage of biologically active substances such as catechins and methylxanthines may differ for different types of green tea depending on the growing conditions of tea plants (*Camellia sinensis*), the period of tealeaves collection, the production process and other factors.

Objective: To evaluate the pharmacological activity of a methylxanthine fraction isolated from a specific type of green tea called Bancha.

Tasks:

1. Isolation and analysis of methylxanthine fraction from Bancha tea leaves;
2. HPLC analysis of the isolated fraction;
3. In vitro cytotoxicity assay (MTT assay);
4. In vivo study for antioxidant, anti-inflammatory and antiobesity activity of the isolated fraction and of synthetic methylxanthine;
5. In vitro study for antitumor activity of the isolated fraction and of synthetic methylxanthine in cell lines;
6. HPLC analysis of blood samples for quantification of methylxanthines and their metabolites.

Materials:

1. Plant material;
2. Antioxidant assay kit;
3. Kits for evaluation of anti-inflammatory activity;
4. Animals for in vivo study of antioxidant, anti-inflammatory and antiobesity activity;
5. Cell cultures for evaluation of antitumor activity;
6. A synthetic derivative of methylxanthines;

7. Reference substances of some methylxanthine metabolites.

Methods:

1. Liquid-liquid extraction and use of chromatographic methods to separate individual fractions from the total extract;
2. High-performance liquid chromatography (HPLC) for the qualitative and quantitative analysis of the isolated methylxanthine fraction and for the analysis of methylxanthines and their metabolites in blood samples;
3. Colorimetric determination of the antioxidant and anti-inflammatory activity of methylxanthines using special assay kits;
4. Biochemical examination of blood serum to assess antiobesity activity;
5. MTT analysis for evaluation of antitumor activity.

Expected results: Observation and evaluation of the antioxidant, anti-inflammatory and antiobesity effects after administration of methylxanthine fraction and synthetic methylxanthine derivative in experimental animals and evaluation of the influence of natural methylxanthines on the biological activity of green tea.

Achieved results:

In fulfillment of the assigned tasks set in project № 20019 “Pharmacological study of methylxanthine fraction isolated from green tea”, the following activities were carried out:

- ❖ Liquid chromatographic methods have been developed for qualitative and quantitative analysis of medicinal substances in biological samples as well as methods for sample preparation in order to evaluate potential pharmacokinetic interactions during simultaneous administration of methylxanthines and other components contained in green tea;
- ❖ A liquid chromatographic method was developed for the qualitative and quantitative analysis of caffeine, catechins (epigallocatechin-3-gallate, catechin) and gallic acid in an extract of Japanese Bancha green tea;
- ❖ Experiments with laboratory animals were conducted to study the potential interactions between Bancha green tea extract as well as various fractions isolated from it, and drugs, substrates of CYP3A4;
- ❖ Qualitative and quantitative analysis of caffeine and paraxanthine (main metabolite of caffeine) in biological samples from rats after repeated intake of methylxanthine fraction isolated from Japanese Bancha green tea was performed;
- ❖ Physiologically based pharmacokinetic (PBPK) models have been developed to predict potential interactions between methylxanthines and drugs in humans.

The obtained results are presented in 3 full-text scientific articles, of which two were published in international journals with an impact factor (IF: 3.117; IF: 0.8), referenced and indexed in world-renowned databases (Scopus). In addition, as a result of the project

activities, a dissertation was successfully defended on the topic “Isolation and analysis of methylxanthine fraction, catechin fraction and total extract of Bancha green tea and study of their influence on the pharmacokinetics of sildenafil in rats”, which is related with the subsequent career growth of a team member.