Summary

„Cytotoxicity of stigmasterol, stigmasterol esters and their thermo-oxidative degradation products in in vitro studies"

Stigmasterol and stigmasterol esters with fatty acids are used as functional additives to food products, which lower the level of total cholesterol and its LDL (*low-density lipoprotein*) fraction in blood. Scientific research has shown that, in addition to its antihypercholesterolemic effect, stigmasterol also has numerous other health-promoting properties important for the prevention and support of the therapy of many diseases. The food enriched with stigmasterol can be intended for direct consumption or after thermal treatment, including cooking, baking, or frying. During the long-term storage and high-temperature processing, degradation products and oxyderivatives of stigmasterol are formed, which have a potentially adverse effect on the human body, including an increased risk of atherosclerosis, cardiovascular diseases, and cancer.

As part of the work, the products of thermal-oxidative transformations of stigmasterol and its esters with linoleic and oleic acid were identified and quantitatively determined. *In vitro* studies assessed the level of their cytotoxicity and genotoxicity to normal human digestive system cells. The mutagenic and promutagenic potential of the analyzed compounds was also determined using mutant bacterial strains of *Salmonella enterica* subsp. *enterica* ser. *typhimurium* and enzymes of the microsomal fraction. The influence of stigmasterol, stigmasterol esters and their derivatives generated during thermal-oxidative treatment on the integrity and functionality of the intestinal barrier *in vitro* was also analyzed.

Based on the chemical analysis, it was found that the conditions of thermal treatment of compounds determine the amount of generated degradation products and oxidative derivatives of stigmasterol. It has been proven that stigmasterol esters are more stable than free stigmasterol when heated at 60 °C (12 h) and 180 °C (8 h), and the degree of unsaturation of the fatty acid carbon chain has a significant impact on the production of derivatives of thermal-oxidative ester transformations.

The results of biological analyses showed that stigmasterol esters are characterized by much less cytotoxicity to the epithelial cells of the small intestine, large intestine and liver than free stigmasterol. Stigmasterol esters and derivatives of their thermal-oxidative transformations at low, physiologically realistic doses (≤40 μg/ml) did not cause cytotoxic

effects in the cells of the digestive system. In contrast, free stigmasterol inhibited cell proliferation by suppressing DNA synthesis, arresting the cell cycle in the G_2/M phase, and directing cells to the caspase-dependent apoptosis pathway. Heating at 180 °C resulted in a decrease in the cytotoxic potential of stigmasterol, which was related to the degradation of the stigmasterol molecule. The negative effect of heating stigmasterol was its increased oxidative reactivity, manifested in the induction of overproduction of reactive oxygen species in cells. Heated linoleic stigmasterol ester was also characterized by similar pro-oxidant activity. Regardless of the heating conditions, stigmasterol and stigmasterol esters did not have genotoxic, mutagenic or pro-mutagenic effects.

The studies conducted on the intestinal epithelium model have shown that stigmasterol may disturb the integrity of the intestinal barrier, cause the intercellular connections to become unsealed, and increase its permeability. Chronic exposure of the intestinal epithelium to stigmasterol linoleate may disrupt its integrity and weaken the expression of genes encoding protein components of intercellular junctions in the epithelium. Unlike linoleate, stigmasterol oleate did not negatively affect the integrity and functionality of the intestinal barrier. The studies proved that among the analyzed compounds, stigmasterol oleate is characterized by the highest stability during the heating process and is the toxicologically safest for digestive system cells.

The obtained data suggest that stigmasterol, stigmasterol compounds, and products of their thermal-oxidative transformations may cause adverse health effects. It should be emphasized that research on these compounds' safety and biological activity should be continued and expanded by applying advanced experimental models, including tissue and organotypic models *in vitro* and animal models *in vivo*.

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