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STUDY OF THE LIVER METABOLIC ACTIVATION OF SOME PLANT PHENOLIC COMPOUNDS

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ABSTRACT

Phenolic compounds form one of the main classes of secondary metabolites. The widespread use of flavonoids and phenolic acids necessitates the study of their metabolism. The aim of this work is to predict the possible metabolites of some plant phenolic compounds by a specialized software *(OECD (Q)SAR Application Toolbox*. Analysis of data reveals that after metabolic activation in liver (observed pathways) for five of the six plant phenolic compounds liver metabolism was not observed. Only for one compound (Luteolin) metabolic activation in liver (observed pathways) was observed.

Keywords: *phenolic compounds, flavonoids, phenolic acids, Quantitative Structure-Activity Relationships*

INTRODUCTION

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known. Despite their wide distribution, the health effects of polyphenols have come to the attention of nutritionists in recent years. The preventive effects of these plant metabolites in terms of cardiovascular, neurodegenerative diseases and cancer are deduced from epidemiologic data as well as *in vitro* and *in vivo* (1,3,6,9,19). Among these compounds flavonoids and phenolic acids constitute one of the most ubiquitous groups of all plant phenolics.

Flavonoids are constituents of fruits, vegetables, plant-derived beverages as tea and wine, as well as components present in dietary supplements. Over 4 000 different naturally occurring flavonoids have been described (11). Phenolic acids (hydroxycinnamica and hydroxvbenzoic acids) belong to the group of phenolic compounds as well, and occur in fruits as esters glycosideas and amides. The most common hydroxycinnamic acids are p-coumaric, caffeic and ferulic acid, while the corresponding hydroxybenzoic acids are p-hydroxybenzoic, gallic, ellagic, 3,4-dihydroxybenzoic, vanillic, and syringic acid (5).

However, the potential toxicity of these two types of phenolic components has not been well studied (7).

In the recent years methods for structural features and properties of a molecule and its impact on biological activity were investigated. One of them

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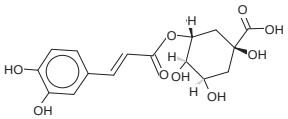
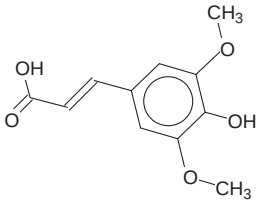
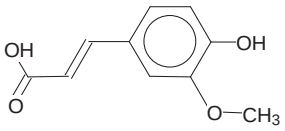
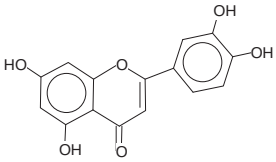
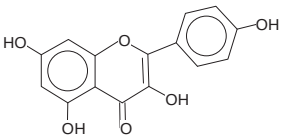
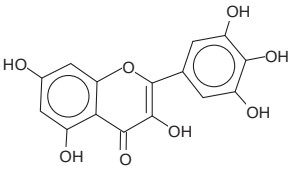
is **Quantitative Structure-Activity Relationship** (QSAR) - a method of studying a series of molecules of different structures with varying observed properties and attempting to find empirical relationships between structure and property or activity. QSAR has been widely used for new drug discovery processes, but also for the prediction of metabolism and toxicity of chemical structures (14).

The aim of this work is to predict the possible metabolites of some plant phenolic compounds by a specialized software *OECD - (Q)SAR Application Toolbox*. Analysis of data reveals that after metabolic activation in liver (observed pathways) for five of the six plant phenolic compounds liver metabolism was not observed. Only for one compound (Luteo-

Table 1. CAS number, name and structure of some phenolic compounds

№	CAS number	Name of compound	Structure of compound
1	327-97-9	Chlorogenic acid	
2	530-59-6	Sinapinic acid	
3	1135-24-6	Ferulic acid	
4	491-70-3	Luteolin	
5	520-18-3	Kaemferol	
6	529-44-2	Myricetin	

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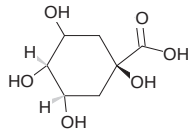
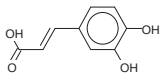
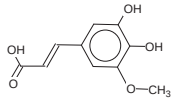
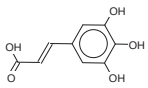
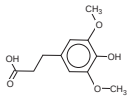
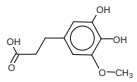
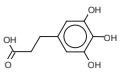
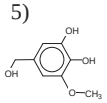
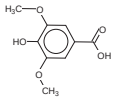
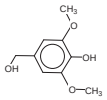
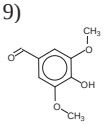
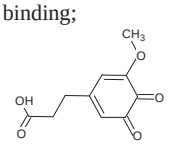
lin) metabolic activation in liver (observed pathways) was observed.

MATERIAL AND METHODS

Compounds. Some investigated phenolic compounds (2) are presented in Table 1.

OECD (Q)SAR Application Toolbox. (Quantitative) Structure-Activity Relationships [(Q)SARs] are methods for estimating the properties of a chemical from its molecular structure and have the potential to provide information on the hazards of chemicals, while reducing time, monetary costs and animal testing currently needed. To facilitate practical application of (Q)SAR approaches in regulatory contexts by governments and industry and to improve their regulatory acceptance, the

Table 2. Probable metabolic activation of some plant phenolic compounds by (Q)SAR Application Toolbox

№	Name of compounds	Observed Liver metabolism	Predicted Liver metabolism
1	Chlorogenic acid	0 metabolites:	2 metabolites: - Protein binding – No binding; - DNA binding - No binding;  1)  2)
2	Sinapinic acid	0 metabolites:	13 metabolites: - Protein binding – Michael-type nucleophilic addition; - DNA binding - No binding;  1)  2) - Protein binding – Schiff base formation; - DNA binding - No binding;  3) - Protein binding – No binding; - DNA binding - No binding;  4)  5)  6)  7)  8)  9)  10)  11)  12) - Protein binding – Michael-type nucleophilic addition and Nucleophilic cycloaddition to diketones; - DNA binding - No binding;  13)

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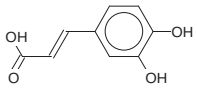
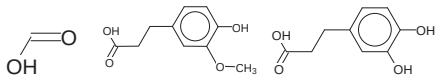
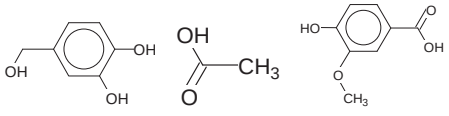
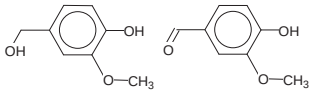
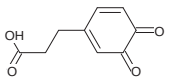
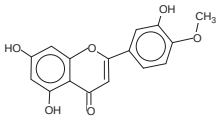
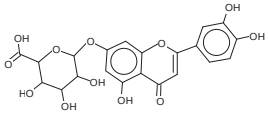
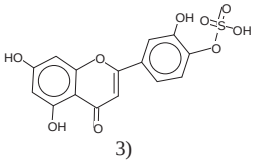
3	Ferulic acid	0 metabolites:	11 metabolites: - Protein binding – Michael-type nucleophilic addition; - DNA binding - No binding;  1) - Protein binding – Schiff base formation; - DNA binding - No binding;  2) - Protein binding – No binding; - DNA binding - No binding;  3) 4) 5)  6) 7) 8)  9) 10) - Protein binding – Michael-type nucleophilic addition and Nucleophilic cycloaddition to diketones; - DNA binding - No binding;  11)	
4	Luteolin	3 metabolites: - Protein binding – Michael-type nucleophilic addition; - DNA binding - No binding;  1)  2)  3)	0 metabolites:	

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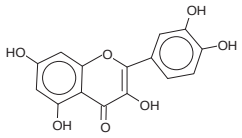
5	Kaemferol	0 metabolites:	1 metabolite: - Protein binding – Michael-type nucleophilic addition; - DNA binding - No binding;  1)
6	Myricetin	0 metabolites:	0 metabolites:

OECD (Q)SAR project has developed various outcomes such as the principles for the validation of (Q)SAR models, guidance documents as well as the QSAR Toolbox (15).

Metabolic pathways documented for 200 organic chemicals in different mammals are stored in a database format that allows easy computer-aided access to the metabolism information. The collection includes chemicals of different classes, with variety of functionalities such as aliphatic hydrocarbons, alicyclic rings, furans, halogenated hydrocarbons, aromatic hydrocarbons and haloaromatics, amines, nitro-derivatives, and multifunctional compounds. *In vivo* and *in vitro* (predominantly, with liver microsomes as experimental systems) studies were used to analyze the metabolic fate of chemicals. Different sources, including monographs, scientific articles and public websites were used to compile the database (10).

RESULTS AND DISCUSSION

The results of the probable metabolic activation in liver (observed and predicted) of some phenolic compounds are presented in Table 2. They are randomly selected because they are a main component in many plants.

The obtained results correspond with an important health care role of these compounds (20). Ferulic, sinapic acids and chlorgenic acids (21) are antioxidants and are reactive towards free radicals such as reactive oxygen species (ROS). Similar effects are also possessed by the mentioned flavonoids luteolin, myricetin and kaemferol (4,8,22). ROS and free radicals are implicated in DNA damage, cancer and accelerated cell aging.

Electrophilic metabolites may not only react with nucleophilic sites in DNA but may also bind to proteins, RNA, and to endogenous substances of lower molecular weight such as glutathione (12). The complexity of the reaction of electrophilic metabolites with the various nucleophilic sites within cells and the reasons why different electrophilic reagents react at different sites have been interpreted on the basis of the concepts of hard and soft electro philes/nucleophiles (hard and soft acids/bases) (13,16,17).

Analysis of data in Table 2 reveals that after metabolic activation in liver (observed pathways) for five of the six plant phenolic compounds by (Q)SAR Application Toolbox liver metabolism was not observed. Only for one compound (Luteolin) of the six metabolic activation in liver (observed pathways) was observed. The Luteolin has three observed metabolites and 0 predicted metabolites in the liver. The observed metabolites have no DNA binding but some of these metabolites have protein binding. The possible mechanism of protein binding for the observed metabolites is Michael-type nucleophilic addition.

One (Myricetin) of the six phenolic compounds was not metabolically activated in liver. The Chlorogenic acid was metabolically activated in liver (observed and predicted metabolism) but its two predicted metabolites were not active – no protein binding.

Kaemferol is a phenolic compound which has one predicted metabolite. The possible mechanism of protein binding for the predicted metabolite is Michael-type nucleophilic addition. The Sinapinic acid has thirteen predicted metabolites. Predicted metabolites have no DNA binding but some of these metabolites have protein binding. Possible mechanisms of protein binding for the observed metabolites are Michael-type nucleophilic addition, Schiff base formation and Nucleophilic cycloaddition to diketones. Ferulic acid has eleven predicted metabolites. The predicted metabolites have no DNA binding but some

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of these metabolites have protein binding. Probable mechanisms of protein binding of the predicted metabolite for Ferulic acid are Michael-type nucleophilic addition, Schiff base formation and Nucleophilic cycloaddition to diketones.

CONCLUSION

Plant phenols play an important role in human health. There is numerous data on the beneficial effects during their application. All this requires an assessment of potential adverse effects on hepatic metabolism and the ability to induce liver injury.

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