THE USE OF GOLDENROD GENERAL (*SOLIDAGO VIRGAUREA*) PREPARATIONS DOES NOT INFLUENCE THE METABOLISM OF CONCOMITANTLY ADMINISTERED DRUGS

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Purpose: *Solidago virgaurea* „goldenrod" is a known medicinal plant that has been used since ancient times (1, 2). The genus *Solidago* is interesting in terms of both biological effects and the presence of many interesting secondary metabolites – flavonoids, phenolic acids and glucosides, polysaccharides and others (3). *Solidago virgaurea* is known to exhibit diuretic, antiinflammatory and newly described antitumoral, antimicrobial, sedative and hypotensive effects (1, 2). Primarily flavonoids and phenolic diglucoside – leiocarposide belong to secondary metabolites having primarily a positive effect on diuresis (3). Our objective was to investigate whether *Solidaginis virgaureae herba* extract, decoction and leachate affect the properties of phase I biotransformation enzymes, namely cytochromes P450 in human liver microsomal fraction and also in primary cultures of human hepatocytes.
Methods: Different concentrations of *Solidaginis virgaureae herba* extract, decoction and leachate, expressed as mg of dry weight in mL of water (0.042 mg/mL; 0.083 mg/mL and 0.167 mg/mL) were studied. Enzyme activities and protein expression were determined using HPLC and Western blotting, respectively.
Results: Activity and protein expression of CYP1A2, CYP2A6, CYP2C9, CYP2D6 and CYP3A4 were only little influenced by *Solidaginis virgaureae herba* preparations, either in human hepatocytes as well as in human liver microsomal fraction in all studied concentrations.
Conclusion: Based on the results of the enzyme activities and the amount of protein can be expected that the *Solidaginis virgaureae herba* preparations do not significantly affect the metabolism of concomitantly administered drugs and their use probably does not result in drug interactions.

Key words: *Solidaginis virgaureae herba*, cytochromes P450, primary cultures of human hepatocytes, human liver microsomal fraction, drug metabolism.

Introduction

Herbal preparations are more and more used to treat mostly the common diseases; in fact, they displace or supplement the conventional medication (4). *Solidago* represents one of the most populated genera belonging to the family of *Asteraceae* comprising 120 species mostly native to Northern America (5, 6). From there, *Solidago* species successfully had spread to the rest of the world (7). In the literature it may be found that *Solidago* plants may exhibit diuretic, antiseptic, choleretic and healing effects (5). The only *Solidago* species native in the Czech Republic is *Solidago* virgaurea, goldenrod general (8). This herbal drug is included in Czech Pharmacopoeia 2009 (9) and has been used since antiquity (1, 2). *Solidago virgaurea* herba has been used to treat the diseases of the urogenital tract, nephrolithiasis, and diseases of the prostate; bloom and leaves have been used as a natural coloring material thanks to their yellow color. This plant has been recently re-discovered in modern phytotherapy (3). It is known not only for its diuretic and antiinflammatory effects but

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for anticancer, antimicrobial, sedative and antihypertensive effects (1, 2). It is probable, than there is not a single compound responsible for all the effects described; on the contrary, there were several components isolated and characterized which may contribute to the beneficial effects of this plant (10). Secondary metabolites, namely, flavonoids and a phenolic diglucoside – leiocarposide have been shown to have a positive effect on diuresis (3).

Drugs as well as natural compounds foreign to the human body (xenobiotics) are subjected, after entering the body, to metabolic processes, or biotransformations, during which a variety of structurally different metabolites are formed and subsequently excreted. Xenobiotics present in the food may however act on the activity of the respective enzymes of biotransformation and, hence, affect the metabolism of drugs given to a patient. The most important enzymes of drug and, more generally, of xenobiotic metabolism are cytochromes P450 (CYP). The most prominent form of CYPs, which is expressed in human liver and intestine and which is known to metabolize the majority of common drugs with known biotransformation pathways, is the form named CYP3A4 (CYPs, in total 57 forms known in humans, are divided according to similarity of their structures to families and subfamilies and labeled by combination of numbers and letters). There are also other CYP forms contributing to metabolism of xenobiotics incl. drugs, as CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A5 or CYP3A7 (11, 12). Broad substrate specificity of drug metabolizing CYP enzymes, i.e. the fact that more enzymes may metabolize the same xenobiotic (which is hence a substrate of these enzymes), is, in principle, an advantage, as in case that one enzyme does not act correctly or not at all, there is another CYP enzyme which may substitute and "help" the body to eliminate the foreign substances. On the other hand, the same is a principle of unwanted drug interactions when one compound interacts with the same enzyme and inhibits the metabolism of another drug metabolized by the same enzyme (13). Another reason for drug interactions may be an induction of a specific CYP enzyme after drug application or after ingestion of a compound in the diet (14). Because of this reason (drug interactions), this paper deals with investigation of possibility that commonly used extract, infusion or leach from goldenrod general (*Solidaginis virgaureae herba*), can influence the activities and expression of enzymes (proteins) in human liver microsomal fraction or in human hepatocytes.

Methods

Preparation of samples tested (extract, infusion or leach) from goldenrod general

Plant extract obtained from Walmark (Prague, CZ) has been prepared according to the producer by extraction to the mixture of organic solvents in 10:1 extraction ratio. The resulting extract contained a minimal amount of resting solvent (max. 0.05 %). This extract which according to the statement of the producer contributes to proper function of the urinary bladder and lower urinary tract is one of components of the Urinal Akut™ product of the producer. One tablet of this preparation contains 30 mg of the dry extract.

Solidaginis virgaureae herba for preparation of the infusion and leach has been obtained from Valdemar Grešik-Natura inc. (Děčín, CZ). For preparation of the infusion, 0.67 g of this product was immersed into 50 mL of cold distilled water. After boiling the content was left for 15 min; filtered and the solution was transferred into Eppendorf tubes and stored frozen at -20 °C.

The leach was prepared from the same amount of the product which has been sprayed by 50 mL of boiling distilled water and left for 15 min; filtered and transferred into Eppendorf tubes and stored frozen at -20 °C until used.

Standardization of samples tested to phenolic diglucoside – leiocarposide

Standardization of the natural material tested was performed on the basis of quantification of the active substance, leiocarposide. Determination of the content of this compound in the samples has been performed by the HPLC method with UV detection using the Prominence HPLC System (Shimadzu, Tokyo, Japan) (3).

Human liver microsomal fraction

Human liver microsomal fraction of liver homogenate has been prepared by the standard procedure using differential centrifugation (15). The use of livers from liver donors has beet approved by Ethical Committee of the Medical Faculty and Faculty Hospital Olomouc according to the 285/2000 Col. Law.

Primary cultures of human hepatocytes

Human hepatocytes were isolated by two-phase collagen perfusion method (16) from human livers of four multi-organ donors (LH63, LH64, LH65, LH66); use of the hepatocytes was approved by the same Ethical Committee as above. Hepatocytes were seed onto the cultivation plates and incubated with the extract, infusion and leach from the *Solidago virgaurea* at concentrations of 0.042 mg/mL; 0.083 mg/mL and 0.167 mg/mL for 24 h.

To study the metabolic activity of the cultured human hepatocytes and to exclude the possible toxicity of the samples studied a cytotoxicity test with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The principle of this test is a reduction of the yellow water-soluble tetrazolium salt by mitochondrial enzymes to a violet formazan derivative insoluble in water. After dissolution of this derivative in organic solvent, the concentration of the product formed is determined spectrophotometrically at 540 nm (17).

Determination of enzyme activities of selected CYP forms

Enzyme activities were determined in human liver microsomal fraction as well as in primary cultures of human hepatocytes according to standard procedures described in (18): CYP1A2, phenacetin O-deethylation; CYP2A6, coumarin 7-hydroxylation; CYP2C9, warfarin 7-hydroxylation; CYP2D6, bufuralol 1´-hydroxylation; CYP3A4, testosterone 6beta-hydroxylation. Enzyme activities were assessed in two parallel determinations in duplicates in microsomal fraction and in four samples from human hepatocytes from different donors. All the enzyme activities were analyzed using the HPLC Prominence System (Shimadzu, Tokyo, Japan) with the UV or fluorescence detection.

Determination of protein expression of selected CYP enzymes by Western blotting

Proteins of liver samples were separated by electrophoresis (SDS-PAGE, 10% separa-

Fig. 1. *Cytotoxity test for a) extract, b) infusion and c) leach from Solidago virgaurea in concentrations 0.042 mg/mL; 0.083 mg/mL and 0.167 mg/mL. Control, distilled water. Data are expressed as per cent of control as averages ± S. D. from two experiments. Values do not significantly differ at p > 0.05*

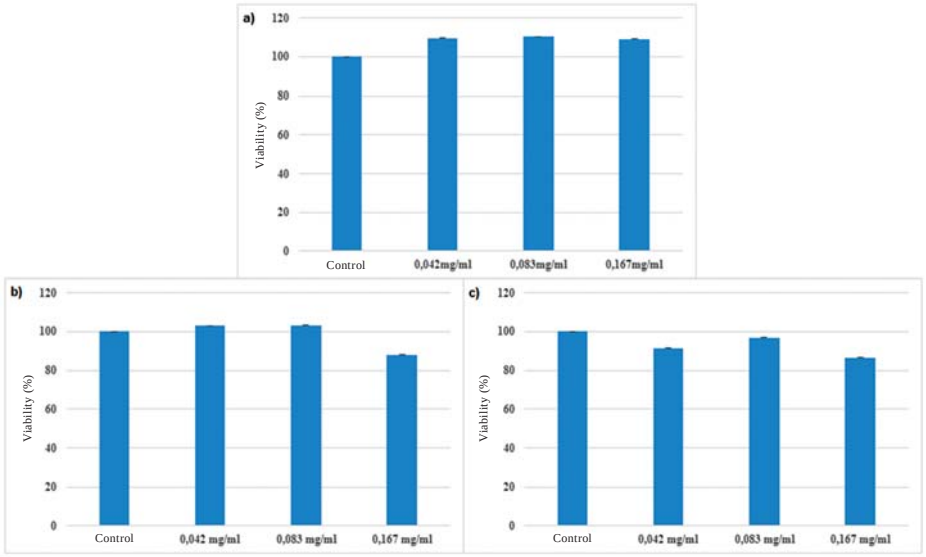
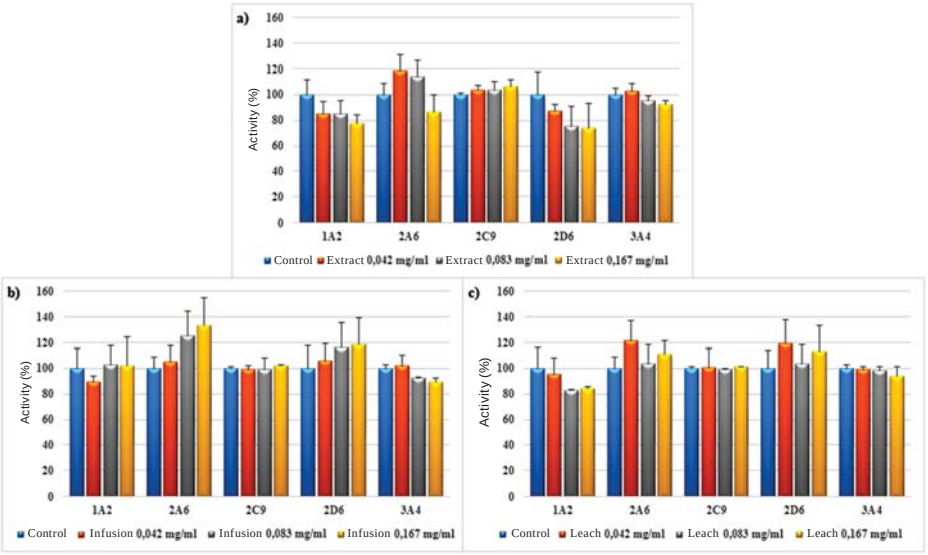


Fig. 2. *Effect of a) extract, b) infusion and c) leach from Solidago virgaurea in concentrations 0.042 mg/mL; 0.083 mg/mL and 0.167 mg/mL on activities of CYP1A2, CYP2A6, CYP2C9, CYP2D6 and CYP3A4 in human liver microsomal fraction. Activities are expressed as averages ± S. D. from two independent experiments performed in duplicates. Control, distilled water. Inhibition expressed as per cent of control. Data are not significantly different at p > 0.05*



tion gel) and subsequently transferred onto nitrocellulose membranes (0.45 micrometers) by Trans-Blot™ Turbo Transfer System (Bio-Rad, Palo Alto, CA, U.S.A.). Immunodection of the CYP enzymes was performed using mouse primary antibodies of the CYP1A2, CYP2A6, CYP2D6 and CYP3A4 forms; rabbit antibodies were used for CYP2C9 (Acris Antibodies, Herford, Germany). Secondary antibodies (with horseradish peroxidase) were anti-mouse (Santa Cruz Biotechnology, Dallas, TX, U.S.A.) and anti-rabbit (Acris Antibodies, Herford, Germany). Signal was detected using a chemiluminiscence kit (Amersham Biosciences, Amersham, UK). Signal intensity of selected proteins was evaluated using a C-DiGit™ Blot Scanner (Li-Cor, Lincoln, NE, U.S.A.).

Statistical analysis

Statistical analysis of data was performed using the Student´s t-test (significance level 0.05) by Microsoft Excel and Statistica 12 (Systat Software, San Jose, CA, U.S.A.)

Results

To study the possible effect of compounds present in goldenrod (*Solidago virgaurea*) on the most important CYP forms, two *in vitro* approaches were used: First, primary cultures of human hepatocytes were used to follow the possible changes in expression and activity of the respective CYP enzymes; second, inhibition of respective CYP enzyme activities in human liver microsomes by goldenrod compounds was used.

At first, the toxicity of the compounds tested by MTT test on human hepatocytes. Results show that the goldenrod preparations in the concentrations used are not toxic and can be used in the following experiments with hepatocytes and microsomal fraction (Fig. 1).

Influence of the Solidago extract, infusion and leach on activities of CYP enzymes in human liver microsomal fraction

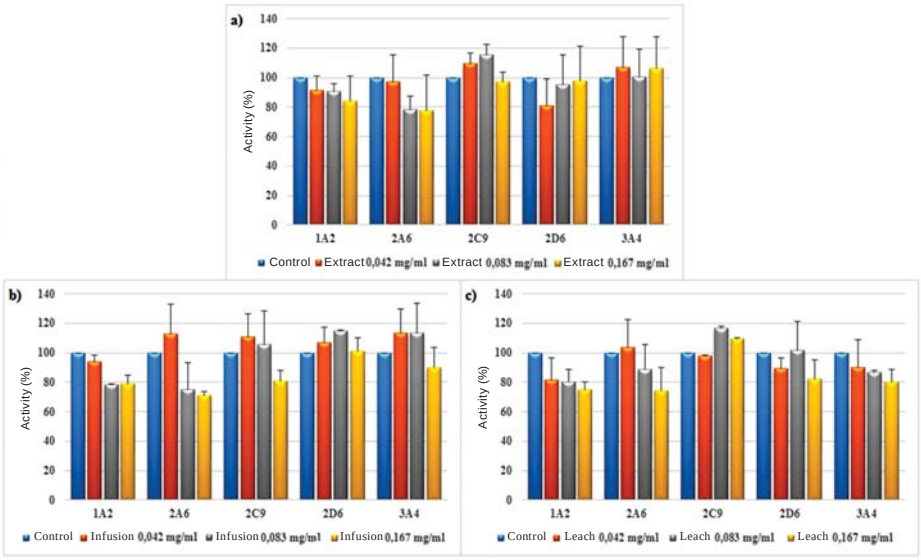
Activities of five main CYP enzymes involved in metabolism of xenobiotics were determined using their specific substrates. Effect of various *Solidago* preparations on these activities was studied; results were quantified using the HPLC and were presented in Fig. 2A, 2B and 2C. There were no significant changes in activities of all the CYP enzymes by goldenrod preparations at all concentrations used indicating no significant influence of goldenrod (*Solidago*) on the human liver microsomal drug-metabolizing enzymes.

Influence of the Solidago extract, infusion and leach on expression of CYP proteins and on their respective enzyme activities in primary cultures of human hepatocytes

Primary cultures of human hepatocytes represent a more complex system than the microsomal fraction of liver homogenate as they preserve the structural organization of the cell. To study the possible changes in the expression and enzyme activities of CYP proteins, the cells were incubated with the extract, infusion and leach from the *Solidago virgaurea* at concentrations of 0.042 mg/mL; 0.083 mg/mL and 0.167 mg/mL for 24 h as stated in the Methods. After that time, specific substrates of individual CYP enzyme forms were added to the medium. he results are displayed in Fig. 3. The results obtained show that after application of *Solidago* preparations

in all three concentrations to human hepatocytes, no significant changes in the respective enzyme activities of CYP1A2, CYP2A6, CYP2C9, CYP2D6 and CYP3A4 were found. The data obtained by checking the enzyme activities correspond well to results on the expression of the CYP proteins by Western Blotting (Fig. 4). Again, there were no prominent changes in the protein expression found.

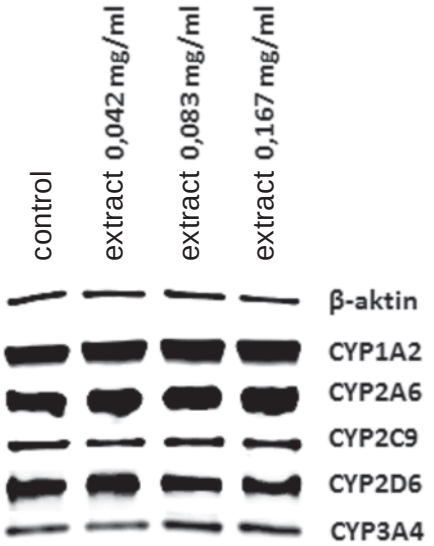
Fig. 3. *Effect of a) extract, b) infusion and c) leach from Solidago virgaurea in concentrations 0.042 mg/mL; 0.083 mg/mL and 0.167 mg/mL on activities of CYP1A2, CYP2A6, CYP2C9, CYP2D6 and CYP3A4 in primary cultures of human hepatocytes. Primary cultures were incubated for 24 h with Solidago preparations in the concentrations as above. Results do not exhibit significant differences (p > 0.05)*



Discussion

There is a growing interest in population worldwide regarding the use various plant preparations and food supplements, mirroring the expansion of the alternative approaches and of sales of nutraceuticals and functional foods. Also, the consumption of drugs based on herbal medicines steadily increases (19, 20). Herbal formulations are also used as food supplements, teas, tonics, and as preventive agents (21). Goldenrod (*Solidago virgaurea*), dandelion (*Taraxacum officinale*, *resp. Taraxacum sect. Ruderalia*), are well known for their diuretic effect and are used in modern phytotherapy. Ethanolic extract from dandelion has been found to increase the frequency of urination in humans (22); *in vitro* studies on activities of rat CYP enzymes indicated a lowering of activity of CYP1A2 and CYP2E1 forms (21). It has been found that also the extract from parsley (*Petroselium crispum*) known from folk and complementary medicine acts as a strong diuretic (23); again, a lowering of the liver CYP activities in mice has been observed and these findings were confirmed also in vivo (24). Green tea, a rather popular beverage, is also, as an extract, used as a component of

Fig. 4. *Representative Western blots of human liver enzymes CYP1A2, CYP2A6, CYP2C9, CYP2D6 and CYP3A4 in primary cultures of human hepatocytes after incubation with Solidago extract in all three concentration used. Control, beta actin*



food supplements (25). It has been shown recently that its consumption has a diuretic effect in man; however, it may influence the activities of liver CYP enzymes in mice leading to a slight decrease of their activities in low doses but to a significant increase of their activities after consumption of high doses (25). A study dealing with effects of various herbal preparations used as herbal medicines or food supplements on potential induction of expression of genes of the CYP1A and CYP3A enzymes indicated a weak however significant induction of the CYP3A4 gene by extract from *Solidago virgaurea* in LS180 cells of human colon carcinoma (27). However, in the cancer cells model, an alteration of regulation of expression of genes corresponding to enzymes of biotransformation as CYP3A4, in comparison to non-cancer cultures of primary human hepatocytes used in this study, may take place.

Infection of urinary tract is, in fact, the most common bacterial disease in humans (28). Herbal preparations with diuretic effects may however be able to influence the activities of the enzymes of biotransformation and hence also the pharmacokinetics and efficacy of the drugs used to treat this condition. This is why also this study has been performed studying whether the preparations of *Solidago* (which is, e.g., one of components of a popular food supplement Urinal Akut™) may influence the activity of enzymes of drug metabolism. In the literature, a recommendation to avoid using the *Solidago* preparations by patients exhibiting an allergy to this herb and by patients with renal failure may be found; it is also strongly recommended to avoid the use of it by pregnant and breastfeeding women even for short time (10).

Our *in vitro* study has been primarily focused on the possible influence of *Solidago* preparations on the activities of human liver microsomal CYP1A2, CYP2A6, CYP2C9, CYP2D6 and CYP3A4 enzymes. Also, the use of human hepatocytes might well indicate an induction of the activity and expression of the CYP enzymes. The results show that the preparations of *Solidago virgaurea* does not significantly influence the enzyme activities and expression of the respective proteins. It may be, hence, reasonably expected that the use of an extract, infusion or leach obtained from this herb most probably has no significant effect on the metabolism of concomitantly used drugs metabolized by the respective CYP enzymes. This fact is, however, an advantage for the use of *Solidago* preparations as a herbal medicine or a food supplement.

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REFERENCES

1. Kalemba D. Constituents of the essential oil of Solidago virgaurea L. Flavour Fragr J 1998; 13(6): 373–376.
2. Dobjanschi L, Antonescu A, Zdrinca M, et al. HPLC-MS analysis of flavonoids obtained from Solidago sp. (Asteraceae). Analele Universitatii din Oradea, Fascicula: Protectia Mediului 2012; 19: 75–79.
3. Thiem B, Wesolowska M, Skrzypczak L, Budzianowski J. Phenolic compounds in two Solidago L. species from in vitro culture. Acta Pol Pharm 2001; 58(4): 277–281.
4. Apáti P, Szentmihályi K, Kristó ST, et al. Herbal remedies of Solidago-correlation of phytochemical characteristics and antioxidative properties. J Pharm Biomed Anal 2003; 32(4–5): 1045–1053.
5. Jiang T, Huang BK, Qin LP. A survey of chemical and pharmacological studies on Solidago. Zhong Xi Yi Jie He Xue Bao 2006; 4(4): 430–435.
6. Demir H, Acik L, Bali EB, Koc LY, Kaynak G. Antioxidant and antimicrobial activities of Solidago virgaurea extracts. Afr J Biotechnol 2009; 8(2): 274–279.
7. Szymura M, Szymura TH. Soil preferences and morphological diversity of goldenrods (Solidago L.) from south-western Poland. Acta Soc Bot Pol 2013; 82(2): 107–115.
8. Pyšek P, Danihelka J, Sádlo J, et al. Catalogue of alien plants of the Czech Republic (2nd edition): checklist update, taxonomic diversity and invasion patterns. Preslia 2012; 84: 155–255.
9. Czech Pharmacopoeia 2009, Solidaginis virgaureae herba (Nať zlatobýlu obecného) 6.0:1893 (díl 3, str. 3153–3154). Grada, Praha 2009.
10. Yarnell E. Botanical medicines for the urinary tract. World J Urol 2002; 20(5): 285–293.
11. Ghose R. Clinical consequences of altered drug disposition in obesity. J Clin Trials 2012; 1: e107.
12. Anzenbacher P, Anzenbacherová E. Cytochromes P450 and metabolism of xenobiotics. Cell Mol Life Sci 2001; 58(5–6): 737–747.
13. Pelkonen O, Turpeinen M, Hakkola J, Honkakoski P, Hukkanen J, Raunio H. Inhibition and induction of human cytochrome P450 enzymes: current status. Arch Toxicol 2008; 82(10): 667–715.
14. Lin JH, Lu AY. Inhibition and induction of cytochrome P450 and the clinical implications. Clin Pharmacokinet 1998; 35(5): 361–390.
15. Phillips IR, Shephard EA, Ortiz de Montellano PR, eds. Cytochrome P450 protocols. 3rd ed. New York, NY: Springer Science + Business Media, 2013: 987.
16. Pichard L, Gillet G, Fabre I, et al. Identification of the rabbit and human cytochromes P-450IIIA as the major enzymes involved in the N-demethylation of diltiazem. Drug Metab Dispos 1990; 18(5): 711–719.
17. Sieuwerts AM, Klijn JG, Peters HA, Foekens JA. The MTT tetrazolium salt assay scrutinized: how to use this assay reliably to measure metabolic activity of cell cultures in vitro for the assessment of growth characteristics, IC50-values and cell survival. Eur J Clin Chem Clin Biochem 1995; 33(11): 813–823.
18. Chang TK, Waxman DJ. Catalytic assays for human cytochrome P450: an introduction. Methods Mol Biol 2006; 320: 73–83.
19. Ohnishi N, Yokoyama T. Interactions between medicines and functional foods or dietary supplements. Keio J Med 2004; 53(3): 137–150.
20. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 2013; 4: 177.
21. Maliakal PP, Wanwimolruk S. Effect of herbal teas on hepatic drug metabolizing enzymes in rats. J Pharm Pharmacol 2001; 53(10): 1323–1329.
22. Clare BA, Conroy RS, Spelman K. The diuretic effect in human subjects of an extract of Taraxacum officinale folium over a single day. J Altern Complement Med 2009; 15(8): 929–934.
23. Kreydiyyeh SI, Usta J. Diuretic effect and mechanism of action of parsley. J Ethnopharmacol 2002; 79(3): 353–357.
24. Jakovljevic V, Raskovic A, Popovic M, Sabo J. The effect of celery and parsley juices on pharmacodynamic aktivity of drugs involving cytochrome P450 in their metabolism. Eur J Drug Metab Pharmacokinet 2002; 27(3): 153–156.
25. Matoušková P, Bártíková H, Boušová I, et al. Effect of defined green tea extract in various dosage schemes on drug-metabolizing enzymes in mice in vivo. J Funct Foods 2014; 10: 327–335.
26. Chacko SM, Thambi PT, Kuttan R, Nishiqaki I. Beneficial effects of green tea: a literature review. Chin Med 2010; 5(13): 1–9.
27. Brandin H, Viitanen E, Myrberg O, Arvidsson AK. Effects of herbal medicinal products and food supplements on induction of CYP1A2, CYP3A4 and MDR1 in human colon carcinoma cell line LS180. Phytother Res 2007; 21(3): 239–244.
28. Nicolle LE. Epidemiology of urinary tract infections. Clin Microbiol Newsletter 2002; 24(18): 135–140.