

EXPERIMENTAL PAPER

Impact of *Panax ginseng* and *Ginkgo biloba* extracts on expression level of transcriptional factors and xenobiotic-metabolizing cytochrome P450 enzymes

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## Summary

**Introduction:** Despite widespread use of *Panax ginseng* and *Ginkgo biloba*, the data on the safety as well as herb-drug interactions are very limited. Therefore, we postulate that *P. ginseng* and *G. biloba* may modulate the activity and content of cytochrome P450 isozymes involved in the biotransformation of diverse xenobiotic substances. **Objective:** The aim of our study was to determine the influence of herbal remedies on the expression level of CYP enzymes and transcriptional factors. **Methods:** Male Wistar rats were given standardized *Panax ginseng* (30 mg/kg p.o.) or standardized *Ginkgo biloba* (200 mg/kg p.o.) for 3 and 10 days. The expression in liver tissue was analyzed by real-time PCR method. **Results:** Our results showed a decrease of CYP3A1 (homologue to human CYP3A4) mRNA level after *P. ginseng* extract treatment. The CYP2C6 (homologue to human CYP2C9) expression was also reduced. Additionally, after 10 days of the treatment with *P. ginseng* an increase of CYP1A1 (homologue to human CYP1A1) and CYP1A2 (homologue to human CYP1A2) expression was observed. Moreover, *G. biloba* extract also caused an increase of expression level for CYP1A1, CYP2C6, CYP3A1 and CYP3A2. **Conclusion:** Our findings suggest that herbal extracts can modulate the expression of transcriptional factors and CYP enzymes involved in xenobiotic metabolism and chemical carcinogenesis.

*Key words:* cytochrom P450, expression, herbal extracts, real-time PCR, drug metabolism, chemical carcinogenesis

## INTRODUCTION

Many herbal medicines often used in prevention of civilization diseases as well as an additive co-medication during therapy with synthetic drugs, can influence the activation of nuclear receptors and CYP enzymes leading to herb-drug interactions and the appearance of undesirable side effects.

Ginseng (*Panax ginseng*) is one the most widely used herbal remedies. It is well known since ancient times as a stimulant, tonic diuretic and digestive aid. This plant includes the active compounds, such as saponins, polysaccharides, flavonoids and volatile oils. Ginseng's saponins generally called ginsenosides

are mainly responsible for physical and mental performance as well as providing resistance to stress and disease. The main compounds involved in many pharmaceutical actions of ginseng are ginsenosides Rb1 and Rg1 that have both stimulatory and inhibitory effects on the central nervous system (CNS) [1]. These active compounds possess mild sympathomimetic activity and may interact with monoamine oxidase inhibitors [2]. A number of studies report that increased consumption of ginseng products reduced the risk of most cancers, as compared with ginseng non-intakers. It results from activities of ginsenosides that show cytotoxic and inhibitory growth effects against tumor cells via different mechanisms [3, 4]. Moreover, ginseng may lower blood sugar levels by increasing insulin production and the sensitivity of the organism to insulin. In addition, *P. ginseng* has positive effects on the cardiovascular system by diverse mechanisms involving nitric oxide, reducing platelet adhesion, modifying vasomotor function and influencing ion channels [5, 6]. Numerous researches claim that ginseng may interact with oral anticoagulants, hypoglycaemic agents, corticosteroids and antiplatelet agents [6].

As a source of herbal medicine, *Ginkgo biloba* has antioxidant and neuroprotective activity. It contains pharmacologically active constituents, such as flavonol glycosides and terpene lactones (ginkgolides A, B, C and bilobalides) that may be useful in the treatment of Alzheimer's disease and cognitive impairment. *G. biloba* has anti-platelet activities and may cause interactions with other anti-platelet agents (warfarin, aspirin) or herbal remedies possessing similar anti-platelet activities (garlic or ginseng) [7, 8].

Clinical studies suggest that herbal remedies can modulate the activities of cytochrome P450 (CYP450) enzymes and may influence the induction of interactions with synthetic drugs. The cytochrome P450 are heme-containing monooxygenases responsible for the metabolism of xenobiotics (drugs, carcinogens) and food components as well as the synthesis of endogenous compounds. The CYPs most active in drug metabolism are CYP2C, CYP2D and CYP3A subfamilies, especially CYP3A4 isoforms involved in the metabolism of nearly 50% of clinically used drugs. The CYP1A, CYP2A and CYP2E subfamilies metabolize many protoxins and procarcinogens to their ultimate reactive metabolite. However, the metabolism foreign compounds by these enzymes not always may lead to detoxification of these compounds but in some cases initiate the chemicals carcinogenesis.

The regulation of cytochrome P450 gene expression depends on the interaction of the xenobiotics with the receptors. It was shown that the CYP1 subfamily can be induced by the AhR/ARNT pathway in response to xenobiotics, such as PAHs. Similarly, the constitutive androstane receptor (CAR) and pregnane X receptor (PXR) form a heterodimer with the retinoid X receptor (RXR) and transcriptionally activate the promoters of CYP2C9 and CYP3A gene expression. Some studies shows that HNF1 and HNF4 are general regulators supporting the

constitutive expression of major CYPs (CYP2D6, CYP3A4, CYP2C9, CYP1A) in hepatocytes [9]. Studies on the expression and activity of enzymes catalyzing the biotransformation of xenobiotics are important from the perspective of herb-drug interaction and their physiological functions.

Moreover, the changes in the activity of specific CYP450 isoforms may significantly alter the efficacy or toxicity of conventional drugs, whose biotransformation pathways depend on these same isoforms. Therefore, further studies are needed to confirm the potential drug-drug interactions as well as food-drug and herb-drug interactions due to the fact that CYPs catalyze a very large number of substrates including most clinically used drugs in different types of reactions. Hence, the aim of this study was to determine whether standardized extracts of *Panax ginseng* and *Ginkgo biloba* may influence the gene expression of cytochrome P450 and their transcriptional factors in animal model.

## MATERIAL AND METHODS

### Standardized herbal extracts

The standardized extracts of *Panax ginseng* C.A. Meyer (roots; *Araliaceae*) and *Ginkgo biloba* L. (leaves, *Ginkgoaceae*) were used in this study, both obtained from Finzelberg GmbH & Co. KG (Germany). The content of active compounds of selected dry alcoholic extracts was determined using high performance liquid chromatography (HPLC) method, according to procedures included in the *European Pharmacopoeia* [10]. An analysis of dried ethanolic extracts: from dried root of *P. ginseng* (ethanol 60%, v/v) revealed the presence of ginsenosides (27.1%), from leaves of *G. biloba* (ethanol 80%, v/v) the presence of flavonol glycosides (24.5%), terpene lactones (13%) and ginkgolic acids (5.0 µg/g).

### Experimental design

The experiment in rats was performed in accordance with Polish governmental regulations (Decree on Animal Protection Dz. U. 97. 111. 724, 1997) and Local Ethics Committee in Poznań (No. 16/2010). Male Wistar rats weighing 200–250 g were housed in plastic cages in the Department of Pharmacology, Poznan University of Medical Sciences, Poland. The animals were maintained in a climate-controlled room with 12h light/dark cycle and allowed access to a commercial rat chow and tap water *ad libitum*. They were acclimatized for at least a few days prior to experiment. The animals were randomly divided into six groups (ten per

group). Control groups were fed standard rat chow diet and remained untreated for the whole time of the study. Experimental groups received standardized *P. ginseng* (30 mg/kg p.o., once daily), or standardized *G. biloba* (200 mg/kg p.o., once daily) for 3 and 10 days. Two hours after the last administration, rats were decapitated. The samples of liver were immediately frozen in liquid nitrogen and stored at -80°C.

## RNA extraction and cDNA synthesis

Total cellular RNA was isolated from the rat livers with TriPure Isolation Reagent (Roche) according to the manufacturer's protocol. The RNA pellet was washed with 70% ethanol and dissolved in DEPC water. The concentrations and the purity of RNA were determined by the measurement of the absorbance at 260 and 280 nm in a spectrophotometer (BioPhotometer Eppendorf). RNA samples were stored at -80°C. Complementary DNA was synthesized from 2 µg of total RNA in a total volume of 20 µl using the SuperScript™ III First-Strand Synthesis System (Invitrogen) and oligo(dT)<sub>20</sub> primer (Life Technologies). The obtained transcripts were stored at -20°C or used directly for the real-time PCR (RT-PCR).

## Real-time PCR

The level of gene expression in liver tissues was analyzed by real-time quantitative PCR. The primers and RT-PCR conditions used for CYP1A1/2, CYP2D1/2, CYP3A1/2, CYP2E1, CYP2C6 and GAPDH amplifications were described by Mrozikiewicz *et al.* [11]. The primers and RT-PCR conditions used for CAR, PXR, RXR, GR, AHR, HNF-1 and HNF-4 amplifications are described in table 1. All oligonucleotide sequences were synthesized by TIB Molbiol (Poland). Amplicon size and reaction specificity were confirmed by agarose gel electrophoresis and melting curve analysis. RT-PCR was carried out using a LightCycler™ Instrument (Roche, Germany) and a LightCycler DNA Master SYBR Green I kit (Roche, Germany) according to the manufacturer's protocol. GAPDH was used as a housekeeping gene for normalization. The PCR program was initiated with an activation at 95°C for 10 min. Each PCR cycle comprised a denaturation step at 95°C, an annealing step at a specific temperature and an extension step at 72°C. The quantitative PCR was monitored by measuring the increase in fluorescence by the binding of SYBR Green I dye to the generated double-stranded cDNA. The data were evaluated with the Roche LightCycler Run 5.32 software.

Table 1.

Sequences of primers used for the RT-PCR analysis and PCR conditions

Gene	Primer sequence (5' → 3')	Product size (bp)	PCR conditions			
			Cycle	Denaturation	Annealing	Extension
CAR F CAR R	GGA GGA CCA GAT CTC CCT T GAC CGC ATC TTC CAT CTT GT	130	35	95°C, 8 s	58°C, 8s	72°C, 8 s
PXR F PXR R	TCC ACT GCA TGC TGA AGA AG AAC CTG TGT GCA GGA TAGGG	187	35	95°C, 8 s	55°C, 8 s	72°C, 8 s
RXR F RXR R	CCT GAG TTC TCC CAT CAA TG GAC GCC ATT GAG GCC TAG A	190	35	95°C, 8 s	57°C, 7s	72°C, 8 s
GR F GR R	CTG GAA TAG GTG CCA AGG CT CCG TAA TGA CAT CCT GAA GCT	210	40	95°C, 10s	58°C, 8 s	72°C, 8 s
AHR F AHR R	ATAGCTACTCCACTTCAGCC TCATGCCACTTTCTCCAGTC	244	35	95°C, 8 s	52°C, 8 s	72°C, 8 s
HNF-1 $\alpha$ F HNF-1 $\alpha$ R	GCT CGG AAG ATG ACA CGG AT CTT GTT GAG GTG CTG GGA CA	245	35	95°C, 8 s	60°C, 7s	72°C, 8 s
HNF-4 $\alpha$ F HNF-4 $\alpha$ R	CTG GAG GAT TAC ATC AAC GAC GTG TTC TTG CAT CAG GTG AG	164	40	95°C, 8 s	57°C, 7s	72°C, 8 s

F – forward

R – reverse

## Statistical analysis

The results were expressed as mean  $\pm$  SEM. Statistical significance of the difference between the control and experimental group was assessed using ANOVA test. The threshold of significance was  $p < 0.05$ .

## RESULTS

The aim of the study was to assess the influence of standardized extracts of *P. ginseng* and *G. biloba* on the mRNA expression level of the major isozymes of CYPs involved in the biotransformation of xenobiotics. The mRNA transcription level in liver tissues was analyzed with RT-PCR method. As shown in figure 1, no significant changes in activity were observed for CYP1A1/2, CYP2D1 and CYP3A2 after 3 days of *P. ginseng* treatment. However, statistically significant differences for these genes were presented after long-term application. The

use of ethanolic extract resulted in a significant increase of CYP1A1 expression level about 40% ( $p=0.034$ ) compared with control group fed standard diet. Similar effect to CYP1A1 was observed for CYP1A2 (increase of expression about 30%). In case of CYP2D1, it was also observed a significant increase of mRNA expression level about 60% ( $p<0.05$ ) after long application of the extract. However, a weak inductive effect of extract by 22% ( $p=0.048$ ) was only observed for CYP3A2 compared with the control group. In case of CYP2D2, the expression of this gene was inhibited by extract both after 3 and 10 days. The mRNA transcription level of CYP2D2 was decreased by 38% ( $p<0.05$ ) and 22% ( $p<0.05$ ), respectively. In addition, a statistically significant decrease in expression was observed for CYP3A1 both after 3 and 10 days of extract treatment. The level of CYP3A1 mRNA was reduced by 38% ( $p<0.05$ ) and 35% ( $p<0.05$ ), respectively. Moreover, the level of CYP2C6 mRNA was also reduced by 38% ( $p<0.05$ ) after 3 days of treatment, whereas long-term application of *P. ginseng* extract caused an increase of mRNA expression level about 40% ( $p>0.05$ ) compared with the control group fed standard diet.

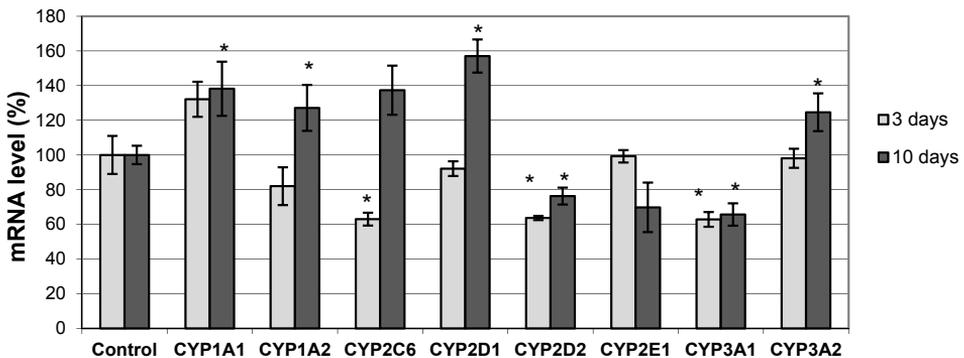


Figure 1.

The influence of *Panax ginseng* on the gene expression of cytochrome P450 in the liver of male rats after 3 and 10 days of treatment. The control group were defined as 100%. Data were presented as mean  $\pm$  SEM of 10 rats in each group. \* $p<0.05$  as compared with the control group.

Furthermore, *G. biloba* extract caused an increase of expression level for CYP1A1 (65%,  $p<0.05$ ), CYP2C6 (115%,  $p<0.05$ ), CYP3A1 (54%,  $p<0.05$ ) and CYP3A2 (38%,  $p<0.05$ ) after 3 days. Additionally, the increase of mRNA level was also observed for CYP2C6 (about 60%,  $p<0.05$ ) after 10 days. A statistically significant decrease in expression was observed for CYP2E1 both after 3 and 10 days of extract treatment (fig. 2). Changes of mRNA levels were also noted for transcriptional factors. These results are presented in table 2.

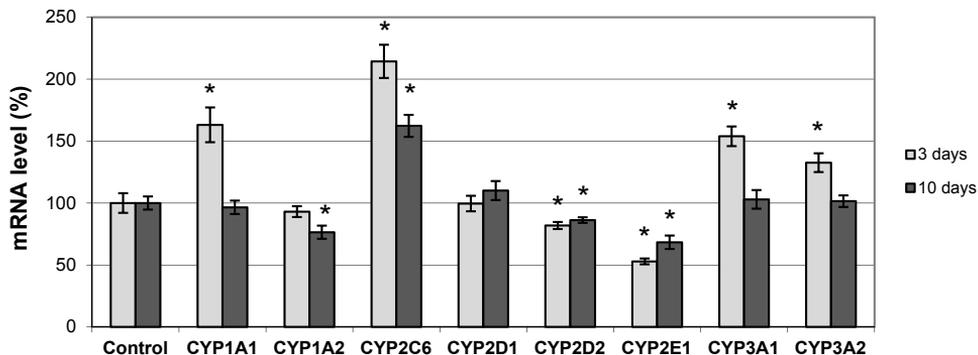


Figure 2.

The influence of *Ginkgo biloba* on the gene expression of cytochrome P450 in the liver of male rats after 3 and 10 days of treatment. The control group were defined as 100%. Data were presented as mean  $\pm$  SEM of 10 rats in each group. \* $p < 0.05$  as compared with the control group.

Table 2.

The mRNA level of transcriptional factors after *Panax ginseng* and *Ginkgo biloba* extracts administration

Gene	3 days		10 days	
	Mean $\pm$ SEM (%)	<i>p</i> -value	Mean $\pm$ SEM (%)	<i>p</i> -value
<i>Panax ginseng</i>				
AHR	86.21 $\pm$ 5.92	NS	114.32 $\pm$ 10.71	NS
PXR	92.44 $\pm$ 5.36	NS	123.83 $\pm$ 8.28	0.023
RXR	76.17 $\pm$ 4.64	0.041	102.64 $\pm$ 6.39	NS
CAR	101.12 $\pm$ 7.92	NS	164.43 $\pm$ 10.67	<0.0001
GR	83.58 $\pm$ 2.82	0.009	116.91 $\pm$ 4.17	0.011
HNF-1 $\alpha$	96.72 $\pm$ 8.26	NS	98.65 $\pm$ 4.51	NS
HNF-4 $\alpha$	83.21 $\pm$ 4.54	0.037	78.01 $\pm$ 7.29	NS
<i>Ginkgo biloba</i>				
AHR	140.32 $\pm$ 11.1	0.022	121.12 $\pm$ 11.48	NS
PXR	82.91 $\pm$ 5.11	0.034	91.03 $\pm$ 3.29	NS
RXR	95.77 $\pm$ 3.25	NS	114.82 $\pm$ 7.36	NS
CAR	83.11 $\pm$ 4.04	0.42	77.32 $\pm$ 9.32	0.32
GR	90.61 $\pm$ 6.28	NS	90.16 $\pm$ 5.28	NS
HNF-1 $\alpha$	208.51 $\pm$ 8.18	<0.0001	145.56 $\pm$ 10.9	0.011
HNF-4 $\alpha$	102.077 $\pm$ 7.12	NS	76.02 $\pm$ 3.41	0.34

The control group were defined as 100%.

## DISCUSSION

Observations from *in vitro* studies on the activity of CYP1A1/2 influenced by *P. ginseng* are different from the results from our earlier *in vivo* study. The use of ethanolic extract resulted in a significant increase of CYP1A1 expression level compared with control group fed standard diet. Similar effect was observed for CYP1A2. *In vitro* study conducted by Chang *et al.* showed that standardized *P. ginseng* extract (G115) decreased human recombinant CYP1A1 *via* competitive inhibition and CYP1A2 *via* linear-mixed inhibition activities in a concentration-dependent manner [12]. Rb1, Rb2, Rc, Rd, Re, Rf and Rg1 at low concentrations had no effect on CYP1 activities, but Rb1, Rb2, Rc, Rd and Rf at higher ginsenoside concentration (50 mg/ml) inhibited these activities. These results indicated that various ginseng extracts and ginsenosides inhibited CYP1 activity in an enzyme-selective and extract-specific manner [12].

No significant changes in the mRNA levels were observed for CYP1A1/2, CYP2D1 and CYP3A2 after 3 days of extract treatment in our study. Three days of administration of the *P. ginseng* extract were too short to get the effect of changes in the level of expression of CYP enzymes. In the study prepared by Ueng *et al.*, treatment with 50 mg/kg of ethanol extract of *P. ginseng* did not affect liver microsomal 7-ethoxyresorufin O-deethylation, pentoxyresorufin O-dealkylation, tolbutamide hydroxylation, nitrosodimethylamine N-demethylation, and nifedipine oxidation activities, which worked as marker activities of Cyp1a, Cyp2b, Cyp2c, Cyp2e1, and Cyp3a, respectively [13]. Meanwhile, 3 days of treatment the level of CYP2E1 produced no inhibition of this gene whereas long application of this extract decrease the mRNA level about 25% ( $p > 0.05$ ). The *in vitro* experiments have also shown that both crude ginseng extract and total saponins inhibited CYP2E1 activity in mice and human microsomes [14].

Also *in vivo* results from studies prepared by Lee *et al.* are in contrary to our results. In the first study subchronic administration of *P. ginseng* extracts (100 mg/kg/day for 14 days from 1 week after a single intraperitoneal injection of 1  $\mu$ g of TCDD/kg of body weight) was examined on the hepatic cytochrome P450-dependent monooxygenase system of guinea pigs pre-exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). It has been shown that *P. ginseng* extracts alone decreased the contents of cytochrome P450 by 33% and may act as an inhibitor of CYP1A [15]. In the second study, the effects of *P. ginseng* extracts on expression of cytochrome P450 (CYP) 1A1 were evaluated in the testis of rats exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). It was observed that the expression of CYP1A1 mRNA were significantly increased in rat testes. There were no significant differences between the control and animals treated with *P. ginseng* extracts. However, a significantly decreased level of DNA damage, decreased CYP1A1 expression and reduced pathological effects were observed in the 2,3,7,8-TCDD with *P. ginseng* extracts treated groups when compared with the TCDD treated group. This study demonstrates that *P. ginseng* extract treatment exhibits

a therapeutic capacity to reduce pathological effects of 2,3,7,8-TCDD *via* the reduction of CYP1A1 mRNA [16]. Furthermore, studies examining the influence of *P. ginseng* on CYP3A activity in humans are limited.

Our results concerning the influence of *G. biloba* extract on the CYP enzymes expression differ depending on the time of extracts administration. In fact, available data for effects of *G. biloba* extract on cytochrome P450 activity *in vitro* and *in vivo* from animal and human studies is inconsistent. In the study conducted by Ribonnet *et al.*, using Caco-2 cells a strong induction of CYP1A1 activity of the *G. biloba* extract after 6 h exposure at the realistic level of 100 µg/ml was demonstrated. In our study, there was an increase of CYP1A1 expression after 3 days of administration [17]. Different results have been observed by Kuo *et al.* who investigated the *in vitro* effect of *G. biloba* extracts on CYP1A-mediated 7-ethoxyresorufin O-dealkylation in hepatic microsomes isolated from rats induced with β-naphthoflavone. The authors observed that *G. biloba* extract competitively inhibited rat hepatic microsomal CYP1A activity but the effect was not due to ginkgolides A, B, C, or J, bilobalide, kaempferol, quercetin, isorhamnetin, or the respective flavonol monoglycosides or diglycosides [18]. Another *in vitro* experiments carried out in human liver microsomes suggested that *G. biloba* extract weakly inhibits human CYP1A2 and CYP3A [19].

In our studies induction of CYP3A enzymes after 3 days of administration of *G. biloba* extract was observed. A dose-dependent induction of cytochrome P450 3A by the *G. biloba* extract at 100–2500 ng/ml for 72 h in human and rat primary hepatocytes has been also observed in a study prepared by Deng *et al.* [20]. In another study, Deng *et al.* observed that bilobalide significantly induced the activity, protein, and mRNA expression of CYP3A1 in a dose-dependent manner [21].

In a clinical trial with 12 healthy volunteers, no significant effect on CYP1A2, CYP2E1, and CYP3A activity for *G. biloba* was found [22]. Another clinical study with 12 healthy volunteers assessed the influence of GBE on CYP2D6 and CYP3A activity using the probe substrates: dextromethorphan and alprazolam, respectively. No differences were observed between baseline and post-GBE treatment [23]. Available scientific data concerning the effect of *G. biloba* extract are very poor, the most common are that *in vitro* studies, and apply only to selected CYP enzymes. Therefore, results obtained by our team contribute to the understanding of the molecular mechanisms implicated in the regulation of metabolism involving the various isoforms of CYP enzymes.

## CONCLUSION

These data suggest that *P. ginseng* and *G. biloba* extracts can modulate the expression of cytochrome P450 enzymes and transcriptional factors thus may participate in xenobiotics metabolism (drugs, procarcinogens, vitamins, food components).

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## REFERENCES

1. Benishin CG. Actions of ginsenoside Rb1 on choline uptake in central cholinergic nerve endings. *Neurochem Int* 1992; 21:1-5. doi: [http://dx.doi.org/10.1016/0197-0186\(92\)90061-U](http://dx.doi.org/10.1016/0197-0186(92)90061-U)
2. Dorman T. Herbal medicine and anesthesia. *Curr Opin Anaesthesiol* 2001; 14:667-669.
3. Wakabayashi C, Murakami K, Hasegawa H, Murata J, Saiki I. An intestinal bacterial metabolite of ginseng protopanaxadiol saponins has the ability to induce apoptosis in tumor cells. *Biochem Biophys Res Commun* 1998; 246:725-730. doi: <http://dx.doi.org/10.1006/bbrc.1998.8690>
4. Entschladen F, Drell TL, Lang K, Joseph J, Zaenker KS. Tumour-cell migration, invasion, and metastasis: navigation by neurotransmitters. *Lancet Oncol* 2004; 5:254-258. doi: [http://dx.doi.org/10.1016/S1470-2045\(04\)01431-7](http://dx.doi.org/10.1016/S1470-2045(04)01431-7)
5. Gillis CN. *Panax ginseng* pharmacology: a nitric oxide link? *Biochem Pharmacol* 1997; 54:1-8. doi: [http://dx.doi.org/10.1016/S0006-2952\(97\)00193-7](http://dx.doi.org/10.1016/S0006-2952(97)00193-7)
6. Izzo AA, Ernst E. Interactions between herbal medicines and prescribed drugs: a systematic review. *Drugs* 2009; 69:1777-1798. doi: <http://dx.doi.org/10.2165/11317010-000000000-00000>
7. Birks J, Grimley EJ. *Ginkgo biloba* for cognitive impairment and dementia. *Cochrane Database Syst Rev* 2007; 2: CD003120. doi: <http://dx.doi.org/10.1002/14651858.CD003120.pub3>
8. Fessenden JM, Wittenbern W, Clarke L. *Ginkgo biloba*. A case report of herbal medicine and bleeding postoperatively from a laparoscopic cholecystectomy. *Am Surg* 2001; 67:33-35.
9. Ribeiro A, Archer A, Beyec JL, Cattin AL, Saint-Just S, Pinçon-Raymond M et al. Nuclear factor-4, a key transcription factor at the crossroads between architecture and function of epithelia. *Recent Patents on Endocrine. Metab Immune Drug Discov* 2007; 1:166-175. doi: <http://dx.doi.org/10.2174/187221407780832000>
10. European Pharmacopoeia. 6th ed/ European directorate for the quality of medicines and healthcare, Strasbourg, 2008; 2:1969-2959.
11. Mrozikiewicz PM, Bogacz A, Karasiewicz M, Mikołajczak PL, Ożarowski M, Seremak-Mrozikiewicz A et al. The effect of standardized *Echinacea purpurea* extract on rat cytochrome P450 expression level. *Phytomed* 2010; 17:830-833. doi: <http://dx.doi.org/10.1016/j.phymed.2010.02.007>
12. Chang TK, Chen J, Benetton SA. *In vitro* effect of standardized ginseng extracts and individual ginsenosides on the catalytic activity of human CYP1A1, CYP1A2, and CYP1B1. *Drug Metab Dispos* 2002; 30: 378-384. doi: <http://dx.doi.org/10.1124/dmd.30.4.378>
13. Yune-Fang U, Yun-Lian L. Effects of ginsenoside Rd and *Panax ginseng* extract on Cyp1a, Cyp2b, Cyp2c, Cyp2e1, and Cyp3a activities in C57BL/6JNarl mice. *J Chin Med* 2012; (3,4):143-152.
14. Nguyen TD, Villard PH, Barlatier A, Elsisi AE, Jouve E, Duc NM et al. *Panax vietnamensis* protects mice against carbon tetrachloride-induced hepatotoxicity without any modification of CYP2E1 gene expression. *Planta Med* 2000; 66:714-719. doi: <http://dx.doi.org/10.1055/s-2000-9603>
15. Lee HC, Hwang SG, Lee YG, Sohn HO, Lee DW, Hwang SY et al. *In vivo* effects of *Panax ginseng* extracts on the cytochrome P450-dependent monooxygenase system in the liver of 2,3,7,8-tetrachlorodibenzo-p-dioxin-exposed guinea pig. *Life Sci* 2002; 71:759-769. doi: [http://dx.doi.org/10.1016/S0024-3205\(02\)01742-3](http://dx.doi.org/10.1016/S0024-3205(02)01742-3)
16. Lee JH, Sul D, Oh E, Jung WW, Jung WW, Hwang KW et al. *Panax ginseng* effects on DNA damage, CYP1A1 expression and histopathological changes in testes of rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Food Chem Toxicol* 2007; 45:2237-2244.

17. Ribonnet L, Callebaut A, Nobels I, Scippo ML, Schneider YJ, De Saeger S et al. Modulation of CYP1A1 activity by a *Ginkgo biloba* extract in the human intestinal Caco-2 cells. *Toxicol Lett* 2011; 202:193-202. doi: <http://dx.doi.org/10.1016/j.toxlet.2011.02.006>
18. Kuo IF, Chen J, Chang TK. Effect of *Ginkgo biloba* extract on rat hepatic microsomal CYP1A activity: role of ginkgolides, bilobalide, and flavonols. *Can J Physiol Pharmacol* 2004; 82:57-64. doi: <http://dx.doi.org/10.1139/y03-133>
19. von Moltke LL, Weemhoff JL, Bedir E, Khan IA, Hartz JS, Goldman P et al. Inhibition of human cytochromes P450 by components of *Ginkgo biloba*. *J Pharm Pharmacol* 2004 56:1039-1044. doi: <http://dx.doi.org/10.1211/0022357044021>
20. Deng Y, Bi HC, Zhao LZ, Wang XD, Chen J, Ou ZM et al. Induction of cytochrome P450 3A by the *Ginkgo biloba* extract and bilobalides in human and rat primary hepatocytes. *Drug Metab Lett* 2008; 2: 60-66. doi: <http://dx.doi.org/10.2174/187231208783478489>
21. Deng Y, Bi HC, Zhao LZ, He F, Liu YQ, Yu JJ et al. Induction of cytochrome P450s by terpene trilactones and flavonoids of the *Ginkgo biloba* extract EGB 761 in rats. *Xenobiotica* 2008; 38:465-481. doi: <http://dx.doi.org/10.1080/00498250701883233>
22. Gurley BJ, Gardner SF, Hubbard MA, Williams DK, Gentry WB, Cui Y et al. Cytochrome P450 phenotypic ratios for predicting herb-drug interactions in humans. *Clin Pharmacol Ther* 2002; 72:276-287. DOI: 10.1067/mcp.2002.126913
23. Markowitz JS, Donovan JL, Lindsay DeVane C, Sipkes L, Chavin KD. Multiple-dose administration of *Ginkgo biloba* did not affect cytochrome P-450 2D6 or 3A4 activity in normal volunteers. *J Clin Psychopharmacol* 2003; 23: 576-581. doi: <http://dx.doi.org/10.1097/01.jcp.0000095340.32154.c6>

## WPLYW EKSTRAKTÓW Z *PANAX GINSENG* I *GINKGO BILOBA* NA POZIOM EKSPRESJI CZYNNIKÓW TRANSKRYPCYJNYCH I ENZYMÓW CYTOCHROMU P450 METABOLIZUJĄCYCH KSENOBIOTYKI

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## Streszczenie

**Wstęp:** Mimo powszechnego stosowania *Panax ginseng* i *Ginkgo biloba* dane dotyczące bezpieczeństwa, a także interakcji pomiędzy preparatami roślinnymi a lekami syntetycznymi są bardzo ograniczone. W niniejszych badaniach założono, iż żeń-szeń oraz miłorząb mogą modulować aktywność i zawartość izoenzymów cytochromu P450 biorących udział w biotransformacji różnych substancji ksenobiotycznych. **Cel:** Określenie wpływu preparatów roślinnych na poziom ekspresji enzymów CYP i ich czynników transkrypcyjnych.

**Metody:** Szczurom rasy Wistar podawano standaryzowany *Panax ginseng* (30 mg/kg) oraz *Ginkgo biloba* (200 mg/kg) przez 3 do 10 dni. Ekspresję w tkance wątrobowej analizowano za pomocą metody PCR w czasie rzeczywistym. **Wyniki:** Uzyskane wyniki wykazały spadek poziomu mRNA CYP3A1 (homolog ludzkiego enzymu CYP3A4) po podaniu ekstraktu z żeń-szenia. Ekspresja genu CYP2C6 (homolog ludzkiego enzymu CYP2C9) również uległa obniżeniu. Dodatkowo, obserwowaliśmy wzrost ekspresji CYP1A1 (homolog ludzkiego enzymu CYP1A1) i CYP1A2 (homolog ludzkiego enzymu CYP1A2) po 10 dniach stosowania *P. ginseng*. Ponadto, ekstrakt z *G. biloba* spowodował również wzrost poziomu mRNA CYP1A1, CYP2C6, CYP3A1 i CYP3A2 w modelu *in vivo*. **Wnioski:** Badania sugerują, że wyciągi roślinne mogą modulować ekspresję czynników transkrypcyjnych i enzymów CYP uczestniczących w metabolizmie ksenobiotyków i chemicznej karcynogenezie.

**Słowa kluczowe:** cytochrom P450, ekspresja, ekstrakty roślinne, real-time PCR, metabolizm leków, chemiczna karcynogeneza