# The influence of soybean extract on the expression level of selected drug transporters, transcription factors and cytochrome P450 genes encoding phase I drug-metabolizing enzymes

## Wpływ ekstraktu sojowego na poziom ekspresji wybranych transporterów leków, czynników transkrypcyjnych i genów cytochromu P450 kodujących enzymy I fazy metabolizmu leków

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## Abstract

***Objective:*** *Soybean phytoestrogens, such as genistein and daidzein, reduce climacteric symptoms and the risk of certain chronic diseases such as cancer and cardiovascular diseases. Despite their widespread use in functional foods and dietary supplements, there is very little data available on their safety and herb-drug interactions, especially with antineoplastic agents. Hence, the aim of our study was to assess the effects of soybean extracts on the expression level of CYP genes and their transcriptional factors. We also investigated the effect of soybean on the mRNA level of transporters, such as P-glycoprotein (MDR1) and multidrug resistance-associated proteins (MRP1, MRP2).*

***Materials and methods:*** *Wistar rats were fed a standardized soybean extract (100 mg/kg, p.o.). cDNA was synthesized from total RNA isolated from different tissues (liver and intestinal epithelium) using reverse transcription. Gene expression level was analyzed by RT-PCR method.*

***Results:*** *We demonstrated a significant increase of CYP1A1 mRNA level (by 89%, p=0.002 and 125%, p=0.004) as compared with the control group. An increase of AHR and CAR expression after 10 days was also observed (by 60%, p<0.001 and 52%, p>0.05, respectively).*

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*Additionally, an inductive effect for CYP2D1 by 22% (p=0.008), Mdr1a by 267% (p<0.0001), Mdr1b by 86% (p<0.0001), Mrp1 by 9-fold (p<0.0001), Mrp2 by 83% (p<0.0001) in the liver and for Mrp2 by 35% (p<0.001) in the intestinal epithelium, was evaluated. A significant decrease of mRNA level was observed for CYP3A1 (human CYP3A4) in the liver and Mdr1b in the intestinal epithelium. Moreover, we also showed a slight decrease in the amount of mRNA for CAR, PXR and ARNT after 3 days.*

***Conclusions:*** *Our results suggest that Glycine max may change the expression level of CYPs, especially CYP3A4 and CYP1A1, involved in biotransformation of xenobiotics (drugs, procarcinogens) and may participate in clinically significant interactions with drugs metabolized by these enzymes. Moreover, an increase of CYP1A1 (homologue to human CYP1A1) mRNA level may not only reduce the carcinogenicity of foreign compounds, but may also activate some compounds to their carcinogenicity. In case of transporters, it is considered that an increase of their expression in the body may lead to increased fetoprotection. Also, it may reduce both, the exposure of sensitive tissues (e.g. brain, placenta) to xenobiotics and treatment effectiveness of certain diseases. Hence, the search for a safe substance that could effectively modulate transporter activity, especially in the treatment of certain hormone -dependent disorders. e.g. osteoporosis and breast cancer, occurring mainly in postmenopausal period, continues.*

Key words: **CYP enzymes** / **transporters** / **soybean** / **interactions** / / **transcriptional factors** / **expression level** /

## Streszczenie

***Cel pracy:*** *Fitoestrogeny sojowe, takie jak genisteina i daidzeina redukują symptomy menopauzy i zmniejszają ryzyko wystąpienia niektórych chorób w tym nowotworów i chorób serca. Pomimo szerokiego zastosowania izoflawonów pod postacią funkcjonalnej żywności i suplementów diety, dane dotyczące bezpieczeństwa jak również interakcji typu preparat roślinny-lek syntetyczny, szczególnie uwzględniając leki przeciwnowotworowe są bardzo ograniczone. Celem badania była ocena wpływu ekstraktu sojowego na poziom ekspresji genów CYP i ich czynników transkrypcyjnych. Zbadano również wpływ soi na poziom mRNA transporterów obejmując glikoproteinę P (MDR1) i białka związane z opornością wielolekową (MRP1, MRP2).*

***Materiał i metody:*** *Szczury rasy Wistar traktowano ekstraktem sojowym (100 mg/kg, p.o.). cDNA syntetyzowano z całkowitego RNA z tkanki wątrobowej i nabłonka jelita stosując odwrotną transkrypcję. Poziom ekspresji genów analizowano wykorzystując metodę RT-PCR.*

***Wyniki:*** *Wykazano znaczny wzrost poziomu mRNA CYP1A1 o 89% (p=0,002) i 125% (p=0,004) w porównaniu do grupy kontrolnej. Wzrost ekspresji AHR i CAR był również obserwowany odpowiednio o 60% (p < 0,001) i 52% (p > 0,05) po 10 dniach stosowania. Dodatkowo, indukcyjny efekt oceniono dla CYP2D1 o 22% (p=0,008), Mdr1a o 267% (p < 0,0001), Mdr1b o 86% (p < 0,0001), Mrp1 9-krotny (p < 0,0001), Mrp2 o 83% (p < 0,0001) w wątrobie i dla Mrp2 o 35% (p < 0,001) w nabłonku jelita. Znaczny spadek poziomu mRNA w wątrobie obserwowano dla CYP3A1 (ludzki CYP3A4) i Mdr1b w nabłonku jelita. Ponadto, wykazano również nieznaczny spadek w ilości mRNA dla CAR, PXR i ARNT po 3 dniach stosowania ekstraktu.*

***Wnioski:*** *Wyniki badania sugerują, że Glycine max może zmieniać poziom ekspresji enzymów CYP, szczególnie CYP3A4 i CYP1A1 związanych z biotransformacją ksenobiotyków (leków, prokancerogenów) oraz może uczestniczyć w klinicznie znaczących interakcjach z lekami metabolizowanymi przez te enzymy. Ponadto, wzrost poziomu mRNA CYP1A1 (ludzki CYP1A1) może nie tylko redukować kancerogenność obcych związków ale może również aktywować niektóre ich kancerogenności. W przypadku transporterów, uważa się, że wzrost ich ekspresji w organizmie może prowadzić do zwiększonej fetoprotekcji, redukcji wrażliwych tkanek (mózg, łożysko) na ekspozycję ksenobiotyków i zmniejszenia efektywności leczenia niektórych chorób. Stąd nadal poszukuje się bezpiecznych substancji, które mogłyby skutecznie modulować aktywność transporterów, szczególnie w leczeniu chorób hormono-zależnych np. osteoporozy czy raka piersi, które występują głównie w okresie postmenopauzalnym.*

Słowa kluczowe: **enzymy CYP** / **transportery** / **soja** / **interakcje** / / **czynniki transkrypcyjne** / **poziom ekspresji** /

## Introduction

Soybean (*Glycine max*) is a rich source of phytoestrogens due to the presence of genistein and daidzein that imitate the action of female sex hormones. These isoflavones have estrogenic properties which are important in the treatment of menopause-associated disorders and prevention of osteoporosis caused by estrogen deficiency in postmenopausal women.

These phytoestrogens, especially genistein, may also offer an alternative to hormone replacement therapy (HRT) owing to their high affinity to estrogen receptors [1, 2]. Natural selective modulators of these receptors are designed not only to reduce troublesome climacteric symptoms, but also to prevent ailments resulting from hormone deficiency [2, 3].

Additionally, epidemiological studies suggest that soy products may lower the risk of certain chronic diseases, i.e. cancer and cardiovascular diseases [4-6].

Clinical data provide compelling evidence for the positive effect of soy isoflavones on improved lipid profiles, including reduction in low density lipid (LDL) and triglycerides, as well as an increase in high density lipid (HDL) levels [7, 8].

Furthermore, soy may modulate the activity of CYP enzymes involved in the metabolism of a variety of xenobiotics (drugs, carcinogens, food components) and synthesis of endogenous compounds such as steroids [9]. The CYP2C, CYP2D and CYP3A subfamilies are the most active CYPs responsible for drug metabolism, while the CYP1A, CYP2A and CYP2E subfamilies metabolize a variety of protoxins and procarcinogens to their ultimate reactive metabolites [10]. High CYP3A4 activity has been suggested to constitute a risk factor for breast cancer [11]. Moreover, tamoxifen is mainly metabolized by CYP2D6 and CYP3A4/5 enzymes that may be modulated by synthetic substances and herbal preparations. Therefore, the profile of the mRNA abundance of CYP enzymes provides important information about possible interactions between herbal medicines and synthetic drugs.

Regulation of cytochrome P450 gene expression depends on interactions between xenobiotics and receptors. The CYP1 subfamily has been demonstrated to 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 [12]. One study shows that HNF1 and HNF4 are general regulators supporting the constitutive expression of major CYPs (CYP2D6, CYP3A4, CYP2C9, CYP1A) in hepatocytes [13]. Studies on the expression and activity of enzymes catalyzing the biotransformation of xenobiotics are important as they shed some light on the herb-drug interactions and their physiological functions.

In addition, herbal substances are known to modulate activity of transporters such as P-glycoprotein (P-gp), and solute carrier family (SLC), as well as the family of proteins involved in multidrug resistance (MRP - multidrug resistance-associated protein) [14, 15] responsible for the transport of xenobiotics (drugs, vitamins) and other endogenous substances (e.g. hormones). The literature on the effects of inhibitors of herbal origin on the level of expression of transporter genes conditioning the phenomenon of multidrug resistance and the blood brain barrier is scant. These studies mainly concern the impact of synthetic drugs (e.g. verapamil, cyclosporine A) and herbal substances on P-gp activity. Results suggest that verapamil and cyclosporine A at higher doses are inhibitors for P-gp. However, their highly adverse side effects prevent their practical application in overcoming the multidrug resistance and the blood-brain barrier [16, 17]. Therefore, the search for a safe substance that could effectively modulate transporter activity in the treatment of various diseases continues.

## Objectives

The aim of our study was to determine the influence of soybean extract on the expression level of CYP enzymes and receptors responsible for their transcriptional regulation. Furthermore, we demonstrated the effect of soy on the mRNA level of xenobiotic transporters, such as P-glycoprotein (MDR1) and proteins associated with multidrug resistance (MRP1, MRP2) in an *in vivo* rat model.

## Materials and Methods

### Soybean extract

Standardized soy extract containing 37% isoflavones expressed as genistein was obtained from Pierre Fabre Sante, France. This powdered extract was kept in a dark and dry place at room temperature until use.

### Animals and treatment

The experiment with Wistar rats (200-250) was performed in accordance with the Polish government regulations (Animal Protection Act 21.01.2005, Dz. U. No 33; 289). The study was approved by the Local Ethics Committee in the Care and Use of Laboratory Animals in Poznan (No 43/2005). The animals were housed in plastic cages at the Department of Pharmacology, Poznan University of Medical Sciences. Rats were housed in climate-controlled quarters with a 12-h light/dark cycle with access to commercial rat chow and tap water *ad libitum*.

The animals were randomly divided into six groups (n=10). The study groups received the standardized soy extract 100 mg/kg p.o., once a day, for 3, 10 and 21 days, while the control group was fed a standard diet. Two hours after the last administration the rats were decapitated. The samples of liver and intestinal epithelium were immediately frozen in liquid nitrogen and stored at -80ºC until use.

### RNA extraction and cDNA synthesis

Total cellular RNA was isolated using TriPure Isolation Reagent (Roche, Germany) according to the manufacturer's protocol. The concentrations and the purity of RNA were determined by measuring the absorbance at 260 and 280 nm in a spectrophotometer (BioPhotometer Eppendorf, USA). RNA samples were stored at -80°C. Complementary DNA was synthesized from 1 µg of total RNA in a total volume of 20 µl using the Transcriptor cDNA First Strand Synthesis Kit (Roche, Germany) and oligo$(dT)_{18}$ primer (Roche, Germany) according to the manufacturer's protocol. The obtained transcripts were stored at -20°C or used directly for the real-time quantitative PCR (RT-PCR).

### Determination of mRNA levels by real-time PCR

The level of mRNA expression in the rat tissues (liver, intestinal epithelium) was analyzed by the RT-PCR method. 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. [18]. The primers and RT-PCR conditions used for CAR, PXR, RXR, GR, AHR, ARNT, HNF-1, HNF-4 and Mdr1b amplifications are described in Table I. The primers for Mrp1 and Mrp2 were used from Sugamo et al. [19], while the primers for Mdr1a were designed by Andersson et al. [20]. 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 TM 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.

### Statistical analysis

The mRNA content for the studied genes was expressed as mean ± SEM. The experimental data were analyzed using the SPSS 17.0 for Windows software. One-way ANOVA test was used to compare mean value. The value of $p<0.05$ was considered as statistically significant.

## Results

The level of mRNA expression in the liver and the intestinal epithelium was analyzed by the RT-PCR method. As shown in Figure 1, the standardized soy extract resulted in a significant increase of CYP1A1 (homologue to human CYP1A1) expression level (by 89%, $p=0.002$ and 125%, $p=0.004$), respectively. An inductive effect was also observed for CYP2D1 (homologue to human CYP2D6) by 22% ($p=0.008$) after 10 days of treatment, as compared to the control group. No significant differences were observed for CYP1A2 (homologue to human CYP1A2), CYP2C6 (homologue to human CYP2C9) and CYP3A2 (homologue to human CYP3A5). In case of CYP3A1 (homologue to human CYP3A4), the mRNA level was reduced by 21% ($p<0.05$) and 40%, respectively. In addition, CYP2E1 (homologue to human CYP2E1) and CYP2D2 (homologue to human CYP2D6) expression was also inhibited by almost 20%.

Moreover, a significant increase of AHR and CAR factors in the mRNA level was observed (by 60, $p<0.001$ and 52%, $p>0.05$, respectively) after 10 days (Figure 2). Additionally, a slight decrease in the amount of cDNA for CAR, PXR and ARNT was noted in a shorter period of soy extract administration as compared to the control group. No significant changes were observed in the mRNA concentration for RXR, GR, HNF-1α and HNF-4α.

Furthermore, we also analyzed the influence of soybean on the mRNA level of transporters in the liver and intestinal epithelium. The inductive effect of the extract was observed for Mdr1a (by 267%, $p<0.0001$), Mdr1b (by 86%, $p<0.0001$), Mrp1 (by 900%, $p<0.0001$), and Mrp2 (by 83%, $p<0.0001$) in the liver and for Mrp2 (by 35%, $p<0.001$) in the intestinal epithelium (Figure 3). In case of Mdr1b, a reduction (by 56%, $p<0.0001$) was demonstrated after 21 days of treatment.

## Discussion

In our study, we investigated the effects of standardized soy extract on mRNA expression of transporters and CYP enzymes involved in detoxification of xenobiotics, as well as changes in the mRNA level of receptors responsible for their transcriptional regulation. The induction or inhibition of CYP isoforms by soybean isoflavones may have a potential impact on the efficacy and safety of synthetic drug use in HRT, especially in alleviating climacteric symptoms and treatment of breast cancer by tamoxifen or other anticancer agents. Hence, the knowledge about the molecular basis of the interactions allows to predict whether compounds derived from medical plants may cause herb-drug interactions or be susceptible to population interindividual variations in drug metabolism.

Our results showed that soybean extract in rats caused a significant induction of CYP1A1 (homologue to human CYP1A1) responsible for carcinogen activation. Thus, we postulate that induction of CYP1A1 may diminish carcinogenicity of foreign compounds because increased metabolism of ingested procarcinogens will lead to faster clearance and reduction of their carcinogenicity. However, such induction of CYP1A1 may also potentially result in an increase in the toxicity and carcinogenicity of procarcinogens. Therefore, it is difficult to explain the mechanism of CYP enzyme activity in the metabolism of procarcinogens and development of carcinogenesis.

**Table I.** Sequences of primers used for the real-time PCR analysis and PCR conditions.

| Gene | Primer sequence (5' → 3') | Product size (bp) | PCR conditions | | | |
|---|---|---|---|---|---|---|
| | | | Cycle | Denaturation | Annealing | Extension |
| CAR F<br>CAR R | GGA GGA CCA GAT CTC CCT T<br>GAC CGC ATC TTC CAT CTT GT | 130 | 35 | 95ºC, 8s | 58ºC, 8s | 72ºC, 8s |
| PXR F<br>PXR R | TCC ACT GCA TGC TGA AGA AG<br>AAC CTG TGT GCA GGA TAGGG | 187 | 35 | 95ºC, 8s | 55ºC, 8s | 72ºC, 8s |
| RXR F<br>RXR R | CCT GAG TTC TCC CAT CAA TG<br>GAC GCC ATT GAG GCC TAG A | 190 | 35 | 95ºC, 8s | 57ºC, 7s | 72ºC, 8s |
| GR F<br>GR R | CTG GAA TAG GTG CCA AGG CT<br>CCG TAA TGA CAT CCT GAA GCT | 210 | 40 | 95ºC, 10s | 58ºC, 8s | 72ºC, 8s |
| AHR F<br>AHR R | ATAGCTACTCCACTTCAGCC<br>TCATGCCACTTTCTCCAGTC | 244 | 35 | 95ºC, 8s | 52ºC, 8s | 72ºC, 8s |
| ARNT F<br>ARNT R | CAG AAC TGT CAG ACA TGG TAC<br>AGT CAG AGA CAT ACA CCA CTC | 246 | 40 | 95ºC, 8s | 57ºC, 7s | 72ºC, 8s |
| HNF-1α F<br>HNF-1α R | GCT CGG AAG ATG ACA CGG AT<br>CTT GTT GAG GTG CTG GGA CA | 245 | 35 | 95ºC, 8s | 60ºC, 7s | 72ºC, 8s |
| HNF-4α F<br>HNF-4α R | CTG GAG GAT TAC ATC AAC GAC<br>GTG TTC TTG CAT CAG GTG AG | 164 | 40 | 95ºC, 8s | 57ºC, 7s | 72ºC, 8s |
| Mdr1b F | TGA CGT GAA TGA CGC TGG | 207 | 40 | 95ºC, 10s | 59 ºC, 8s | 72ºC, 8s |
| Mdr1b R | CAC ATG GCA GAT GAC AAC C | | | | | |

F – Forward; R – Reverse

Ronis et al., demonstrated that consumption of isolated soy protein significantly decreases induction of CYP1A1 and CYP1A2 mRNA as compared to rats fed a diet containing casein. They also focused on the possibility of interactions among soybean, polycyclic aromatic hydrocarbons (PAH) and other dietary components which are known CYP1A-inducers, and the fact that diet may contribute to interindividual differences in CYP1A1 and CYP1A2 expression [21].

Another study reported that genistein and equol did not affect the protein level and activity of CYP1A1/2, CYP2E1 and CYP3A1 in mice [22]. However, these authors in a later study showed that these isoflavones or their metabolites may decrease the protein level and activity CYP1A2. Therefore, they suggest that it is difficult to postulate chemopreventative influence of soybean isoflavones [23].

In our study, we also showed that soybean extract reduced the mRNA level of CYP3A1 (homologue to human CYP3A4) and CYP2D2 (homologue to human CYP2D6). Our study suggests that inhibition of CYP3A4 and CYP2D6 by consuming soy preparations may lead to decreased metabolism of drugs that are substrates for these enzymes.

Similar conclusions are found in a study by Laurenzana et al., where genistein administration resulted in significantly reduced CYP3A and CYP2C mRNA levels in male rats [24]. Another interesting study was conducted by Anderson et al., and showed that hydrolyzed soy extract in the microsomes of human liver led to an inhibition of CYP1A2, CYP2A6, CYP2D6, CYP2C9 and CYP3A4, whereas only some of these enzymes were insignificantly inhibited by unhydrolyzed extract [25].

Furthermore, there are few if any studies on the effects of soy on the expression of transcription factors including nuclear translocator (ARNT) for AHR. Singhal et al., found no effect of genistein and daidzein on the AHR signal transduction pathway [26]. They drew attention to the possibility of regulation of this pathway at the stage of protein XAP2. Hence, in order to fully interpret the presented results, it is important to understand the mechanisms controlling the expression of CYP450 enzymes at the stage of transcription and posttranscriptional modification because constitutive expression of AHR, CAR, HNF1 and HNF4 is not well-understood and requires further studies. We postulate that daily use of standardized extracts of *G. max* at a defined dose may have an inductive effect on the AHR and CYP1A1 enzyme because soybean can be a source of naturally occurring ligands having potential properties of selective modulator for this receptor.

Moreover, Li et al. analyzed the effect of genistein, daidzein and equol on the activity of human and mouse PXR [27]. They showed that genistein and daidzein activate wild-type but not the mutant form of PXR in mice. In the case of human PXR, equol was observed to be a more potent activator for this receptor than genistein and daidzein. Additionally, it was shown that equol as metabolite of daidzein caused an increase of CYP3A4 expression level in human hepatocytes. Currently, there are isolated studies on the effects of soy extract on the expression level of the studied transcription factors. They are needed to fully understand the molecular mechanism of action of herbal preparations in the activation of CYP enzymes and transporters.

In addition, we also evaluated the expression level of genes encoding xenobiotic transporters (Mdr1a, Mdr1b, Mrp1 and Mrp2) under the influence of standardized soybean extract. So far, there is no data on changes in the expression of the studied transporters under the influence of soy. The number of studies focusing only on changes in the activity of some transporters is scarce. Thus, our investigation is a screening method for further

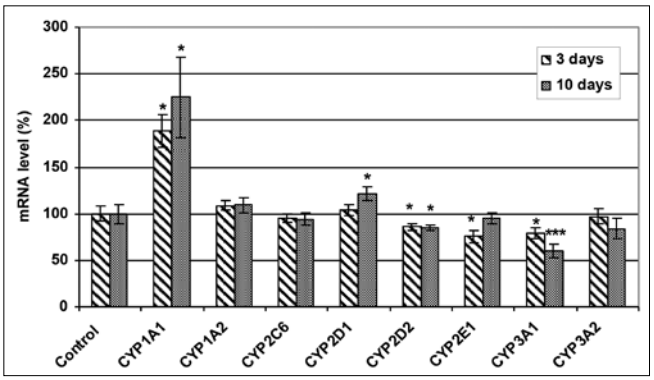


**Figure 1.** The effect of *Glycine max* (100 mg/kg) on the expression level of CYP genes in rat liver after 3 and 10 days of treatment. The control group was defined as 100%. Data were presented as mean ± SEM (n = 10). *p<0.05, **p<0.001, ***p<0.0001 as compared to controls (one-way ANOVA test).

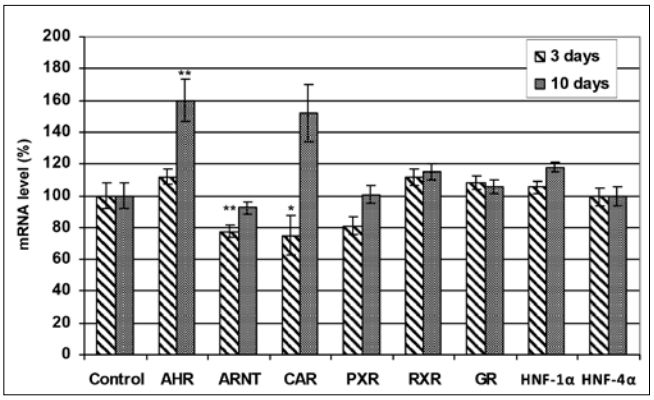


**Figure 2.** The effect of *Glycine max* (100 mg/kg) on the expression level of transcription factors and ARNT in rat liver after 3 and 10 days of treatment. The control group was defined as 100%. Data were presented as mean ± SEM (n = 10). *p<0.05, **p<0.001, ***p<0.0001 as compared to controls (one-way ANOVA test).

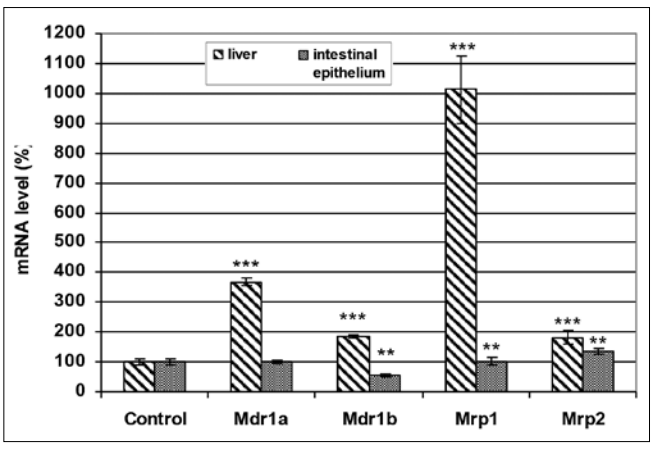


**Figure 3.** The effect of Glycine max (100 mg/kg) on the expression level of genes encoding transmembrane transporters in rat liver and intestinal epithelium after 21 days of administration. The control group was defined as 100%. Data were presented as mean ± SEM (n = 10). *p<0.05, **p<0.001, ***p<0.0001 as compared to controls (one-way ANOVA test).

analysis in the exploration of herbal substances that may modulate the expression of transporters. Analyzing the Mdr1b mRNA profile in the intestinal epithelium, we observed a decrease of its expression level as compared to the control group. A significant increase of mRNA level in the liver tissue was observed for Mdr1a and Mrp1. Our results suggest that the use of soybean extracts in the treatment of some diseases, especially hormone-dependent disorders and cancer diseases, would be effective only in case of drugs that are substrates for MDR1 due to the fact that a decreased MDR1 expression in the intestine increases the oral bioavailability of these drugs. However, studies on the effectiveness of *Glycine max* in reducing expression of MDR1 gene need to be confirmed in clinical trials. Furthermore, increased expression of MDR1 in the body may lead to increased fetoprotection, reduced exposure of sensitive tissues (e.g. brain, placenta) to xenobiotics, and less effective treatment of certain diseases.

Moreover, numerous reports confirmed the existence of a relationship between gene expression and protein activity, so there was no need to analyze the protein level in our study. The analysis of mRNA level with the use of real-time PCR method allows to assess, in a quick and precise way, the effectiveness and safety of pharmacotherapy, as well as the risk of the formation of carcinogenic compounds through induction of CYP1A enzymes.

## Conclusions

Our results show that decreased CYP3A1 (homologue to human CYP3A4) and CYP2D2 (homologue to human CYP2D6) expression may cause an increase in plasma drug levels, leading to undesirable pharmacological effects. Furthermore, we suggest that soybean may reduce the effectiveness of treatment of breast cancer in combination with tamoxifen, which is a prodrug mainly metabolized by CYP2D6 to endoxifen as an active metabolite. Additionally, increased CYP1A1 (homologue to human CYP1A1) mRNA level may not only reduce the carcinogenicity of foreign compounds by increased metabolism of the ingested procarcinogens, but it may also activate some compounds to their carcinogenic metabolites, initiating chemical carcinogenesis. Moreover, AHR expression changes are also important from the point of view of endocrine disorders because an association between AHR and estrogen receptor was demonstrated. High stimulation of AHR pathway due to exposure to dioxins and other procarcinogenic substrates is associated with an adverse effect on health, as it can initiate a cascade of changes in gene expression, leading to intensification of carcinogenicity, teratogenicity, hepatotoxicity and immunosuppression. Hence, the search for AHR antagonists seems to be the key component of chemoprevention.

Additionally, further studies concerning the influence of *Glycine max* on the transcription of transporter genes, taking into account the duration of extract administration, its dose and composition, are needed to assess the safety of soybean application in treatment and prevention.

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