Research Paper

Evaluation of Safety and Interactions with Conventional Drugs of Commercially Available Soybean Extract-containing Dietary Supplements

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Hogervorst *et al.*: Hepatic Safety of Soybean-containing Dietary Supplements

Dietary supplements based on soybean extracts are widely used for a myriad of indications, but there is evidence that isoflavones, components of these supplements affect cytochrome activity. Alteration in pharmacodynamics of cytochrome-substrate drugs after co-administration with dietary supplements based on soybean extracts offers information on clinically significant herb-drug interactions that can cause unanticipated adverse reactions or therapeutic failures. Moreover, herbal drugs can contribute to the development and severity of drug-induced liver injury. Therefore, the aim of this study was to examine the effect of commercially available dietary supplements based on soybean extracts on liver and renal function, oxidative stress and pharmacodynamics of several conventional drugs. After quantification of isoflavones in the supplement using high-performance liquid chromatography with a diode-array detector, pharmacological tests such as hot-plate, rotarod, pentobarbital-induced sleeping time were performed on Swiss albino mice treated with soybean extracts and several central nervous system acting drugs. Liver, renal function, and oxidative status in liver homogenates were determined in healthy and Wistar rats subjected to oxidative stress with carbon tetrachloride. Dietary supplements based on soybean extracts weakened the analgesic activity of codeine, significantly potentiated diazepam-induced motor coordination impairment at 10th and 30th minute after diazepam administration, but had the opposite effect on alprazolam effect. Soybean extracts pretreated group also exhibited significantly shorter pentobarbital sleep induction and sleeping time. Soybean extracts administration did not affect the liver and renal function and ameliorated oxidative stress caused by carbon tetrachloride. Despite exhibiting no negative effects on liver and renal function and demonstrated antioxidant *in vivo* potential, the safety of soybean extracts in addition to conventional drugs is questionable. The results of our study implicate the potential of dietary supplements based on soybean extracts for serious herb-drug interactions.

Key words: Phytoestrogens, soybean extract, drug interactions, cytochrome P-450, conventional drugs, liver function, antioxidant

Soy (*Glycine max*. L.) is a plant that has been used since ancient times due to its high nutritive value and health benefits. To this day, soy and soybeans products, such as tofu, soy milk, soy sauce, miso, tempeh represent an important component of human diet[1]. In addition to their high nutritive value, soybeans also contain biologically active compounds and vitamins, which includes isoflavones, tocopherols, vitamin C, saponins, and sterols[2,3]. Isoflavones are non-steroidal, polyphenolic compounds that are present in unconjugated form (aglycones), and as different types of glycoside conjugates (β-glucosides,

malonyl glucosides and acetyl glucosides). The most important representatives of isoflavones are daidzein, genistein and glycitein[1,4]. Consumption of foods rich in phytoestrogens is reported to be associated with reduced risk of prostate and breast cancer[1]. Soybean

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isoflavones and other phytoestrogens play an important role in reducing the incidence of cardiovascular diseases, hormone-dependent cancers, osteoporosis as well as alleviation of hot flushes[5]. Different nutritional or nutraceutical supplement products have been produced from soybeans with the ones containing vitamin-E, lecithin and isoflavone, being the most popular ones[6]. Soybean-based supplements could become an alternative to the hormone replacement therapy, a treatment of choice to relieve menopausal symptoms and reduce incidence of osteoporosis in postmenopausal women, which is avoided by many women because of the perceived increased risk of breast cancer[6-8].

With an increase in the popularity and use of herbal supplements, potential for adverse drug reactions also increases as these are seldom used alone, and more often as an adjunct to conventional drugs. Conventional drugs that are metabolized by cytochrome P450 (CYP) enzymes are particularly susceptible to herb-drug interactions, which can be attributed to the ability of many herbal compounds to induce or inhibit these enzymes[9]. These potential interactions between herbs and conventional drugs could lead to two major problems, toxicity or failure of the treatment. Roberts-Kirchhoff *et al.* showed that isoflavones were metabolized by microsomal liver enzymes (CYP 450), affecting the activity of these enzymes at the same time[10]. Number of studies showed that soy isoflavones affect the expression and activity of drug metabolizing enzymes[10-12] and multiple studies have demonstrated the regulation of several isoforms of CYP1, 2, and 3 subfamily members by isoflavones in rodent models[13-16]. A study which evaluated the influence of genistein, one of the most active soy isoflavones on cytochrome enzymes reported potentiation of the activity of CYP3A isoenzyme and P-glycoprotein[11]. When administered to experimental animals, soy isoflavones have potentiated gene expression for the synthesis of CYP1A1 and 2D1 and inhibited synthesis of 2D2 and 3A1 isozymes[12]. Li *et al.* determined that the concurrent intake of soy isoflavones with prescription medication may affect the pharmacokinetic profile and hence bioavailability of such co-administered drugs, due to CYP3A4 induction, an enzyme critically important in drug metabolism, being responsible for the metabolism of ~50 % of all prescription drugs[17]. However, it is known that even though during pharmacokinetic interactions significant changes in drugs concentrations can occur,

this must not always be followed with a significant change in the pharmacodynamic response[18]. There is no data if this effect on the enzymatic complex and subsequent changes in pharmacokinetics is related to actual changes in drug effect. Therefore, possible serious clinically relevant herb-drug interactions might occur during co-administration of soybean-based supplements and conventional drugs whose metabolic pathways included isozymes reported to be affected by the main isoflavones in the supplement. To mention a few, benzodiazepines are metabolized via CYP450 enzymatic system. Diazepam oxidative metabolism through demethylation (cytochrome 2C9, 2C19, 2B6, 3A4) and hydroxylation (cytochrome 3A4 and 2C19) results in formation of several active metabolites, while alprazolam biotransformation in CYP 3A4 produces less potent metabolites[19]. In experimental pharmacology, pentobarbital, a well-known hypnotic is used to establish the effect of xenobiotics on pentobarbital-induced sleep as a measure of pharmacodynamics of this drug[20]. Pentobarbital is almost completely metabolized into inactive metabolites through hydroxylation by CYP2B6 and CYP2D6. Changes in codeine activity can also help understand the span of effects soybean supplementation has on microsomal liver enzymes, as codeine metabolism is strongly influenced by the activity of this enzymatic complex. Codeine is transformed into active metabolites through CYP2D6, and these metabolites then undergo glucuronidation and are excreted through urine[21].

Besides the risk of interactions, herbal drugs can also significantly affect liver function, while some may even cause drug-induced liver injury[22]. The main mechanism of drug-induced hepatotoxicity involves the generation of hepatotoxic metabolites and accumulation of reactive oxygen species (ROS). The disturbance in the balance between cellular pro- and antioxidant activities and increased ROS levels damage DNA, proteins, enzymes, and lipids and induce apoptosis and necrosis of hepatocytes[23]. Reports on the effects of soybean-based supplement on liver function are scarce, but as the incidences of liver injuries due to herbal and dietary supplements continue to increase, it is necessary to determine the effect of soybean-derived supplements on liver function, in both normal and pathological conditions. Furthermore, renal dysfunction is common in liver diseases, since concomitant renal and liver dysfunction may share common pathogenic mechanisms. Renal failure can be functional, secondary to liver dysfunction or acute

Research Council (USA). The experimental procedures were approved by Ethics Committee for Animal Use in Experiments of the University of Novi Sad (I-2013-03). The doses of DSU used in the experiment were adapted for the experimentation on mice using FDA guidance[26] according to the recommended human daily dosed for a male with standard weight of 70 kg.

Examination of potential interactions:

All animals were divided into 9 experimental groups, each containing 6 animals, and treated as follows, CONS, saline 1 ml/kg per os (p.o.) for 7 d, where the last dose was applied 30 min before intraperitoneal (i.p.) administration of saline solution on the d 7; CODS, saline 1 ml/kg p.o. for 7 d, where the last dose was applied 30 min before i.p. administration of codeine (30 mg/kg) on d 7; PENS, saline 1 ml/kg p.o. for 7 d, where the last dose was applied 30 min before i.p. administration of pentobarbital (40 mg/kg) on d 7; DIAS, saline 1 ml/kg p.o. for 7 d, where the last dose was applied 30 min before intramuscular (i.m.) administration of diazepam (2 mg/kg) on d 7; ALPS, saline 1 ml/kg p.o. for 7 d, where the last dose was applied 30 min before i.m. administration of alprazolam (2 mg/kg) on d 7; CODDSU, DSU p.o. 170 mg/kg for 7 d, where the last dose was applied 30 min before i.p. administration of codeine (30 mg/kg) on d 7; PENDSU, DSU p.o. 170 mg/kg for 7 d, where the last dose was applied 30 min before i.p. administration of pentobarbital (40 mg/kg) on d 7; DIADSU, DSU p.o. 170 mg/kg for 7 d, where the last dose was applied 30 min before i.m. administration of diazepam (2 mg/kg) on d 7; ALPDSU, DSU p.o. 170 mg/kg for 7 d, where the last dose was applied 30 min before i.m. administration of alprazolam (2 mg/kg) on d 7.

Examination of *in vivo* antioxidative potential:

To determine the effects of DSU on *in vivo* antioxidant capacity, Wistar rats were subjected to pro-oxidative compound, CCl_4. All animals were divided into 4 experimental groups, each containing 6 individuals, and treated as follows: CONS, saline 1 ml/kg p.o. for 7 d; CONCCl$_4$, saline 1 ml/kg p.o for 7 d+a single CCl_4 dose 2 ml/kg i.p. DSU; DSU p.o. for 7 d; CCl$_4$DSU: DSU p.o. for 7 d, where the last dose was applied 1 h before administration of a single CCl_4 dose 2 ml/kg i.p.

DSU in the dose of 170 mg/kg was dissolved in 10 ml of saline and was applied by per oral gavage to laboratory animals. The control group received an equivalent volume of saline solution. Codeine hydrochloride (30 mg/kg) and pentobarbital (40 mg/kg) were administered i.p. 30 min after the last DSU solution intake[20,27]. Diazepam (2 mg/kg) and alprazolam (2 mg/kg) were applied via i.m. injection 30 min after the last DSU solution intake. Alprazolam was suspended in 1 % Tween 80 in water because alprazolam is not freely soluble in saline. CCl_4 was dissolved in olive oil solution to the final concentration of 50 %. All experiments were carried out during the daytime.

Hot plate test:

The hot plate test was performed by placing the animal's individually on hot plate and assessing their response to the thermal stimulus. The temperature of the metal plate enclosed by Plexiglas walls was maintained at 52.5°. The response time was measured in seconds at which the animal licked or flinched one of the hind paws, or jumped off the plate. To prevent tissue damage, a cut-off time was used as a double value of latencies measured before drug application. Response latencies were first determined two times before the application of the tested compound in order to determine a pre-treatment response for each animal, and then 5, 10, 20, 30, 60, 90 min following the drug administration. After responding or reaching the cut-off time, animals were removed from the plate[20,27]. Analgesic effect determined in seconds was expressed as percent of prolongation of measured reaction time compared to control reaction time.

Rotarod performance test:

Evaluation of motoric coordination of experimental animals was performed using the rotarod performance test. Horizontally oriented rotating cylinder was placed on the height of 50 cm, so that animals would be discouraged to jump from it and would not be injured when falling down. Rotation speed was set to 12 rpm. Only those animals able to maintain balance on the rod for 5 min were included in the experiment. The length of time (s) that animals stayed on this rotating rod was measured in the intervals of 10, 20, 30 and 50 min after administration of diazepam and alprazolam (retention time)[20]. A depression of motoric activity was calculated as a percent of time changed before and after the administration of diazepam and alprazolam, according to the following formula: % depression = $100-(A \times 100)/B$, where A is a retention time (s) and B is maximum time (300 s)[27].

Sleeping time test:

Sleeping time test was used to determine potential interactions in the depressive effects on central nervous system between pentobarbital and commercially available DSU[20,27]. After administration of tested substances, animals were observed for onset of sleep. A mouse was considered to be asleep if it was placed on its back and displayed a loss of righting reflex for 5 min. The latency of the loss of the righting reflex after pentobarbital administration (sleep induction time) and the time between the loss and the recovery of the righting reflex, duration (sleeping time) were determined for each animal.

Determination of oxidative status of the liver and serum biochemical parameters:

Following CCl$_4$ administration, animals were sacrificed after 24 h and 1 g liver samples were collected and homogenized with Tris-HCl buffer (pH=7.4) in 1:3 ratio to determine the parameters of oxidative stress. Levels of total liver proteins, lipid peroxidation intensity (LPX), activity of glutation peroxidase (GPX), peroxidase (PX), glutation reductase (GR), catalasis (CAT) and gluthatione reducatase (GR) levels were determined. The procedures were performed on Agilent 8453 UV/Vis (Santa Clara, USA) spectrophotometer. The LPX activity was estimated through measurement of malondialdehyde levels[28]. XOD activity was measured by the rate of the reduction of methylene blue[29]. CAT activity was estimated by determination of the rate of decomposition of H$_2$O$_2$ at 240 nm[30], and PX activity through application of guaiacol as the enzyme's substrate[31]. The activities of GPX and GR were determined by the measuring the decrease in absorbance caused by the oxidation of NADPH at 340 nm[32,33].

Total and direct bilirubin, urea, uric acid and creatinine levels and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, were determined in serum using commercially available kits. All analyses were performed in triplicate on the Olympus AU 400 autoanalyser (Hamburg, Germany) according to the manuals supplied.

Statistical analysis:

The level of significance between the groups was assessed with the Student's t-test for small independent samples using MedCalc 9.2.0.1 software. All data were expressed as a mean±standard deviation (SD). A value of p<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Analysis of DSU used in this study was performed on HPLC (Tables 1 and 2, fig. 1). It has been show

TABLE 1: ISOFLAVONE CONTENT IN DSU AS AGLYCONE EQUIVALENTS

Measured compounds	Content (mg/cps)
Total daidzein	25.59
Total glycitein	3.83
Total genistein	10.67
TI determined	40.09
TI declared	40

DSU is dietary supplement based on soybean extract. TI- total isoflavones

TABLE 2: INDIVIDUAL ISOFLAVONE COMPOSITION OF DSU WITH MASS CORRECTION

Measured compounds	Content (mg/cps)
Daidzein	13.11
Daidzin	18.19
ac-Daidzin	2.47
Glycitein	0.96
Glycitin	3.92
ac-Glycitin	0.65
Genistein	7.47
Genistin	5.12
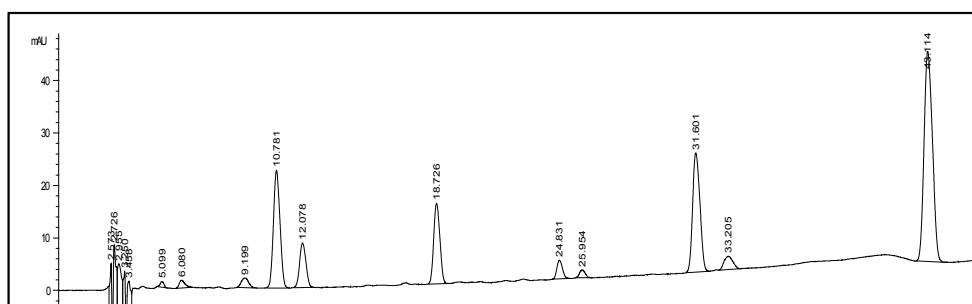
Fig. 1: HPLC chromatogram of DSU
Retention times of daidzin- 10.781, glycitin- 12.078, genistin- 18.726, acetyl daidzin- 24.831, acetyl glycitin- 25.954, daidzein- 31.601, glycitein- 33.205 and genistein- 43.114

that daidzein and its conjugates were present to the highest extent in this dietary supplement (25.59 mg/capsule). The second most frequent was genistein type of isoflavones (10.67 mg/cps). Glycitein was the least present in the analysed supplement (3.83 mg/capsule). It was observed that the content of total isoflavones (40.09 mg/capsule) was in accordance with label claim (40 mg/capsule) with deviation from the declared content of only +0.225 %. The concentration of total isoflavones in supplement analysed in the present study was 87.5 mg per gram of supplement.

Pretreatment with DSU reduced analgesic activity of codeine at the beginning of the experiment, in 5th and 10th min after codeine administration, analgesic effect of codeine was significantly lower in the group pretreated with DSU (CODDSU) compared to the control (p<0,05). On the other hand, 20 min post administration, percentage of pain depression was significantly higher in CODDSU group (Table 3). In other measured time points (30', 60' and 90') pretreatment with DSU has potentiated analgesic effect of codeine, however, this difference was not statistically significant.

The retention time of animals in DIADSU group was significantly shorter in 10th and 30th min after diazepam administration compared to the DIAS group (p<0.05). In other time points, DIADSU and DIAS groups exhibited no statistically significant differences. Unlike with diazepam, seven-day pretreatment with DSU (ALPDSU) significantly prolonged the retention time of animals in 20th and 30th min after alprazolam administration (p<0.05; Table 4).

It was observed that percentage of motoric coordination depression was significantly higher in 10th and 30th min in DIADSU group, compared to the control group that was treated with saline solution (DIAS group), p<0.05. Depression of motoric activity of mice in ALPS group was significantly lower in 20th and 30th min after alprazolam administration than in ALPDSU group (Table 5). In general, animals treated with alprazolam showed a higher rate of motoric coordination depression in all measured time points compared to the animals treated with diazepam. Pentobarbital-induced sleeping time in group pretreated with DSU (PENDSU) was significantly shorter compared to the control group (PENS; Table 6), and PENDSU group also exhibited significant prolongation of pentobarbital-induced sleeping time (p<0.05).

TABLE 3: EFFECT OF DSU ON ANALGESIC ACTIVITY OF CODEINE IN THE HOT-PLATE TEST

Time (min)	Pain depression (%) CODS	Pain depression (%) CODDSU
5	35.11±18.89	10.90±5.57*
10	42.93±11.93	16.63±3.12*
20	31.70±10.82	58.51±7.57*
30	32.35±19.88	37.79±15.39
60	11.16±34.29	17.56±37.53
90	40.52±16.14	42.54±55.21

Pain depression (%, mean±SD) induced by the codeine (30 mg/kg) in animals pretreated with saline solution (10 ml/kg) or DSU (dietary supplement based on soybean extract, 170 mg/kg) for seven days. *P<0.05 compared to the CODS group

TABLE 4: EFFECT OF DSU ON DIAZEPAM- AND ALPRAZOLAM-INDUCED CHANGES RETENTION TIME IN THE ROTAROD TEST

Time (min)	Retention time (s) DIAS	Retention time (s) DIADSU	Retention time (s) ALPS	Retention time (s) ALPDSU
10	296.8±1.92	224.8±49.43*	29.33±12.01	38.83±48.59
20	271.4±60.63	281.8±35.74	77.67±29.04	167.17±52.58[a]
30	293.8±3.03	267.8±49.22*	80.67±28.34	215.83±45.12[a]
50	294.6±3.05	293.6±2.70	103.33±32.5	184.83±54.13

Retention time (S, mean±SD) after diazepam (2 mg/kg) and alprazolam (2 mg/kg) administration in animals pretreated with saline solution (10 ml/kg) or DSU (dietary supplement based on soybean extract, 170 mg/kg) for seven days *p<0.05 compared to the DIAS group, [a]p<0.05 compared to the ALPS group

TABLE 5: EFFECT OF DSU ON DIAZEPAM- AND ALPRAZOLAM-INDUCED COORDINATION IMPAIREMENT IN THE ROTAROD TEST

Time (min)	Motor depression (%) DIAS	Motor depression (%) DIADSU	Motor depression (%) ALPS	Motor depression (%) ALP DSU
10	1.07±0.64	25.07±16.50*	90.22±10.01*	87.06±16.20[b]
20	9.53±20.21	6.07±11.91	74.11±15.24*	44.28±21.20[a,*,b]
30	2.07±1.01	10.73±16.49*	73.11±20.84*	28.06±9.62[a]
50	1.80±1.02	2.13±0.90	65.56±15.56*	38.39±10.23[b]

Depression of motor coordination (%, mean±SD) induced by diazepam (2 mg/kg) and alprazolam (2 mg/kg) in animals pretreated with saline (10 ml/kg) or DSU (dietary supplement based on soybean extract, 170 mg/kg) for seven days. *P<0.05 compared to the DIAS group, [a]p<0.05 compared to the ALPS group, [b]p<0.05 compared to the DIA DSU group

Single dose of CCl$_4$ induced 3.5-fold increase in MDA level when compared to control group. This was accompanied by the significant reduction of GSH concentration (0.7±0.11 nmol vs. 1.68± 0.44 nmol GSH/mg of proteins in control group), and reduced activity of all examined antioxidant enzymes, except GPX for which the increased activity in liver homogenates was noted (fig. 2). Administration of DSU did not enforce oxidative damage on liver cells, the activity of tested antioxidant enzymes did not differ significantly compared to control group. Pretreatment with DSU alleviated the toxic effects of CCl$_4$- the

levels of measured parameters were neared the values of the control group.

Administration of DSU alone did not affect the levels of measured biochemical parameters (Table 7). Treatment of animals with CCl$_4$ induced statistically significant increase in both total and direct bilirubin levels in serum. The impaired excretory function caused by CCl$_4$ was exacerbated after the co-treatment with DSU. AST and ALT activities were increased 25- and 20-fold, respectively in CONCCl$_4$ group compared to saline-treated group. The treatment of animals with DSU attenuated these parameters of hepatotoxicity significantly, even though the reduction was not to physiological levels. The values of both total and direct bilirubin in the group of animals treated with supplement alone were not significantly elevated in relation to control. In addition, the concentration of total and direct bilirubin was significantly lower in the DSU-treated group than in the group of animals treated

TABLE 6: EFFECT OF DSU ON PENTOBARBITAL (40 mg/kg)-INDUCED SLEEP TIME

PARAMETER	PENS	PENDSU
Induction time (min)	7.20±3.49	4.80±0.79*
Sleep duration time (min)	43.60±18.78	96.60±36.38*

Pentobarbital (40 mg/kg)-induced sleep time (min, mean±SD) in animals pretreated with saline (10 ml/kg) or DSU (dietary supplement based on soybean extract, 170 mg/kg) for seven days. *P<0.05 compared to the PENS group
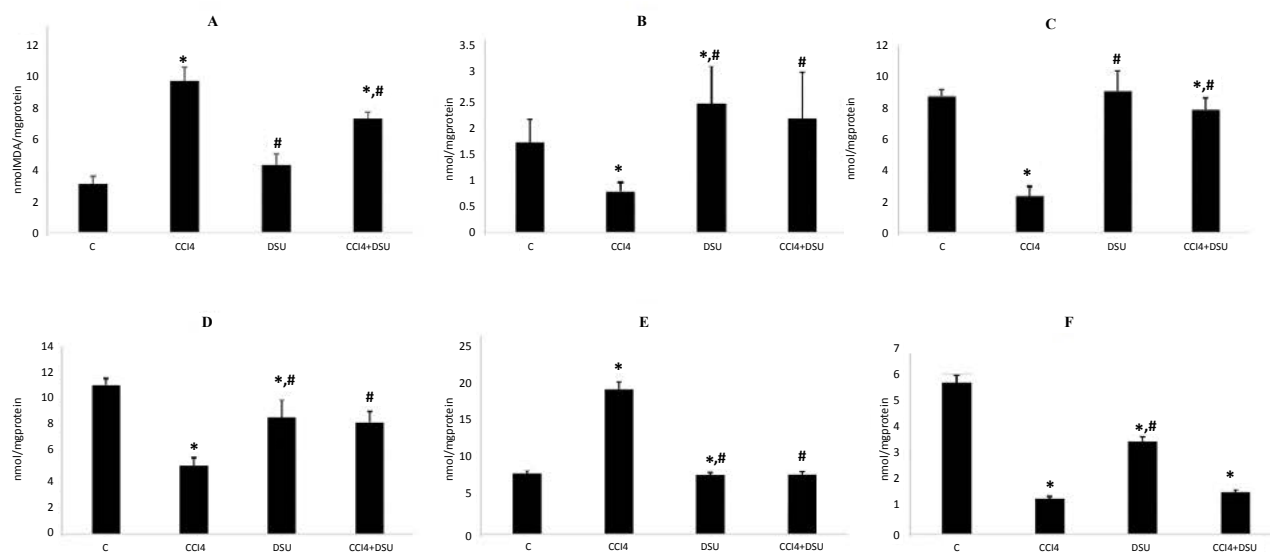
Fig. 2: *In vivo* antioxidant activity of DSU
(A) Levels of lipid peroxidation, (B) reduced glutathione, (C) catalase, (D) peroxidase, (E) glutathione peroxidase and (F) glutathione reductase in rat liver homogenates. *Significantly different from CONS group; #significantly different from CONCCl$_4$ group. CONS control group, saline 1 ml/kg p.o, CONCCl$_4$ saline 1 ml/kg p.o., single dose CCl$_4$ 2 mg/kg i.p., DSU soybean-based supplement 170 mg/kg for 7 d, DSUCCl$_4$ soybean-based supplement 170 mg/kg for 7 d, single dose CCl$_4$ 2 mg/kg i.p.; MDA, malondialdehyde

TABLE 7: EFFECT OF DSU PRETREATMENT ON SERUM BIOCHEMICAL, LIVER AND RENAL PARAMETERS

Parameter	CONS	CONCCL$_4$	DSU	CCL$_4$+DSU
Bilirubin total (mM)	2.16±0.17	4.21±0.42*	2.67±0.12#	5.24±0.74*#
Bilirubin direct (mM)	0.38±0.09	1.38±0.31*	0.62±0.08#	2.78±0.34*#
AST	117.40±5.10	2827.80±510.50*	138.70±8.10#	1512.10±121.30*#
ALT	48.50±1.30	983.70±231.50*	59.60±3.80#	817.30±173.20*
Urea	7.31±0.31	7.04±0.21	7.78±0.29	6.29±0.27#
Creatinine	46.10±1.72	54.10±0.64*	48.20±1.12	47.80±0.86#
Uric acid	58.90±2.31	102.40±13.10*	61.70±3.24#	77.40±7.31

DSU is dietary supplement based on soybean extract. *P<0.05 compared to control, #p<0.05 compared to CONCCL$_4$

only with CCl_4. But, both direct and total bilirubin concentrations were significantly higher in the group treated with the combination of CCl_4 and DSU in comparison to CCl_4 alone. Serum concentrations of urea, creatinine and uric acid, as biomarkers of renal function, were also examined. Administration of DSU for 7 d did not the affect the hepatic and renal function. These biochemical parameters were increased in CCl_4-treated group, indicating its nephrotoxicity, but the co-administration of DSU lowered the levels of these parameters.

In the present study, selected isoflavones were quantified and their effect on liver and renal function, their potential to ameliorate liver injury induced by pro-oxidant agent CCl_4 and herb-drug interactions with commercial DSU. Based on the patient information sheet, this supplement is used for relief of menopausal symptoms such as hot flashes, insomnia and nervousness. The beneficial effects of DSUs been attributed to presence of isoflavones, the most examined group of phytoestrogens from a nutritional and health point of view, with daidzein and genistein having the greatest effect on oestrogen receptors[3,34-36]. The analysis of examined DSU also confirmed that daidzein and genistein were present in the highest amount in the supplement.

As mentioned before, there is evidence that isoflavones affected the expression and activity of several drug-metabolizing enzymes[10-12], which implied a risk of clinically significant interactions between DSU and conventional drugs metabolized through these enzymes. Our results showed that DSU administration affected pharmacodynamics of all tested conventional drugs. Pretreatment with DSU had notably potentiated pharmacodynamic effect of diazepam and had shown an increased impairment of motor coordination in the rotarod test. The opposite effect on alprazolam activity was observed. Furthermore, statistically significant decrease of time of induction and prolongation of sleep duration after pretreatment with DSU was detected in the pentobarbital-induced sleeping time test[37]. The obtained results were in accordance with current literature data, confirming that soybean isoflavones have the ability to potentiate the change the activity of microsomal enzymes and in this way activate the drug metabolism, thus changing the pharmacological profile of studied central nervous system depressors[11,17,38]. It is suggested that soybean isoflavones, inhibit microsomal enzymes responsible for degradation of pentobarbital

to inactive metabolites, thus potentiating its hypnotic effect while having opposite effect on the isoforms responsible for the benzodiazepine metabolism. DSU treatment influenced pharmacodynamics of codeine in an interesting manner. Following 7 d treatment with commercially available DSU, analgesic effect of codeine in 5th and 10th min of the hot-plate test decreased. Contrary to that, in 20th min and afterwards, the analgesic effect of codeine in the experimental group became stronger compared to control group. Decrease of codeine's analgesic effect at the beginning of hot plate test can be explained by inhibition of codeine metabolism via CYP2D6 by soybean extract, and subsequent decrease in the production of its active metabolites[39,40], while the following potentiation of analgesia can be the consequence of elevated glucuronyltransferase activity. Glucuronidation of codeine produces morphine-6-glucuronide, which analgesic effect is even stronger than morphine[40].

The results of liver and renal function tests, demonstrated that 7 d pretreatment with DSU alone did not negatively affect the liver and renal excretory function. The values of biochemical parameters measured in serum did not differ between the control and DSU-treated group and supplementation exhibited no negative effects on excretory capacity and liver function of treated animals. Pretreatment with DSU also exhibited protective effects on the extent of parenhimatose organ damage induced by CCL_4. Supplementation had positive effect on serum transferase levels, implicating better preservation of liver cellular integrity that can be explained by the marked *in vivo* antioxidant activity of tested supplement. As noticeable in fig. 2, DSU showed ability to prevent CCl_4-induced increase in MDA level, one of the well-known secondary products of lipid peroxidation, an indicator of cell membrane injury. The accumulation of ROS after CCL_4 challenges the endogenous antioxidant capacities, leading to depletion of PX, GSH, CAT and GR levels. The administration of DSU restored the levels of these enzymes to those of control group. The hepatoprotective potential of soy-derived products has been previously documented. Kim *et al.* examined the effects of fermented soybean extract on tert-butyl hydroperoxide-induced oxidative stress in HepG2 cells and rat liver. The fermented soy extract demonstrated antioxidant activity and the ability to decrease the serum levels of clinical markers for liver function[41]. Tofu made from fermented soy also exhibited protective effects in CCl_4-induced liver

injury[42], and soyasaponins-rich extract from soybean prevented the increases in serum transaminases and markers of oxidative stress in alcohol-induced liver damage[43]. Besides the ability to restore the antioxidant capacity in liver tissue homogenates, the levels of AST and ALT were significantly lower in animals treated with DSU compared to CONCCl$_4$, even though the DSU did not restore these parameters to physiological levels. Nephroprotective effects of DSU were also noted, as there was statistically significant decrease in urea and creatinine levels compared to CONCCl$_4$ group.

Despite all these effects, supplementation failed to prevent CCl$_4$-induced increase in bilirubin levels. High levels of both direct and total bilirubin with concomitant physiological levels of aminotransferases, indicative of cholestatic liver injury, were measured and especially pronounced in the CCl$_4$DSU group. Cholestasis occurring after CCl$_4$ administration appeared to be mediated through changes in the activity of several efflux transporters. One of these efflux pumps, a MRP3 transporter, responsible for the occurrence of conjugated hyperbilirubinemia[44], was shown to be major factor in phytoestrogens disposition[45], which might explain the aggravation of already disrupted bilirubin secretion noted in CCl$_4$ and DSU-treated animals.

Despite several beneficial effects noted in animals subjected to oxidative stress, large potential for interactions renders this supplement suitable for further examination with regard to safety and clinically relevant interactions. Administration of DSU concomitantly to several central nervous system acting drugs significantly changed pharmacological profiles of conventional drugs, depending on their metabolic pathways and biotransformation.

Commercially available DSU significantly changed pharmacological profile of diazepam, alprazolam, pentobarbital and codeine. This suggested that isoflavones modify the activity of microsomal enzymes, there by affecting the metabolic processes and subsequent pharmacological effects of the conventional drugs studied. Hence, the risk of possible side effects and interactions between these conventional drugs and dietary supplements based on soybean extract cannot be excluded. Our study confirmed that there is a need for further research in this field to obtain new preclinical, safety and toxicity data, with a special focus on interactions with conventional drugs. This would ensure safe use of DSUs by the patients, especially taking into consideration its therapeutic benefit and increased use by the patients.

Conflict of interest:

The authors declare that they have no competing interests.

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