

EFFECTS OF *CYCLAMEN TROCHOPTERANTHUM* ON HEPATIC DRUG-METABOLIZING ENZYMES

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Abstract - The modulatory effects of the *Cyclamen trochopteranthum* tuber extract on hepatic drug-metabolizing enzymes, including aniline 4-hydroxylase (A4H; CYP2E1), ethoxyresorufin *O*-deethylase (EROD; CYP1A), methoxyresorufin *O*-demethylase (MROD; CYP1A), caffeine *N*-demethylase (C3ND; CYP1A2) aminopyrene *N*-demethylase (APND; CYP2C6), and erythromycin *N*-demethylase (ERND; CYP3A1), were examined *in vivo* in rats. The activities of all of these enzymes were induced by the cyclamen extract. In addition, Western-blot and RT-PCR results clearly showed that CYP2E1, CYP1A1/CYP1A2 and CYP2C6 protein and mRNA levels were substantially increased by four different doses of cyclamen. Although, the CYP3A1 protein level was increased significantly, the mRNA level was not changed. These results indicate that cyclamen tuber extract might have a potential not only to inhibit and/or induce the metabolism of certain co-administered drugs but also influence the development of toxicity and carcinogenesis due to the induction of the cytochrome P450-dependent drug-metabolizing enzymes.

Key words: *Cyclamen trochopteranthum*, drug-metabolizing enzymes, complementary and alternative herbs, drug interaction potential, cytochrome P450

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INTRODUCTION

In recent years, the use of complementary and alternative therapies mostly depending on herbs and herbal preparations has grown substantially. Their usage generally increases in areas in which conventional methods have failed to provide satisfactory solutions to the treatment of diseases, such as in cancer and HIV infection. Many herbs and herbal preparations are natural and so they are considered as safe. However, the lack of critical information regarding the toxicity, allergenicity, mutagenicity and possible drug interactions of these compounds constitutes a serious issue with respect to their use. It is well established that many herbs and herbal remedies such as garlic, green tea, grapefruit, curcumin, echinacea, ginseng, ginkgo, kava kava, urtica

and ginger interact pharmacokinetically with drugs by modulating both the activities and expression of drug-metabolizing enzymes, particularly cytochrome P450s (Delgoda and Westlake, 2004; Yang and Raner, 2005; Ozkarsli et al., 2008; Agus et al., 2009). Thus, *in vitro* and *in vivo* studies clarifying pharmacokinetic interactions, altered drug concentrations and enhanced bioactivation of drugs to reactive intermediates, which might be attributable to the induction and/or inhibition of cytochrome P450s by co-administered herbs, have important clinical significance (Zhou et al., 2003).

The cytochromes P450 (CYP) are a superfamily of hemoproteins that play critical roles in the bioactivation and detoxification of a wide variety of xenobiotic substances. Besides, they also have

roles in the metabolism and synthesis of endogenous compounds. They are mainly located in the smooth endoplasmic reticulum membranes as well as in mitochondrial inner membrane. Multiple isozymes of P450 show different substrate specificities and affinities toward both endogenous and exogenous compounds. Among these, P450s, CYP3A, CYP2C, CYP2D, CYP1A, CYP2E subfamilies have received a great deal of attention in recent years because of their ability to metabolize various pharmaceutical agents, and because of their role in carcinogenicity (Sen and Arinc, 1998; Chung et al., 2004). It is well established that CYP1A and CYP2E enzymes are mainly involved in carcinogen metabolism while CYP3A, CYP2D and CYP2C enzymes are mainly responsible for drug metabolisms.

The CYP3A subfamily is considered to have the greatest overall impact on human pharmacotherapy because it is the most abundantly expressed CYP in the human liver and small intestine. It possesses metabolic activity towards an extremely broad spectrum of xenobiotic substrates including antibiotic, anti-arrhythmics, sedatives, immune system modulators, calcium channel blockers, HIV-directed antiviral agents and HMG CoA reductase inhibitors (Sugimoto et al., 2006; de Wildt et al., 2007). The CYP2C subfamily accounts for approximately 20% of the total liver cytochrome P450 content in humans. It is responsible for the metabolism of approximately 20% of all clinically administered drugs, including anti-coagulants, anti-diabetic agents, non-steroidal anti-inflammatory drugs, anti-convulsants (Carlile et al., 1999). Cytochrome P4502E, the ethanol inducible form of P450, can metabolize many low molecular weight endogenous and exogenous compounds such as acetone, long chain fatty acids, benzene, chloroform, pyridine, acetaminophen, chlorzoxazone, trimethadione and acetylsalicylic acid (aspirin) (Lieber, 1999; Klotz and Ammon, 1998). The CYP1A subfamily is responsible for the metabolism of pharmaceuticals and well-known human carcinogens (Adamson et al., 1996; Kim and Guengerich, 2005; Ma and Lu, 2007).

The Turkish flora includes around 12 000 plant species. About one third of these plants are endemic and most of these are used as medicines and spices in Turkey. Cyclamen are a genus of twenty species within the family *Primulaceae*. *Cyclamen trochop-teranthum* is one of the endemic species of cyclamens in Turkey. It is a species that grows naturally in the south-western part of Turkey, especially in Antalya, Mugla, Denizli, Burdur, and Isparta. Many cyclamen species are widely used as ethno-medicine for the treatment of hemorrhoids and eczema and expelling digestive tract worms. It was reported that cyclamen is also used in Turkish folk medicine against infertility (Calis et al., 1997). The cyclamen extract shows interesting spermicidal, anti-microbial, anti-inflammatory and anti-nociceptive activities and is used in rhinosinusitis (Primorac et al., 1985; Mahasneh and El-Oqlah, 1999; Speroni et al., 2007). Despite their wide usage and diverse biological activities, there is no available information about the effect of cyclamens on xenobiotic metabolism and possible drug interaction potential. In this respect; the aim of this study is to determine the effect of the ethanol extracts of cyclamen tubers on hepatic CYP450 isozymes.

MATERIALS AND METHODS

Chemicals

The following chemicals were purchased from Sigma-Aldrich Chemical Company (St Louis, Missouri, USA): acrylamide, aniline, anti-rabbit IgG-ALP conjugate, bovine serum albumin (BSA), Folin phenol reagent, glycerol, glycine, HEPES, β -NADPH, phenol, anti-rabbit IgG-ALP conjugate, caffeine, TRIS, phenylmethylsulphonyl fluoride (PMSF), potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium dodecyl sulfate (SDS), sodium potassium tartrate. Anti-rat CYP1A2, CYP2C6, CYP2E1 and CYP3A1 antibodies were from Abcam (Abcam PLC, Cambridge, UK). Iblot Transfer Stack was purchased from Invitrogen Corporation, (Carlsbad, CA, USA). All other chemicals and solvents were obtained from commercial sources at the highest grade of purity available.

Preparation of the Cyclamen trochopteranthum tuber extract

The fresh tubers of *Cyclamen trochopteranthum* used in the present study were collected from Denizli, Turkey. The tubers (200 g) were first peeled, cut into 5 mm × 5 mm cubes. These tubers were extracted two times with ethanol at 55°C. The ethanol extract was then dried under a vacuum in a rotary evaporator and the remaining material was ground into a fine powder. The powder was then *lyophilized and stored at -80°C until usage*.

Standardization of Cyclamen trochopteranthum tuber extract

The total concentration of phenolic compounds in the extracts was determined using a series of gallic acid standard solutions (0.05-0.3 mg/mL) as described by Singleton and Rossi (1965). Results were expressed as milligrams of total phenolics content per grams of extract as gallic acid equivalents (GAE).

Animals and treatment

Healthy male Wistar rats, about 12 weeks old and weighing 200–250 g, were obtained from the University Animal House. They were housed in small cages at an ambient temperature of $22 \pm 1^\circ\text{C}$, on a 12 h light/dark cycle and were fed commercial rat food with water *ad libitum*. All experimental procedures with the animals were performed under appropriate regimes with veterinary services within the licensed projects. Since no data are available on the consumption habit of cyclamen we had carried out preliminary pilot studies involving the dose-response activities to find out the right doses without any toxic side effects (data not shown). In addition, the extract was given to the animals in drinking water in order to correspond to human use. After a 10 day adaptation period, the rats were divided into five groups consisting of 4-8 animals each. The first group consisted of control animals administered water without extract. The other groups were treated with cyclamen extract at doses of 0.1, 0.2, 0.5 and 1.0 mg/ml in drinking water for 10 consecutive days. At the end of the ex-

perimental period and following 16 h of fasting, the rats were killed; the livers were removed, rinsed with cold physiological saline and stored at -80°C until analyses.

Microsome preparation

Tissues were homogenized in 4 part homogenization solution [1.15% KCl containing 3 mM EDTA, 0.5 mM PMSF, 0.3 mM ϵ -aminocaproic acid, 0.15 mM butylated hydroxytoluene, 0.025% Triton X-100] using a tissue homogenizer with a Teflon pestle at 4°C. Subcellular fractions of rat tissues were prepared by standard differential centrifugation with calcium aggregation as described by Sen and Kirikbakan (2004). The amount of protein in individual fractions was measured using the method of Lowry et al. (1951) with BSA as the standard.

Enzyme assays

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) activities were determined with an autoanalyzer using Audit diagnostics AST, ALT and LDH enzyme kits. The microsomal cytochrome P450-dependent aniline 4-hydroxylase (A4H) activities of rat microsomes were determined by measuring the quantity of p-aminophenol formed, as described by Imai et al. (1966). Aminopyrene N-demethylase (APND), erythromycin N-demethylase (ERND) and caffeine N-demethylase (C3ND) activities were determined by measuring the quantity of formaldehyde formed, according to the method of Nash (1953) and modified by Cochin and Axelrod (1959). Ethoxyresorufin O-deethylase (EROD), methoxyresorufin O-demethylase (MROD) activities were assayed as described by Sen and Arinc (1998).

Gel electrophoresis and Western blotting

SDS-PAGE and Western blotting were performed as described previously (Sen and Arinc, 1998). Briefly, 120 μg protein samples were separated on 8.5% polyacrylamide gels using the discontinuous buffer system of Laemmli (1970). Proteins were transferred to

a nitrocellulose membrane by the iBlot dry blotting system (20 V, 12 min), using iBlot gel transfer stacks. Following transfer, the membranes were blocked using 5% non-fat dry milk in TBST (20 mM Tris-HCl, pH 7.4, 400 mM NaCl and 0.1% (v/v) Tween 20) for 60 min and incubated with rabbit polyclonal anti-rat CYP1A2, CYP2C6, CYP2E1 or CYP3A1 antibodies (diluted 1:1000 in blocking solution) for 120 min at room temperature. The membranes were then washed with TBST (3 × 5 min), incubated with the secondary antibody (ALP-conjugated anti-rabbit IgG at a 1:5000 or 1:10000 dilution) for 60 min and again washed with TBST (3 × 5 min). Visualization of the bands was carried out using the NBT/BCIP substrate system. The final images were photographed by using computer-based gel imaging instrument (DNR LightBIS Pro Image Analysis System, Israel). Protein bands were quantified using Scion Image Version Beta 4.0.2 software.

RNA isolation and RT-PCR of CYP mRNAs

Total RNA was extracted from 100 mg rat livers using Trizol reagent. Extracted RNA was quantified spectrophotometrically at 260/280 nm and the integrity was checked using 1% agarose gel. For cDNA synthesis, 2.5 µg of RNA was incubated at 70°C for 10 minutes with 0.5 µg of oligo(dT). After 5 min on ice, 50 U Moloney murine leukemia virus reverse transcriptase, 1 mM dNTPs and 5X reaction

buffer were added to the previous mixture and incubated at 42°C for 60 min. The reaction was stopped by heating to 70°C for 10 min and the cDNA was stored at -80°C for further use.

Semi-quantitative two-step RT-PCR assay was performed by using gene specific primers. The oligo sequences used as forward and reverse primers for rat CYP450 isozymes were based on those reported in Agus et al. (2009). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin were used as a housekeeping gene. Preliminary control experiments (data not shown) were carried out to verify the RT-PCR conditions allowed for linear amplification of PCR products. The PCR products were analyzed by electrophoresis on 1.5% agarose gels containing ethidium bromide. The intensity of the bands was measured using Scion Image Version Beta 4.0.2 software. Levels of mRNA for CYP genes were determined by measuring the band intensity of the RT-PCR product on each agarose gel and are reported relative to GAPDH or β-actin expression.

Statistical analysis

Statistical analyses were performed by using the Minitab statistical software package. All results were expressed as means with their Standard Error of Means (SEM). Comparison between two groups was

Table 1. Blood serum LDH, AST and ALT enzyme activities in control and cyclamen treated rats.

	Lactate Dehydrogenase (LDH) Unit/min/mg protein	Change (Fold)	Aspartate Aminotransferase (AST) Unit/min/mg protein	Change (Fold)	Alanine Aminotransferase (ALT) Unit/min/mg protein	Change (Fold)
Control	11.08 ± 0.96	--	1.51 ± 0.14	--	1.36 ± 0.09	--
0.1 mg/ml Cyclamen	12.62 ± 2.9	1.1 X ↑	1.55 ± 0.38	1.02 X ↑	1.38 ± 0.08	1.01 X ↑
0.2 mg/ml Cyclamen	11.74 ± 0.34	1.06 X ↑	1.57 ± 0.07	1.04 X ↑	1.51 ± 0.04	1.11 X ↑
0.5 mg/ml Cyclamen	13.68 ± 0.74	1.23 X ↑	1.68 ± 0.12	1.11 X ↑	1.54 ± 0.07	1.13 X ↑
1.0 mg/ml Cyclamen	22.6 ± 7.0*	2.04 X ↑	2.74 ± 0.70*	1.81 X ↑	1.72 ± 0.04*	1.26 X ↑

*Significantly different from the respective control value $p < 0.05$

Table 2. Stimulation of Erythromycin *N*-demethylase and Aminopyrene *N*-demethylase activities in liver of cyclamen treated rats..

	Erythromycin <i>N</i> -demethylase (nmol HCHO/min/mg prot.)	Change (Fold)	Aminopyrene <i>N</i> -demethylase (nmol HCHO/min/mg prot.)	Change (Fold)
Control	0.03± 0.0033 (N=8)	--	0.023 ± 0.003 (N=8)	--
0.1 mg/ml <i>Cyclamen</i>	0.066 ± 0.011* (N=4)	2.20 X ↑	0.026 ± 0.005 (N=4)	1.13 X ↑
0.2 mg/ml <i>Cyclamen</i>	0.0780 ± 0.004*** (N=4)	2.60 X ↑	0.025 ± 0.002 (N=4)	1.09 X ↑
0.5 mg/ml <i>Cyclamen</i>	0.0420 ± 0.006 (N=4)	1.40 X ↑	0.031 ± 0.004 (N=4)	1.35 X ↑
1.0 mg/ml <i>Cyclamen</i>	0,050 ± 0.007* (N=4)	1.67 X ↑	0.046 ± 0.002** (N=4)	2.00 X ↑

*Significantly different from the respective control value $p < 0.05$

**Significantly different from the respective control value $p < 0.005$

***Significantly different from the respective control value $p < 0.0001$

Table 3. Changes of Aniline 4-hydroxylase, Ethoxyresorufin *O*-deethylase, Methoxyresorufin *O*-demethylase and Caffeine *N*-demethylase activities in liver of cyclamen treated rats.

	Aniline 4-hydroxylase (nmol HCHO/min /mg prot.)	Change (Fold)	Ethoxyresorufin <i>O</i> -deethylase (pmol Resorufin/ min/mg prot.)	Change (Fold)	Methoxyresorufin <i>O</i> -demethylase (pmol Resorufin/ min/mg prot.)	Change (Fold)	Caffeine <i>N</i> -demethylase (nmol HCHO/min/mg prot.)	Change (Fold)
Control	0.15± 0.008 (N=8)	--	0.60 ± 0.07 (N=8)	--	3.57 ± 0.6 (N=8)	--	0.049 ± 0.007 (N=8)	--
0.1 mg/ml <i>Cyclamen</i>	0.33 ± 0.011* (N=4)	2.20 X ↑	1.76 ± 0.36* (N=4)	2.93 X ↑	7.91 ± 1.5* (N=4)	2.21 X ↑	0.077 ± 0.008* (N=4)	1.57X ↑
0.2 mg/ml <i>Cyclamen</i>	0.20 ± 0.004 ** (N=4)	1.33 X ↑	1.23± 0.033* (N=4)	2.05 X ↑	8.19± 1.8* (N=4)	2.29 X ↑	0.056 ± 0.003 (N=4)	1.14 X ↑
0.5 mg/ml <i>Cyclamen</i>	0.18 ± 0.008* (N=4)	1.20 X ↑	0.91 ± 0.09* (N=4)	1.52X ↑	7.85 ± 1.2* (N=4)	2.20X ↑	0.098 ± 0.008* (N=4)	2.00 X ↑
1.0 mg/ml <i>Cyclamen</i>	0.29 ± 0.01*** (N=4)	1.93 X ↑	0.35 ± 0.07* (N=4)	1.86 X ↑	10.39 ± 0.4*** (N=4)	2.91 X ↑	0.075 ± 0.006* (N=4)	1.53 X ↑

*Significantly different from the respective control value $p < 0.05$

**Significantly different from the respective control value $p < 0.005$

***Significantly different from the respective control value $p < 0.0001$

performed by Student's *t*-test and $p < 0.05$ was chosen as the level for significance. Statistical comparisons between five groups were assessed by one-way analysis of variance (ANOVA). When *F* ratios were significant ($p < 0.05$), one-way ANOVA was followed by Tukey's post hoc test for comparisons of multiple group means.

RESULTS

Cyclamen tuber extract was applied to rats in drinking water at four different doses. Control and treated rats showed no significant differences in food consumption or body weight (data not shown). As shown in Table 1, blood serum AST, ALT and LDH

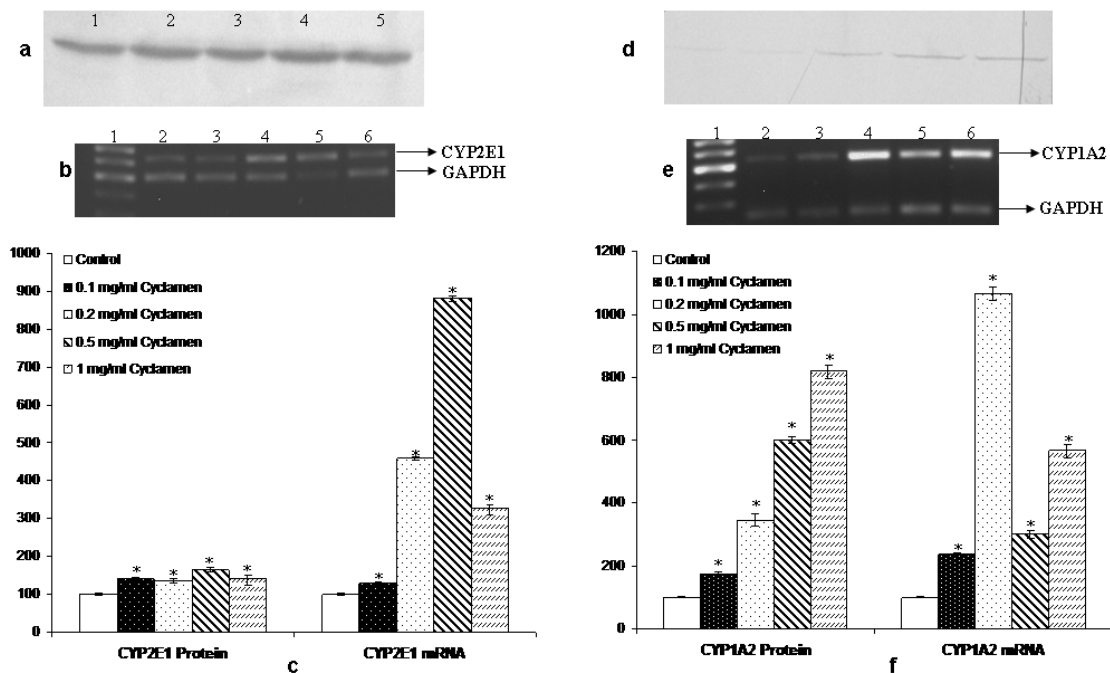


Fig. 1. The expression levels of CYP2E1 and CYP1A2 proteins and mRNAs in control rats and rats treated with *C. trochopteranthum*. Treatments were carried out as described in Materials and Methods. **a.** Representative immunoblot analysis of liver microsomal CYP2E1 proteins in experimental groups. Lane 1, control; Lanes 2 to 5 different doses of cyclamen treatment. **b.** Representative agarose gel showing the effect of treatments on regulation of CYP2E1 mRNA expressions in liver, analyzed by RT-PCR. Lane 1, 1 KB DNA ladder; Lane 2, CYP2E1 in control; Lanes 3, 4, 5 and 6, CYP2E1 in cyclamen-treated rats. **c.** Comparison of CYP2E1 protein and mRNA levels among experimental groups. The bar graphs represent the mean intensity of the bands obtained from Western blot and/or RT-PCR results. **d.** Representative immunoblot analysis of liver microsomal CYP1A2 proteins in experimental groups. Lane 1, control; Lanes 2 to 5 different doses of cyclamen treatment. **e.** Representative agarose gel showing the effect of treatments on regulation of CYP1A2 mRNA expressions in liver, analyzed by RT-PCR. Lane 1, 1 KB DNA ladder; Lane 2, CYP1A2 in control; Lanes 3, 4, 5 and 6, CYP1A2 in cyclamen-treated rats. **f.** Comparison of CYP1A2 protein and mRNA levels among experimental groups.

Experiments were repeated at least three times. Results are presented as mean \pm standard deviation. * $p < 0.05$.

activities were increased compared to the control rats. With the highest dose of cyclamen treatment the increases of AST, ALT and LDH activities were found to be significant ($p < 0.05$) whereas other doses were not (Table 1).

The total phenolic content of the *C. trochopteranthum* tuber extract was determined by the method of Singleton and Rossi (1965) and the results are expressed as mg per g of gallic acid equivalents (GAE) to standardize the extract. The amount of total phenolic compounds found in the extract of *Cyclamen*

trochopteranthum tuber extract was 16.4 ± 0.5 mg GAE per g extract.

As presented in Table 2, hepatic CYP3A1-associated ERND activity was increased 2.20-, 2.60-, 1.40- and 1.67-fold in cyclamen-treated rats when compared to control ones. These increases were found to be significant ($p < 0.05$) in cyclamen-treated rats at a dose of 0.1, 0.2, and 1.0 mg/ml whereas at that of 0.5 mg/ml they were not (Table 2). On the other hand, 0.1, 0.2 and 0.5 mg/ml cyclamen extract treatment did not change the CYP2C6-

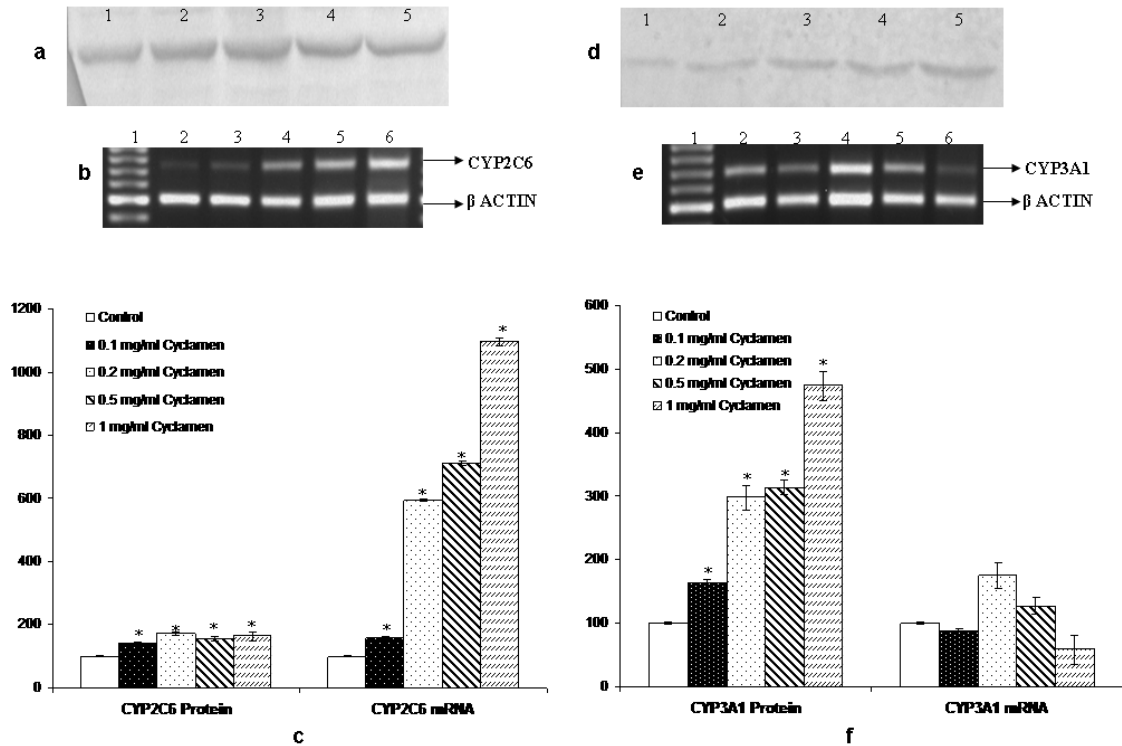


Fig. 2. The expression level of CYP2C6 and CYP3A1 protein and mRNAs in control rats and rats treated with *C. trochopteranthum*. Treatments were carried out as described in Materials and Methods. **a.** Representative immunoblot analysis of liver microsomal CYP2C6 proteins in experimental groups. Lane 1, CYP2C6 protein in control; Lanes 2 to 5 CYP2C6 protein in different doses of cyclamen treatment. **b.** Representative agarose gel showing the effect of treatments on regulation of CYP2E1 mRNA expressions in liver, analyzed by RT-PCR. Lane 1, 1 KB DNA ladder; Lane 2, CYP2C6 in control; Lanes 3, 4, 5 and 6, CYP2C6 in cyclamen-treated rats. **c.** Comparison of CYP2C6 protein and mRNA levels among experimental groups. The bar graphs represent the mean intensity of the bands obtained from Western blot and/or RT-PCR results. **d.** Representative immunoblot analysis of liver microsomal CYP3A1 proteins in experimental groups. Lane 1, CYP3A1 proteins in control; Lanes 2 to 5, CYP3A1 proteins in different doses of cyclamen treatment. **e.** Representative agarose gel showing the effect of treatments on regulation of CYP3A1 mRNA expressions in liver, analyzed by RT-PCR. Lane 1, 1 KB DNA ladder; Lane 2, CYP3A1 in control; Lanes 3, 4, 5 and 6, CYP3A1 in cyclamen-treated rats. **f.** Comparison of CYP3A1 protein mRNA levels among experimental groups. The bar graphs represent the mean intensity of the bands obtained from Western blot and/or RT-PCR results

Experiments were repeated at least three times. Results are presented as mean \pm standard deviation. * $p < 0.05$.

associated APND activity significantly in rat liver (Table 2). In contrast, in the 1 mg/ml group this activity was increased around 2-fold with respect to control rats.

The effect of the cyclamen tuber extract on CYP2E1-associated A4H activity is presented in Table 3. Cyclamen extract treatment at a dose of 0.1, 0.2, 0.5 and 1.0 mg/ml in drinking water for 10 consecutive days caused a statistically significant 2.20-, 1.33-, 1.20- and 1.93-fold increase in A4H activity in

the liver, respectively. CYP1A-associated EROD and MROD activities in control and cyclamen-treated rats are given in Table 3. As can be seen, EROD activity was increased 2.93-fold ($p < 0.05$) in the lowest dose (0.1 mg/ml) of cyclamen extract. When the dose of cyclamen was increased, the extent of EROD induction decreased and was finally inhibited 1.86-fold ($p < 0.05$) at the highest dose of cyclamen extract (1.0 mg/ml). On the other hand, treatment of rats with four different concentrations of cyclamen extract caused 2.21-, 2.29-, 2.20- and 2.91-fold in-

creases in MROD activity with respect to the control (Table 3). The effect of different concentrations of cyclamen extract on CYP1A2-dependent C3ND activity was also determined throughout in this study. 0.1, 0.2, 0.5 and 1.0 mg/ml cyclamen extract in drinking water caused a statistically significant 1.57-, 1.14-, 2.0- and 1.53-fold increase in the C3ND activity in liver, respectively.

Activation of catalytic activities was generally consistent with the protein levels of related CYP isoforms in rat liver microsomes that were prepared from control and cyclamen-treated rats (Figs. 1 and 2). The densitometric scanning of Western blot results showed that hepatic CYP2E1 protein level was increased significantly 1.5-fold in the cyclamen-treated rats relative to the control animals. Similarly, CYP1A2 protein level was increased (1.75-8.20-fold) by increasing doses of cyclamen extract (Fig. 1). Moreover, cyclamen treatment caused induction of the CYP2C6 protein level (Fig. 2). Also, 1.65 to 4.76-fold induction of the CYP3A1 protein level was observed as a result of different doses of cyclamen treatment with respect to control rats (Fig. 2).

The effect of cyclamen extract on the mRNA levels of CYP isozymes was also determined throughout in this study. The relative CYP2E1 mRNA level was increased up to 8.8-fold in the cyclamen-treated rats compared to the control animals (Fig. 1). Similarly, CYP1A2 levels were increased significantly 2.35-, 10.65-, 3.00- and 5.65-fold in cyclamen-treated rats at a dose of 0.1, 0.2, and 1.0 mg/ml 0.1 mg/ml, respectively (Fig. 1). Similar to CYP2E1 and CYP1A2, CYP2C6 mRNA levels were increased significantly, from 1.60- to 10.98-fold, as a result of the cyclamen treatment (Fig. 2). On the other hand, the cyclamen treatment did not change the relative CYP3A1 mRNA level with respect to the control rats (Fig. 2).

DISCUSSION

Interest in herbal remedies is on the increase in the Western world. However, organ toxicities of the

heart, liver, blood, kidneys, central nervous system, skin and carcinogenesis due to adverse drug reactions and poisonings associated with the use of herbal medicines have also increasingly been reported. (Deng, 2002; Klepser and Klepser, 1999). The most important factor that is mainly involved in the development of these undesired effects is both the induction and inhibition of CYP450-dependent drug-metabolizing enzymes of many herbs (Zhou et al., 2003). In this study, for the first time, the inductive effect of cyclamen on P450 isozymes was shown by an increase of associated enzyme activities in parallel to significant protein and mRNA induction in rat liver.

The amount of total phenolics was found to be 16.4 ± 0.5 mg GAE per g dry extract. Phenolic acids have repeatedly been implicated as natural antioxidants in fruits, vegetables, and other plants. The high phenolic content suggests that the cyclamen extract has a reasonably high antioxidant activity.

It is well established that, LDH, AST and ALT alone or in combination are primarily recommended for the assessment of hepatocellular injury in rodents and non-rodents in non-clinical studies. In this study, the activities of LDH, AST and ALT in the blood serum were increased in cyclamen-treated rats with respect to controls. The observed increases in serum might be an indication of mild tissue damage due to cyclamen treatment.

Cyclamen extract treatment caused an increase in CYP3A-associated ERND activity in rat liver microsomes. Similar to the observation in this study, the induction of liver CYP3A-associated enzyme activity by various plant extracts such as St John's wort extract and Ginkgo biloba extract, was shown (Dürr et al., 2000). The results of this study show that drug interactions and clinical toxicity are likely to occur if the cyclamen extract is taken simultaneously with a broad spectrum of drugs. The effects of cyclamen extract on CYP3A1 protein and mRNA levels were investigated in order to determine the underlying mechanism of the increase in CYP3A1-associated enzyme activity. Although the CYP3A1 protein lev-

el was increased significantly, the mRNA level was not changed. Different posttranslational increases in CYP3A have been reported (Zangar et al., 1997). Analogous to their results, our studies suggest point to an increase in CYP3A1 activity and protein content by protein stabilization.

Treatment with the cyclamen extract caused a significant increase in CYP2C6-associated APND activity only at the highest dose. On the other hand, both the CYP2C6 protein and mRNA level were induced by cyclamen in four different doses. Similarly, Kuo et al. (2006) has shown that a CYP2C-inducing agent is present in the ethyl acetate extract of *Salvia miltiorrhiza*. Thus, *C. trochopteranthum* might possess CYP2C-inducing agents. The results of the present study showed that alterations of drug clearance and clinical drug toxicity could be observed due to induction CYP2C in people who used cyclamen as a herbal remedy.

Intake of the cyclamen tuber extract at doses of 0.1, 0.2, 0.5 and 1.0 mg/ml in drinking water for 10 consecutive days increased A4H activity in the liver. Densitometric analysis of Western blots showed that the hepatic CYP2E1 protein level was increased significantly in the cyclamen-treated rats relative to the control animals. Some other herbs or chemical compounds isolated from herbs caused the induction of not only CYP2E1 catalytic activity but also its protein level (Bray et al., 2002). Cytochrome P4502E1 has received a great deal of attention in recent years because of its vital role in the activation of many low molecular weight toxic chemicals such as benzene, CCl₄, nitrosamines and pyridine (Guengerich et al., 1991; Arinc et al., 2000). The results of the present work indicate that cyclamen could stimulate the metabolic activation of *N*-nitrosodimethylamine, pyridine, benzene (and other toxic chemicals metabolized by CYP2E1) by inducing CYP2E1 which results in increased amounts of reactive metabolites formation. This may in turn further potentiate the risk of organ toxicity, mutagenesis and malignant transformation in the liver of these subjects. Administration of the cyclamen extract increased the CYP2E1 mRNA level too. The

regulation of CYP2E1 expression is complex, involving transcriptional, post-transcriptional, and post-translational events with polymorphism playing a role (Song, 1995). Therefore, the observed mRNA increase resulting from cyclamen treatment could be either transcriptional or post-transcriptional; this remains to be elucidated.

Among all cytochrome P450 isoforms, CYP1A holds priority due to its role in the metabolism of carcinogens, mutagens and environmental pollutants. In this study, CYP1A1/CYP1A2-associated enzyme activities, EROD, MROD and C3ND, were increased as a result of cyclamen treatment. CYP1A2 protein and mRNA levels were also increased during treatment with four different doses of cyclamen extract. Similar to cyclamen, many herbs and their extracts, such as *Salvia miltiorrhiza*, caused the induction of CYP1A-related enzyme activities and protein levels (Kuo et al., 2006). This work indicates that the induction of CYP1A by cyclamen stimulates the metabolic activation of benzo(a)pyrene, aromatic and heterocyclic amines. As a result of this metabolic activation, organ toxicity, mutagenesis and carcinogenesis may be observed. It is well established that the Aryl hydrocarbon Receptor (AhR) acts as a transcription factor to regulate multiple genes including the CYP1A family. Therefore, the induction of CYP1A by cyclamen administration may be caused by the upregulation of the AhR.

In conclusion, based on the observed alterations in CYP450 activities, cyclamen would be expected to change the disposition of other essential medications, including antibiotics, anti-arrhythmics, immune modulators, antihistamines, calcium channel blockers, HMG CoA reductase inhibitors, steroids, proton pump inhibitors, antiepileptics, and NSAIDs. On the basis of its nondiscriminatory effects on a number of important drug-metabolizing enzymes, the cyclamen extract could in principle affect the actions of many medicines. Therefore, until further clinical drug interaction experiments are completed, the co-administration of drugs with the cyclamen preparation should be avoided. Moreover, the results of the present study indicate that

the cyclamen extract stimulates the metabolic activation of the toxic chemicals metabolized by not only CYP2E1 but also CYP1A1/1A2 by inducing these enzymes which results in increased amounts of reactive metabolite formation. This may in turn further potentiate the risk of toxicity and carcinogenesis in these subjects. Therefore some precautions should be taken in terms of reduction of cyclamen usage.

Conflict of Interest - The authors declare that there is no conflict of interest.

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