

Abri Cantongnesis Modulates Detoxifying Enzymes to Ameliorate Hepatotoxicity in Rats

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Abstract

Abri cantongnesis in combination with herbs are used for treatment of diseases. However, the detail of its pharmacological activities is lacking. In this study, the chemoprotective effects of the active fraction of Abri cantongnesis on toxicant-induced liver toxicity in rats were studied. The Abri cantongnesis extract was orally administered to SD rats for three days before treatment of rats with trichloroethylene once for two days. Rat urines and hepatic enzymes were collected for analysis. The results showed that CYP 1A and CYP 2E1 and glutathione transferase increased over 45% while the activity of diaphorase increased by 30%. These enzyme activities were increased with the extract concentration. However, only UGT showed a decrease in activity upon treatment with Abri cantongnesis. The treatment of rats with Abri cantongnesis caused an increase in catalytic activities of CYP 1A and CYP 2E1, GST and diaphorase in the rat. The results show that Abri cantongnesis enhanced elimination of trichloroethylene and its metabolites; thus the treatment of Abri cantongnesis can reduce liver toxicity in rats. The findings suggest that Abri cantongnesis has chemopreventive effects on toxicants-induced hepatotoxicity in rats. The Abri cantongnesis can be used as a chemo-protective agent for the liver.

Keywords: Abri cantongnesis; Trichloroethylene; Carcinogenesis; Cytochrome P450s

Introduction

The composition of herbal medicines for treatment of liver disorders contains Abri cantongnesis. However, the detail pharmacological activity of Abri cantongnesis is lacking. Abri cantongnesis is a member of the family Leguminosae of which active saponins are commonly found. Though the combination of Abri cantongnesis and other herbs has been used for treatment of liver disorders, it has not been used alone for treatment of disease. Ingestion of appropriate quantities of Abri cantongnesis can enhance hepatic functions in man and experimental animals [1,2]. Hepato-protective properties of Abri cantongnesis have been reported [3]. However, the pharmacological activity of Abri cantongnesis in the liver remains unclear. Trichloroethylene (TCE) is known to cause toxic effects in the liver. It was reported that TCE undergoes metabolism by two major pathways, namely, cytochrome P450-mediated oxidation and conjugation with glutathione (GSH) [4-6]. Differing rates and extent of TCE metabolism have been implicated as being responsible for the hepato-carcinogenic effects of TCE in mice and rats. Previous study reported that the metabolites of TCE, trichloroacetate (TCA) and/or dichloroacetate could induce hepatic tumors in mice [7-9]. If the formation of these metabolites can be attenuated, tumor formation can be inhibited. Several P450s have been implicated in TCE bio-activation, including CYP2E1 and CYP1A2 in man and lab animals [4,10,11]. In animal studies, the administration of TCE produced liver toxicity in the rat and its toxicity may be potentiated by alcohol consumption [12].

The aim of present study was to investigate effects of Abri cantongnesis on the trichloroethylene toxicity in the rat.

Methods and Materials

Chemicals

Trichloroethylene, trichloroacetic acid, trichloroethanol, *p*-nitrophenol (*p*-NP), β -nicotinamide adenine dinucleotide phosphate tetrasodium salt (NADPH), 2,6-dichlorophenol-indophenol (DCPIP), glutathione (GSH), uridine 5'-diphosphoglucuronic acid trisodium salt

(98%, UDPGA), were purchased from Sigma Chemical Co (St. Louis, MO). Other chemicals and solvents were of analytical reagent grade.

Animals, dosing and sample collection

Male Sprague-Dawley rats (150-180 g) were obtained from the Laboratory Animal Service Center of the Chinese University of Hong Kong. Rats were housed under controlled conditions (12 h light-dark cycles, 22°C, 60% humidity). They were fed a standard rodent chow and had water *ad libitum*. Rats were administered orally with Abri cantongnesis extract for three days, followed by TCE (2.4 g/kg) for two days. Urinary samples were collected at 24 and 48 h after TCE administration. The liver and blood were used for enzyme assays after rats were sacrificed. The serum was used for measurement of the activity of Glutamic Oxalacetic Transferase (GOT) and Glutamic Pyruvic Transferase (GPT).

Preparation of microsomal fractions

The liver homogenate 20% (w/v) in 0.1M tris-sucrose buffer (pH 7.4) was centrifuged at 10,000 g for 20 min. The supernatant was centrifuged at 105,000 g for 60 min. The pellets were resuspended in 0.1 M tris-sucrose buffer (pH 7.4) and centrifuged again at 105,000 g for 60 min. The microsomes were resuspended in 50 mM phosphate buffer containing 1 mM EDTA and 10% glycerol (pH 7.4) and stored frozen at -70°C until use. The protein content of rat liver microsomes was measured by Bradford's method [13].

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EROD assay (CYP 1A)

The microsomal P450 content was quantified according to the described method with modifications [14]. The reactions were done in 96-well plate. The fluorescent intensity was measured by the Cytfluor instrument for resorufin [15]. The reaction assay contained microsomal protein (20 µg), the substrate 7-ethoxyresorufin (2 µM), Tris-NaCl buffer (50 mM Tris base and 0.1 M NaCl, pH 7.8), and was started by the addition of NADPH 100 µM. The fluorescence of the assay was measured at an excitation wavelength of 590 nm and emission wavelength at 530 nm for 10 min at 2-minutes interval. The blank contained no enzyme. The reaction rate was determined based on standard curve using 1.25 nmol to 250 nmol resorufin in the reaction medium. The specific activity of the reaction was expressed in nmol resorufin formed/min/mg protein.

p-Nitrophenol assay (CYP 2E1)

The p-nitrophenol hydroxylation activity was measured according to the previous method with modifications [16]. A total of 500 µl of the assay mixture contained 100 mM potassium phosphate buffer pH 7.4, 100 µM p-nitrophenol, 1 mM NADPH and microsomal protein (200 g/ml). The assay was performed at 37°C for 0, 5, 10 and 20 min in the dark and terminated by the addition of 100 µl of 20% (w/v) trichloroacetic acid. After centrifugation at 10,000 g for 5 min, 0.5 ml of the supernatant was mixed with 0.25 ml of 2M NaOH. The absorbance of p-Nitrocatechol was determined at 546 nm. All experiments were performed in triplicate.

Glutathione S-transferase assay

The GST activity was measured with 1-Chloro-2,4-Dinitrobenzene (CDNB) according to the described method [17]. The reaction mixture contained 100 mM phosphate buffer (pH 6.5) and 1 mM GSH, and started by the addition of 1 mM CDNB.

UDP-glucuronosyl transferase assay

The UGT activity was determined with p-nitrophenol as substrate by the established methods [18,19]. Microsomes (0.8 mg) were treated with 560 µg Emulgen 911 in 75 mM Tris-HCl buffer, pH 7.3; 5 mM MgCl₂, 2 mM UDP-glucuronic acid and 0.6 mM p-nitrophenol in a total volume of 0.4 mL and stirred continuously at 4°C for 30 min. The reaction was incubated at 37°C for 0, 5, 10 and 20 min in the dark, and was terminated by the addition of 20% (w/v) trichloroacetic acid to a final concentration of 3% (w/v). After centrifugation, 70 µL of supernatant was transferred to 1 mL of 0.5 M NaOH solution. The p-nitrophenol glucuronidation was measured by the change in absorbance at 400 nm with respect to time.

DT-diaphorase assay

DT-diaphorase activity was measured according to the described method [20]. The reaction mixture contained 50 mM Tris-HCl, pH 7.5, 0.08% Triton X-100, 0.05 mM NADPH and 40 µM DCPIP. The assay was started by the addition of 100 µg protein of rat liver microsomes. The reduction of DCPIP was measured at 600 nm. An extinction coefficient of 21.0 mM⁻¹cm⁻¹ at 600 nm for DCPIP was used. The DT-diaphorase activity was expressed as nmol min⁻¹ mg⁻¹ protein.

Glutamic oxalacetic transferase (GOT) and glutamic pyruvic transferase (GPT) assay

The assay methods were the same as those described by Reiman and Frankel in the Sigma Diagnostics Transaminase reagents.

Preparation of Abri cantongnesis

Small pieces (1 kg) of dried *Abri Cantongnesis* (SIPI-12-008-AC) were boiled in water (2000 mL) for 2 h. The mixture were filtered and concentrated under reduced pressure. The concentrated extract was dried with freeze dryer. About 85 g of the crude extract was obtained and extracted in a small volume (40 ml) of absolute ethanol.

Analysis of the metabolites of TCE in rat urine

GC/MS analysis was conducted to determine the metabolites of TCE in rat urine. The GC-MS system consisted of a Hewlett-Packard (Palo Alto, CA, USA) HP6890 gas chromatography, a 5973 series mass selective detector, a HP 6890 auto-injector, a G1512A auto sampler controller and a Vectra XM series 45/166 computer using HP G1701AA MS Chem Station software (Version A.01.00). The capillary column was an HP-5 5% phenyl methyl siloxane (30 m x 0.25 mm I.D) with 0.50 µm film thickness. The carrier gas was set at a flow rate of 1.0 ml/min and a head pressure of 13.8 psi on the column. One µl of the sample in hexane-dichloromethane (1:1) was injected using splitless mode. Temperature of the injection port and mass selective detector interface were set at 150°C and 280°C, respectively. The temperature gradient of the GC oven was programmed to start at 60°C for 4 min, raised to 200°C at 50°C/min and held at the final temperature for 2 min. An Electron Impact (EI) ionization mode with the ionization of 70 eV was used. Perfluoro tributylamine was used for the calibration of the MS detector.

The derivatization procedure was done according to the published method [21] with modifications. Urine sample (200 µL) and internal standard (DCA, 200 mM in methanol, 5 L) were mixed with 500 µL of water-sulfuric acid-methanol (6:5:1) in tightly-capped glass tube. The mixture was heated at 70°C for 10 min. After cooling to room temperature, 500 µL of hexane-dichloromethane (1:1) was added to the mixture and vortexed for 10 min before centrifugation at 600 g for 5 min. The organic layer was used for GC-MS analysis. Chemical structures of the metabolites of TCE were identified using a full mass spectra scan mode in comparison with the authentic compounds.

Results

Hepatic enzyme activity

The TCE metabolites were measured by GC-MS. Treatment of rats with different concentrations of Abri cantongnesis produced significant effects on both cytochrome P450s and Phase II enzymes (Figure 1). Abri cantongnesis treatment produced a remarkable increase in the enzyme activity in the liver. CYP 2E1 and CYP1A1 exhibited a considerable increase (P < 0.01). The GST activity was considerably elevated while diaphorase was increased noticeably. The relative changes in enzyme activity were summarized in Table 1. Abri cantongnesis treatment decreased the GOT (Figure 2) and GPT (Figure 3) levels of TCE-treated rats. The activity of CYP 1A1 and CYP 2E1 increased up to 53% and 45% in Abri cantongnesis treated rats relative to those in the un-treated groups (Table 1). The increase was dose-dependent but represented a downward change in activity at dose > 5 kg.

TCE metabolites

The ability of Abri cantongnesis to influence the activities of hepatic drug-metabolizing enzymes was recognized by the change in metabolite level. Trichloroethanol (TCEOH) and Trichloroacetate (TCA) were increased after Abri cantongnesis treatment (Figure 4).

However, no conjugated forms of TCE metabolites were observed. The levels of TCEOH and TCA in the urine from both the treated and un-treated rats were compared. Figure 4 showed the treatment of rats with the extract on the metabolite formation. The concentration of Abri cantongnesis affected the production of metabolite (Figure 5). Considerable changes were observed in the liver weight between the two groups (Figure 6).

Discussion

The effects of Abri cantongnesis on TCE-induced liver toxicity in the rat were demonstrated in the study. Abri cantongnesis reduced the level of metabolite formation in the rat liver. The active fraction of Abri cantongnesis ameliorated the enzyme activity in the liver. Previous studies of TCE toxicity in animal and human livers showed that CYP 2E1 was more important isoform in TCE-induced carcinogenicity in rats and mice, although CYP 1A1 may also contribute. It is therefore believed that TCE activation by P450s and its toxicity can be attenuated by herbal medicines. In this study, CYP 2E1 and CYP 1A1 were both found to be responsible for the conversion of TCE to TCEOH and TCA. The activities of P450s were significantly enhanced by the Abri

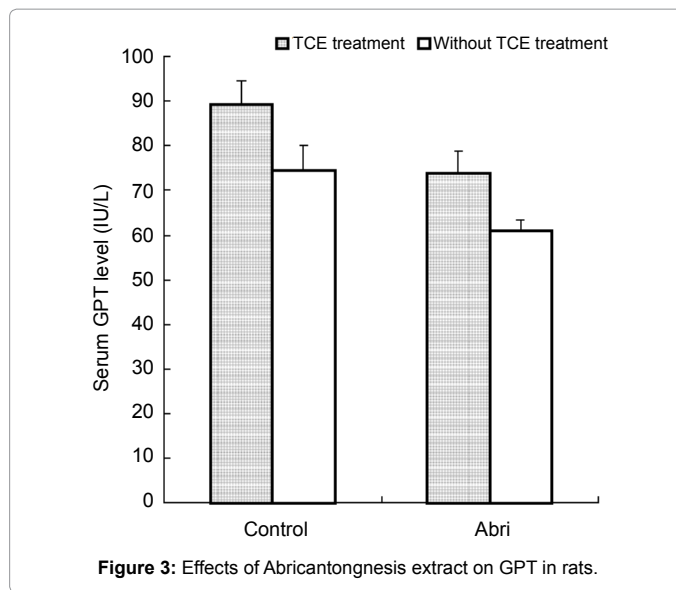


Figure 3: Effects of Abri cantongnesis extract on GPT in rats.

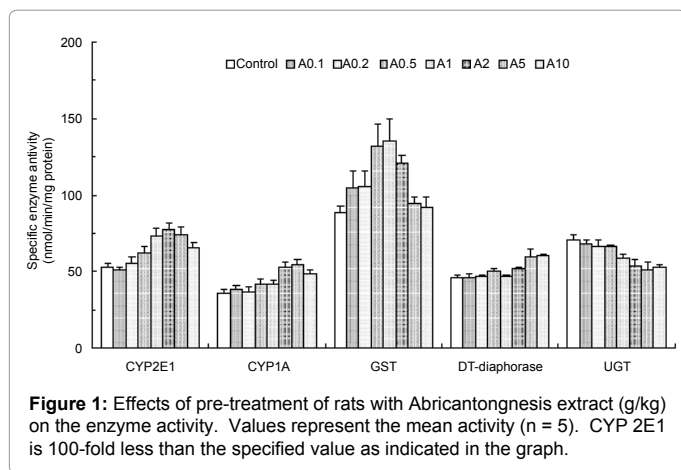


Figure 1: Effects of pre-treatment of rats with Abri cantongnesis extract (g/kg) on the enzyme activity. Values represent the mean activity (n = 5). CYP 2E1 is 100-fold less than the specified value as indicated in the graph.

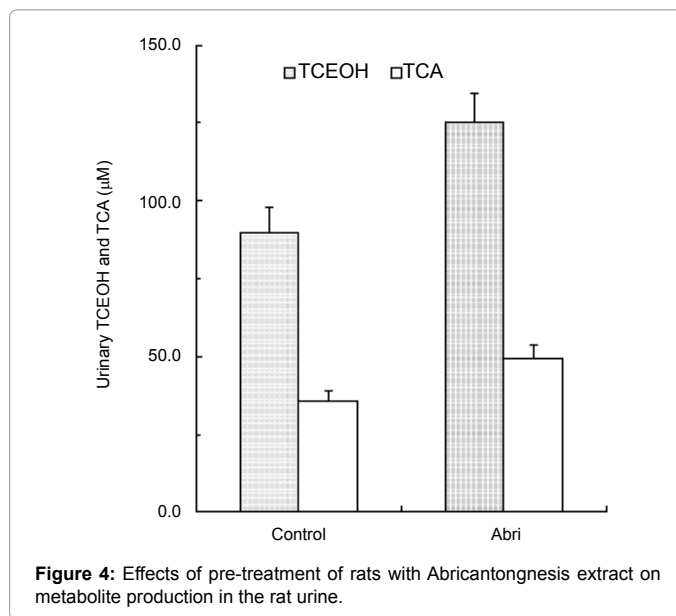


Figure 4: Effects of pre-treatment of rats with Abri cantongnesis extract on metabolite production in the rat urine.

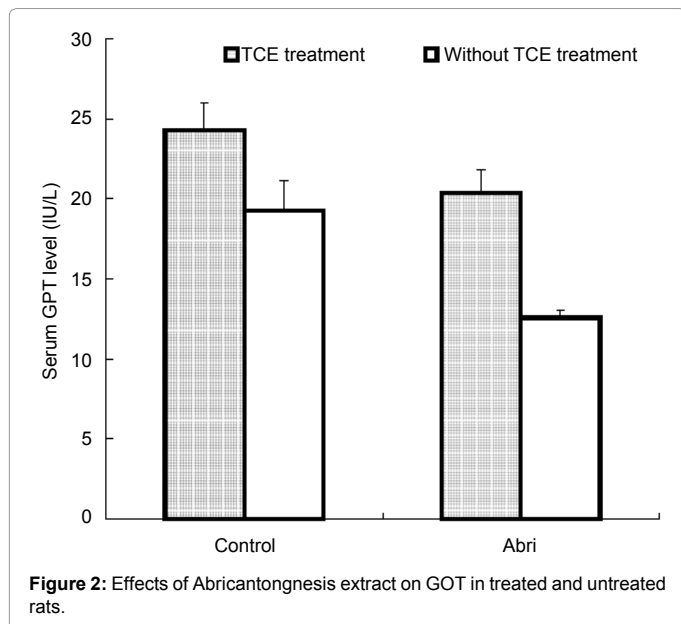


Figure 2: Effects of Abri cantongnesis extract on GOT in treated and untreated rats.

cantongnesis treatment (Table 1). The severity of TCE-induced hepatotoxicity in the rat can be abrogated by the treatment of rats with the active fraction. In as much as the difference between the treated and the un-treated rats with Abri cantongnesis is the biologic action of Abri cantongnesis on CYP 1A1, CYP 2E1 and the other hepatic enzymes, among which only UGT decreased in activity ($P < 0.01$) [4,5]. The present findings provide supporting experimental evidence that Abri cantongnesis can reduce liver toxicity related to TCE metabolism compared with the un-treated rats, which showed physiological changes in the liver manifested as increased plasma concentrations of the liver enzymes, GOT and GPT.

Early studies have demonstrated that P450-mediated TCE metabolism produced TCEOH and TCA, which are believed to be responsible for the subsequent TCE-induced carcinogenicity. Selective hydroxylation of TCE is more closely associated with TCE-induced hepato-toxicity than GST-mediated metabolic pathway. Treatment of

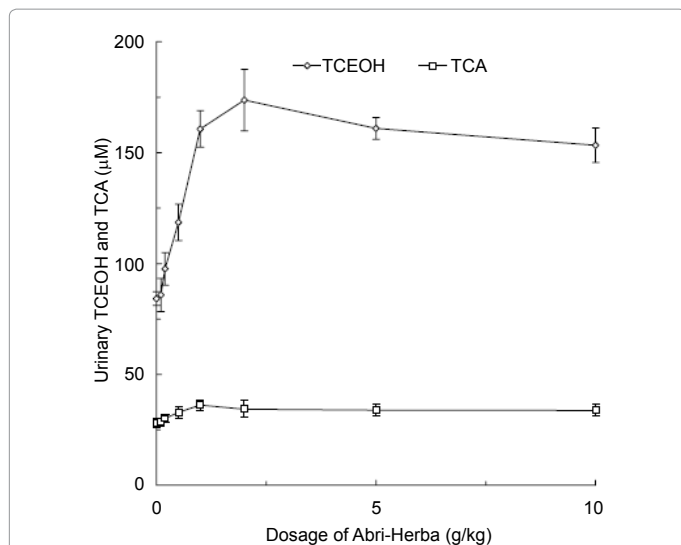


Figure 5: Changes of the metabolite level with the dosage of Abri cantongnesis.

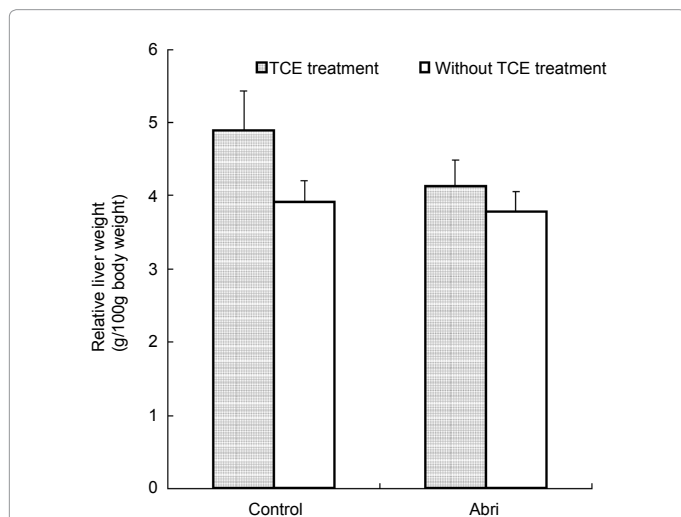


Figure 6: Effects of pre-treatment of rats with Abri cantongnesis extract on the rat liver weight.

the rat with the Abri cantongnesis extract ameliorated the detoxifying enzyme activities. The increase of the drug-metabolizing enzyme activity in the treated group of rats suggest that the chemo-protective roles of Abri cantongnesis in reduction of toxicant-induced liver toxicity. The studies also demonstrated that these enzymes were responsible for activating TCE. Besides detoxifying enzymes, previous studies reported that GSH is important in TCE detoxification [22,23]. After treatment of rats with Abri cantongnesis, the increase in GST activity contributed to the elimination of toxic metabolites of TCE through conjugation reaction; thus, TCE-induced hepato-toxicity can be reduced in the rat. It is possible that the activation of TCE could be mediated to some extent by both GST and CYP 1A1 and CYP 2E1 which are the major enzymes associated with TCE metabolism.

In summary, the present studies demonstrate the therapeutic benefits of Abri cantongnesis in TCE-induced liver toxicity in rats through activation of enzymes in the rat. The findings suggest a functional significance of CYP 2E1 and CYP 1A1 in TCE-induced

Dosage (g/kg)	CYP2E1	CYP1A	GST	DT-diaphorase	UGT
0.1	-3.78 ± 0.20	7.06 ± 0.58	17.99 ± 0.74	0.22 ± 0.01	-4.11 ± 0.19
0.2	3.78 ± 0.11	3.95 ± 0.32	18.78 ± 1.95	1.97 ± 0.13	-5.52 ± 0.23
0.5	17.15 ± 1.27	17.80 ± 1.41	49.32 ± 4.00	10.28 ± 0.22	-5.52 ± 0.36
1	38.67 ± 2.53	18.64 ± 1.35	53.17 ± 5.42	2.41 ± 0.08	-16.29 ± 0.18
2	45.94 ± 3.02	49.15 ± 2.51	36.20 ± 4.30	13.35 ± 0.27	-24.22 ± 1.17
5	40.31 ± 2.38	52.82 ± 3.42	7.24 ± 0.43	30.63 ± 0.62	-27.05 ± 2.36
10	23.55 ± 1.64	36.16 ± 2.70	4.07 ± 0.19	33.04 ± 2.61	-24.93 ± 2.21

Table 1: Relative percentage change in enzyme activity in rats after pre-treatment with Abri extract compared with the control.

toxicity. Comparison of the treated with the un-treated rats, Abri cantongnesis provides an alternative medicine for prevention and treatment for toxicant-induced liver toxicity. The results show experimental evidence of the pharmacological activities of Abri cantongnesis as a potential chemo-protective agent for the liver.

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