Natural Product Sciences
10(4) : 182-189 (2004)

Inhibitory Effect of *Ligularia fischeri* var. *spiciformis* and Its Active Component, 3,4-Dicaffeoylquinic Acid on the Hepatic Lipid Peroxidation in Acetaminophen-Treated Rat

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Abstract – To find the action mechanism of the MeOH extract (LFS) of *Ligularia fischeri* var. *spiciformis* herbs (Compositae) and its active component, 3,4-dicaffeoylquinic acid (DCQA) on antihepatotoxicity, the effect was investigated on hepatic lipid perxodation and drug-metabolizing enzyme activities in acetaminophen-treated rat. Pretreatment with 250 mg/kg LFS (*p.o.*) and 10 mg/kg DCQA (*p.o.*) significantly decreased hepatic lipid peroxidation caused by acetaminophen injection. Further, LFS and DCQA inhibited hepatic microsomal enzyme activation such as hepatic P-450 cytochrome b_5, aniline hydroxylase and aminopyrine N-demethylase, suggesting that the two substances might effectively prevent the metabolic activation or scavenge electrophilic intermediates capable of causing hepatotoxicity. Both LFS and DCQA increased hepatic glutathione content and glutathione reductase activity, indicating that both resultantly prevented hepatotoxicity via antioxidative mechanism. Therefore, it was found that LFS had antihepatotoxicity based on the antioxidative action of DCQA.
Keywords – *Ligularia fischeri* var. *spiciformis*, 3,4-dicaffeoylquinic acid, antihepatotoxic, lipid peroxidation

Introduction

Ligularia fischeri var. *spiciformis* (Compositae) is an endemic perennial herb, which is used as a mountain-edible vegetable and a folkloric medicine in Korea (Nakai, 1943). This herb is medicinally used to treat jaundice, hepatic failure, scarlet fever and arthritis (Choi, 1991). We previously reported the isolation of (+)-intermedeol, 6-oxoeremophilenolide, spiciformisins a and b, and monocyclosqualene (Park *et al.*, 2000; Lee *et al.*, 2002) together with a differentiation-inducing effect of intermedeol on HL-60 cells (Jeong *et al.*, 2002).

In our successive studies on antihepatotoxic principles of this plant, it has been found that this plant contains 3,4-dicaffeoylquinic acid (DCQA) responsible for antihepatotoxic action in CCl_4-, D-galactosamine-, α-naphthylisothiocyanate- and DL-ethionine-induced rat (Park, 2002). Although Basnet *et al.* (1996) have reported the antihepatotoxicity of dicaffeoylquinic acids isolated from propolis in primary cultured hepatic cells, the action mechanism is unclear. Xenobiotics capable of causing lipid peroxidation, hepatic damage and cancer seriously change hepatic drug-metabolizing enzyme activities and resultantly cause lipid peroxidation. A very high dose of acetaminophen, one of antipyretics, produces the same biochemical properties as the xenobiotics, and therefore it increases hepatic microsomal enzyme activities and decreases free radical-scavenging ones. Further, overdose of acetaminophen depletes hepatic glutathione and destroys hepatic cells (Miners *et al.*, 1984).

In this communication, the experimental results are reported that the MeOH extract of *Ligularia fischeri* var. *spiciformis* (LFS) and the active component DCQA prevent the serious enzyme activity changes of hepatic drug-metabolizing enzymes involved in the biochemical process for acetaminophen metabolism: e.g., the activities of hepatic microsomal enzymes such as cytochrome P-450, cytochrome b_5, aniline hydroxylase and aminopyrine

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N-demethylase, and of hepatic cytosolic enzymes such as sulfotransferase and UDP-glucotransferase were investigated.

Materials and Methods

Plant material – The leaves of *Ligularia fischeri* var. *spiciformis* were collected in July 1999, in Pyongchang, Kangwondo, South Korea, and the plant was identified by Dr. K. O. Yoo (Department of Biology, Kangwon National University, Chuncheon, Korea). A voucher specimen (KW-980814) is deposited in the herbarium of Department of Biology, Kangwon National University. This plant was air-dried avoiding sunlight and pulverized for the experiment.

Extraction, fractionation and isolation – The plant material (2.0 kg) was extracted three times with hot MeOH under reflux and the filtered extract was evaporated on a rotatory evaporator under reduced pressure to give MeOH extract (526 g). This was suspended in H_2O and then partitioned successively with diethyl ether, EtOAc and *n*-BuOH to give diethyl ether fraction (25 g), EtOAc fraction (63 g), and *n*-BuOH fraction (45 g).

A part (15 g) of EtOAc fraction was subjected to column chromatography over silica gel (300 g, 5 × 50 cm, Merck Art 7734) with eluting solvent of $CHCl_3$-MeOH-H_2O (65:35:10, lower phase) led the isolation of DCQA (1.95 g). A detailed isolation procedure is not described here. The isolated compound was identified as 3,4-dicaffeoylquinic acid (DCQA) on the basis of physico-chemical and spectroscopic data (Basnet *et al.*, 1996). The structure of DCQA was shown in Fig. 1.

Animals – Four 4 week-old Sprague-Dawley male rats were purchased from Korea BioLink Experimental Animal Co. (Eumseong, Korea) and adapted them in a constant condition (temperature: 20 ± 2°C, dampness: 40 – 60%, light/dark cycle: 12 hr) for two weeks or more. Twenty-four hours before the experiment, only water was offered to the animals.

Enzyme source – After exsanguinations of rats, the liver was excised, a slice was removed for microscopic investigation and the rest blotted on a filter paper was sliced with scissors in an iced 0.1 mM potassium phosphate buffer (KP buffer, pH 7.4) and homogenized in a Potter-Elvehjem Teflon glass homogenizer with 4 volume of KP buffer per 1 g of liver tissue. Liver homogenate was assayed for total glutathione content. Microsomes were assayed for cytochrome P-450, Cytochrome b_5, aminopyrine *N*-demethylase and aniline hydroxylase activities. Cytosoles were assayed for glutathione reductase, γ-glutamylcysteine synthetase and glutathine *S*-transferase activities.

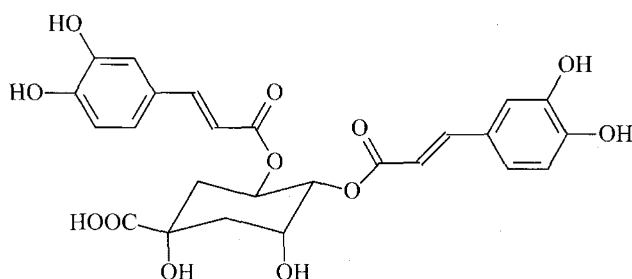

Fig. 1. Structure of 3,4-dicaffeoylquinic acid (DCQA) isolated from *L. fischeri* var. *spiciformis*.

Preparation of test samples – A MeOH extract and an isolate, 3,4-dicaffeoylquinic acid (DCQA) were dissolved in 10% tween 80 and diluted with saline for animal experiment. The rat of an untreated group was offered with the vehicle only. Based on our previous report regarding the antihepatotoxicity assay results, the extracts were orally administered with 100 and 250 mg/kg dose once a day for 2 weeks, and DCQA with 10 mg/kg were done for 2 weeks.

Measurement of TBARS – Thiobarbituric acid reactive substances (TBARS) in the liver were measured as a marker of lipid peroxidation by using the modified method described by Ohkawa *et al.* (1979). An aliquot (0.4 ml) of 10% liver homogenate in 0.9% NaCl was added to 1.5 ml of 8.1% SDS, 1.5ml of 20% acetate buffer (pH 3.5) and 1.5 ml of 0.8% TBA solution. The mixture was then heated at 95°C for 1 h, cooled, and extracted with 5.0 ml of *n*-butanol-pyridine (15:1), and the absorbance of the *n*-butanol-pyridine layer was measured at 532 nm to determine the level of TBARS.

Measurement of hepatic microsomal enzyme activities – 1) Cytochrome P-450: The content was estimated by using the method of Omura and Sato (1964). Microsomes were resuspended in 0.1 M potassium phosphate buffer (pH 7.5), containing 1% Triton X-100. Resuspended microsomes were equally divided into sample and reference cuvettes. First both cuvettes should be saturated with sodium dithionite, then the sample cuvette was saturated with carbon monoxide. The cytochrome P-450 concentration was measured spectrophotometrically and a millimolar extinction coefficient of $\varepsilon = 91\ mM^{-1}\ cm^{-1}$ was used for the calculations.

2) Cytochrome b_5: Activity was determined based on the method described in Omura and Sato (1964). Absorbances were measured at the wavelengths of the reduced- and oxidized forms of cytochrome b_5. In brief, the diluted microsomal solution with 0.1 M potassium phosphate buffer (pH 7.0) was adjusted so that the final

NADH concentration becomes 0.2 mM, and then the absorbance was measured at 424 nm and 490 nm using spectrophotometer. The activity was calculated using the molar extinction coefficient 185 $mM^{-1}cm^{-1}$.

3) Aniline hydroxylase: Activity was determined according to the Bidlack and Lowrys method (Bidlack and Lowry, 1982). The reaction mixture contained 1 mM aniline (added in methanolic solution), 0.1 M potassium phosphate buffer (pH 7.4), 1 mM NADPH and 0.5 – 1 mg protein in a final volume of 1.5 ml. Incubation was carried out for 20 minutes at 37°C with moderate shaking and was stopped by adding 20% of trichloroacetic acid. After centrifugation, 10% Na_2CO_3 and 0.2 N NaOH/2% phenol were added to the obtained supernatant. This solution was reacted at 37°C and resulting blue color was measured at 630 nm after standing for 30 minutes. The enzyme activity was expressed as the amount (nmole) of *p*-aminophenol of mg protein produced per minute.

4) Aminopyrine *N*-demethylase: Activity was determined according to Nashs method (Nash, 1953), in microsomes by measuring formaldehyde formation in a reaction mixture containing 17 mM 4-dimethylaminoantipyrine, 2 mM $MgCl_2$, 2 mM glucose-6-phosphate, 0.2 mM NADP, 2U glucose-6-phosphate dehydrogenase, 100 mM phosphate buffer (pH 7.5) in a final volume of 1.5 ml. Incubation was carried out for 15 minutes at 37°C and stopped by adding 10% trichloroacetic acid. After centrifugation a 1 ml aliquot of the supernatant was taken out and reaction with Nash reagent (contained ammonium acetate 15.4 g, acetic acid 0.29 ml, acetylacetone 0.2 ml in 100 ml) was performed for 30 minutes in 60°C. Sample absorbance was measured at 415 nm.

Measurement of the hepatic enzyme activities involved in conjugation reaction – 1) Determination of UDP-glucuronyl transferase activity: The enzyme activity of UDP-glucuronyl transferase (UDPGA) activity was determined according to the Reinke's method (Reinke *et al.*, 1986). In brief, 1.0 ml solution containing 10 mM phosphate buffer (pH 7.0), 1 mM *p*-nitrophenol, 3 mM UDPGA, 1 mM $MgCl_2$, 0.02% bovine serum albumin, 0.05% triton X-100 and enzyme solution (20 – 100 µg protein) were reacted at 37°C for 5 min. The reaction was terminated by adding 0.25 ml of 0.6 N $HClO_4$ and centrifuged. The supernatant was added with 0.5 ml of 1.6 M glycine buffer (pH 10.4) and the absorbance was taken at 436 nm wavelength. The unit was expressed as nmoles of *p*-nitrophenol removing 1 mg protein per minute.

2) Measurement of sulfotransferase activity: According to the method described by Dawson and Bridges (1981), 2.0 ml reaction mixture was prepared by adding the solutions, 0.25 mM *p*-nitrophenol, 2 mM K_2SO_4, 5 mM ATP, 0.01 mM $MgCl_2$ and enzyme solution in 0.25 mM Tris-HCl buffer solution (pH 7.4). This mixture was incubated at 37°C for 30 min and ended the reaction by standing in 100°C for 2 min. 0.2 ml Glycine buffer (pH 10.4) was added to the supernantant obtained from the centrifugation. The absorbance was measured at 400 nm using spectrophotometer and the enzyme activity was calculated from the calibration curve. The unit was expressed as *p*-nitrophenol nmol/mg protein/min.

3) Glutathione *S*-transferase: The enzyme activity of glutathione *S*-transferase was determined according to the Habig's method (Habig *et al.*, 1974). In brief, 75 µl of 40 mM reduced glutathione was added to 0.1 M potassium phosphate buffer (pH 6.5) in the test tube and then added 100 µl enzyme solution. In the blank, the reaction was terminated by 20% trichloroacetic acid. The mixture was reacted at 25°C for 5 min and followed by adding 25 µl of 2,4-dinitrochlorobenzene (substrate). The reaction was terminated with 20% trichloroacetic acid and centrifuged. The absorbance of each supernatant obtained from the centrifugation of test- and blank reactant was measured at 340 nm. The activity (nmole) was calculated using a molar absorption coefficient (9.6 $mM^{-1}cm^{-1}$).

Measurement of hepatic glutathione content, γ-glutamylcysteine synthetase (γ-GCS) activity and glutathione reductase activity-1) γ-GCS: Based on the method described by Richman and Meister (1975), 3.5 ml reaction mixture was prepared by adding 0.1 M tris HCl buffer (pH 8.0), 8.9 mM L-glutamic acid, 0.94 mM EDTA, 3.2 mM $MgCl_2$, 1.35 mM ATP and enzyme mixture (100 – 300 µl protein) and then incubated at 37°C for 10 min. The absorbance of the reaction mixture was measured at 600 nm using UV spectrophotometer.

2) Glutathione reductase: Based on the method described by Mize and Langdon (1962), 3.5 ml reaction mixture was prepared by adding 0.1 M potassium phosphate buffer (pH 7.5), 0.94 mM EDTA, 4.6 mM oxidized glutathione, 0.16 mM NADPH and the enzyme mixture and then incubated at 37°C form 10 min. The absorbance was measured at 340 nm to find the reduced NADPH amount.

Histopathological assay – The liver picked out from the rat was set in 10% buffered neutral formalin for 24 h and washed with H_2O, and then dehydrated with alcohol solution successively of 60 – 100% concentration. This was embedded with paraffin and the tissue section (4 µm thickness) was prepared using rotary microtome from the block. This was again dyed with hematoxylin-eosin and examined under microscope. Histopathological scores

were shown by the five grade of the tissue: e.g., 0, mild (–); 1, mild (±); 2, mild (+); 3, moderate (+); 4, severe (+).

Statistics – Statistical significance between experimental groups were analyzed with the Duncans new multiple range test.

Results

Hepatic TBARS of the acetaminophen-treated group was shown to be approximately 5-folder higher than of the untreated group. Pretreatment with 250 mg/kg LFS and 10 mg/kg DCQA which were orally administered for 2 weeks significantly decreased heptic lipid peroxide by 36.2% and 76.6%, respectively (Fig. 2). This result suggested that DCQA, an active constituent of LFS, inhibits hepatic lipid peroxidation induced by acetaminophen.

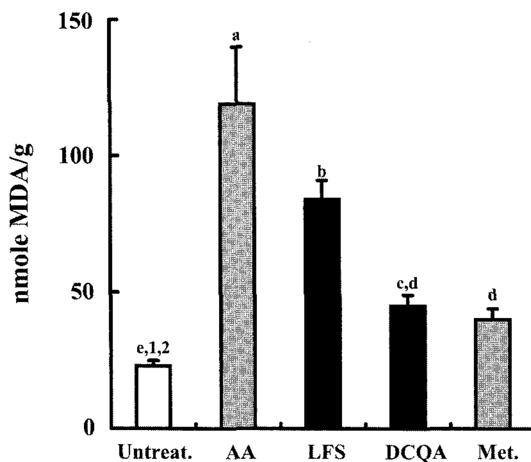

Fig. 2. Effect of pretreatment with *L. fischeri* var. *spiciformis* extract (LFS) and 3,4-dicaffeoylquinic acid (DCQA) on the hepatic lipid peroxide content in acetaminophen-induced rats. Rats were orally preadministered with AA: acetaminophen, the MeOH extract of *Ligularia fisheri* var *spiciformis* (LFS, 250 mg/kg, *p.o.*), 3,4-dicaffeoylquinic acid (DCQA, 10 mg/kg, *p.o.*), and methionine (Met, 100 mg/kg, *p.o.*) daily for two weeks and phenobarbital (75 mg/kg) daily for four days, and then intraperitoneally injected acetaminophen (AA, 800 mg/kg) for once a day. Rats were decapitated 24 h after sample treatments.
[1]Values represent mean ± S.D. for eight experiments. [2]Data followed by the same alphabet (a-e) are not statistically significant by Duncan's new multiple range test from the untreated group.

To find the mechanism on the anti-lipid peroxidation of LFS and DCQA, the activities of microsomal enzymes such as cytochrome P-450, cytochrome b_5, aniline hydroxylase and aminopyrine N-demethylase were measured. As shown in Table 1, treatment with acetaminophen alone considerably increased all hepatic microsomal enzyme activities tested in this experiment. Pretreatment with LFS, DCQA and methionine prevented the increase of these four enzyme activities.

To find the effect of LFS and DCQA on the phase 2 reaction of hepatic drug-metabolizing enzymes, hepatic sulfotransferase- and UDP-glucuroylransferase activities were observed in acetaminophen-treated rats. Since test samples showed no significant inhibition of the two

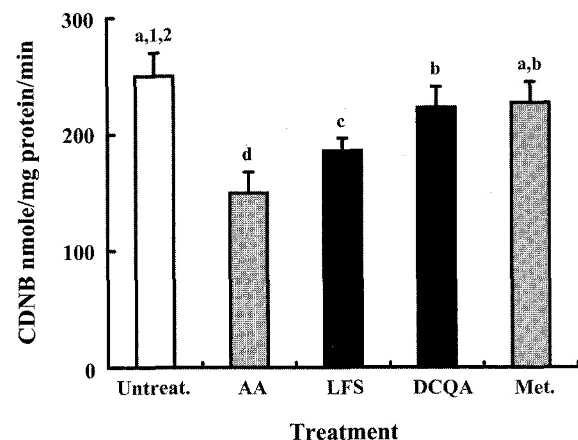

Fig. 3. Effect of pretreatment with LFS and DCQA on the hepatic glutathione S-transferase activity in acetaminophen-induced rats. [1]Values represent mean ± S.D. for eight experiments. [2]Data followed by the same alphabet (a-d) are not statistically significant by Duncan's new multiple range test from the untreated group.

Table 1. Effect of preteatment with LFS and DCQA on the hepatic microsomal enzyme system in acetaminophen-induced rats

Treatment	Dose (mg/kg)	Activity			
		Cyto P450*	Cyto b_5*	AH**	AD***
Untreated	–	1.20±0.09[e,1,2)]	0.33±0.03[e]	1.48±0.17[d]	3.86±0.33[d]
AA	800	2.93±0.11[a]	1.62±0.10[a]	2.80±0.13[a]	9.26±0.58[a]
AA+LFS	250	2.29±0.12[b]	1.23±0.03[b]	2.19±0.11[b]	7.27±0.39[b]
DCQA	10	1.68±0.08[c]	0.64±0.05[c]	1.82±0.13[cd]	5.63±0.42[c]
Methionine	100	1.47±0.05[d]	0.53±0.04[d]	1.70±0.09d	4.45±0.42[d]

[1)]Values are mean ± S.D. for eight experiments. 2) Data followed by different superscript (a-e) are statistically sighificant by Duncans new multiple range test from untreated group ($p < 0.05$).
*nmole/mg protein, **Aniline hydroxylase: *p*-aminophenol nmol/mg protein/min, ***Aminopyrine *N*-demethylase: HCHO nmole/mg protein/min

enzyme activities induced by acetaminophen and the data are not shown. After the observation of no effect of LFS and DCQA on the hepatic sulfotransferase activity and UDP-glucuronylransferase activity, hepatic cytosolic glutathione *S*-transferase activity wihch is involved in phase 2 reaction was investigated. As shown in Fig. 3, pretreatment with LFS, DCQA and methionine increased glutathione *S*-transferase activity induced by acetaminophen treatment by 30.1%, 65.6%, and 72%, respectively.

Hepatic glutathione concentration, glutathione reductase activity and γ-glutatmylcysteine synthetase activities were investigated and the results were shown in Table 2. Treatment with acetaminophen considerably decreased hepatic glutathione concentration, glutathione reductase activity and γ-glutamylcysteine synthetase activity when compared with those of an untreated group. Pretreatment

Table 2. Effect of preteatment with LFS and DCQA on the hepatic glutathione concentration and glutathione biosynthesis enzyme activity in acetaminophen-induced rats

Treatment	Dose (mg/kg)	Content	Activity	
		Glutathione*	GR**	γ-GCS***
Untreated		5.41±0.64[a,1,2)]	31.2±3.13[a]	15.37±2.67[a]
AA	250	2.48±0.22[d]	16.7±2.11[cd]	8.64±0.48[c]
AA+LFS	250	2.93±0.29[d]	24.3±2.50[bc]	8.92±0.61[c]
DCQA	10	4.26±0.40[bc]	26.1±1.98[b]	9.02±0.98[c]
Methionine	100	5.01±0.55[ab]	18.3±1.54[de]	12.21±1.92[b]

[1)]Values are mean ± S.D. for eight experiments. 2) Data followed by different superscript (a-e) are statistically significant by Duncans new multiple range test from untreated group ($p<0.05$).
*μmole/g of tissue, **glutathione reductase: glutathione nmole/mg protein/min, ***γ-glutamylcysteine synthetase: Pi nmole/mg protein/min

Table 3. Histopathological scrore of the pretreatment with LFS and DCQA in acetaminophen-induced hepatitis rats

Treatment	Dose (mg/kg)	Case	Centrilubular degen			Lubular Acidophil. Degen.			Fatty change			Total score
			Mild	Mod.	Sev.	mild	Mod.	Sev.	mild	Mod.	Sev.	
Untreated		1	–	–	–	–	–	–	–	–	–	0
		2	–	–	–	–	–	–	–	–	–	0
		3	+	–	–	–	–	–	+	–	–	1
		4	+	–	–	–	–	–	–	–	–	1
		5	–	–	–	–	–	–	–	–	–	0
AA		1	–	+	–	–	–	–	–	–	–	5
		2	±	–	–	+	–	–	+	–	–	4
		3	–	–	±	+	–	–	+	–	–	4
		4	–	–	+	–	–	–	+	–	–	5
		5	–	+	–	+	–	–	–	–	–	4
AA+LFS	250	1	–	–	–	+	–	–	±	–	–	4
		2	±	–	–	±	–	–	±	–	–	4
		3	+	–	–	–	–	–	+	–	–	3
		4	+	–	–	–	±	–	+	–	–	2
		5	+	–	–	+	–	–	+	–	–	3
AA+DCQA	10	1	±	–	–	+	–	–	–	–	–	2
		2	+	–	–	+	–	–	–	–	–	3
		3	±	–	–	+	–	–	±	–	–	2
		4	–	–	–	–	–	–	+	–	–	2
		5	+	–	–	–	±	–	±	–	–	2
AA+Met.	100	1	±	–	–	–	–	–	+	–	–	2
		2	±	–	–	–	–	–	+	–	–	2
		3	–	–	–	–	–	–	+	–	–	1
		4	–	–	–	±	–	–	+	–	–	1
		5	–	–	–	–	–	–	–	–	–	0

0, mild (–); 1, mild (±); 2, mild (+); 3, moderate (+); 4, severe (+).

with LFS and DCQA increased glutathione concenration and glutathione reductase activity and both test samples showed no significant increase in γ-glutamylcysteine synthetase activity. Oral administration of LFS increased glutathione concentration induced by acetaminophen by 15.4% and that of DCQA did by 60.8%. Pretreatment with methionine (100 mg/kg, *p.o*) prevented hepatic glutathione depletion and the decrease of γ-glutamylcysteine activity by 86.3% and 53.0%, respectively, but it showed no significant increase in glutathione reductase activity.

Table 3 shows the histopathological data on the hepatic tissue examined by a microscope in rats. Histopathological lesions like centrilubular degenerative, lubular acidophilic degenerative and fatty change were observed under microscopic examination at the control (total score 4 – 5). Pretreatments with LFS and DCQA reduced the total score caused by acetaminophen by 2 – 4 and 2, respectively.

Discussion

We previously found that LFS contains DCQA as an antiheptotoxic princple in CCl_4, D-galactosamine, α-naphthylisothiocyanate and DL-ethionine-treated rats (Park, 2002). In our successive studies, treatment with LFS exhibited no significant change of body weight and organs up to 500 mg/kg dose indicating that LFS is adequate to study the antihepatotoxicity (data not shown). Furthermore, treatment with 100, 250, and 500 mg/kg dose decreased serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) dose-dependently (data not shown). Based on our previous results, doses of 250 mg/kg LFS and 10 mg/kg DCQA were used in this experiment.

After acetaminophen had been revealed to be one of metabolites of acetanilid and phenacetin, it became a widely used analgesic/antipyretic therapeutic (Miners *et al.*, 1984). However, excessive dose of acetaminophen causes lethal toxicity on liver and kidney in human and animals. Covalent bonding of acetaminophen to macromolecules in hepatic tissues by the cytochrome P-450 action produces intermediates with highly reactive aryls, and therefore it induces hepatic glutathione depletion. At least 50% decrease of hepatic glutathione content leads to the hepatotoxicity accompanied by hepatic tissue necrosis (Clissoid, 1986; Prescott and Critchley, 1983). On consideration of the latest article on acetaminophen, common dose forms sulfate- and glucuronic acid conjugates of acetaminophen. However, excessive dose produces acetaminophen mercapturate and acetaminophen cysteine by the reaction with hepatic glutathione and excretes to urine. The lack of detoxification ability resulted from the glutathione depletion produces very high reactive substances which covalently bind cellular nucleophilic macromolecules and cause hepatic necrosis (Lin and Levy, 1986; Smilkstein *et al.*, 1988).

Lipid peroxidation is caused by a variety of free radicals, which is produced by intrinsic or extrinsic electrophilic subastances. It also causes toxic actions, aging, carcinogenicity and immunologically inhibitory action (Ahokas *et al.*, 1985). Therefore, the effects of LFS and DCQA were investigated on the lipid peroxidation and the enzyme activities involved in acetaminophen metabolism in the rat.

Anti-lipid peroxidation of the plant material and active component DCQA against hepatic tissues suggest the antihepatotoxicity caused by acetaminophen. Based on the general hepatic reaction started from phase 1 reactions by the enzymes in hepatic smooth endoplasmic reticulum, cytochrome P-450, aminopyrine *N*-demethylase and aniline hydroxylase (Reddy *et al.*, 1981), the effect of pretreatment with LFS and DCQA were investigated on the hepatic drug-metabolizing enzyme activities. LFS and DCQA inhibited hepatic TBARS caused by acetaminophen, indicating that both samples prevented hepatic damage. Furthermore, LFS and DCQA inhibited hepatic microsomal enzyme activities such as cytochrome P-450, cytocrome b_5, aminopyrine *N*-demethylase and aniline hydroxylase, which are involved in phase 1 reaction, induced by acetaminophen treatment.

On the other hand, the enzyme activities involved in phase 2 reaction were also investigated on glutathione *S*-tranferase, sulfotransferase and UDP-glucuronyltransferase. At common dose of acetaminophen hepatic microsomal UDP-glucuronyltransferase and cytosolic sulfotransferase act on acetaminophen metabolism whereas at the overdose glutathione *S*-transferase does in the metabolism (Fried, 1975). Treatment with LFS and DCQA significantly increased glutathione *S*-transferase activity induced by acetaminophen but not reached the value of the untreated group. However, no test samples showed significant change of UDP-glucuronyltransferase- and sulfotransferase activities.

Glutathione is necessarily required for the final detoxification process against electrophilic substances, reactive oxygen species and lipid peroxides (Schulz *et al.*, 1962; Dodds and Foord, 1970). And γ-glutamylcysteine synthetase (γ-GCS, a glutathione biosynthetic enzyme) and glutathione reductase acting on an oxidized form of glutathione are responsible for the maintenance of cellular glutathione concentration. To find the attenuation

mechanism of glutathione content, the effect of LFS and DCQA were observed on γ-GCS, a rate-limiting enzyme for glutathione biosynthesis, and glutathione reductase (Klassen and Fitzgerald, 1974). The former enzyme activity was not significantly influenced by test samples whereas the latter was considerably increased when compared with acetaminophen-treated group. This suggested that the increase of glutathione *S*-transferase should be attributed to a high hepatic glutathione content. It was also found that the maintenance of glutathione content by test samples might possibly depend not on γ-GCS but on glutathione reductase activity. Since the prevention of hepatic cellular necrosis by pretreatment with LFS and DCQA in the histological examination, these antihepatotoxicities were confirmed.

Therefore, it was suggested that hepatic damage caused by acetaminophen could be prevented by the inhibitory effect of LFS and DCQA on hepatic microsomal enzymes and on cellular maintenance of glutathione.

Acknowledgements

This study (203016-02-1-SB010) was supported by Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

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(Accepted August 10, 2004)