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Hepatoprotective Effects of *Arctium lappa* Linne on Liver Injuries Induced by Chronic Ethanol Consumption and Potentiated by Carbon Tetrachloride

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Key Words
Arctium lappa · Ethanol · Carbon tetrachloride · Liver injury

Abstract
Arctium lappa Linne (burdock) is a perennial herb which is popularly cultivated as a vegetable. In order to evaluate its hepatoprotective effects, a group of rats (n = 10) was fed a liquid ethanol diet (4 g of absolute ethanol/80 ml of liquid basal diet) for 28 days and another group (n = 10) received a single intraperitoneal injection of 0.5 ml/kg carbon tetrachloride (CCl_4) in order to potentiate the liver damage on the 21st day (1 day before the beginning of *A. lappa* treatment). Control group rats were given a liquid basal diet which did not contain absolute ethanol. When 300 mg/kg *A. lappa* was administered orally 3 times per day in both the 1-day and 7-day treatment groups, some biochemical and histopathological parameters were significantly altered, both in the ethanol group and the groups receiving ethanol supplemented with CCl_4. *A. lappa* significantly improved various pathological and biochemical parameters which were worsened by ethanol plus CCl_4-induced liver damage, such as the ethanol plus CCl_4-induced decreases in total cytochrome P-450 content and NADPH-cytochrome c reductase activity, increases in serum triglyceride levels and lipid peroxidation (the deleterious peroxidative and toxic malondialdehyde metabolite may be produced in quantity) and elevation of serum transaminase levels. It could even restore the glutathione content and affect the histopathological lesions. These results tended to imply that the hepatotoxicity induced by ethanol and potentiated by CCl_4 could be alleviated with 1 and 7 days of *A. lappa* treatment. The hepatoprotective mechanism of *A. lappa* could be attributed, at least in part, to its antioxidative activity, which decreases the oxidative stress of hepatocytes, or to other unknown protective mechanism(s).

Arctium lappa Linne, a perennial herb, has been cultivated as a vegetable for a long time in Taiwan and Japan [26]. Its root has long been used as a diuretic and antipyretic [17]. It is now a very popular health drink in Taiwan for hypertension, gout, arteriosclerosis, hepatitis and other inflammatory disorders [20]. A recent study demonstrated that it possesses an inhibitory effect on the binding of platelet-activating factor to rabbit platelets [16]. A des-

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mutagenic factor has been isolated from the root of burdock [25]. This factor is resistant to heat and proteolytic enzymes. The partially purified desmutagenic principle had a molecular weight of greater than 300,000 and showed characteristics of a polyanionic substance [25]. Lin et al. [22] reported that *A. lappa* has anti-inflammatory and free radical-scavenging activities. It has also been reported to have hepatoprotective effects on carbon tetrachloride (CCl_4)- and acetaminophen-induced liver damage [23]. In a biotransformation study, the structural transformation of arctiin, a major component of the seeds of *A. lappa,* was investigated using rat gastric juice (pH 1.2–1.5) and rat large intestinal flora in vitro [27]. Quantitative analysis of arctiin and its metabolite was carried out by high-performance liquid chromatography. Arctiin was stable in rat gastric juice and was rapidly transformed to arctigenin by the rat large intestinal flora, followed by conversion to the major metabolite, 2-(3″,4″-dihydroxybenzyl)-3-(3′,4′-dimethoxybenzyl)-butyrolactone. This experiment suggested that in the course of metabolism of arctiin, the glycosidic bond is first cleaved, followed by demethylation of the phenolic methoxy group in the alimentary tract [27].

Alcohol is commonly consumed for its mood-altering effects, and therefore it is often used as a psychoactive drug and also as a food. Alcohol is metabolized by alcohol dehydrogenase in the cytosol, by the cytochrome P-450 enzyme in endoplasmic reticula and by catalase in peroxisomes, and thus the highly active metabolite acetaldehyde is produced [1]. It has been known for many years that chronic ethanol consumption induces its own oxidative metabolism in rat hepatic microsomes, and that the metabolites produced damage the hepatocytes [15]. CCl_4 is an extensively used industrial solvent. CCl_4-induced liver damage is the best-characterized animal model of xenobiotic-induced free radical-mediated hepatotoxicity [30]. It is metabolized and activated by hepatic microsomal cytochrome P-450-mediated reactions to the reactive trichloromethyl free radical ($^{\cdot}CCl_3$), which may damage hepatocytes by covalently binding to polyunsaturated fatty acids on cellular membranes during lipid peroxidation [6, 28]. Ethanol can greatly induce the production of hepatic microsomal cytochrome P-450 [3, 18, 19], which can potentiate the formation of $^{\cdot}CCl_3$. Therefore, it is strongly suspected that chronic ethanol consumption potentiates CCl_4-induced hepatotoxicity [8, 20]. In the present study, the hepatoprotective effects and the mechanisms of action of *A. lappa* on liver injuries induced by chronic ethanol consumption and potentiated by CCl_4 were investigated.

Materials and Methods

Chemicals

n-Butanol, carrageenan (C-1013), casein, *L*-cysteine, ethyl linoleate, ferric chloride, a glutamic-pyruvic transaminase (GPT) kit, a glutamic-oxaloacetic transaminase (GOT) kit, *dl*-methionine, sodium dodecyl sulfate, thiobarbituric acid and *DL*-α-tocopherol were all purchased from Sigma Chemical (St. Louis, Mo., USA). Absolute ethanol, formalin, sucrose, olive oil and corn oil were all analytical grade, and all were purchased from Nihon Shiyaku Industries (Osaka, Japan).

Animals

Male Wistar strain rats (5 weeks old, body weight 160–180 g) were purchased from the Animal Center of National Taiwan University. They were kept for at least 1 week on a commercial solid diet (Fu-So, Taipei, Taiwan) under environmentally controlled conditions (25 ± 1°C, 55 ± 5% humidity) with free access to food and water. A 12-hour light/dark cycle was maintained, and hard wood chips were used as bedding. After being fed the commercial solid diet and water for 1 week, the rats were switched to a liquid basal diet for 4 weeks [11] in the ethanol group, the ethanol plus CCl_4 group and the *A. lappa* therapeutic group; one group was used as the blank and was fed the commercial solid diet during the entire experimental period for comparison and to check whether the liquid basal diet interfered with normal growth and normal hepatic functioning of the growing rats. The Lieber-DeCarli liquid basal diet containing alcohol as 35% of total calories was used [21]. Animals of the control group were given the liquid basal diet containing sucrose instead of alcohol.

Diets

The liquid basal diets were freshly prepared as described by DeCarli and Lieber [11]. Thirty-five percent of the calories of the liquid basal diets was supplied from fat (corn oil:olive oil = 1:4). The mineral mixture (AIN-76) and vitamin mixture (AIN-76A) were purchased from ICN Biochemical Company (Cleveland, Ohio, USA).

In the ethanol diet group (n = 10), 5 ml of absolute ethanol dissolved in 80 ml of the ethanol diet was put into the feeding bottles; the rats were fed this for 4 weeks. When the ethanol diet was supplied in the feeding bottles, the water bottles were removed from the cages. This was to ensure that each rat would consume the 80 ml of the ethanol diet each day. The average number of calories accounted for by ethanol in the ethanol diet was maintained at 35% during this experiment.

The control group (n = 10) was given the liquid basal diet for 4 weeks, which had the same constituents as the ethanol diet except for no ethanol.

In order to supply the same number of calories, 5 ml of absolute ethanol and 5 g of sucrose were dissolved in the 80 ml of the liquid basal diet for the ethanol diet group, whereas in the control group, 12 g of sucrose were dissolved in 80 ml of the liquid basal diet.

Formulation of the Liquid Basal Diet and the Ethanol Diet Groups

The constituents of the liquid basal diet and the ethanol diet were formulated as shown in table 1, according to the method described by DeCarli and Lieber [11].

Preparation of Crude Water Extract of A. lappa

One hundred grams of roots of *A. lappa* was ground into fragments and boiled with 1 liter of distilled water in a Chinese herbal decocter for 1 h. The extract was filtered, and the residue was boiled and filtered again. The filtrates were mixed well, put into freeze-drying bottles, cooled to below −60 °C and transformed into powder with the freeze-dryer; the yield was 24.67%. When orally administered to the rats, the crude water extract powder was dissolved in normal saline with an administration dose of 300 mg/1.0 ml normal saline/kg body weight of the rat [23].

Animal Groupings

The experimental animals were divided into the following groups.

(1) Blank group: in order to investigate whether the liquid basal diet interfered with normal growth and normal liver function, rats in the blank group (n = 10) were fed the commonly used commercial solid diet for the entire experiment. This blank group was used to compare the liver functions in the liquid basal diet group and the normal commercial solid diet group.

(2) Control group: control group rats (n = 10) were fed 80 ml of the liquid basal diet daily for the entire experiment.

(3) Ethanol group: this groups of rats (n = 10) were fed a diet with 4 g of absolute ethanol/80 ml of liquid ethanol for 3 weeks.

(4) Ethanol plus CCl_4 group: this group of rats (n = 10) was fed a diet with 4 g of absolute ethanol/80 ml of liquid ethanol for 3 weeks. Then, the liver injury was potentiated by a single intraperitoneal administration of CCl_4 (0.5 ml in 1.0 ml of corn oil/kg body weight) on the 21st day (1 day before the beginning of the *A. lappa* treatment).

(5) Therapeutic groups: 10 rats of the ethanol group and of the ethanol plus CCl_4 group were individually administered 300 mg/kg p.o. *A. lappa* crude water extract 3 times (every 4 h, at 9.00, 13.00 and 17.00 h) on the 28th day, as the 1-day treatment group. Another 10 rats each from the ethanol group and the ethanol plus CCl_4 group were administered 300 mg/kg p.o. *A. lappa* 3 times (every 4 h, at 9.00, 13.00 and 17.00 h) per day from day 22 to 28, as the 7-day treatment group.

All of the experimental rats were weighed every 7 days, and they were sacrificed after light anesthetization with ethyl ether on the 29th day. Blood was collected by cardiac puncture, and livers were immediately excised. Part of the liver was used for histological examination, while the other part of the same lobe of the liver was homogenized in 4 volumes of a 1.15% KCl solution.

The homogenate was centrifuged at 9,000 *g* for 20 min to separate the nuclear and mitochondrial fractions. The supernatant fraction was ultracentrifuged at 100,000 *g* for 60 min. The microsomal pellets obtained were suspended in 0.1 *M* potassium phosphate buffer (pH 7.4) and stored at −70 °C until the microsomal enzyme assay was conducted.

Assessment of Liver Function

All animals were lightly anesthetized with ethyl ether, and blood was collected by cardiac puncture. The blood samples were allowed to coagulate at room temperature for 1 h. Serum was separated by centrifugation at 4 °C and 12,000 rpm for 5 min. Serum levels of GOP, GPT and triglyceride were measured using a CH-100 autoanalyzer (Texas International Laboratory) and Sigma GOT, GPT and triglyceride optimized reagents (Sigma Chemical Company) according to the method described by Bergmeyer et al. [2] for GOT and GPT and Bucolo and David [4] for triglyceride.

Table 1. Formulation of the liquid basal diet and the ethanol diet

	Liquid basal diet (amounts in 80 ml)	Ethanol diet (amounts in 80 ml)
Casein, g	3.47	3.47
Sucrose, g	12.00	5
Absolute alcohol, g	–	4 (equal to 5 ml)
L-Cysteine, mg	41.00	41.00
DL-Methionine, mg	25.00	25.00
Oil (olive:corn = 4:1), g	2.00	2.00
Ethyl linoleate, mg	212.00	212.00
Vitamin mixture, mg	407.00	407.00
DL-α-Tocopherol, mg	2.00	2.00
Mineral mixture, mg	813.00	813.00
Carrageenan, mg	212.00	212.00

Both the liquid basal diet and the ethanol diet were freshly prepared. The vitamin and mineral mixtures were stored in a refrigerator before use. Both casein and the mineral mixture are water insoluble, so they were placed directly into a steel bowl in their original powder form, then they were placed in the rats' cages, so the rats could eat them ad libitum. The other constituents were dissolved in distilled water, and 2 liters was prepared in a volumetric bottle at a time. It was mixed thoroughly, then divided into the feeding bottles. According to the average feeding amount, each rat consumed 80 ml per day.

Assessment of Lipid Peroxidation Activity (in vivo)

In order to evaluate the inhibitory activity of *A. lappa* on a lipid peroxidation-generated assay system, a portion of rat liver tissue from the various groups was sliced and homogenated (13,000 rpm, 3 min) with 25 m*M* Tris-HCl buffer (pH 7.2; 10% w/v) [36]. In a glass test tube, 0.1 ml of the liver homogenates of various groups was incubated with shaking for 1 h at 37 °C in Tris-HCl buffer (pH 7.2). After 1.5 ml of 1.0% thiobarbituric acid and 1.5 ml of 20% acetic acid were added, the mixture was further incubated for 1 h at 95 °C. The upper layer of the mixture was collected, and the level of malondialdehyde produced was measured using spectrophotometric analysis at 532 nm [35].

Hepatic Glutathione Concentration

The total hepatic reduced glutathione concentration was determined by the method of Tietze [33]. Each liver was excised and homogenized with phosphate buffer (pH 7.5) containing 10 m*M* EDTA and centrifuged at 4 °C and 10,000 *g* for 10 min. The supernatant was used for the total glutathione level assay.

Microsomal Enzyme Activity Assays

Microsomal cytochrome P-450 activity and b5 content were determined by the method of Omura and Sato [28] using an extinction coefficient of 91 cm^{-1} mM^{-1} for the absorbance difference between 450 and 490 nm. NADPH-cytochrome c reductase activity was determined by the method of Phillips and Langdon [29], measured by observing the increase in light absorbance at 550 nm produced by reduction of cytochrome c and calculated as the change in absorbency at 550 nm/min/mg of microsomal protein. Protein con-

Table 2. Improvements in lipid, lipid peroxidative product and some hepatic enzymes with *A. lappa* (300 mg/kg, p.o.) treatment after chronic ethanol (EtOH; 4 g of absolute ethanol/80 ml of liquid diet)-induced liver damage in rats

Assay	Blank	Control	EtOH	EtOH + *A. lappa* (1-day treatment)	EtOH + *A. lappa* (7-day treatment)
Liver weight, g	4.87±0.18	4.80±0.12	5.18±0.17	5.04±0.11	5.04±0.13
Triglyceride, mg/dl	38.07±4.80	39.15±0.82	126.65±23.86	53.98±2.96*	47.84±5.72**
Glutathione, μmol/g liver	11.0±0.34	11.55±1.22	8.86±0.39	9.40±0.59	9.15±0.33
Malondialdehyde, nmol/mg protein	0.91±0.24	0.97±0.24	1.84±0.14	0.74±0.06***	0.41±0.07***,#
Cytochrome P-450, nmol/mg protein	0.49±0.03	0.50±0.03	0.57±0.02	0.67±0.03*	0.64±0.01**
Cytochrome b5, nmol/mg protein	0.31±0.01	0.29±0.02	0.30±0.01	0.33±0.01	0.35±0.01**
NADPH-cytochrome c-reductase nmol cytochrome c reductase/ mg protein/min	21.73±0.91	20.99±1.12	25.99±1.46	23.59±0.09	21.55±1.13*

The control group was given the liquid basal diet without ethanol. Values are presented as the mean ± SE for male Wistar rats (n = 10). Statistical significance of differences was determined by one-way analysis of variance coupled with Dunnett's test. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to the ethanol group. # $p < 0.01$ for the 7-day *A. lappa* treatment group compared with the 1-day *A. lappa* treatment group.

Table 3. Improvement in lipid, lipid peroxidative product and some hepatic enzymes with *A. lappa* (300 mg/kg, p.o.) treatment after chronic ethanol (EtOH; 4 g of absolute ethanol/80 ml of liquid diet) plus CCl_4 (0.5 ml/kg, i.p.)-induced liver damage in rats

Assay	EtOH	EtOH + CCl_4	EtOH + CCl_4 + *A. lappa*	
			1-day treatment	7-day treatment
Liver weight, g	5.18±0.17	5.37±0.09	5.34±0.16	4.99±0.23
Triglyceride, mg/dl	126.65±23.86	240.40±53.43#	107.46±17.06*	73.93±4.99**
Glutathione, μmol/g liver	8.86±0.39	6.62±0.30	7.85±0.23**	7.51±0.16*
Malondialdehyde, nmol/mg protein	1.84±0.14	2.75±0.16	0.44±0.04**	0.38±0.02***
Cytochrome P-450, nmol/mg protein	0.57±0.02	0.24±0.03	0.54±0.05***	0.55±0.01***
Cytochrome b5, nmol/mg protein	0.30±0.01	0.18±0.02#	0.29±0.01**	0.33±0.01***
NADPH-cytochrome c-reductase nmol cytochrome c reductase/mg protein/min	25.99±1.46	16.42±1.09	25.53±1.17**	25.57±0.79***

Values are presented as the mean ± SE for male Wistar rats (n = 10). Statistical significance of differences was determined by one-way analysis of variance coupled with Dunnett's test. # $p < 0.05$ for the ethanol plus CCl_4 group compared with the ethanol group. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ for the *A. lappa* treatment group compared with the ethanol plus CCl_4 group.

centrations of the microsomal suspensions were determined by the method of Lowry et al. [24].

Histopathological Examination

After blood was taken, part of the hepatic tissue was immediately collected from the same lobe of the liver and fixed in a 10% neutral formalin solution for at least 1 week. Subsequently, hepatic tissue was dehydrated in a series of ethanol solutions from 75 to 100% before being embedded in paraffin. Cross-sections (5 μm thick) were stained with hematoxylin and eosin for photomicroscopic assessment.

Grading for Liver Morphological Changes

To quantify the morphological changes, liver sections were graded for changes such as centrilobular necrosis or cytoplasmic vacuolation using the following arbitrary scale: + = slight (below 10% change); ++ = moderate (below 25%); +++ = marked (25–50%); ++++ = intense (overall change). Results are reported as the mean of all observations made by all observers.

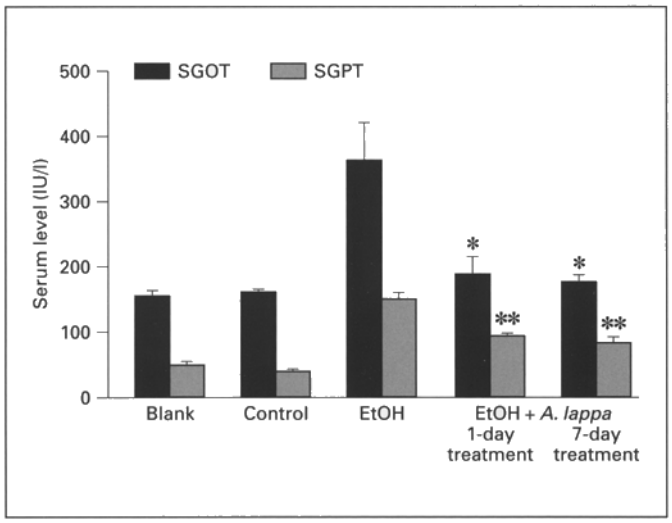

Fig. 1. Hepatoprotective effect of *A. lappa* (300 mg/kg, p.o.) on chronic ethanol (EtOH; 4 g of absolute ethanol/80 ml of liquid diet)-induced liver damage in rats. The control group was given a liquid basal diet without ethanol. Values are presented as the mean ± SE for male Wistar rats (n = 10). Statistical significance of differences was determined by one-way analysis of variance coupled with Dunnett's test. * $p < 0.05$ and ** $p < 0.01$ compared to the ethanol group.

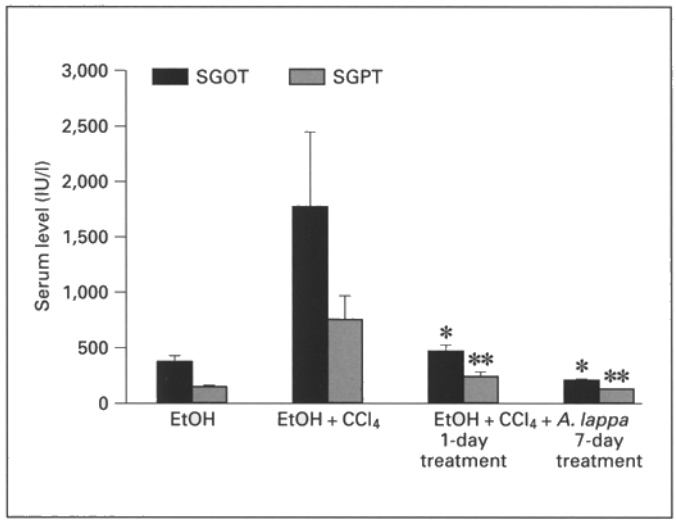

Fig. 2. Hepatoprotective effect of *A. lappa* (300 mg/kg, p.o.) on chronic ethanol (EtOH; 4 g of absolute ethanol/80 ml of liquid diet) administration supplemented with CCl_4 (0.5 ml/kg, i.p.)-induced liver damage in rats. Values are presented as the mean ± SE for male Wistar rats (n = 10). Statistical significance of differences was determined by one-way analysis of variance coupled with Dunnett's test. * $p < 0.05$ and ** $p < 0.01$ for the *A. lappa* treatment group compared with the ethanol plus CCl_4 group.

Data Analysis

All experimental data are shown as the mean ± SE. The statistical significance of differences between control and treated animals was evaluated using one-way analysis of variance coupled with Dunnett's test. p values of 0.05 or less were considered statistically significant.

Results

Liver Weights of Each Experimental Group

After the rats were sacrificed, their livers were excised and weighed. It was found that although administration of ethanol and ethanol plus CCl_4 slightly increased the liver weight, no significant difference was observed between liver weights of all groups during the entire experimental period (table 2, 3).

Ethanol- and Ethanol plus CCl_4-Induced Liver Injury

Chronic ethanol (4 g of absolute ethanol/80 ml of liquid basal diet) consumption for 28 days may injure liver cells and lead to elevation of serum levles of GOT (SGOT) and GPT (SGPT) and triglyceride levels. Intraperitoneal injection of CCl_4 (0.5 ml/kg) on the 28th day potentiated the ethanol-induced liver damage, hence further significantly increasing SGOT ($p < 0.001$), SGPT ($p < 0.001$) and triglyceride ($p < 0.05$) levels and significantly decreasing the cytochrome b5 level ($p < 0.05$) (table 3).

One-Day and Seven-Day A. lappa Treatment

Absolute ethanol (4 g of absolute ethanol/80 ml of liquid basal diet)-induced and absolute ethanol plus CCl_4 (0.5 ml/kg)-potentiated elevations in SGOT, SGPT and triglyceride levels were significantly reduced in rats treated for 1 and 7 days with *A. lappa* (fig. 1, 2, table 2, 3). These phenomena were also confirmed by histopathological examinations (fig. 3), but there were no biochemical or histological differences observed between 1- and 7-day *A. lappa* treatments (fig. 1, 2, table 2, 3). In an attempt to elucidate the possible hepatoprotective mechanism(s) of *A. lappa* on ethanol-induced and CCl_4-potentiated liver injuries, a possible restorative effect of *A. lappa* on hepatotoxin-induced changes in glutathione, cytochrome P-450, cytochrome b5 content and NADPH-cytochrome c reductase activity was investigated (table 2, 3).

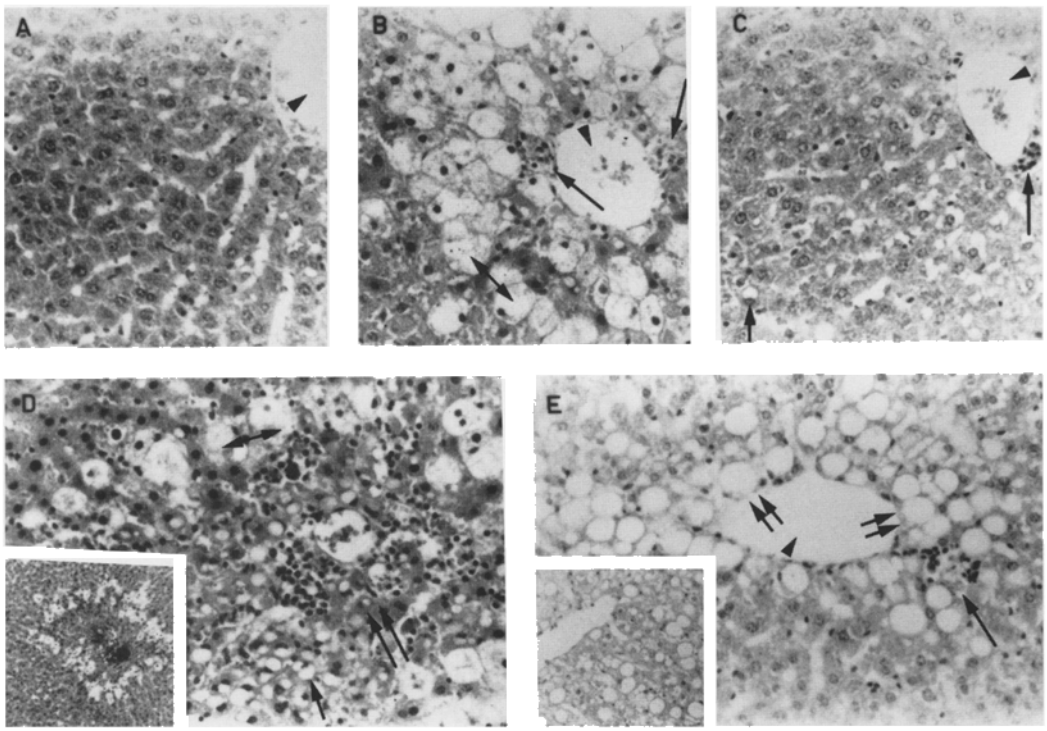

Fig. 3. Photomicrographs of liver sections taken from the control (**A**), ethanol plus CCl_4 plus *A. lappa* (300 mg/kg, p.o.) (**B**), ethanol plus *A. lappa* (300 mg/kg, p.o.) (**C**), ethanol plus CCl_4 (**D**) and ethanol (**E**) groups. HE. ×200. In the lower left corner of **D**, note the wreath-like appearance of the ballooning change in the hepatocytes around the hemorrhagic area of the central vein. Arrowhead = Central vein; long single arrow = inflammatory cell; long double arrow = hemorrhagic necrosis with inflammatory cell; short single arrow = microvesicular change; short double arrow = macrovesicular change; double-headed arrow = ballooning change.

Glutathione

Ethanol administration significantly decreased ($p < 0.05$) the glutathione level from 11.0 ± 0.34 μmol/g liver (blank group) and 11.5 ± 1.22 μmol/g liver (control group) to 8.86 ± 0.39 μmol/g liver. After treatment with *A. lappa,* the glutathione level returned to 9.40 ± 0.59 μmol/g liver (in the 1-day *A. lappa* treatment group) and 9.15 ± 0.33 μmol/g liver (in the 7-day *A. lappa* treatment group). The ethanol-induced glutathione decrease was further significantly decreased ($p < 0.001$) to 6.62 ± 0.30 μmol/g liver by 0.5 ml/kg CCl_4. After treatment with *A. lappa,* the decrease in glutathione could be significantly restored to 7.85 ± 0.23 μmol/g liver ($p < 0.01$, in the 1-day treatment group) and 7.5 ± 0.16 μmol/g liver ($p < 0.05$, in the 7-day treatment group); however, the 7-day treatment did not show a significantly better improvement effect compared to the 1-day treatment group.

Malondialdehyde

Malondialdehyde is one of the deleterious lipid peroxidative metabolites. From our results, it was found that *A. lappa* could significantly decrease the formation of malondialdehyde, regardless of whether it was in livers of the ethanol- (table 2) or the ethanol plus CCl_4-intoxicated rats (table 3). After ethanol administration, the liver malondialdehyde level significantly increased ($p < 0.01$) from 0.91 ± 0.24 nmol/mg protein (blank group) and 0.97 ± 0.24 nmol/mg protein (control group) to 1.84 ± 0.14 nmol/mg protein. However, after 300 mg/kg *A. lappa* was administered orally, the liver malondialdehyde level decreased to 0.74 ± 0.06 nmol/mg protein ($p < 0.001$, in the 1-day treatment group) and 0.41 ± 0.07 nmol/mg protein ($p < 0.001$, in the 7-day treatment group) (table 2). Obviously, the 7-day treatment was better than the 1-day treatment ($p < 0.05$; table 2). After potentiating the ethanol-induced liver damage with 0.5 ml/kg CCl_4, the liver malondialdehyde level further significantly increased ($p < 0.01$) to 2.75 ± 0.16 nmol/mg protein, but after 300 mg/

kg of *A. lappa* was administered orally, the liver malondialdehyde level significantly decreased to 0.44 ± 0.04 nmol/mg protein ($p < 0.01$, in the 1-day treatment group) and to 0.38 ± 0.02 nmol/mg protein ($p < 0.001$, in the 7-day treatment group) (table 2). Seven-day treatment did not show a better improvement compared to 1-day treatment (table 3).

Cytochrome P-450 and NADPH-Cytochrome c Reductase

After ethanol administration, cytochrome P-450 (0.57 ± 0.02 nmol/mg protein) and NADPH-cytochrome c reductase (25.99 ± 1.46 nmol cytochrome c reductase/mg protein/min) levels significantly increased ($p < 0.05$), when compared with those of the blank and control groups. Although 1-day treatment (23.59 ± 0.09 nmol cytochrome c reductase/mg protein/min) with 300 mg/kg *A. lappa* did not significantly reduce the NADPH-cytochrome c reductase level to the normal blank (21.73 ± 0.91 nmol cytochrome c reductase/mg protein/min) or control (20.99 ± 1.12 nmol cytochrome c reductase/mg protein/min) levels, 7-day treatment (21.55 ± 1.13 nmol cytochrome c reductase/mg protein/min) significantly reduced ($p < 0.05$) the ethanol-induced NADPH-cytochrome c reductase elevation (25.99 ± 1.46 nmol cytochrome c reductase/mg protein/min) nearly to that of the normal blank (21.73 ± 0.91 nmol cytochrome c reductase/mg protein/min) and control (20.99 ± 1.12 nmol cytochrome c reductase/mg protein/min) levels (table 2).

After potentiation of the ethanol-induced liver injury with 0.5 ml/kg CCl_4, it was found that cytochrome P-450 (0.24 ± 0.03 nmol/mg protein) and NADPH-cytochrome c reductase levels (16.42 ± 1.09 nmol cytochrome c reductase/mg protein/min) were obviously and significantly further decreased compared to those of the ethanol group. However, with 1-day (25.53 ± 1.17 nmol cytochrome c reductase/mg protein/min; $p < 0.01$) and 7-day (25.57 ± 0.79 nmol cytochrome c reductase/mg protein/min; $p < 0.001$) *A. lappa* treatments, the NADPH-cytochrome c reductase level significantly recovered to nearly that of the ethanol group (25.99 ± 1.46 nmol cytochrome c reductase/mg protein/min). However, when the cytochrome P-450 and NADPH-cytochrome c reductase levels were compared between the 1-day and 7-day treatment groups, no significant differences could be observed (table 2, 3).

Cytochrome P-450 levels showed significant further elevation after 1-day (0.67 ± 0.03 nmol/mg protein) and 7-day (0.64 ± 0.01 nmol/mg protein) *A. lappa* administration, when compared to those of the ethanol group (0.57 ± 0.02 nmol/mg protein) (table 2). One-day (0.54 ± 0.05 nmol/mg protein) and 7-day (0.55 ± 0.01 nmol/mg protein) *A. lappa* administration were able to recover the CCl_4-potentiated (0.24 ± 0.03 nmol/mg protein) reduction in cytochrome P-450, causing a significant elevation nearly to the levels of the ethanol group (0.57 ± 0.02 nmol/mg protein) (table 3).

Histopathological Examination

The hepatoprotective effect of *A. lappa* was further confirmed by histopathological examination. Livers of ethanol-treated rats showed macrovesicular and microvesicular changes in hepatocytes, located mainly around the central areas, with focal mild inflammatory cell infiltration; fatty changes in hepatocytes mainly around the central vein could also be observed.

Ethanol pretreatment followed by CCl_4 supplementation produced massive hemorrhagic necrosis with marked inflammatory cell infiltration like a wreath in and around the central area, and many ballooning and microvesicular changes in hepatocytes were also noted. After treatment of the ethanol group with *A. lappa,* microvesicular changes in hepatocytes and a small amount of inflammatory cell infiltration could still be observed; binuclear cells increased, but macrovesicular fatty changes were not observed and neither ballooning nor necrotic cells could be found.

In the group administered ethanol plus CCl_4, oral administration of *A. lappa* significantly decreased the necrosis and the ballooning degeneration, so that it could not be observed, but some microvesicular changes and inflammatory cell infiltration could still be found around the central vein. The phenomenon of increasing binuclear cells indicated that the damaged hepatocytes were regenerating after the administration of *A. lappa.*

Discussion

In recent years, the crude water extract of *A. lappa,* named *A. lappa* tea, has become a promising and important beverage, because it was found that *A. lappa* exhibits anti-inflammatory and free radical-scavenging effects [22], and hence it can be used to prevent possible free radical-induced tissue damage.

In the present study, liver injuries induced by chronic ethanol consumption and potentiated by CCl_4 in Wistar rats were used as the main experimental model.

Serum levels of transaminases (SGOT and SGPT), triglyceride, hepatic glutathione, cytochrome P-450, cyto-

chrome b5, NADPH-cytochrome c reductase and malondialdehyde production levels and liver histopathological changes were used as indicators of the hepatoprotective and therapeutic efficacy of *A. lappa.*

The root of *A. lappa* is often cooked as *A. lappa* tea in Taiwan and Japan. As we know, alcoholic beverages are among the most popular and available beverages worldwide, but chronic ethanol consumption often leads to liver injuries, so we have tried to search for other beverages to substitute for alcohol which can overcome the ethanol-induced liver damage as the main direction of our research efforts. Perhaps *A. lappa* tea can achieve this worthy objective.

The major organ which metabolizes alcohol is the liver [12]. There are three main pathways for the metabolism of ethanol, each located in a different subcellular compartment: the alcohol dehydrogenase pathway in the cytosol, the microsomal ethanol-oxidizing system located in endoplasmic reticula and catalase in the peroxisomes. Among these pathways, the alcohol dehydrogenase pathway is the major metabolic pathway during the early stage of chronic alcohol liver injury.

In alcohol dehydrogenase-mediated oxidation of ethanol, hydrogen is transferred from the substrate to the cofactor nicotinamide adenine dinucleotide (NAD), resulting in excess conversion to its reduced form (NADH) with the production of acetaldehyde [9, 10]. The excess production of NADH alters the redox state in the liver and, in turn, leads to a variety of metabolic abnormalities. The elevated ratio of NADH to NAD increases the concentration of α-glycerophosphate and suppresses the citric acid cycle, which favors accumulation of hepatic triacylglycerol by trapping fatty acids [13].

In order to metabolize the ingested ethanol, the NADPH-cytochrome c reductase in liver cells is induced and a large amount is generated, so its serum level obviously increases. When ethanol is chronically consumed, the necessary metabolic enzyme cytochrome P-450 is induced and gradually generated [3, 16, 17]. Through the cytochrome P-450 metabolic pathway, the by-product reactive oxygen species (such as superoxide and the hydroxyl radical) of alcoholic metabolism are generated in great amounts, thus damaging liver cells [31].

Activation of the cytochrome P-450 enzyme can increase the metabolism of some xenobiotics, such as CCl_4, to toxic metabolites (such as $^{\bullet}CCl_3$) [8, 14, 34], further damaging liver cells.

In 1996, Lin et al. [22] reported that *A. lappa* possesses free radical-scavenging activity. The IC_{50} values of *A. lappa* extract on superoxide and hydroxyl radical-scavenging activity were 2.06 and 11.8 mg/ml, respectively [22]. In the present study, it was found that *A. lappa* could protect liver cells from ethanol- and ethanol plus CCl_4-induced liver damage. Perhaps the hepatoprotective effect of *A. lappa* can be attributed to its antioxidant effect and free radical-scavenging activity, thus eliminating the deleterious effects of toxic metabolites from ethanol and CCl_4 and inducing liver cell regeneration.

The pathological changes induced by chronic alcohol ingestion, such as a fatty liver and hypertriglyceridemia, may be alleviated by ingestion of *A. lappa.* Binuclear liver cells were also found in *A. lappa*-treated groups, which indicated the regeneration of hepatocytes (fig. 3).

Although interesting results were obtained in the present study, and the LD_{50} (by the oral route) of *A. lappa* was even higher than 2,000 mg/kg in rats, further studies are necessary to determine the hepatoprotective effect of *A. lappa* on damaged hepatocytes. The obvious hepatoprotective effect of *A. lappa* on ethanol- and ethanol plus CCl_4-induced liver injuries may be developed into clinical applications. Fractions of *A. lappa* should be purified, then the exact hepatoprotective mechanism(s) of *A. lappa* and/or its main chemical constituents should be investigated. The development of an effective remedy for alcohol-induced liver damage is proceeding in our laboratory.

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