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Effects of Yam Peel Extract against Carbon Tetrachloride-Induced Hepatotoxicity in Rats

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ABSTRACT: The phenolic acid and flavonoid profiles in yam peel extract were determined by HPLC. Quercetin, hesperidin, and apigenin were predominant components in yam peel extract. Male Wistar rats were orally treated with yam peel extract (100.02, 266.72, and 433.42 mg/kg) or silymarin (200 mg/kg) daily, with administration of CCl_4 (1 mL/kg, 20% CCl_4 in olive oil) twice a week. Yam peel extract for 8 weeks significantly reduced the impact of CCl_4 toxicity on the serum markers of liver damage, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). The overall potential of the antioxidant system was significantly enhanced by the yam peel extract supplements as the plasma and hepatic thiobarbituric acid reactive substances (TBARS) levels were lowered, whereas the hepatic superoxide dismutase (SOD) and catalase (CAT) activities and glutathione peroxidase (GSH-Px) protein level were elevated. Yam peel extract decreased the level of nitric oxide (NO) production, tumor necrosis factor-alpha (TNF-α), and nuclear factor-kappa B (NF-κB) in CCl_4. These results point out that yam peel extract can inhibit lipid peroxidation, enhance the activities of antioxidant enzymes, and decrease the TNF-α/NF-κB level, nitric oxide production, and inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expressions. Therefore, it was speculated that yam peel extract protects rats from liver damage through its anti-inflammation capacity.

KEYWORDS: *yam peel extract, antioxidant activity, carbon tetrachloride, hepatoprotective, hepatotoxicity*

INTRODUCTION

Among the various health problems suffered by people in Taiwan, liver diseases, including hepatocellular carcinoma, fibrosis, cirrhosis, and hepatitis, appear to be some of the most serious.[1] Hepatotoxins, such as ethanol, acetaminophen, and carbon tetrachloride (CCl_4), sparked liver injury, which is characterized by varying degrees of hepatocyte degeneration and cell death.[1] Vitaglione et al.[2] suggested that reactive oxygen species (ROS) including superoxide and hydroxyl radicals, known to play an important role in liver disease's pathology and progression, have been proved to associate with the intoxication by CCl_4.[3] Documented evidence suggested that CCl_4 has been commonly used as a hepatotoxin in experimental hepatopathy.[4] Covalent binding of the metabolites of CCl_4, trichloromethyl free radicals, to cell proteins is considered to be the initial step in a chain of events that eventually lead to membrane lipid peroxidation and finally to cell death.[5] Many studies reported that natural antioxidants are efficacious in preventing oxidative stress-related liver pathologies due to particular interactions and synergisms.[2] ROS production is linked with oxidative stress, which is defined as an imbalance in the generation of oxidants and the antioxidant defense.[6] With regard to the central role of ROS in liver disease and pathology, antioxidants might prevent hepatic damage through scavenger activity and increase the activity of intracellular antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT). There is much evidence indicating that natural substances from edible and medicinal plants exhibit strong antioxidant activity that could act against hepatic toxicity caused by various toxicants.[7]

A major defense mechanism involves antioxidant enzymes, including SOD, CAT, and GSH-Px, which convert active oxygen molecules into nontoxic compounds. One such candidate is yam peel extract, which was chosen in the present study.

Yams are a major food crop in West Africa, the Caribbean, islands of the South Pacific, Southeast Asia, India, and parts of Brazil.[8] Nutritionally, yam constitutes a better source of ascorbic acid and protein than cassava.[9] It is estimated that there are more than 600 species in the world, 93 of which are found in China and 14 in Taiwan.[10] Yam tubers provide much of the carbohydrates in many tropical countries. In addition, it is used in Chinese herbal medicine and was first described in the Shennong Benchao Jing as the "Chinese yam". Chinese yam is commonly used in Chinese medicine to strengthen stomach function, improve anorexia, and eliminate diarrhea.[11] In Taiwan, yam is also used as tonic nourishment and is considered a potential functional food containing antioxidant activity.[12,13] It has been reported that a number of polyphenolic compounds extracted from food or fruit can protect against liver injury, because they exhibit one or a combination of

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antioxidant, antifibrotic, immunomodulatory, or antiviral activities.[14] In recent years, therefore, there has been a substantial increase in the use of so-called complementary dietary polyphenols and alternative therapies to treat patients with liver disease.

These activated macrophages released inflammatory mediators including tumor necrosis factor-alpha (TNF-α)/nuclear factor-kappa B (NF-κB), and nitric oxide (NO) that have been implicated in liver damage induced by a number of different toxicants.[15] Therefore, in the present study, we investigated the activity of yam peel extract against CCl_4-induced oxidative stress and hepatotoxicity in the rats for 8 weeks; hepatic GSH-Px and thiobarbituric acid reactive substances (TBARS) levels as well as activities of aspartate transferase (AST), alanine transferase (ALT), alkaline phosphatase (ALP), TNF-α/NF-κB, and nitric oxide in serum and CAT, SOD, GSH-Px, iNOS, and COX-2 in liver tissues were measured to monitor liver injury. The extent of CCl_4-induced liver injury was also analyzed through histopathological examination.

MATERIALS AND METHODS

Preparation of Yam (*Dioscorea alata*) Peel Extract. Fresh yam peels obtained from a local yam chip-making unit were washed three times with tap water and then dried at 70 °C for 5 h in a "cross-flow dryer". The dried peel was ground in a multimill and passed through a 0.5 mm mesh sieve to obtain a fine powder. Freeze-dried extract of yam peel was prepared from cold water extract of yam peel powder. Half a gram of yam peel powder was homogenized with 10 mL of distilled water for 5 min. The homogenate was centrifuged at 10000 rpm for 10 min. The supernatant was filtered through Whatman no. 1 filter paper, and the resultant extract was lyophilized to dryness in vacuo. The lyophilized powder yield of 8.00−11.50% was stored in a dark bottle at 4 °C until use.

Treatment. Male Wistar rats, weighing 220−260 g, were randomly divided into seven groups with each consisting of eight rats. Group A basal diet did not include the addition of yam peel extract and had a formulation based on American Institute of Nutrition (AIN) and was fed for a period of 8 weeks; group B basal diet, with the addition of yam peel extract, 16% at doses of 266.72 mg/kg, was fed for a period of 8 weeks. To induce hepatotoxicity, animals of groups C−F were given carbon tetrachloride (CCl_4) at a dose of 0.1 mL/100 g body weight (20% v/v in olive oil) twice a week for a period of 8 weeks. Groups A and B received olive oil and saline and served as vehicle control animals. After CCl_4 intoxication, group C served as control CCl_4. Groups D−F were administered yam peel extract in the diet for 6% at a dose of 100.02 mg/kg, 16% at a dose of 266.72 mg/kg, and 26% at a dose of 433.42 mg/kg; group G served as positive control and was given silymarin in the diet for 12% at a dose of 200 mg/kg, respectively, daily for a period of 8 weeks. During weeks 2 and 4, blood was obtained by tail vein puncture 6 h after administration. At week 8, the rats were weighed and anesthetized with diethyl ether. Blood was obtained by heart puncture with syringes.

Plasma was collected by centrifugation (1000g × 15 min) from blood and analyzed using a Merck VITALAB Selectra Biochemical Autoanalyzer (Merck, Darmstadt, Germany) to determine AST, ALT, and ALP. Livers of the rats were quickly excised and weighed. Both relative ratios of liver weight to body weight were obtained. The liver was stored at −40 °C for glutathione peroxidase (GSH-Px) and TBARS determinations.

Determination of Total Phenolics Content (TPC) and Total Flavonoids Content (TFC). TPC was determined using a modified version of the Folin−Ciocalteu method.[16] Yam peel extract (0.4 mL; 2 mg/mL) was added to 1.0 mL of Folin−Ciocalteu reagent, and the mixture was kept at room temperature for 5 min. Five milliliters of sodium carbonate (1 M) was added to the mixture and the whole mixed gently. The total volume of the mixture was adjusted to 10 mL with distilled water. After the mixture had been kept at room temperature for 1 h, the absorbance was read at 760 nm. The standard calibration (0.02−0.12 mg/mL) curve was plotted using gallic acid. TPC was expressed as gallic acid equivalents (GAE) per gram yam peel extract (mg GAE/100 g).

TFC was determined according to the method proposed by Jia et al.[17] with a slight modification. One milliliter of yam peel extract (2 mg/mL) was placed in a 10 mL volumetric flask, and 0.4 mL of 5% sodium nitrite solution was added. Six minutes later, 0.4 mL of 10% aluminum nitrate was added. After 6 min, 4 mL of 4% sodium hydroxide was added, and the total was made up to 10 mL with methanol. The solution was mixed well again, and the absorbance was measured against a blank at 510 nm 15 min later. Catechin was used as the standard for a calibration curve. The TFC was expressed as the catechin equivalents (CE) per gram yam peel extract (mg CE/100 g).

Extraction of Phenolic Acids and Flavonoids Compounds. Briefly, to 1 g of freeze-dried yam peel extract was added a solution containing methanol (containing 2 g/L of 3,5-di-*tert*-butyl-4-hydroxytoluene) and 10% acetic acid in a ratio of 85:15, and the mixture was ultrasonicated for 30 min. Deionized water was then added, and the samples were vortexed. The clear upper layer was collected, evaporated under vacuum in a rotary evaporator, redissolved in methanol solution, and kept at 20 °C. The residues were redissolved in deionized water containing 1% ascorbic acid, 0.42% ethylenediaminetetraacetic acid sodium salt (EDTA), and 5 mL of 10 M NaOH, flushed with nitrogen, and stirred overnight with a magnetic stirrer at room temperature. Samples were then adjusted to pH 2 with concentrated HCl and extracted with a mixture of diethyl ether and ethyl acetate in a 1:1 ratio. The organic layer was collected, evaporated to dryness as before, redissolved in methanol, and kept at 20 °C. Concentrated HCl was added to the remaining solution and heated at 85 °C for 30 min. Following cooling, samples were extracted with diethyl ether and ethyl acetate in a ratio of 1:1. The organic layer was collected, evaporated to dryness as before, and redissolved in methanol. All methanol extracts were combined and filtered through a membrane filter (0.45 μm) and analyzed for total phenolic acid and flavonoid compounds.

Identification of Phenolic Acids and Flavonoids by HPLC. Phenolic acids and flavonoids were separated on a Lichrospher 100 RP-18 reverse-phase column (5 μm, 25 × 0.3 cm i.d., Merck) using a Hitachi HPLC system (model L-6200) with a model L-4000 UV−vis detector. For separation of phenolic acids a solvent system consisting of water/methanol/acetic acid (83:15:2, v/v/v) was used as mobile phase (isocratic) at a flow rate of 1 mL/min and was monitored at 280 and 320 nm. Phenolic acid standards such as caffeic acid, *p*-coumaric acid, chlorogenic acid, salicylic acid, ferulic acid, gallic acid, ellagic acid, vanillic acid, syringic acid, and gentisic acid standards (Sigma Co. St. Louis, MO, USA) were used for identification and quantification. Flavonoids were separated using a gradient system. Solvent A consists of water adjusted to pH 2.8 with acetic acid, and solvent B consists of 100% acetonitrile. The gradient program used was as follows: 1−5 min, 10% B; 5−31 min, 33% B; 31−43 min 67% B; 43−60 min, 100% A. Flavonoids such as catechin, rutein, fisetin, apigenin, hesperidin, naringin, puerarin, myricetin, and quercetin standards (Sigma Co.) were used for identification and quantification.

Antioxidant Activities. Appropriate liver tissues were dissected, weighed, immersed in liquid N_2 within 60 s of death, and kept frozen at −70 °C. Prior to enzyme determinations, thawed tissue samples were homogenized in 20 volumes of ice-cold 50 mM phosphate buffer (pH 7.4) and centrifuged at 3200g for 20 min (three times) at 5 °C. The supernatant was used for antioxidant enzyme determinations.

CAT Activity. The mitochondria pellet was dissolved in 1.0 mL of a 0.25 M sucrose buffer. Ten microliters of the mitochondria solution was added to a cuvette containing 2.89 mL of a 50 mM potassium phosphate buffer (pH 7.4), and then the reaction was initiated by adding 0.1 mL of 30 mM H_2O_2 to make a final volume of 3.0 mL at 25 °C. The decomposition rate of H_2O_2 was measured at 240 nm for 5 min on a spectrophotometer. A molar extinction coefficient of 0.041 mM^{-1} cm^{-1} was used to determine the CAT activity. The activity was defined as μmol H_2O_2 decrease/mg protein/min.

SOD Activity Assays. One hundred microliters of the cytosol supernatant was mixed with 1.5 mL of a Tris−EDTA−HCl buffer (pH 8.5) and 100 μL of 15 mM pyrogallol and then incubated at 25 °C for 10 min. The reaction was terminated by adding 50 μL of 1 N HCl, and the activity was measured at 440 nm. One unit was determined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. The activity was expressed as units/mg protein.

Levels of GSH-Px Measurement. GSH-Px levels were measured using the glutathione assay kit (Calbiochem, San Diego, CA, USA). An equal volume of ice-cold 10% metaphosphoric acid was added to the liver preparations. Supernatants were collected after centrifugation at 1000 rpm for 10 min and analyzed for GSH-Px per the manufacturer's instructions. Total GSH-Px in the samples was normalized with protein.

TBARS Concentration. Lipid peroxidation activities in the liver and plasma were assayed by measurement of MDA, an end-product of peroxidized fatty acids, and TBA reaction product. The sample of 20% liver homogenate was mixed with 1.0 mL of 0.4% TBA in 0.2 N HCl and 0.15 mL of 0.2% BHT in 95% ethanol. The samples were incubated in a 90 °C water bath for 45 min. After incubation, the TBA−MDA adduct was extracted with isobutanol. The isobutanol extract was mixed with methanol (2:1) prior to injection into the system of HPLC. The supernatant was examined by using the HPLC system at an excitation of 515 nm and an emission of 550 nm on a Hitachi fluorescence detector (Japan).

Total Protein Assay. Protein content in each sample was determined by a bicinchoninic acid (BCA) protein assay kit (Pierce).

Measurement of Serum TNF-α and NF-κB Level by ELISA. Serum levels of TNF-α and NF-κB were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Biosource International Inc., Camarillo, CA, USA) according to the manufacturer's instruction. TNF-α and NF-κB were determined from a standard curve. The concentrations were expressed as pg/mL.

Measurement of Nitric Oxide/Nitrite Level. NO production was indirectly assessed by measuring the nitrite levels in serum determined by a calorimetric method based on the Griess reaction. Serum samples were diluted four times with distilled water and deproteinized by adding $^{1}/_{20}$ volume of zinc sulfate (300 g/L) to a final concentration of 15 g/L. After centrifugation at 10000*g* for 5 min at room temperature, 100 μL of supernatant was applied to a microtiter plate well, followed by 100 μL of Griess reagent (1% sulfanilamide and 0.1% *N*-1-naphthylethylenediamine dihydrochloride in 2.5% polyphosphoric acid). After 10 min of color development at room temperature, the absorbance was measured at 540 nm with a Micro-Reader (Molecular Devices, Sunnyvale, CA, USA). By using sodium nitrite to generate a standard curve, the concentration of nitrite was measured by absorbance at 540 nm.

Western Blot Analysis. Liver tissues were homogenized in lysis buffer (0.6% NP-40, 150 mM NaCl, 10 mM HEPES (pH 7.9), 1 mM EDTA, and 0.5 mM PMSF) at 4 °C. Fifty micrograms of protein was fractionated on 10% SDS−polyacrylamide gels and transferred onto nitrocellulose membranes (Millipore, Bedford, MA, USA). Membranes were incubated with primary antibodies overnight at 4 °C using 1:1000 dilution of goat polyclonal anti-rabbit iNOS, COX-2, and β-actin antibodies. The membranes were washed three times, and the immunoreactive proteins were detected by enhanced chemiluminescence (ECL) using hyperfilm and ECL reagent (Amersham International plc., Buckinghamshire, UK). The results of Western blot analysis were quantified by measuring the relative intensity compared to the control using Kodak Molecular Imaging software (version 4.0.5, Eastman Kodak Co., Rochester, NY, USA) and represented in the relative intensities.

Histopathological Examination. The livers were preserved in 10% buffered formalin for at least 24 h, dehydrated with a sequence of ethanol solutions, and processed for embedding in paraffin. Sections of 5−6 mm in thickness were cut, deparaffinized, rehydrated, stained with hematoxylin and eosin (H&E) for the estimation of hepatocyte necrosis and vacuolization, as well as Masson trichrome stain and Sirius red stain for hepatocyte fibrosis, and subjected to photomicroscopic examination. The histological scoring of hepatic damage and fibrosis were expressed using the following score system: 0, no histopathologic change; 1≤, mild histopathologic change; 2≤, moderate histopathologic change; 3≤, severe histopathologic change.

Statistical Analysis. All data were analyzed by one-way ANOVA. Duncan's new multiple-range test was used to resolve the difference among treatment means. All statistical analyses were performed using the statistical software SPSS 11.0 (SPSS Ltd., Surrey, UK). A P value <0.05 was considered statistically significant. Ratio values were not arcsin transformed before statistical analysis.

RESULTS

TPC, TFC, and HPLC Analysis. Individual phenolic compounds of yam peel extract were determined by HPLC. The results of quantitative determination are shown in Table 1. Phenolic acids (ferulic acid, chlorogenic acid, *p*-coumaric acid, caffeic acid, gallic acid, sinapic acid, ellagic acid, salicylic acid, vanillic acid, gentisic acid) and flavonoid compounds (apigenin, quercetin, hesperidin, naringin, myricetin, puerarin, catechin, rutin, fisetin) were present in yam peel extract. The content of quercetin (21.2 mg/100 g) is the highest among identified phenolic compounds, followed by hesperidin and apigenin. The TFC (CE) in yam peel extract of 92.6 mg/100 g was higher than TPC (GAE) in yam peel extract of 16.8 mg/100 g. However, only 19 individual phenolic compounds were identified and quantified in yam peel extract in our study.

Effect of Yam Peel Extract on CCl_4-Induced Liver Injury in Rats. The effect of various doses of yam peel extract on serum biochemical markers in CCl_4-intoxicated rats was studied (Figure 1). After a single injection of CCl_4, serum activities of AST, ALT, and ALP enzymes in the CCl_4-treated groups (group C-G) were significantly increased ($P < 0.05$), as compared to the normal control group (group A), respectively. Treatment of animals with different doses of yam peel extract for 8 weeks significantly reduced the activities of serum AST, ALT, and ALP as compared to the group of CCl_4-treated alone. The positive control drug, silymarin, at a dose of 200 mg/kg also reduced the levels of serum AST, ALT, and ALP. The histological observations supported the results obtained from serum enzyme assays. Liver sections from control rats showed normal lobular architecture and hepatic cells with a well-preserved cytoplasm and well-defined nucleus and nucleoli (Figure 4). When compared with the normal liver tissues of vehicle controls, liver tissue in the rats treated with CCl_4 revealed extensive liver injuries characterized by moderate to severe hepatocellular hydropic degeneration and necrosis around the central vein, lipidosis, hepatic fibrosis, and cholangiocyte hyperplasia.

Effects of Yam Peel Extract on Antioxidant Enzymes in Liver. The hepatic antioxidant enzyme activities of SOD and CAT were decreased in the liver of rats treated with CCl_4; however, the activities of SOD and CAT were restored by yam peel extract (groups D−F). As shown in Figure 2, the hepatic GSH-Px level was markedly lower in CCl_4-intoxicated rats. However, the GSH-Px level was significantly increased by yam peel extract (groups D−F) treatment when compared with the CCl_4 group. Administration of silymarin (group G) did not significantly increase ($P > 0.05$) the activities of SOD, CAT, and GSH-Px, respectively, as compared to the CCl_4-treated group. Expected increases of the hepatic and serum lipid peroxidative indices in the CCl_4-treated model group confirmed that oxidative damage has been induced (Figure 2) after treatment with various doses of yam peel extract (groups D−F); the levels of TBARS in the liver and plasma were significantly lower than those in the CCl_4-treated model group. Silymarin

Table 1. Levels of Phenolic Acids and Flavonoids in Yam Peel Extract[a]

compound	contents[b] (mg/100 g freeze-dried sample)
total phenolic acids (GAE)[c]	16.8 ± 0.6
total flavonoids (CE)[d]	92.6 ± 1.2
ferulic acid	1.6 ± 0.6
	(1.6 ± 0.3)
chlorogenic acid	1.3 ± 0.5
	(1.3 ± 0.2)
p-coumaric acid	3.1 ± 0.3
	(3.0 ± 0.1)
caffeic acid	2.2 ± 0.5
	(2.1 ± 0.2)
gallic acid	1.7 ± 0.2
	(1.7 ± 0.3)
sinapic acid	0.6 ± 0.1
	(0.6 ± 0.2)
ellagic acid	0.3 ± 0.1
	(0.3 ± 0.1)
salicylic acid	0.2 ± 0.1
	(0.2 ± 0.1)
vanillic acid	0.3 ± 0.1
	(0.3 ± 0.1)
gentisic acid	0.2 ± 0.1
	(0.2 ± 0.1)
apigenin	11.5 ± 1.1
	(11.2 ± 0.5)
quercetin	21.2 ± 1.5
	(20.7 ± 1.2)
hesperidin	16.5 ± 1.2
	(16.1 ± 1.3)
naringin	8.5 ± 0.1
	(8.3 ± 0.3)
myricetin	6.9 ± 0.5
	(6.7 ± 0.2)
puerarin	7.2 ± 0.3
	(7.0 ± 0.3)
catechin	5.3 ± 0.2
	(5.2 ± 0.3)
rutein	6.2 ± 0.2
	(6.1 ± 0.2)
fisetin	7.5 ± 0.3
	(7.3 ± 0.3)

[a]The detection limits for different phenolic acids and flavonoids are as follows: gallic acid, 8.7 μg/g; caffeic acid, 7.3 μg/g; ferulic acid, 5.2 μg/g; chlorogenic acid, 3.5 μg/g; myricetin, 5.5 μg/g; *p*-coumaric acid, 3.3 μg/g; sinapic acid, 2.2 μg/g; apigenin acid, 3.7 μg/g; hesperidin, 3.2 μg/g, naringin, 2.8 μg/g; ellagic acid, 3.6 μg/g; salicylic acid, 5.1 μg/g; quercetin, 6.5 μg/g; vanillic acid, 2.6 μg/g; gentisic acid, 5.3 μg/g; puerarin, 8.5 μg/g; catechin, 2.5 μg/g; rutein, 1.5 μg/g; fisetin, 6.5 μg/g. $n = 6$. [b]Data represent the mean ± SD. Data in parentheses represent percentages. [c]GAE, gallic acid equivalent. [d]CE, catechin equivalent.

(group G) also inhibited the elevation of TBARS levels upon CCl_4 administration.

Effect of Yam Peel Extract on the Serum Levels of TNF-α, NF-κB, and NO in Rats. As shown in Figure 3, the CCl_4 treatment caused a significant ($p < 0.01$) increase in the level of TNF-α/NF-κB in the serum when compared with the control group. The pretreatment of yam peel extract (groups D−F) significantly decreased TNF-α/NF-κB levels when compared to the CCl_4-treated model group. Mice treated with silymarin (group G) also showed a significant ($p < 0.05$) decrease in TNF-α/NF-κB levels in serum compared with the CCl_4-treated model group. As shown in Figure 3, the production of NO in model group serum was significantly increased in the CCl_4-treated model group compared to the control group. However, pretreatment with yam peel extract reduced NO production in the CCl_4-treated model group. For example, NO production in the control group was significantly increased in the CCl_4-treated model group. However, the NO production in the CCl_4-treated model group was significantly ($p < 0.05$) decreased from yam peel extract (groups D−F) treatment, respectively. Mice treated with silymarin (group G) also showed a significant ($p < 0.05$) decrease of NO production in serum compared with the CCl_4 group.

Effect of Yam Peel Extract on Activities of iNOS and COX-2 in Liver. Changes of the activation of iNOS and COX-2 by yam peel extract in the CCl_4-treated model group (Figure 3) were investigated. The relative intensities of bands obtained from Western blot were calculated with the use of the Kodak Molecular Imaging software. The results showed that CCl_4 treatment stimulates increased activation of iNOS and COX-2. For example, in the CCl_4 treatment group, the relative intensity of iNOS and COX-2 was increased compared to the control. However, the treatment of yam peel extract decreased the iNOS and COX-2 expression in the CCl_4-induced model group. Namely, the relative intensities about iNOS and COX-2 expressions were reduced by yam peel extract, respectively, compared to CCl_4 treatment alone.

Histopathology of the Liver. The histological observations supported the results obtained from serum enzyme assays. Liver sections from control rats showed normal lobular architecture and hepatic cells with a well-preserved cytoplasm and well-defined nucleus and nucleoli (Figure 4). The results of hepatic histopathological examination are shown in Table 2. When compared with the normal liver tissues of vehicle controls, liver tissue in the rats treated with CCl_4 revealed extensive liver injuries characterized by moderate to severe hepatocellular hydropic degeneration and necrosis around the central vein, lipidosis, hepatic fibrosis, and cholangiocyte hyperplasia. However, the histopathological hepatic lesions induced by administration of CCl_4 were only remarkably ameliorated in central lobular necrosis, hepatic lipidosis, and hepatic fibrosis by treatment with yam peel extract and silymarin.

DISCUSSION

Phenolic agents occur widely and are found in many plants, including a variety of vegetables, fruits, and medicinal plants. Recently, the role of phenolic compounds of foods and beverages in the prevention of free radical-mediated diseases has attracted great interest. They possess a variety of antioxidant properties, which can be ascribed to a broad range of pharmacological activities. Inhibition of free radical-induced damage caused by antioxidant supplementation has become an attractive therapeutic strategy for reducing the risk of liver disease. From the overall results, it was concluded that yam peel extract exhibits free radical-scavenging activity, which could have a beneficial effect against oxidative liver damage induced by CCl_4. Because the phytochemical analysis of apple peel extract showed the presence of polyphenols, mainly chlorogenic acid, procyanidin B_2, and phloridzin, and the antioxidant or hepatoprotective activities of such constituents are well-known,[13]

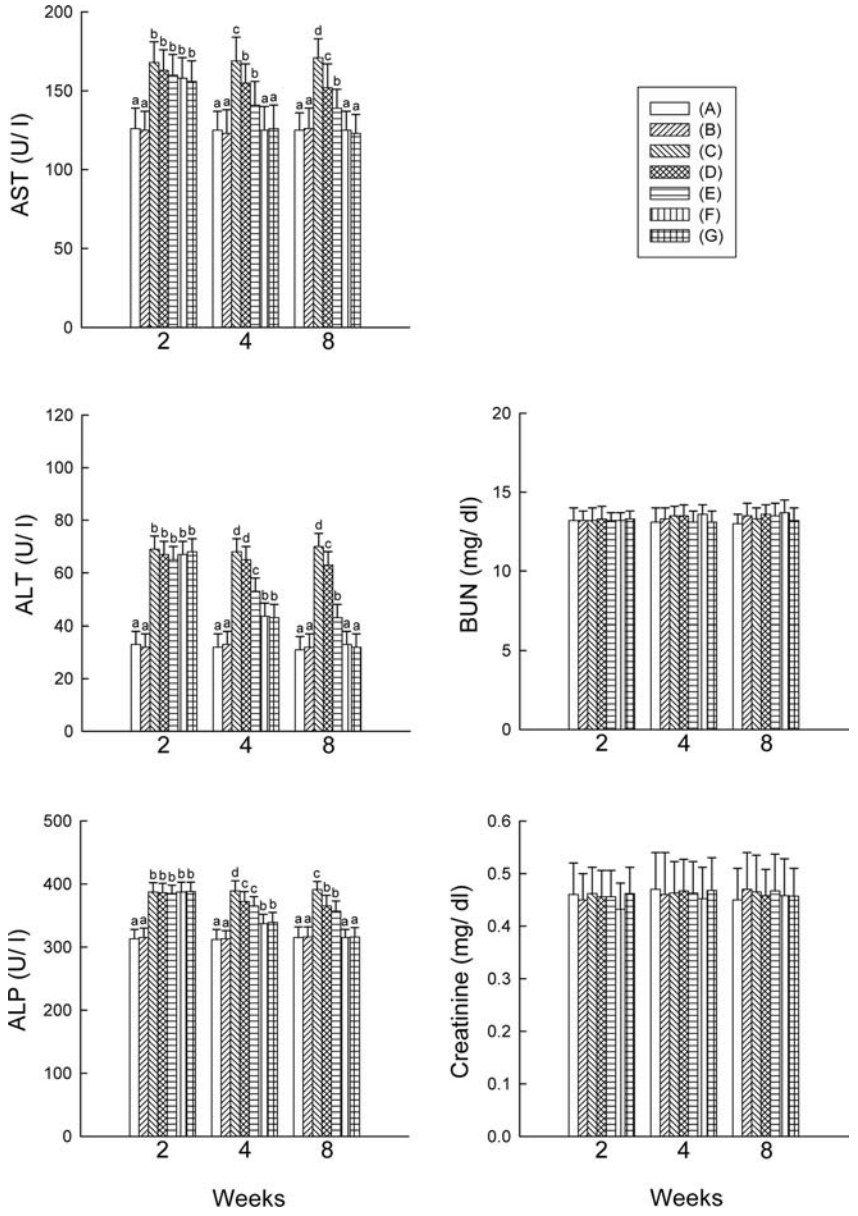

Figure 1. Effect of CCl_4 and yam peel extract in levels of BUN, creatinine, AST, ALT, and ALP of rats. Values in the same week with different letters (a–d) are significantly different ($p < 0.05$).

there has been speculation that these constituents might be responsible for the observed hepatoprotective effects.

In the present study, the capability of yam peel extract to protect against CCl_4-induced hepatotoxicity and oxidative stress was investigated. CCl_4 is a mild analgesic and antipyretic agent.[5] It is also known that its oxy metabolite can produce significant hepatic toxicity through the depletion of GSH-Px level in the liver. This active metabolite reacts with liver macromolecules to induce lipid peroxidation and causes hepatic cell death, resulting in an elevation of serum enzymes AST, ALT and ALP.[18]

We studied further other underlying mechanisms responsible for this hepatoprotective action of yam peel extract in the animal. The protective effect of yam peel extract was accompanied with a partial prevention of GSH-Px depletion in the liver tissue. It is considered that hepatic GSH-Px represents an enzyme reserve of the liver, which is responsible for reducing the hepatotoxicity induced by the active metabolites of CCl_4. As GSH-Px is also a crucial determinant of tissue susceptibility to oxidative damage,[19] the partial protection of yam peel extract on GSH-Px reserves provide an additional action not only to remove the active metabolites of CCl_4 but also to scavenge free radicals, which are involved in lipid peroxidation.

CCl_4 also increased lipid peroxidation, and as a result the hepatic TBARS level was elevated.[20] Yam peel extract treatment prevented this effect, indicating that yam peel extract was able to attenuate the lipid peroxidation induced by CCl_4.

Hepatocellular necrosis leads to elevations of serum AST, ALT, and ALP activities and an increased incidence and severity of histopathological hepatic lesions in rats. The present study revealed a significant increase in the activities of AST, ALT, and ALP on exposure to CCl_4, indicating considerable hepatocellular injury. Administration of yam peel extract attenuated the increased levels of the serum enzymes (AST, ALT, and ALP) induced by CCl_4 and caused a subsequent

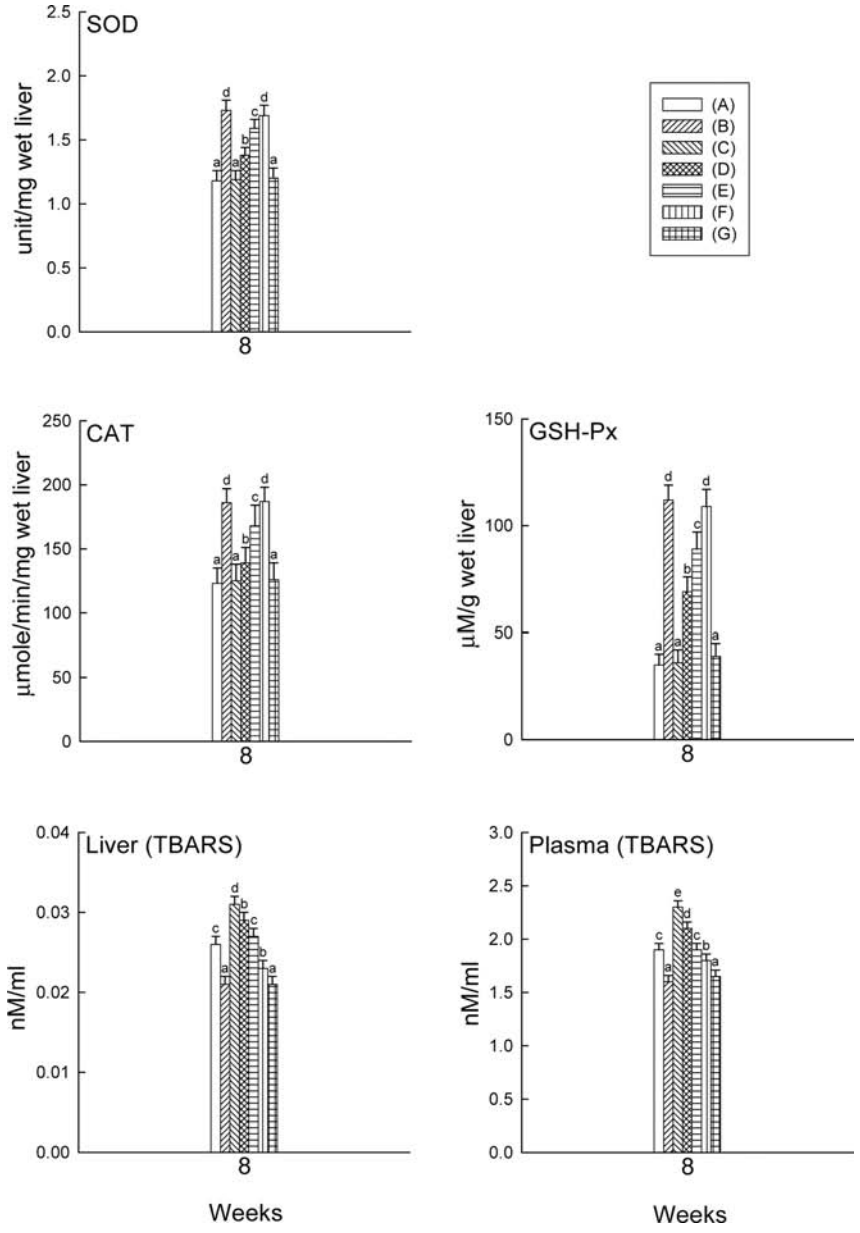

Figure 2. Effect of CCl_4 and yam peel extract on the activities of SOD, CAT, and GSH-Px and TBARS concentrations of rats. Values in the same week with different letters (a–e) are significantly different ($p < 0.05$).

recovery toward normalization comparable to the control group, and the good hepatoprotective effect is comparable with that of silymarin. Silymarin, an antioxidant flavonoid, has been used to treat hepatotoxicity diseases in clinical practice for at least two decades.[21] Other phenolic compounds, such as hesperetin,[22] quercetin,[23] and caffeic acid phenethyl ester,[24] also have been proved to possess a hepatoprotective effect in mice against liver damage induced by lipid peroxidation. Moreover, a considerable amount of polyphenol extracts are from natural products, such as propolis extract.[25] Among identified and quantified phenolic compounds, owing to superior antioxidant activities, quercetin and hesperetin have been investigated and proved to possess hepatoprotective effects in mice.[22,23,26]

The effect of yam peel extract was further confirmed by histopathological examinations. Yam peel extract offers a hepatoprotection effect in central lobular necrosis, hepatic lipidosis, and cholangiocyte hyperplasia than other lower doses in rats. It has been hypothesized that one of the principal causes of CCl_4-induced liver injury is formation of lipid peroxides by free radical derivatives of CCl_4. Thus, the antioxidant activity or the inhibition of the generation of free radicals is important in protection against CCl_4-induced hepatopathy. The body has an effective defense mechanism to prevent and neutralize the free radical induced damage by a set of endogenous antioxidant enzymes such as SOD and catalase. These enzymes constitute a mutually supportive defense team against ROS.[2] Lipid peroxidation, a ROS-mediated mechanism, has been implicated in the pathogenesis of various liver injuries and subsequent liver fibrogenesis in experimental animals.[27] The significant non-dose-dependent decrease in the hepatic lipid hydroperoxide confirmed that treatment with yam peel extract could effectively protect against the hepatic lipid peroxidation induced by CCl_4. Hence, it is possible that the mechanism of hepatoprotection of yam peel extract may be due to its antioxidant activity. Moreover, the observed reduced activities of SOD and CAT

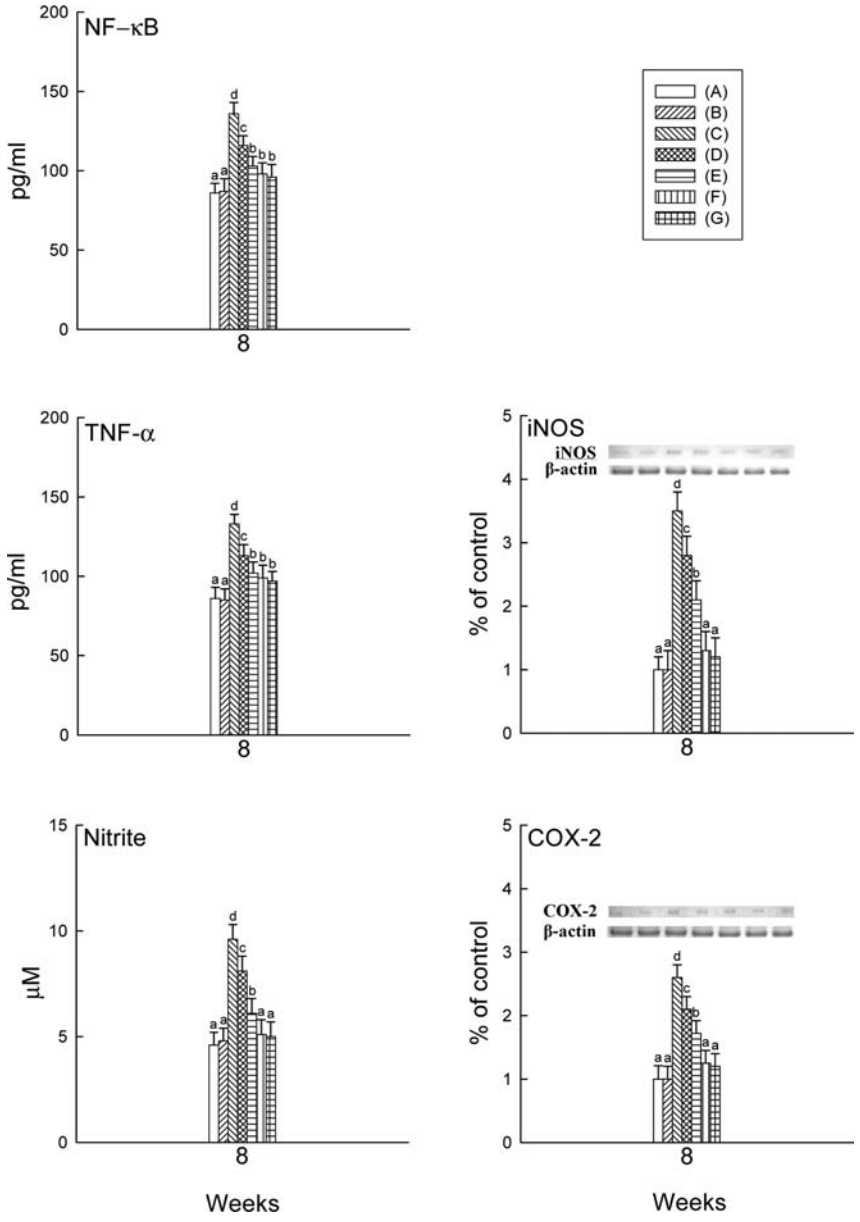

Figure 3. Effect of CCl_4 and yam peel extract on the activities of iNOS, COX-2, and TNF-α and NF-κB and NO concentrations of rats. Values in the same week with different letters (a−d) are significantly different ($p < 0.05$).

point out the hepatic damage in the rats administered CCl_4. The animal groups treated with yam peel extract showed an increase in the level of SOD and CAT, which indicated the antioxidant activity of the yam peel extract. GSH-Px acts as an enzymatic antioxidant both intracellularly and extracellularly in conjunction with various enzymatic processes that reduce hydrogen peroxide and hydroperoxides. The depletion of hepatic GSH-Px has been shown to be associated with an enhanced toxicity to chemicals, including CCl_4.[28] In the present study, a decrease in hepatic tissue GSH-Px level was observed in the CCl_4-treated groups. The increase in hepatic GSH-Px levels in the rats treated with yam peel extract may be due to de novo GSH-Px synthesis or GSH-Px regeneration.

The inflammatory processes contribute to a number of pathological events after exposure to various hepatotoxins. Kupffer cells release pro-inflammatory mediators either in response to necrosis or as a direct action by the activated hepatotoxins, which are believed to aggravate CCl_4-induced hepatic injury.[29] TNF-α, a pleiotropic pro-inflammatory cytokine, is rapidly produced by macrophages in response to tissue damage. Whereas low levels of TNF-α may play a role in cell protection, excessive amounts cause cell impairment. An increase in the TNF-α level has been directly correlated with the histological evidence of hepatic necrosis and the increase in the serum aminotransferase levels.[30] DeCicco et al.[31] have reported the stimulation of TNF-α production in both serum and liver following CCl_4 administration, and it is suggested that CCl_3 activates Kupffer cells to release TNF-α. The cell signaling of TNF-α is speculated as follows: when in contact with its ligand, TNF-α mediates its receptors to form trimers, the binding of which causes a conformational change serving as a platform for protein bioeffects. Following the binding, NF-κB pathways can be initiated and activated.[32] NF-κB is a heterodimeric transcription factor that translocates to the nucleus and then mediates the transcription of a vast array of proteins, resulting in cell survival and proliferation and

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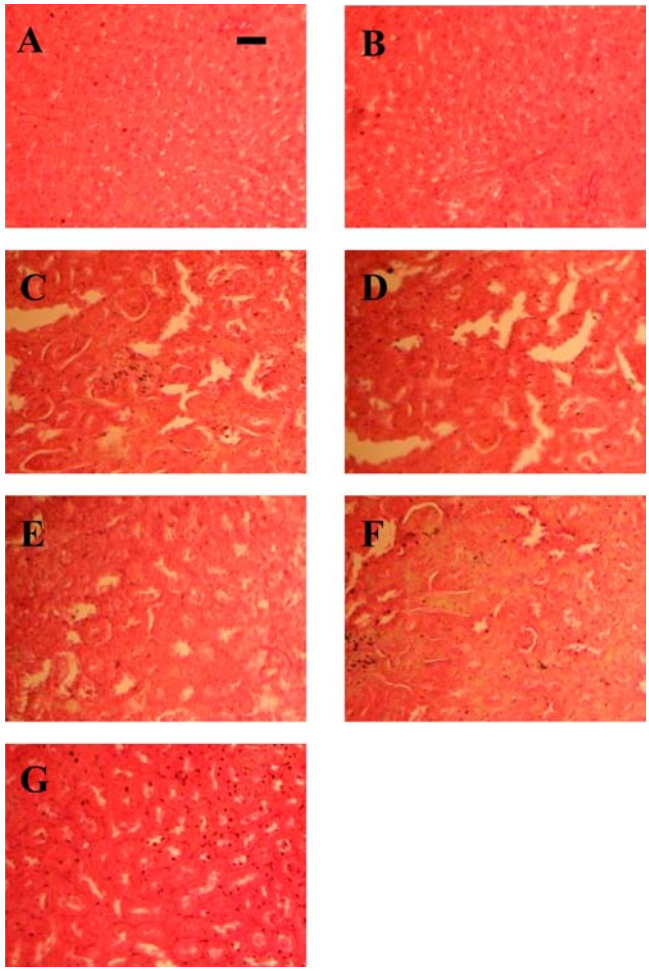

Figure 4. Microscopic cross section of liver lobules in rat after 8 weeks (×400 H&E). Bar represents 0.01 mm.

inflammatory response.[33] TNF-α also stimulates the release of cytokines from macrophages and induces the phagocyte oxidative metabolism and nitric oxide production.[31] Nitric oxide is a highly reactive oxidant that is produced through the action of iNOS and plays a role in a number of physiological processes, such as vasodilation, neurotransmission, and nonspecific host defense.[34] Nitric oxide can also exacerbate oxidative stress by reacting with ROS, particularly with the superoxide anion, and forming peroxynitrite. This study confirmed a significant increase in the serum TNF-α/NF-κB protein expression after CCl_4 administration. These alterations were attenuated by yam peel extract pretreatment (Figure 3), which suggests that yam peel extract suppresses TNF-α/NF-κB protein secretion and/or enhances its degradation.

Yam peel extract blocked the reduction of serum NO level in CCl_4-treated mice. There are two possible explanations for the observed decrease in NO levels after CCl_4 treatment in our study. First, gene expression of NOS was reduced. Second, the NOS system (enzyme protein, substrates, or cofactors) was damaged, thus decreasing NO production. Third, NO usage increased after CCl_4 treatment. It is possible that another mechanism of protective action of yam peel extract against CCl_4-induced hepatotoxicity is due to the increased NO production. Several studies have found that NO protected against CCl_4-induced liver injury using a NOS knockout mice or a NOS inhibitor.[35] The mechanism underlying the protective effects of NO in CCl_4-induced hepatotoxicity has not been elucidated and may be related to its antioxidant properties. NO has also been shown to interfere directly with the progression of lipid peroxidation, which may contribute to its protective actions in the present work. NO is a short-lived signaling molecule capable of regulating many physiological and pathological processes. Neuronal NOS (nNOS) and endothelial NOS (eNOS) are constitutively expressed; iNOS is triggered in many cell types by cytokines such as TNF-α or interferon-γ. Endogenous NO, produced by an early and transient activation of constitutive NOS, protects both hepatocytes and endothelial cells against reperfusion injury in the liver.[36] However, iNOS expression usually occurs after inflammatory responses. iNOS has been implicated as a mediator of cellular injury at sites of inflammation, including liver ischemia/reperfusion injury. Under this circumstance, NO reacts with superoxide and generates ROS, thereafter modifying bioorganic molecules. ROS lead to extracellular matrix (ECM) degradation and leucocyte migration across ECM proteins.[37]

In the current study, treatment with the yam peel extract partially prevented TBARS in liver and plasma. This action could alleviate the injurious action of oxidative stress and inflammation on the liver. Higher TPC and TFC (especially abundant hesperetin and quercetin) were observed in yam peel extract. Also, flavonoids exhibit favorable anti-inflammatory and hepatoprotective effects. Flavonoids could prevent chemical-induced dyslipidemia, inflammatory response, and mitochondrial oxidative damage of rat hepatocytes[38−42] and therefore remarkably high antioxidant activity and anti-inflammation capacity, superior free radical-scavenging ability, and inhibition of lipid peroxidation contributed to the hepatoprotective effect in rats against liver damage induced by CCl_4.

Table 2. Effects of Yam Peel Extract on Hepatic Histopathology of Liver Damage in Rats Treated with CCl_4[a]

	design of treatment						
	control	yam peel extract	CCl_4	CCl_4 + yam peel extract			CCl_4 + silymarin
parameter	(A)	(B)	(C)	(D)	(E)	(F)	(G)
hepatocellular hydropic degeneration	0.0 ± 0.0	0.0 ± 0.0	3.8 ± 0.3[b]	2.1 ± 0.2[c]	1.8 ± 0.2[c]	1.2 ± 0.2[c]	1.3 ± 0.2[c]
central lobular necrosis	0.0 ± 0.0	0.0 ± 0.0	3.6 ± 0.2[b]	2.2 ± 0.1[c]	1.6 ± 0.2[c]	1.1 ± 0.2[c]	1.2 ± 0.2[c]
hepatic lipidosis	0.0 ± 0.0	0.0 ± 0.0	3.7 ± 0.2[b]	2.2 ± 0.3[c]	1.7 ± 0.1[c]	1.2 ± 0.1[c]	1.1 ± 0.3[c]
hepatic fibrosis	0.0 ± 0.0	0.0 ± 0.0	3.6 ± 0.2[b]	2.3 ± 0.2[c]	1.8 ± 0.3[c]	1.1 ± 0.2[c]	1.2 ± 0.1[c]
cholangiocyte hyperplasia	0.0 ± 0.0	0.0 ± 0.0	3.8 ± 0.3[b]	2.1 ± 0.1[c]	1.5 ± 0.3[c]	1.2 ± 0.1[c]	1.1 ± 0.2[c]

[a]Values are expressed as the mean ± SD (n = 8) in each group. 0, no histopathologic change; 1≤, mild histopathologic change; 2≤, moderate histopathologic change; 3≤, severe histopathologic change. [b]P < 0.05 indicate statistically significantly different from control. [c]Significant difference at P < 0.05 levels compared with the CCl_4.

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Notes

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REFERENCES

(1) Wu, J.; Danielsson, A.; Zern, M. A. Toxicity of hepatotoxins: new insights into mechanisms and therapy. *Expert Opin. Invest. Drugs* **1999**, *8*, 585−607.

(2) Vitaglione, P.; Morisco, F.; Caporaso, N.; Fogliano, V. Dietary antioxidant compounds and liver health. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 575−586.

(3) Slater, T. F.; Sawyer, B. C. The stimulatory effects of carbon tetrachloride on peroxidative reactions in rat liver fractions in vitro. Inhibitory effects of freeradical scavengers and other agents. *Biochem. J.* **1971**, *123*, 823−828.

(4) Hsu, Y. W.; Tsai, C. F.; Chang, W. H.; Ho, Y. C.; Chen, W. K.; Lu, F. J. Protective effects of *Dunaliella salina* − a carotenoids-rich alga, against carbon tetrachloride-induced hepatotoxicity in mice. *Food Chem. Toxicol.* **2008**, *46*, 3311−3317.

(5) Recknagel, R. O.; Glende, E. A.; Dolak, J. A.; Waller, R. L. Mechanisms of carbon tetrachloride toxicity. *Pharmacol. Ther.* **1989**, *43*, 139−154.

(6) Upur, H.; Amat, N.; Blazekovic, B.; Talip, A. Protective effect of *Cichorium glandulosum* root extract on carbon tetrachloride-induced and galactosamine-induced hepatotoxicity in mice. *Food Chem. Toxicol.* **2009**, *47*, 2022−2030.

(7) Zeashan, H.; Amresh, G.; Singh, S.; Rao, C. V. Hepatoprotective activity of *Amaranthus spinosus* in experimental animals. *Food Chem. Toxicol.* **2008**, *46*, 3417−3421.

(8) Okonkwo SNC. The botany of the yam plant and its exploitation in enhanced productivity of the crops. In *Advances in Yam Research; The Biochemistry and Technology of the Yam Tuber*; Osuji, G., Ed.; Biochemical Society Nigeria and ASUT: Enugu, Nigeria, 1985; pp 3−29.

(9) Onayemi, O. Some chemical factors acting the quality of processed yam. *J. Food Sci.* **1986**, *51*, 161−168.

(10) Liu, S. Y.; Chang, T. W.; Lin, Y. K.; Chen, S. F.; Wang, J. Y.; Zu, G. L.; Wang, S. C. Studies on the varietal characters, production potential, phytochemical properties, and antioxidant effect of *Dioscorea* spp. *J. Agric. Res.* **1999**, *8*, 1−22.

(11) Lee, S. C.; Tsai, C. C.; Chen, J. C.; Lin, J. G.; Lin, C. C.; Hu, M. L.; Lu, S. Effects of "Chinese yam" on hepatonephrotoxicity of acetaminophen in rats. *Acta Pharmacol. Sin.* **2002**, *23*, 503−512.

(12) Hsu, C. K.; Yen, J. Y.; Wei, J. H. Protective effects of the crude extracts from yam (*Dioscorea alata*) peel on *tert*-butylhydroperoxide-induced oxidative stress in mouse liver cells. *Food Chem.* **2011**, *126*, 429−434.

(13) Yeh, Y. H.; Lee, Y. T.; Hwang, D. F. Yam (*Dioscorea alata*) inhibits of hypertriglyceridemia and liver enlargement of hypercholesterol diet in rats. *J. Chinese Med.* **2007**, *18*, 77−86.

(14) Yang, J.; Li, Y.; Wang, F.; Wu, C. Hepatoprotective effects of apple polyphenols on CCl_4-induced acute liver damage in mice. *J. Agric. Food Chem.* **2010**, *58*, 6525−6531.

(15) Celik, I.; Temur, A.; Isik, I. Hepatoprotective role and antioxidant capacity of pomegranate (*Punica granatum*) flowers infusion against trichloroacetic acid exposed in rats. *Food Chem. Toxicol.* **2009**, *47*, 145−149.

(16) Wolfe, K.; Wu, X.; Liu, R. H. Antioxidant activity of apple peels. *J. Agric. Food Chem.* **2003**, *51*, 609−614.

(17) Jia, Z.; Tang, M.; Wu, J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radical. *Food Chem.* **1999**, *64*, 555−559.

(18) Weber, L. W.; Boll, M.; Stampfl, A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit. Rev. Toxicol.* **2003**, *33*, 105−136.

(19) Ischiropoulos, H.; Zhu, L.; Beckman, J. S. Peroxynitrite formation from macrophage-derived nitric oxide. *Arch. Biochem. Biophys.* **1992**, *298*, 446−451.

(20) Szymonik-Lesiuk, S.; Czechowska, G.; Stryjecka-Zimmer, M.; Slomka, M.; Madro, A.; Celinski, K.; Wielosz, M. Catalase, superoxide dismutase, and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication. *J. Hepato-Biliary Pancreat. Surg.* **2003**, *10*, 309−315.

(21) Shaker, E.; Mahmoud, H.; Mnaa, S. Silymarin, the antioxidant component and *Silybum marianum* extracts prevent liver damage. *Food Chem. Toxicol.* **2010**, *46*, 803−806.

(22) Tirkey, N.; Pilkhwal, S.; Kuhad, A.; Chopra, K. Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. *BMC Pharmacol.* **2005**, *5*, 2.

(23) Lee, E. S.; Lee, H. E.; Shin, J. Y.; Yoon, S.; Moon, J. O. The flavonoid quercetin inhibits dimethylnitrosamine-induced liver damage in rats. *J. Pharm. Pharmacol.* **2010**, *55*, 1169−1174.

(24) Albukhari, A. A.; Gashlan, H. M.; EI-Beshbishy, H. A.; Nagy, A. A.; Abdel-Naim, A. B. Caffeic acid phenethyl ester protects against tamoxifen-induced hepatotoxicity in rats. *Food Chem. Toxicol.* **2009**, *47*, 1689−1695.

(25) Bhadauria, M.; Nirala, S. K.; Shukla, S. Multiple treatment of propolis extract ameliorates carbon tetrachloride induced liver injury in rats. *Food Chem. Toxicol.* **2008**, *46*, 2703−2712.

(26) Cheng, N.; Ren, N.; Gao, H.; Lei, X.; Zheng, J.; Cao, W. Antioxidant and hepatoprotective effects of *Schisandra chinensis* pollen extract on CCl_4-induced acute liver damage in mice. *Food Chem. Toxicol.* **2013**, *55*, 234−240.

(27) Niemela, O.; Parkkila, S.; Yla-Herttuala, S.; Halsted, C.; Witztum, J. L.; Lanca, A.; Israel, Y. Covalent protein adducts in the liver as a result of ethanol metabolism and lipid peroxidation. *Lab. Invest.* **1994**, *70*, 537−546.

(28) Alía, M.; Horcajo, C.; Bravo, L.; Goya, L. Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats. *Nutr. Res. (N.Y.)* **2003**, *23*, 1251−1267.

(29) Badger, D. A.; Sauer, J. M.; Hoglen, N. C.; Jolley, C. S.; Sipes, I. G. The role of inflammatory cells and cytochrome P450 in the potentiation of CCl_4-induced liver injury by a single dose of retinol. *Toxicol. Appl. Pharmacol.* **1996**, *141*, 507−519.

(30) Bruccoleri, A.; Gallucci, R.; Germolec, D. R.; Blackshear, P.; Simeonova, P.; Thurman, R. G.; Luster, M. I. Induction of early-immediate genes by tumor necrosis factor α contribute to liver repair following chemical-induced hepatotoxicity. *Hepatology* **1997**, *25*, 133−141.

(31) DeCicco, L. A.; Rikans, L. E.; Tutor, C. G.; Hornbrook, K. R. Serum and liver concentrations of tumor necrosis factor α and interleukin-1β following administration of carbon tetrachloride to male rats. *Toxicol. Lett.* **1998**, *98*, 115−121.

(32) Wajan, H.; Pfizenmaier, K.; Scheurich, P. Tumor necrosis factor signaling. *Cell Death Differ.* **2003**, *10*, 45−65.

(33) Brasier, A. R. The NF-κB regulatory network. *Cardiovasc. Toxicol.* **2006**, *6*, 111−130.

(34) Yen, G. C.; Lai, H. H.; Chou, H. Y. Nitric oxide-scavenging and antioxidant effects of *Uraria crinita* root. *Food Chem.* **2001**, *74*, 471−478.

(35) Wink, D. A.; Cook, J. A.; Pacelli, R.; DeGraff, W.; Gamson, J.; Liebmann, J.; Krishna, M.; Mitchell, J. B. The effect of various nitric oxide-donor agents on hydrogen peroxide-mediated toxicity: a direct correlation between nitric oxide formation and protection. *Arch. Biochem. Biophys.* **1996**, *331*, 241−248.

(36) Liu, D. Z.; Liang, Y. C.; Lin, S. Y.; Lin, Y. S.; Wu, W. C.; Hou, W. C.; Su, C. H. Antihypertensive activities of a solid-state culture of

Taiwanofungus camphoratus (Chang-Chih) in spontaneously hypertensive rats. *Biosci., Biotechnol., Biochem.* **2007**, *71*, 23−30.
(37) Huang, G. J.; Deng, J. S.; Huang, S. S.; Shao, Y. Y.; Chen, C. C.; Kuo, Y. H. Protective effect of antrosterol from *Antrodia camphorata* submerged whole broth against carbon tetrachloride-induced acute liver injury in mice. *Food Chem.* **2012**, *132*, 709−716.
(38) Hu, Q. H.; Zhang, X.; Pan, Y.; Li, Y. C.; Kong, L. D. Allopurinol, quercetin and rutin ameliorate renal NLRP3 inflammasome activation and lipid accumulation in fructose-fed rats. *Biochem. Pharmacol.* **2012**, *84*, 113−125.
(39) Liu, S.; Hou, W.; Yao, P.; Li, N.; Zhang, B.; Hao, L.; Nussler, A. K.; Liu, L. Heme oxygenase-1 mediates the protective role of quercetin against ethanol-induced rat hepatocytes oxidative damage. *Toxicol. In Vitro* **2012**, *26*, 74−80.
(40) Tang, Y.; Gao, C.; Xing, M.; Li, Y.; Zhu, L.; Wang, D.; Yang, X.; Liu, L.; Yao, P. Quercetin prevents ethanol-induced dyslipidemia and mitochondrial oxidative damage. *Food Chem. Toxicol.* **2012**, *50*, 1194−1200.
(41) Kebieche, M.; Lakroun, Z.; Lahouel, M.; Bouayed, J.; Meraihi, Z.; Soulimani, R. Evaluation of epirubicin-induced acute oxidative stress toxicity in rat liver cells and mitochondria, and the prevention of toxicity through quercetin administration. *Exp. Toxicol. Pathol.* **2009**, *61*, 161−167.
(42) Yousef, M. I.; Omar, S. A. M.; El-Guendi, M. I.; Abdelmegid, L. A. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. *Food Chem. Toxicol.* **2010**, *48*, 3246−3261.