Journal of the Formosan Medical Association (2012) 111, 550—559

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ORIGINAL ARTICLE

Green tea extract supplementation ameliorates CCl_4-induced hepatic oxidative stress, fibrosis, and acute-phase protein expression in rat

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Received 7 March 2011; received in revised form 28 April 2011; accepted 1 June 2011

KEYWORDS
acute phase proteins;
carbon tetrachloride;
catechins;
fibrosis;
liver;
oxidative stress

Background/Purpose: We evaluated the long-term effects of green tea extract (GTE) supplementation on oxidative stress, biliary acute phase protein expression, and liver function in CCl_4-induced chronic liver injury.
Methods: We evaluated the antioxidant activity of GTE in comparison with those of vitamin C, vitamin E, and β-carotene *in vitro* by using an ultrasensitive chemiluminescence analyzer. Chronic liver injury was induced by intraperitoneally administering carbon tetrachloride (CCl_4) (1 mL/kg body weight, twice weekly) to female Wistar rats for 8 weeks. The effects of low (4 mg/kg body weight per day) and high (20 mg/kg body weight per day) doses of intragastric GTE on CCl_4-induced liver dysfunction and fibrosis were examined by measuring the bile and blood reactive oxygen species levels and biochemical parameters by using Western blot and two-dimensional polyacrylamide gel electrophoresis techniques.
Results: GTE has greater scavenging activity against O_2^-, H_2O_2, and Hypochlorous acid (HOCl) *in vitro* than vitamin C, vitamin E, and β-carotene do. *In vivo*, CCl_4 markedly increased bile and blood reactive oxygen species production, lipid accumulation, number of infiltrated

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doi:10.1016/j.jfma.2011.06.026

leukocytes, fibrosis, hepatic hydroxyproline content, and plasma alanine aminotransferase and aspartate aminotransferase activities, and reduced plasma albumin levels. Two-dimensional polyacrylamide gel electrophoresis revealed that CCl_4 increased the acute-phase expression of six biliary proteins and decreased hepatic B-cell lymphoma 2 (Bcl-2), catalase, and CuZn superoxide dismutase protein expression. GTE supplementation attenuated CCl_4-enhanced oxidative stress, levels of biochemical parameters, pathology, and acute-phase protein secretion, and preserved antioxidant/antiapoptotic protein expression.

Conclusion: GTE supplementation attenuates CCl_4-induced hepatic oxidative stress, fibrosis, acute phase protein excretion, and hepatic dysfunction via the antioxidant and antiapoptotic defense mechanisms.

Introduction

Hepatocellular apoptosis, hepatic inflammation, and fibrosis are prominent features of chronic liver disease.[1] Experimental and clinical data from several studies have suggested that oxidative stress plays a critical role in chronic liver damage and hepatic fibrosis.[2]

Carbon tetrachloride (CCl_4) is a widely used industrial solvent, and it is the best-characterized animal model of xenobiotic-induced, oxidative stress-mediated hepatotoxicity.[3] The symptoms of CCl_4-induced chronic liver injury are similar to those of chronic liver injury in humans.[4] CCl_4 induces the production of several types of reactive oxygen species (ROS) via cytochrome P450, thereby causing liver injury.[5] These ROS can bind to polyunsaturated fatty acids, forming alkoxy and peroxyl radicals to produce lipid peroxide, cause cell membrane damage, cause changes in enzyme activity,[6] and consequently induce hepatic injury, inflammation, apoptosis, and necrosis.[7,8]

Since antioxidants have antiviral, anti-inflammatory, antifibrogenic, and anticarcinogenic potential, their use has been proposed as an adjunctive therapy for various liver diseases. Antioxidants such as green tea extracts (GTE) containing (+)-catechin (C), (−)-epicatechin, (+)-gallocatechin, (−)-epigallocatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate (EGCG) are popular and are recognized to exert protective effects against bladder hyperactivity,[9] cancer,[10] lipogenesis,[11] inflammation,[10,12] atherosclerosis,[12] and acute liver injury.[8] GTE inhibited inflammation and apoptosis through decrease in ROS production, translocation of nuclear factor-κB and activated protein-1, and expression of intercellular adhesion molecule-1.[7,9] GTE showed dose-dependent, palliative effects against hemodialysis-enhanced plasma H_2O_2 and Hypochlorous acid (HOCl) activities, lipid peroxide (phosphatidylcholine hydroperoxide) production, and C-reactive protein and proinflammatory cytokine expression in end-stage renal disease patients.[12] Furthermore, GTE is more effective in scavenging plasma H_2O_2 and HOCl activity compared to vitamins C and E.[13] Data have suggested that GTE administration prevents the development of hepatic fibrosis in rats with dimethylnitrosamine or CCl_4-induced[14] liver injury[15] and inhibits the number of glutathione *S*-transferase placental form- and gamma-glutamyl transpeptidase-positive hepatic foci and areas in aflatoxin B1-initiated and CCl_4-promoted hepatocarcinogenesis.[16] EGCG, the primary active component of GTE, exhibited anti-inflammatory, antioxidant, and immunosuppressive effects, improved liver dysfunction, reduced liver inflammatory infiltration and hepatocyte apoptosis, and abrogated tumor necrosis factor-alpha and interferon-gamma expression at the protein level in plasma.[17] EGCG also attenuated hepatic hydroxyproline content and hepatic stellate cell activation, as well as matrix metalloproteinase-2 activity and protein expression.[18]

Proteomic analysis of bile fluid holds promise as a technique for the identification of biomarkers in various kinds of liver diseases. Our previous studies found that altered biliary secretion profiles of several acute-phase proteins directly indicate oxidative stress affecting intracellular trafficking in hepatocytes.[8] GTE supplementation reduced hepatotoxicity-induced oxidative stress and liver dysfunction and normalized the alteration of several acute-phase protein expressions. The differentially displayed proteomes suggested the potential application of proteomic analysis for bile fluid analysis.

In this study, we performed proteomic analysis of bile fluid by using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) techniques to identify disease-associated biomarkers in the control, CCl_4-treated, and CCl_4 plus GTE-treated groups. We also evaluated the effects of chronic GTE supplementation on CCl_4-induced oxidative injury, inflammation, and fibrosis in rats. Our data showed that safe dosage of GTE protects the liver against CCl_4 injury via antioxidative, anti-inflammatory, antiapoptotic, and antifibrotic processes.

Materials and methods

Animals

Twenty-four female Wistar rats (weight, 220—255 g) were purchased and housed at the Experimental Animal Center, National Taiwan University, at a constant temperature and with a consistent light cycle (light from 7:00 AM to 6:00 PM). Food and water were provided *ad libitum*. All surgical and experimental procedures were approved by the Institutional Animal Care and Use Committee of National Taiwan University College of Medicine and College of Public Health and were in accordance with the guidelines of the National Science Council of the Republic of China (NSC, 1997).

To induce chronic liver injury, we injected CCl_4 (Showa Chemical Co., Ltd., Tokyo, Japan) intraperitoneally for 8 weeks. We divided the animals into the following four

groups ($n = 6$ each): control group, in which olive oil was intraperitoneally administered at a dose of 1 mL/kg body weight; CCl_4 treatment group, in which CCl_4 solution was intraperitoneally administered (CCl_4 in olive oil in a ratio of 1:1; dose, 1 mL/kg body weight, twice weekly); CCl_4 plus high-dose GTE (HGT) treatment group, in which CCl_4 solution was intraperitoneally administered and GTE was intragastrically administered (20 mg/kg body weight per day); and CCl_4 plus low-dose GTE (LGT) treatment group, in which CCl_4 solution was intraperitoneally administered and GTE was intragastrically administered (4 mg/kg body weight per day). Decaffeinated GTE catechins containing 328 mg/g of EGCG, 152 mg/g of (−)-epicatechin gallate, 148 mg/g of gallocatechin gallate, 132 mg/g of (−)-epicatechin, 108 mg/g of (−)-epigallocatechin, 104 mg/g of (+)-gallocatechin, and 44 mg/g of C were purchased from Numen Biotech Co., Ltd. (Taipei, Taiwan).

On the day of the experiment, the animals were anesthetized by subcutaneously administering 1.2 g/kg urethane (Sigma, St. Louis, MO, USA). Their arterial blood pressure and bile flow were measured.[8] At the end of each experiment, the animals were sacrificed by overdose of anesthetics. Bile, plasma, and liver were stored at −70 °C until analysis.

In vivo chemiluminescence recording of ROS activity

The ROS generation in response to 8 weeks of CCl_4-induced liver injury was measured in bile and whole blood samples via a modified chemiluminescence detection method as described previously.[7,8] In brief, 0.2 mL of blood or bile samples were mixed with 0.5 mL of 0.1 mmol/L lucigenin or 0.2 mmol/L luminal, and were analyzed using a Chemiluminescence Analyzing System (CLD-110, Tohoku Electronic Inc. Co., Sendai, Japan). Each assay was performed in triplicate and was expressed as the chemiluminescence count per 10 seconds.

To compare the antioxidant potentials of catechins, vitamin C, vitamin E, and β-carotene, we evaluated their inhibitory effects on $O_2^{-\cdot}$, H_2O_2, and HOCl activity *in vitro*. We evaluated 0.2 mL of each test sample for O_2^- activity induced by 0.1 mL xanthine (final concentration, 100 μM) plus 0.1 mL xanthine oxidase (final concentration, 2.5 mU/mL) in 0.5 mL of 0.1 mmol/L lucigenin solution. We also evaluated 0.2 mL of the test sample on 0.1 mL of 0.003% H_2O_2 in 0.2 mmol/L luminol solution. All $O_2^{-\cdot}$, H_2O_2, and HOCl activities were detected using a Chemiluminescence Analyzing System (CLD-110, Tohoku Electronic Inc. Co.).

Biochemical analysis

The plasma alanine aminotransferase (ALT) and aspartate aminotransferase levels were assayed using commercial kits (Sigma-Aldrich, St. Louis, MO, USA). The concentration of plasma albumin was measured using a clinical chemistry analyzer (Fuji Dri-Chem 4000, Tokyo, Japan). Hydroxyproline content was measured as described previously.[19] Biliary transferrin and haptoglobin concentrations were measured using the Rat Transferrin ELISA Kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and Rat Haptoglobin ELISA Kit (Wako Pure Chemical Industries), respectively.

Two-dimensional electrophoresis

Bile is secreted by hepatocytes; therefore, proteomic profiles of bile may provide specific information and protein markers of the hepatic responses to CCl_4-induced injury and GTE effects. The collected bile was analyzed via two-dimensional electrophoresis (2-DE) gel techniques as described previously.[8]

Peptide analysis via mass spectrometry

The method of analysis has been reported in detail elsewhere.[8] Matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry (MALDI-TOF MS) analysis was conducted using a Voyager DE-STR workstation (PerSeptive Biosystems, Framingham, MA, USA) equipped with a 337-nm nitrogen laser. The peptide mass fingerprint data were compared to those in the NCBInr protein database using the Mascot searching tool (http://www.matrixscience.com). The following search parameters were included: Database, NCBInr; taxonomy, *Homo sapiens*; enzyme, trypsin; peptide charge, 1+; instrument, MALDI-QUAD-TOF.

Western blot analysis

We measured the expression of transferrin, haptoglobin, B-cell lymphoma 2 (Bcl-2), catalase, and CuZn superoxide dismutase (SOD) in the total protein content from the liver samples. Protein concentration was determined using a Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA). Ten micrograms of protein was electrophoresed and evaluated via Western immunoblotting and densitometry as described previously.[7,8]

Antibodies raised against rat transferrin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), haptoglobin (Abcam, Cambridge, UK), Bcl-2 (Transduction Laboratories, Inc., Lexington, KY, USA), CuZn SOD (Stress Marq Biosciences Inc., Victoria, Canada), catalase (Chemicon International Inc., Temecular, CA, USA), and β-actin (Sigma-Aldrich) were used. Proteins on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels were transferred to nitrocellulose filters and stained as described previously.[7,8] The density of the band with the appropriate molecular mass was determined semiquantitatively via densitometry by using an image-analyzing system (Alpha Innotech, San Leandro, CA).

Liver histology assessment

The right liver lobe was cut and fixed in 4% buffered neutral formalin at 4 °C. Liver specimens were embedded in paraffin and stained with both hematoxylin and eosin. Liver histology was assessed by a single observer blinded to the experimental protocol and biochemical results. The severity of hepatocyte inflammation, as indicated by leukocyte infiltration and fibrosis, was estimated as an indicator of liver damage. Liver fibrosis scores were as follows: 0, no fibrosis; 1, perivenular and/or pericellular fibrosis; 2, septal fibrosis; 3, incomplete cirrhosis; and 4, complete cirrhosis. The tissue slices were blindly scored by

two expert pathologists. The degree of fibrosis was expressed as the mean of 10 different fields on each slide.

Statistical analysis

All values were expressed as mean [standard error of the mean (SEM)]. Within-groups differences were evaluated using the paired *t* test. One-way analysis of variance was used for establishing differences between groups. Inter-group comparisons were made using Duncan's multiple-range test. Differences were regarded as significant if $p < 0.05$ was attained.

Results

Comparison of the antioxidant activities of GTE, vitamin C, vitamin E, and β-carotene

The scavenging activities of GTE, vitamin C, vitamin E, and β-carotene against O_2^{-} are shown in Fig. 1A—1D. All four antioxidants inhibited O_2^{-} at a dose of 1 μg/mL in a dose-dependent manner as follows: GTE (86.3%) > vitamin C (83.1%) > vitamin E (23.1%) > β-carotene (3.4%).

The antioxidant activity against H_2O_2 is shown in Fig. 2A—2D. All four antioxidants inhibited H_2O_2 at a dose of 1 μg/mL in a dose-dependent manner as follows: GTE (98.9%) > vitamin C (86.5%) > β-carotene (67.5%) > vitamin E (40.7%).

The antioxidant response against HOCl is shown in Fig. 3A—3D. GTE and vitamin C displayed dose-dependent inhibition of HOCl activity, but β-carotene and vitamin E did not. The inhibition of HOCl activity at 1 μg/mL of each antioxidant was in the following order: GTE (97.5%) > vitamin C (95.3%) > vitamin E (71.1%) > β-carotene (12.2%). According to the above findings, GTE is the most efficient antioxidant scavenging O_2^{-}, H_2O_2, and HOCl.

CCl_4 increased bile and blood ROS production and plasma ALT and hepatic hydroxyproline levels

In comparison to the levels in controls, the biliary O_2^{-} (lucigenin counts; Fig. 4A) and H_2O_2 (luminol counts; Fig. 4B) and the blood O_2^{-} (lucigenin counts, Fig. 4C) and H_2O_2 (luminol counts; Fig. 4D) levels were consistently and significantly elevated after 8 weeks of CCl_4 injury. The increased levels of bile and blood ROS were significantly reduced by treatment with both low and high doses of GTE. We also found that CCl_4 produced a significant increase in plasma ALT (Fig. 4E) and aspartate aminotransferase (Fig. 4F) levels, as well as a significant decrease in plasma albumin level (Fig. 4G). CCl_4 increased the hepatic hydroxyproline content (Fig. 4H). CCl_4 plus both low and high doses of GTE showed a significant decrease in enzyme

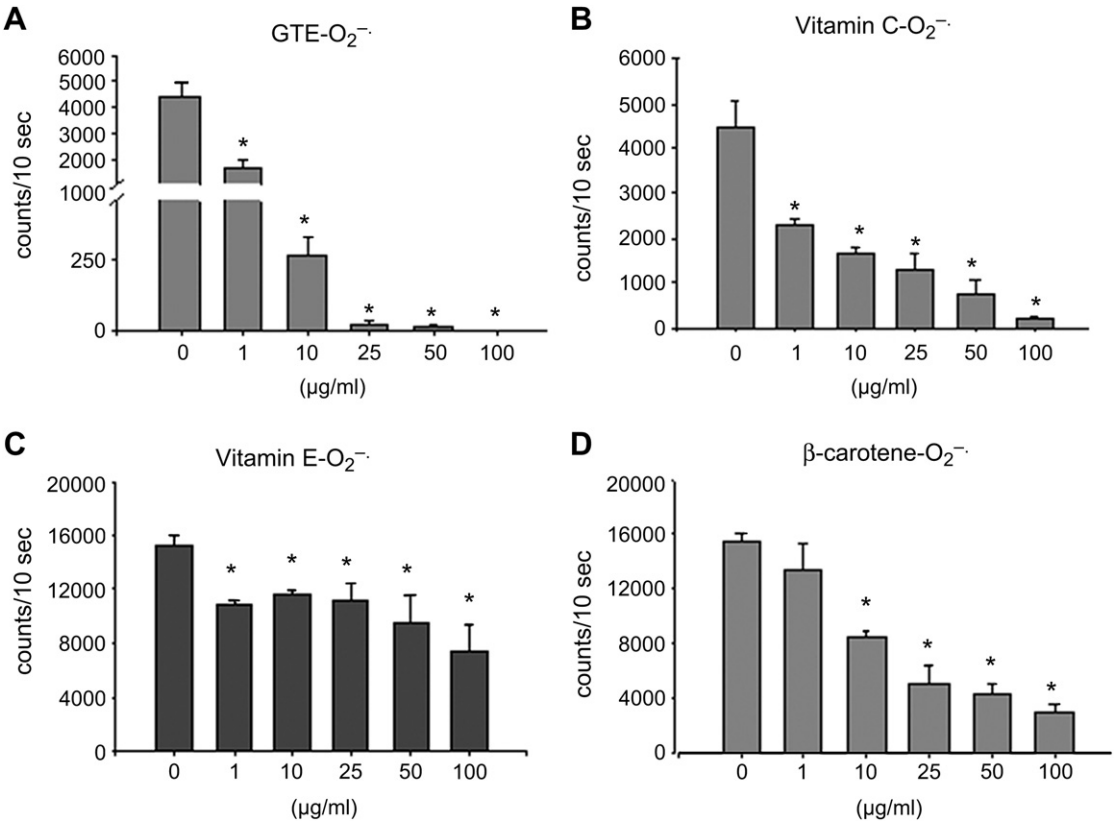


Figure 1 Antioxidant effects of green tea extract (GTE), vitamin C, vitamin E, and β-carotene on O_2^{-} activity. Different concentrations (1, 10, 25, 50, and 100 μg/mL) of antioxidants scavenged O_2^{-} in a dose-dependent manner. Each dose was tested six times. The values are displayed as means (SEM). *$p < 0.05$ versus 0 μg/mL.

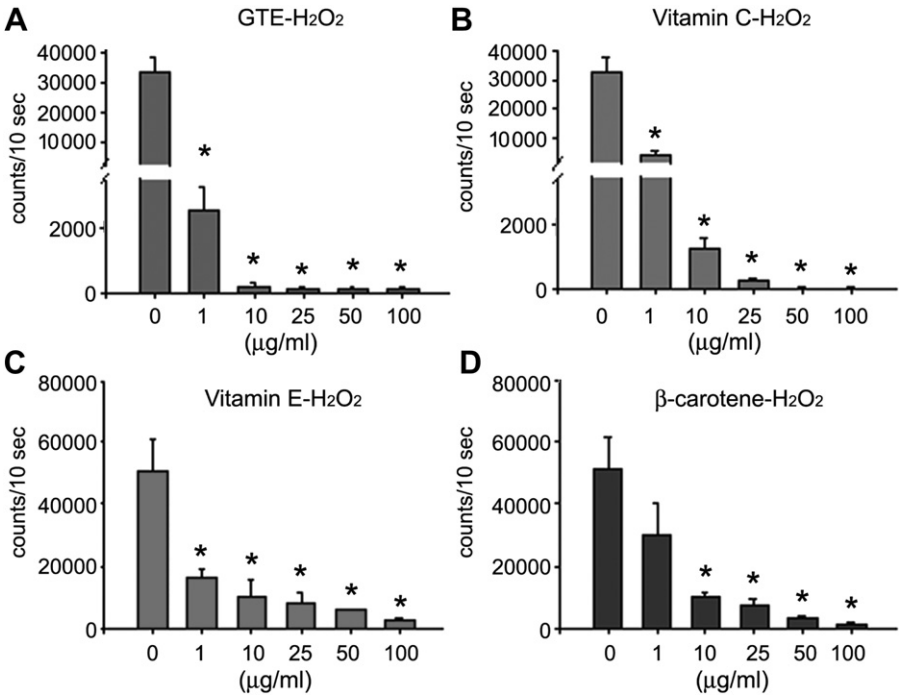


Figure 2 Antioxidant effects of green tea extract (GTE), vitamin C, vitamin E, and β-carotene on H_2O_2 activity. Different concentrations (1, 10, 25, 50, and 100 μg/mL) of antioxidants scavenged H_2O_2 in a dose-dependent manner. Each dose was tested six times. The values are displayed as means (SEM). *$p < 0.05$ versus 0 μg/mL.

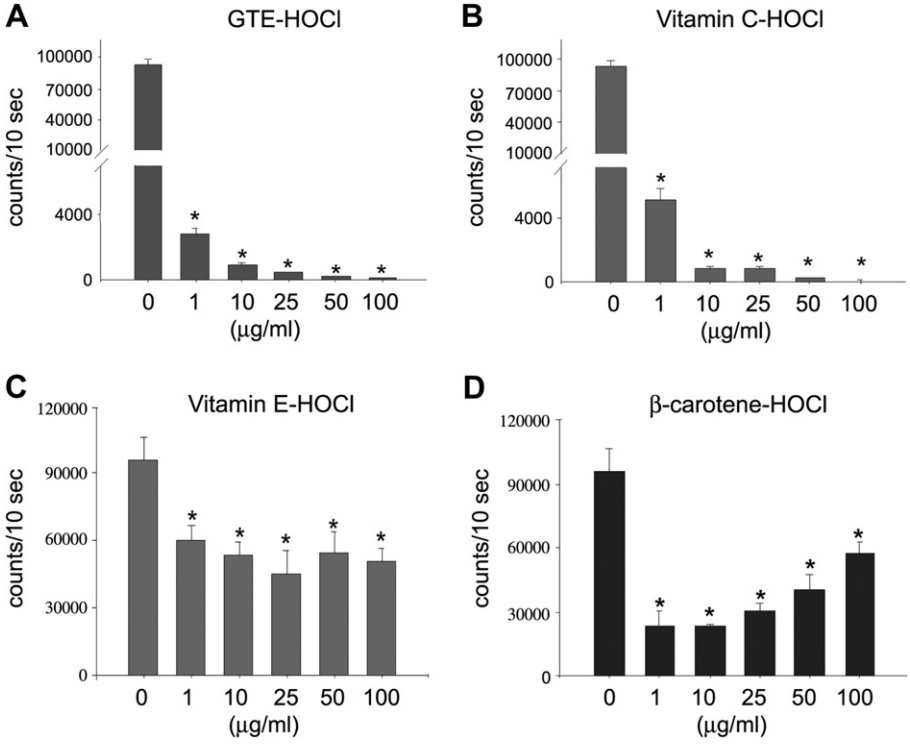


Figure 3 Antioxidant effects of green tea extract (GTE), vitamin C, vitamin E, and β-carotene on Hypochlorous acid (HOCl) activity. Different concentrations (1, 10, 25, 50, and 100 μg/mL) of antioxidants scavenged $O_2^{-\cdot}$ in a dose-dependent manner. Each dose was tested six times. The values are displayed as means (SEM). *$p < 0.05$ versus 0 μg/mL.

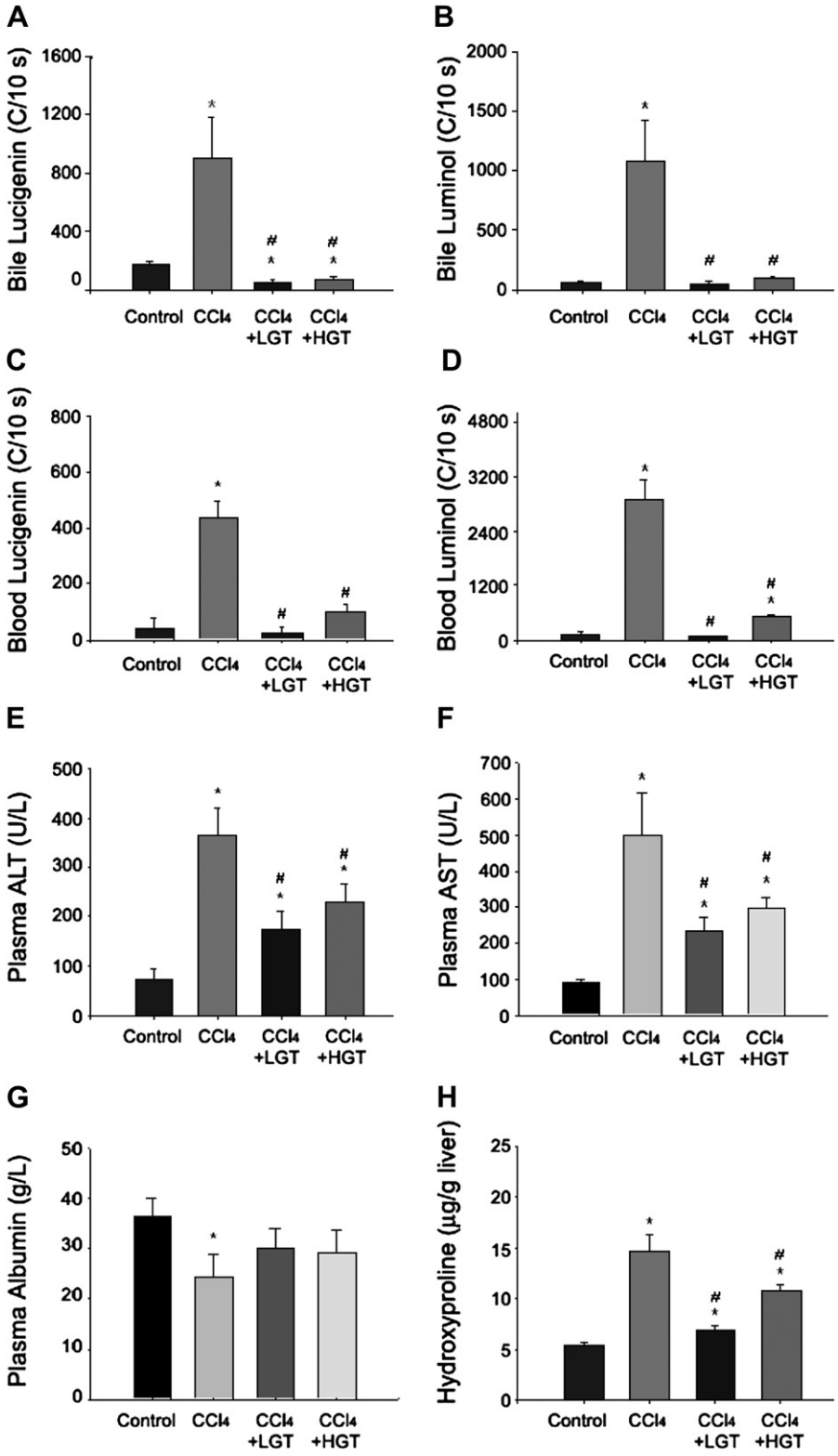


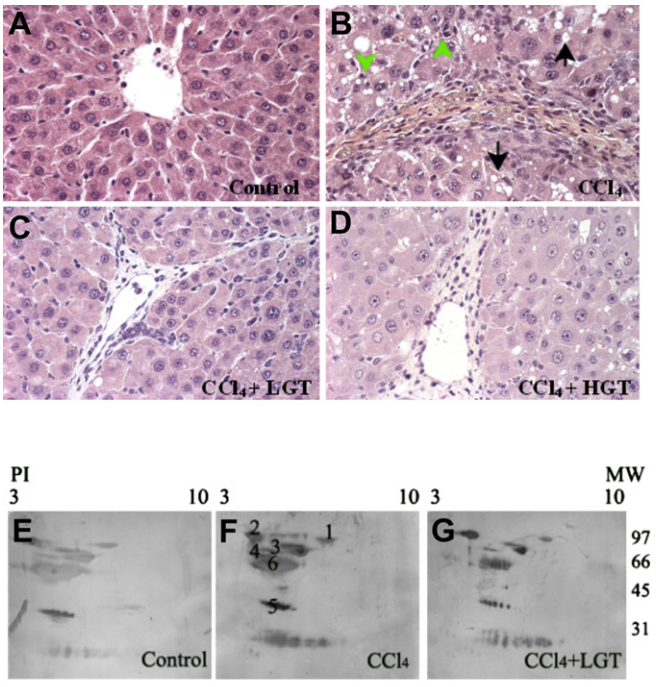
Figure 4 Effect of green tea extract (GTE) on oxidative stress and biochemical parameters in CCl_4-injured rats. Low doses (LGT) and high doses (HGT) of GTE reduced (A, B) lucigenin and(C, D) luminol chemiluminescence levels indicating the bile and blood plasma (E) ALT and (F) aspartate aminotransferase (AST) levels, and (H) hepatic hydroxyproline content; further, they recovered the reduced (G) plasma albumin level in CCl_4-injured rats. $^*p < 0.05$ versus control group. $\#p < 0.05$ versus CCl_4-injured group.

levels and a significant increase in albumin levels. GTE did not show dose-dependent effects in this study.

Influence of CCl_4 and GTE on morphology and bile proteomics

Marked lipid accumulation, fibrosis, necrosis, and leukocyte infiltration were found in CCl_4-treated livers (Fig. 5B) when compared to control livers (Fig. 5A). Both low (Fig. 5C) and high doses of GTE (Fig. 5D) attenuated the morphologic changes. Quantitative data describing the increased leukocyte infiltration (Fig. 5I) and fibrosis scores (Fig. 5 J) are indicated; both low and high doses of GTE significantly decreased CCl_4-induced leukocyte infiltration and fibrosis.

For proteomic analysis, 5-μL bile samples from control rats ($n = 3$), CCl_4-treated rats ($n = 3$), and GTE-treated CCl_4 rats ($n = 3$ each, LGT and HGT dose groups) were



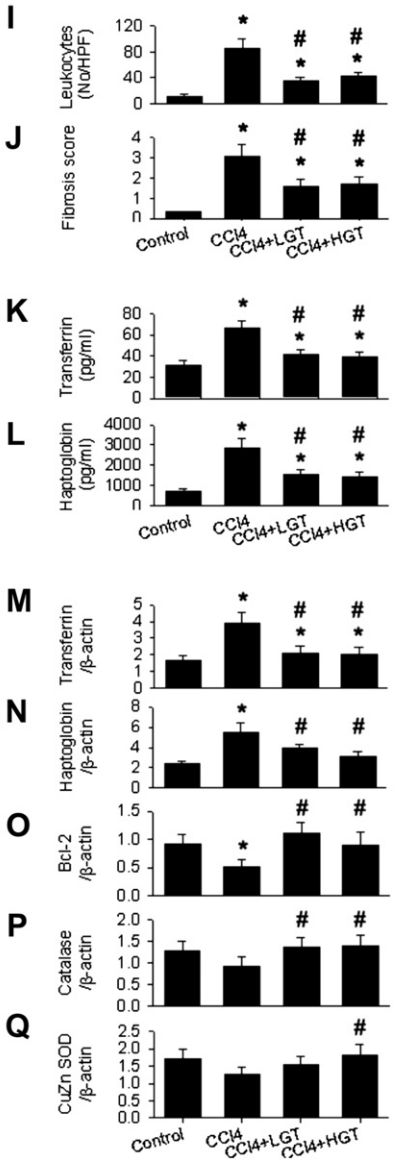


Figure 5 Effects of green tea extract (GTE) on CCl_4-induced pathologic findings, 2-D PAGE electrophotogram of bile, and Western blot analysis. (A—D) Marked lipid accumulation (black arrows), leukocyte infiltration (green arrow heads), and fibrosis are noted in (B) CCl_4-injured liver when compared to (A) control liver, (C) low dose (LGT), or (D) high dose (HGT) GTE-treated livers. The increases in (I) leukocyte infiltration and (J) fibrosis score caused by CCl_4 injury are significantly suppressed by both low and high doses of GTE. (E—G) Electrophotograms were created with silver nitrate staining, and the approximate molecular weight (MW) and PI values are indicated. There were six proteins (spots 1 to 6), with the most striking differences between the (E) control and (F) CCl_4 bile proteome. LGT depressed CCl_4-enhanced protein expression in (G) the bile. (H—Q) CCl_4 significantly increased (K) biliary transferrin and (L) haptoglobin concentrations, and the increased transferrin and haptoglobin levels were depressed by GTE treatment. CCl_4 also enhanced transferrin and haptoglobin expression in the bile and decreased Bcl-2, catalase, and CuZn SOD expression in the liver, as shown by (H) Western blot. LGT and HGT supplementation significantly depressed (M) CCl_4-enhanced transferrin and (N) haptoglobin protein expression and restored the expression of the (O) antioxidant proteins Bcl-2, (P) catalase, and (Q) CuZn SOD. *$p < 0.05$ compared with control. #$p < 0.05$ compared with CCl_4 injury alone.

subjected to 2-DE analysis, and the proteins were visualized with silver staining. Fig. 5E—5 G illustrate the representative silver-stained 2-DE gels of the control, CCl_4, and CCl_4 plus LGT treatment. The six identified major proteins included transferrin (spot 1), polymeric immunoglobin A receptor (spot 2), acute-phase α-1 protein (spot 3), kallikrein-binding protein (spot 4), haptoglobin (spot 5), and IgA α-chain (spot 6). After 8 weeks of CCl_4 injury, the expression of these six biliary proteins was upregulated. Enzyme-linked immunosorbent assay analysis confirmed the increased biliary transferrin (Fig. 5 K) and haptoglobin (Fig. 5L) levels. GTE supplementation decreased the CCl_4-enhanced acute-phase protein secretion. Western blot (Fig. 5H) and statistical analysis showed that CCl_4 markedly increased biliary transferrin (Fig. 5 K) and haptoglobin (Fig. 5L) levels and decreased hepatic Bcl-2 (Fig. 5O), catalase (Fig. 5P), and CuZn SOD (Fig. 5Q) protein expression. Both low and high doses of GTE reduced acute-phase protein expression and preserved antioxidant and antiapoptotic protein expression after CCl_4 injury.

Discussion

A previous study has described plant flavonoids with hepatoprotective qualities, which were primarily attributed to their ability to scavenge $O_2^{-\cdot}$, H_2O_2, $HO^{\bullet}$, and HOCl production from rat liver microsomes.[20] Our study compared the antioxidant capabilities of GTE, vitamin C, vitamin E, and β-carotene against $O_2^{-\cdot}$, H_2O_2, and HOCl activity. The antioxidant capability of 1 μg of each antioxidant against $O_2^{-\cdot}$, H_2O_2, and HOCl was in the following order: GTE $>$ vitamin C $>$ vitamin E $>$ β-carotene. These *in vitro* data further confirmed previous findings that GTE is more efficient than vitamins C and E in palliating hemodialysis-induced plasma H_2O_2 and HOCl activities, lipid peroxidate (phosphatidylcholine hydroperoxide) production, and C-reactive protein and proinflammatory cytokine expression in end-stage renal disease patients.[12,13] According to our findings, GTE is the most efficient antioxidant to scavenge $O_2^{-\cdot}$, H_2O_2, and HOCl, and it may have therapeutic potential in CCl_4-induced oxidative stress.

In this study, we applied a well-established enhanced chemiluminescence method to measure the generation of various types of ROS.[7,8,12,13] This method has been successfully adapted to measure the amounts of ROS in cultured cells, bile, whole blood, and urinary bladder, liver, and kidney cells. Our evidence indicated that the enhanced levels of $O_2^{-\cdot}$, H_2O_2, and HOCl *in vitro* and the bile and blood O_2^{-} and H_2O_2 levels after CCl_4 injury were significantly depressed by GTE.

The causes of hepatic injury include alcoholism, intoxication, viral infection, and immunodeficiency. CCl_4 chemical-induced hepatic damage is a popular model used to investigate the chemopreventive function of phytochemicals or nutraceuticals. After the metabolism of CCl_4, a large amount of ROS is produced. In this study, during the 8 weeks of CCl_4 injury, the expressions of Bcl-2, CuZn SOD, and catalase protein were significantly lower than those in the controls (Fig. 5H). CCl_4 increases the oxidant/antioxidant ratios in the liver. According to the free radical theory, modulation of this phenomenon is one of the possible strategies to inhibit or mitigate the damage induced by toxic radicals. Therefore, we evaluated the antioxidant and hepatoprotective efficacy of GTE. Intake of antioxidants like vitamin E could increase the antioxidant capacity in serum and attenuate CCl_4-induced liver injury.[21] In our study, the antioxidant defense mechanism of Bcl-2, CuZn SOD, and catalase was markedly preserved, even enhanced, after 8 weeks of GTE supplementation in the CCl_4-treated livers. Our data suggested that GTE, via catechins, not only directly exhibited its anti-inflammatory and antioxidant characteristics as described earlier, but also modulated the physiological environment, especially by inhibiting oxidative damage and upregulating antioxidant and antiapoptotic status.

In our previous study, the GTE dose showed no significant effect on ROS level, biochemical parameters, and inflammation.[9] The ingestion of GTE increased the plasma concentration of several catechins. According to our previous report, the concentrations of rat plasma epigallocatechin gallate (10.1 ± 0.3 or 13.2 ± 1.2 ng/mL), epicatechin gallate (5.1 ± 0.6 or 6.3 ± 0.9 ng/mL), and gallocatechin gallate (4.9 ± 0.7 or 5.7 ± 0.8 ng/mL) were similar between the low and high GTE dose groups.[9] We suggest that GTE doses of 4 and 25 mg/kg body weight may have a similar influence on the reduction of CCl_4-induced liver injury.

In response to various types of oxidative stress and toxicity, several acute-phase proteins are synthesized and released from the damaged liver.[22—28] In our bile proteomics analysis, we have identified six acute phase proteins,[8] which regulate intracellular trafficking in the hepatocytes via endosomal transcytotic and lysosomal function.[22—28] These acute phase proteins, like polymeric IgA receptor and transferrin, are upregulated, whereas haptoglobin is depressed in acute liver injury.[8] According to the present data, CCl_4 toxicity increases the levels of transferrin, polymeric IgA receptor, major acute-phase α-1 protein precursor, kallikrein binding protein, and haptoglobin as measured via 2D-PAGE proteomics, suggesting a disruption of intracellular trafficking integrity in the hepatocytes after CCl_4-induced toxicity. In addition, CCl_4 enhances the expression of biliary haptoglobin, which is an inflammation-inducible plasma protein.[28] After long-term high and low-dose GTE supplementation, the enhanced expression of these proteins is greatly diminished. We suggest that GTE can protect against CCl_4-induced injury by attenuating several upregulated acute phase proteins.

In addition to exacerbating oxidative stress, CCl_4 triggers apoptosis, inflammation, and increased collagen gene and protein expression, subsequently leading to liver fibrosis and cirrhosis.[1] Furthermore, CCl_4 causes necrosis, mononuclear cell infiltration, steatosis, foamy degeneration of hepatocytes, and cirrhosis.[29] Our histopathologic observations in livers subjected to CCl_4 injury (Fig. 5) are in agreement with the findings of previous studies.[1, 29]

Secondary liver injury occurs from inflammatory processes initiated by the activation of Kupffer cells, which release chemoattractants and neutrophil activators.[30] Kupffer cell activation increases ROS amounts and plays a critical role in hepatocellular injury.[7,8] The enhanced ROS can further activate hepatic stellate cells, a process characterized by the enhanced production of extracellular matrix and accelerated proliferation. Because hepatocellular apoptosis, hepatic

inflammation, and fibrosis are prominent characteristics in chronic liver diseases, Liu et al[1] found that the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling and CD68-double-positive apoptotic Kupffer cells located in the portal or fibrotic septa area were situated close to hepatic stellate cells. Thus, antioxidant activity and the inhibition of free radical generation are important in protecting the liver from CCl_4-induced damage.[31] The antioxidants may inhibit Kupffer cell activation and may secondarily depress hepatic stellate cell activation and collagen accumulation. However, further studies will be required to validate this hypothesis.

Both low and high doses of GTE significantly reduced plasma ALT levels and hepatic hydroxyproline content in CCl_4-treated rats. We also found that CCl_4-induced pathologic changes in lipid accumulation, leukocyte infiltration, and fibrosis were ameliorated by GTE supplementation. This beneficial effect may be due to GTE's antioxidant, antiapoptotic, anti-inflammatory, and antifibrogenic effects.

In contrast to GTE's beneficial potential, another report has indicated the hepatotoxic effects of high oral doses of EGCG (750 or 1500 mg/kg) in mice.[32] These doses increased plasma ALT levels and produced moderate to severe hepatic necrosis associated with oxidative stress, including increased levels of hepatic lipid peroxidation, plasma 8-isoprostane, interleukin-6, monocyte chemoattractant protein-1, and hepatic metallothionein protein. However, the dosages we used (4 and 20 mg/kg) were significantly lower. Overdose of EGCG or GTE should be avoided due to this hepatotoxicity.

In conclusion, GTE supplementation attenuates CCl_4-enhanced hepatic oxidative stress, fibrosis, acute phase protein excretion, and hepatic dysfunction via the antioxidant, anti-inflammatory, and antiapoptotic defense mechanisms.

Acknowledgments

This work was supported, in part, by the National Science Council (NSC 95-2320-B-002-022), National Taiwan University Hospital (NTUH 94S104), and Kuang-Tien General Hospital, Taiwan. We thank the Third Core Laboratory of the Department of Medical Research in National Taiwan University Hospital for the equipment availability.

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