Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862

Article

Epigallocatechin-3-gallate reduces hepatic oxidative stress and lowers CYP-mediated bioactivation and toxicity of acetaminophen in rats

Hsien-Tsung Yao[1]*, Chien-Chun Li[2] and Chen-Hui Chang[1]

[1]Department of Nutrition, China Medical University, 91 Hsueh-shih Road, Taichung 404, Taiwan; u104059001@cmu.edu.tw (C.-H. C)
[2]School of Nutrition, Chung Shan Medical University, 110 Sec,1, Jianguo North Road, Taichung 40201, Taiwan; licc@csmu.edu.tw (C.-C. Li)
* Correspondence: htyao@mail.cmu.edu.tw; Tel.: +886-4-22053366 ext. 7526; Fax: +886-4-22062891

Received: date; Accepted: date; Published: date

Abstract: Epigallocatechin-3-gallate (EGCG) is the most abundant polyphenol in green tea. In this study, the effects of dietary EGCG on oxidative stress and the metabolism and toxicity of acetaminophen in liver were investigated. Rats were fed the diets with (0.54 %) or without EGCG supplementation for four weeks and were then intraperitoneally injected with acetaminophen (1g/kg). Results showed EGCG lowered hepatic oxidative stress and cytochrome P450 (CYP) 1A2, 2E1, and 3A, and UDP-glucurosyltransferase activities prior to acetaminophen injection. After acetaminophen challenge, the elevations in plasma alanine aminotransferase activity and histological changes in liver were ameliorated by EGCG treatment. EGCG reduced acetaminophen-induced apoptosis by increasing the Bax/Bcl2 ratio in liver. EGCG mildly increased autophagy by increasing the LC3B II/I ratio. Lower hepatic acetaminophen-glutathione and acetaminophen-protein adducts contents were observed after EGCG treatment. EGCG increased glutathione peroxidase and NAD(P)H quinone 1 oxidoreductase activities and reduced organic anion-transporting polypeptides 1a1 expression in liver after acetaminophen treatment. Our results indicate that EGCG may lower oxidative stress and reduce the metabolism and toxicity of acetaminophen. The reductions in CYP-mediated acetaminophen bioactivation and uptake transporter, as well as enhanced antioxidant enzyme activity, may limit the accumulation of toxic products in liver and thus lower hepatotoxicity.

Keywords: epigallocatechin-3-gallate; acetaminophen; cytochrome P-450; bioactivation; apoptosis; autophagy; hepatotoxicity.

1. Introduction

Studies have shown that intake of green tea or green tea polyphenols (GTPs) can reduce the development and progression of various diseases such as cancer, cardiovascular disease, and neurodegenerative diseases [1,2]. The principal hypothesis associated with the putative benefits of green tea is linked to the strong free radical scavenging and antioxidant and anti-inflammatory properties of these polyphenol compounds [3-5]. In addition, GTPs can change drug metabolism by modulating drug-metabolizing enzymes and transporters [6]. These actions may change the fate of drug metabolism and toxicity. Because of the many polyphenolic components in GTPs, discrepancies exist concerning their effects on drug metabolism and toxicity [6].

Among the various tea polyphenols, epigallocatechin-3-gallate (EGCG) is the most abundant and active polyphenol in green tea. Recently, the beneficial effects of green tea on health promotion focused on EGCG [2]. Studies have shown that EGCG can protect the liver from thioacetamide and triptolide-induced hepatotoxicity [7,8]. However, some studies have also shown that high-dose EGCG

Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862

administration to animals (through an intragastric tube or intraperitoneal injection) can cause oxidative damage to the liver [9-11]. To our knowledge, the dose and route by which EGCG is given to animals can be important factors in determining whether oxidative damage will occur. To date, various commercial EGCG products are on the market worldwide. However, little is known about the effect of dietary EGCG on oxidative stress and drug-metabolizing systems, especially its effect on the metabolism and toxicity of prescribed drugs such as acetaminophen (*N*-acetyl-p-aminophenol, APAP). Therefore, it is of considerable importance to evaluate the interactions between EGCG and APAP and their effects on hepatotoxicity.

APAP is widely used as an over-the-counter analgesic and antipyretic agent. APAP overdose is now the most common cause of acute hepatic failure in many countries [12]. APAP is metabolized primarily by sulfation and glucuronidation reactions to generate the nontoxic metabolites APAP-glucuronate and APAP-sulfate [13]. An overdose can induce severe hepatotoxicity as a result of an increase in the cytochrome P-450 (CYP)-mediated bioactivation of APAP to a highly reactive metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI), which exerts its toxicity by covalent binding to cellular macromolecules [14]. In addition, NAPQI also reacts with glutathione (GSH), leading to cellular GSH exhaustion, mitochondrial damage, and cell apoptosis in the liver [15,16]. The removal of damaged organelles, including mitochondria, by autophagy can protect the hepatocytes against APAP-induced mitochondrial damage and subsequent necrosis [17]. The other way to lower APAP toxicity is to facilitate the excretion of glucuronate, sulfate, GSH conjugates, and oxidative stress products from the liver by increasing expression of membrane transporters such as multi-drug resistance-associated protein (Mrp)2/3 or reduced uptake transporters such as organic anion-transporting polypeptide (OATP) 1a1 and OATP 1b2 [18-20].

Administration of GTPs has been shown to provide protection against APAP-induced liver injury [21]. In our pilot study, supplementation with EGCG (0.54%, w/w) in the diet for one week had an inhibitory effect against APAP-induced liver injury in rats [22]. However, the reactive oxygen species (ROS) level in the liver might also have been increased by EGCG treatment (supplementary data 1), suggesting the oxidative stress was mildly increased by short-term exposure of EGCG. In addition, the mechanism by which EGCG lowers APAP-induced liver damage is still not clear. In the present study, rats were fed a diet containing EGCG for a longer time (four weeks) to investigate the effects of EGCG on oxidative stress, drug-metabolizing enzymes, and membrane transporters in liver. Then, the effects of EGCG on the metabolism and toxicity of APAP in liver were investigated.

2. Materials and Methods

2.1. Materials

Testosterone, methoxyresorufin, resorufin, *p*-nitrophenol, 4-nitrocatechol, NADPH, GSH, 1-chloro-2,4-dinitrobenzene, and heparin were obtained from Sigma (St. Louis, MO, USA). 6-β-Hydroxytestosterone was purchased from Ultrafine Chemicals (Manchester, UK). All other chemicals and reagents were of analytical grade and were obtained commercially. EGCG was purchased from Huzhou Ruzhou Rongkai Foliage Extract Co. LTD (Huzhou, China). The purity of the EGCG used was >99% as determined by high-performance liquid chromatography (HPLC).

2.2. Animal studies

Experiment I. Male Sprague-Dawley (SD) rats (aged six weeks) obtained from BioLASCO in Ilan, Taiwan, were used to investigate the effect of dietary EGCG on oxidative stress and the activities of drug-metabolizing enzymes and the expressions of membrane transporters in liver. Rats were fed a standard laboratory chow powder diet (Purina Laboratory Chow 5001) without (control group) or with 0.18% EGCG (1X EGCG group) or 0.54% EGCG (3X EGCG group) for four weeks. Each group consisted of 5 rats. The daily dose of EGCG was about 460 mg/kg body weight (BW) in rats fed a

Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862

laboratory diet containing 0.54% EGCG, which was approximately equivalent to the dose used in previous studies that found that EGCG did not change liver function [22,23]. The rats were all housed in plastic cages in a room kept at 23 ± 1°C with 60 ± 5% relative humidity and a 12-h light-dark cycle. Food and drinking water were available *ad libitum* for four weeks.

At the end of the experiment, fresh feces was collected for determining microbial β-glucuronidase activity. Then, rats were sacrificed and blood was collected by exsanguination via the abdominal aorta while the rats were under carbon dioxide (70:30, CO_2/O_2) anesthesia. Heparin was used as the anticoagulant agent, and the plasma was separated from the blood by centrifugation (1750 × *g*) at 4°C for 20 min. Plasma alanine aminotransferase (ALT) activity was measured immediately by use of commercial kit (Randox Laboratories, Antrum, UK). The liver samples from each animal were stored at -80°C. Microsomes preparation and enzyme assays were performed within two weeks of liver collection.

Experiment II. To investigate the effects of EGCG on the metabolism and toxicity of APAP, male SD rats (six weeks old) were randomly divided into three groups with six rats in each group. The animals in Groups 1 (control group) and 2 were fed a standard laboratory chow powder diet. The animals in Group 3 were fed the same diet fortified with 0.6% EGCG. The daily dose of EGCG was about 511 mg/kg BW. At the end of four weeks feeding period, food was withdrawn for 12 h. A single 1000 mg/kg BW dose of APAP, as a solution in polyethylene glycol 400/water (50/50, v/v), was intraperitoneally injected into each animal in Groups 2 and 3. At 12 h after the APAP dose, the animals were sacrificed. The separated plasma, liver, and urine samples (collected in metabolic cage) were used to determine APAP and its conjugates. Part of the liver samples was excised and fixed in 10% neutral formalin followed by dehydration in ascending grades of alcohol, clearing in xylene, and embedding in paraffin wax. Liver sections (5-μm thickness) were stained with hematoxylin and eosin (H&E) for histological examination [24].

This study was approved (No. 102-70-N) by the Animal Center Management Committee of China Medical University. The animals were maintained in accordance with the guidelines for the care and use of laboratory animals [25].

2.3. Drug-metabolizing enzyme activity assays

Two-step centrifugation was used to prepare liver microsomes and cytosol preparations according to the method reported previously [26]. The formation of metabolites from various CYP enzyme reactions was determined by high performance liquid chromatography (HPLC)/mass spectrometry (MS) [26]. Methoxyresorufin (5 μM), *p*-nitrophenol (50 μM), and testosterone (60 μM) or midazolam (2.5 μM) were respectively used as the probe substrates for methoxyresorufin *O*-demethylation (CYP1A2), *p*-nitrophenol 6-hydroxylation (CYP2E1), testosterone 6β-hydroxylation (CYP3A), and midazolam 1-hydroxylation (CYP3A). Enzyme activities were expressed as pmol of metabolite formation/min/mg protein.

Microsomal UDP-glucuronosyltransferase (UGT) activity was determined by using *p*-nitrophenol as the substrate where the rate of formation of *p*-nitrophenol glucuronic acid was measured by HPLC/MS [27]. Cytosolic sulfotransferase activity was determined by using phosphoadenosine 5-phosphosulphate as the substrate and *p*-nitrophenol as the acceptor of sulfate, and the rate of formation of adenosine 3,5-diphosphate was measured by HPLC/MS [28]. Glutathione *S*-transferase (GST) [29] and NAD(P)H quinone 1 oxidoreductase (NQO1) [30] activities were determined spectrophotometrically.

2.4. Determination of oxidative stress in liver

Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862

Liver homogenate was prepared by homogenizing each gram of liver with 10 mL of ice-cold 1.15% KCl and centrifuging the homogenate at 10,000 ×*g* for 15 min at 4°C. The resulting supernatant was used to determine the oxidative markers including GSH, lipid peroxide, ROS, and glutathione peroxidase activity. The GSH content in liver homogenates was determined by HPLC/MS [31]. GSH peroxidase activity was determined spectrophotometrically according to the method of Mohandas et al [32]. Liver thiobarbituric acid–reactive substance (TBARS) content was determined by the method of Uehiyama and Mihara [33]. ROS production was measured according to the method of Ali et al. [34] by determining the fluorescent product of dichlorofluorescein.

2.5. Determinations of APAP and APAP conjugates in plasma, liver, and urine

Plasma, urine, and liver homogenate samples were diluted and extracted by use of acetonitrile and were then analyzed by LC/MS [35]. To determine hepatic APAP-protein adducts, liver homogenate was filtered through a Nanosep centrifugal device (Pall Life Sciences, Ann Arbor, MI, USA) with a membrane molecular weight cutoff of 30 kDa to remove low molecular weight compounds with the potential to interfere in the assay. The filtrate was then digested for 16 hours with proteases to free the APAP-cysteine from APAP-protein adducts [35]. The resulting APAP-cysteine level was determined by HPLC/MS [36].

2.6. In vitro APAP-GSH formation

To evaluate the susceptibility of APAP-GSH formation in rat liver microsomes from each group, amounts of 20 mM APAP and 5 mM GSH were incubated with liver microsomes (1 mg/mL protein) containing 2 mM NADPH, 1.5 mM $MgCl_2$, and 45 mM potassium phosphate buffer (pH 7.4). The reaction was incubated at 37°C for 60 min. An electrophilic metabolite, NAPQI, conjugated with GSH to form APAP-GSH during the reaction. The formation of APAP-GSH in the incubation was then added with an equal volume of ice-cold isopropanol to stop the reaction. The APAP-GSH formation was determined by HPLC/MS [37].

2.7. Immunoblotting analysis

Liver homogenate was prepared by homogenizing each gram of liver with 10 mL of ice-cold 1.15% KCl and centrifuging the homogenate at 10,000 ×*g* for 15 min at 4°C. The resulting supernatant was used to determine the protein expressions of BCL2-associated X protein (Bax), B-cell lymphoma 2 (Bcl2), and microtubule-associated protein light chain 3B (LC3B I/II). The supernatant was used as a cellular protein for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis [26]. Plasma membrane was prepared by use of a cell membrane protein extraction kit (Bio-Kit, Miaoli, Taiwan). After electrophoresis, the separated proteins were transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA). The membrane was then blocked with 5% nonfat milk, followed by probing with primary antibodies against CYP1A2, CYP2E1, CYP3A, Bax, Bcl2, LC3B I/II, Mrp2/3, p-glycoprotein (p-gp), and Oatp1a1 [38]. The membranes were then probed with the horseradish peroxidase-labeled secondary antibody. The bands of target proteins were visualized and quantitated as described by Yen et al [38].

2.8. Fecal β-glucuronidase activity

Fecal β-glucuronidase activity was determined by the method of Yao and Chiang [39]. Nitrophenyl-β-D-glucuronide was used as the substrate. Fecal β-glucuronidase activity was expressed as nmol p-nitrophenol formation/min/mg protein.

2.9. Statistical analysis

Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862

Statistical differences among groups were calculated by using one-way ANOVA (SAS Institute, Cary, NC, USA). The differences were considered to be significant at $p<0.05$ as determined by independent-sample *t*-tests.

3. RESULTS

3.1. Drug-metabolizing enzyme activity, oxidative stress, membrane transporters, and liver function index in normal rats

In experiment I, we evaluated the effects of supplementation with EGCG for four weeks on oxidative stress, drug-metabolizing enzyme activities and membrane transporters in the liver of normal rats. Table 1 shows the effects of EGCG on drug-metabolizing enzyme activity and oxidative stress in the liver. Rats fed the 3X (0.54%) EGCG diet significantly lower ($p<0.05$) the activities of CYP3A, CYP2E1, and CYP1A2 in liver than that of rats fed the control diet. UGT and GST activities were also reduced in 3X EGCG group ($p<0.05$). In addition, GSSG content was lower and the GSH/GSSG ratio was higher in the liver of rats fed the 1X or 3X EGCG diet than in animals fed the control diet ($p<0.05$). Hepatic TBARS and ROS levels were also lower after 3X EGCG treatment ($p<0.05$). These results indicated that EGCG may reduce drug metabolism and oxidative stress in the liver. No significant difference in plasma ALT activity was observed among the groups, indicating that rats fed the 3X EGCG diet for four weeks caused no hepatotoxicity.

Table 1. Drug-metabolizing enzyme activities, oxidative stress, and liver function index in the liver of rats fed EGCG-containing diets for four weeks

	Control	1X EGCG	3X EGCG
Phase I enzymes (pmol/min/mg protein)			
Testosterone 6β-hydroxylase (CYP3A)	525.2±63.0	535.7±44.2	281.1±75.3 *
Nitrophenol 6-hydroxylase (CYP2E1)	304.7±31.0	308.3±38.4	208.9±28.2 *
Methoxyresorufin O-demethylase (CYP1A2)	34.5±0.3	35.9±5.3	24.5±2.9 *
Phase II enzymes (nmol/min/mg protein)			
UDP-glucurosyltransferase	34.5±0.3	35.9±5.3	24.5±2.9 *
Sulfotransferase	1.3±0.0	1.3±0.0	1.3±0.0
Glutathione S-transferase	209.1±10.0	178.1±9.4 *	158.2±6.4 *
Oxidative stress status			
GSH (nmol/mg protein)	46.2±0.9	43.8±3.2	42.9±1.7
GSSG (nmol/mg protein)	0.7±0.1	0.3±0.0 *	0.3±0.1 *
GSH/GSSG	69.1±9.6	163.8±27.6 *	139.6±38.1 *
GSH peroxidase (nmol/min/mg protein)	83.0±4.1	87.0±16.3	88.1±7.9
TBARS (nmol/g protein)	116.5±18.0	98.3±0.4	75.6±6.9 *
ROS (nmol/mg protein)	1.0±0.1	0.7±0.0 *	0.7±0.1 *
Liver function index			
Alanine aminotransferase (U/L)	22.4±3.2	23.7±4.0	21.0±4.4

[a]Results are expressed as the mean±S.D. of five rats in each group. *Significantly different from control, $p<0.05$. 1X EGCG: 018% EGCG in the diet; 3X EGCG: 0.54% EGCG in the diet.

Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862

Figure 1 shows the immunoblots of Mrp2/3 and P-glycoprotein in the liver of control and EGCG-treated rats. Mrp2 protein expression was mildly lower in rats fed the 3X EGCG diet than in the control group (p<0.05). EGCG had no significant effect on the expression of Mrp3 protein in rat liver (p>0.05). Rats fed the 1X EGCG diet caused a little increase in P-glycoprotein expression (p<0.05).

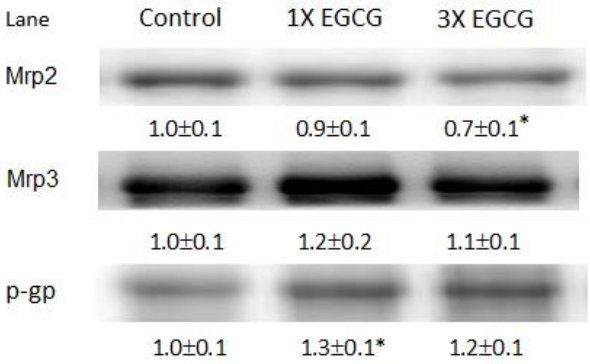


Figure 1. Effects of EGCG on membrane transporters in the liver of rats. Densitometry quantitation of membrane transporter protein (Mrp2/3 and P-glycoprotein, p-gp) levels from rats fed the EGCG diet for four weeks. Each lane represents the pooled liver membrane protein from 5 to 6 individual rats per group. The protein band was quantified by densitometry, and the level of the control was set at 1. *Significantly different from control group, p< 0.05.

On the other hand, in this study, EGCG supplementation reduced (p<0.05) fecal microbial β-glucuronidase activity (Control group: 79.6±32.1 nmol/min/mg protein; 3X EGCG group: 31.3±16.3 nmol/min/mg protein). There were no significant differences (p>0.05) in food intake, body weight, or liver weight between rats fed the EGCG-containing diet and animals fed the control diet.

In experiment II, the effect of EGCG on the metabolism and toxicity of APAP was evaluated. Rats were fed a normal diet or a normal diet containing EGCG (0.6%) for four weeks. Histological examination of H&E stained liver sections was conducted 12 h after APAP challenge to confirm the pattern of hepatotoxicity and compare the extent of liver damage between the control and the EGCG-fed animals (Figure 2A, 2B, 2C). Plasma ALT activity was significantly increased 12 h after APAP treatment compared with that in control animals (p<0.05) (Figure 2D). However, plasma ALT activity was significantly lower (p<0.05) in rats treated with EGCG after APAP treatment. Morphological findings were consistent with plasma transaminase observations. The APAP-induced histopathological changes in the liver came with significant degeneration and necrosis of hepatocytes in the centrilobular region and with perivenular inflammatory infiltrates (Figure 2B). These APAP-induced histopathological changes were significantly ameliorated by EGCG treatment (Figure 2C). These results indicate that the hepatotoxicity induced by APAP treatment in rats was improved by EGCG.

To investigate the effects of EGCG supplementation on oxidative stress in liver, the hepatic GSH, GSH peroxidase, and TBARS were determined. As shown in Figure 2E and 2F, the GSH level in liver was dramatically decreased (p<0.05) and GSH peroxidase activity was lower (p<0.05) in the APAP group than in the control group. EGCG had no significant effect on hepatic GSH content after APAP treatment (p>0.05). However, EGCG increased (p<0.05) GSH peroxidase activity after APAP treatment. There was no significant difference on hepatic TBARS content (nmol/g protein**)** among the groups (Control group: 179.9±58.9; APAP group: 205.6±75.9; APAP+EGCG group: 221.5±72.2). In this study, there were no significant differences (p>0.05) in food intake, liver weight, or body weight in the APAP group compared with the untreated control group.

Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862

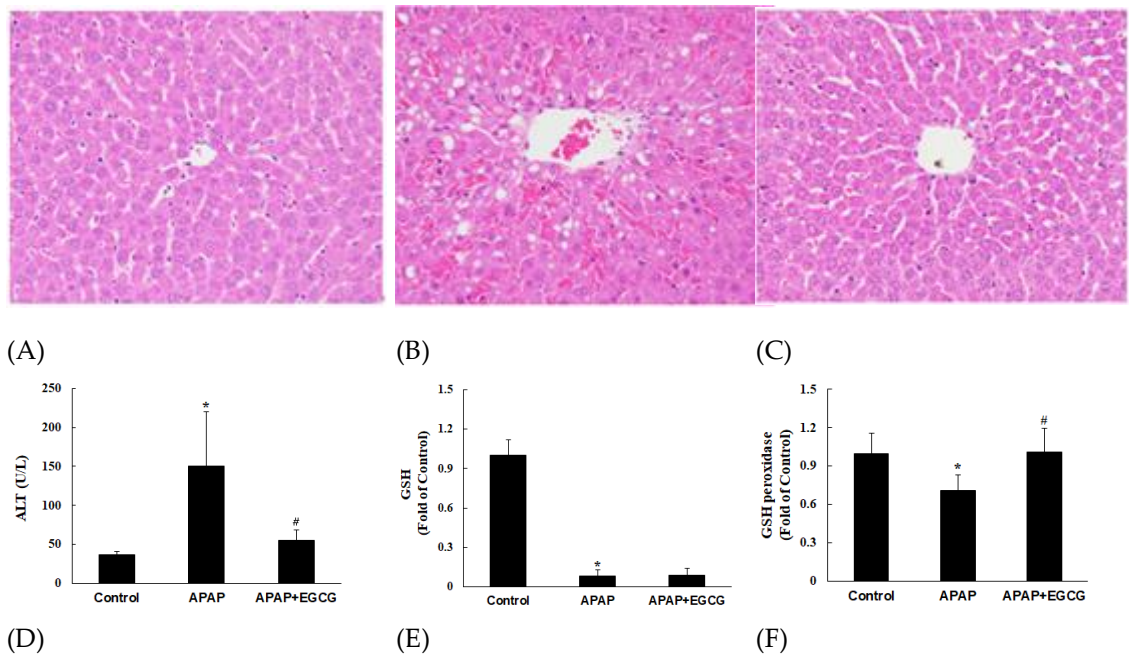
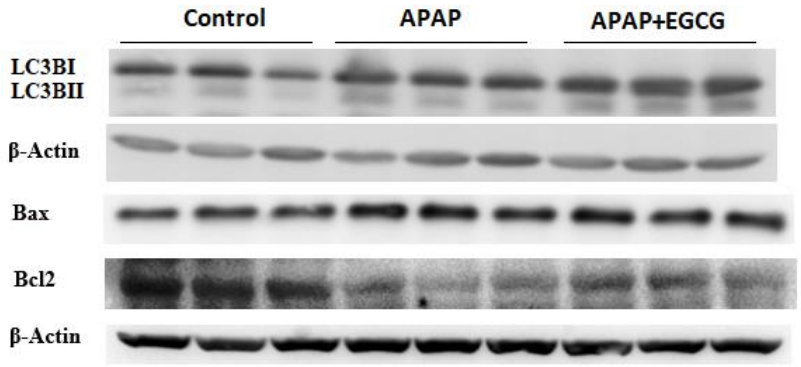


Figure 2. Effects of EGCG supplementation (0.6%) on APAP-induced hepatotoxicity in rats. Histopathological examination of livers was shown in (A) Control group, (B) APAP group, and (C) APAP+ EGCG group. H&E stain, 400x. Normal architecture of liver was found in the control group (A). Multifocal necrosis was graded as slight (2) in B (APAP group) and minimal (1) in C (APAP+ EGCG group). Plasma ALT activity and hepatic GSH and GSH peroxidase activity were shown in Figure 2D, 2E and 2F, respectively. *Significantly different from control group, $p< 0.05$. #Significantly different from APAP group, $p< 0.05$.

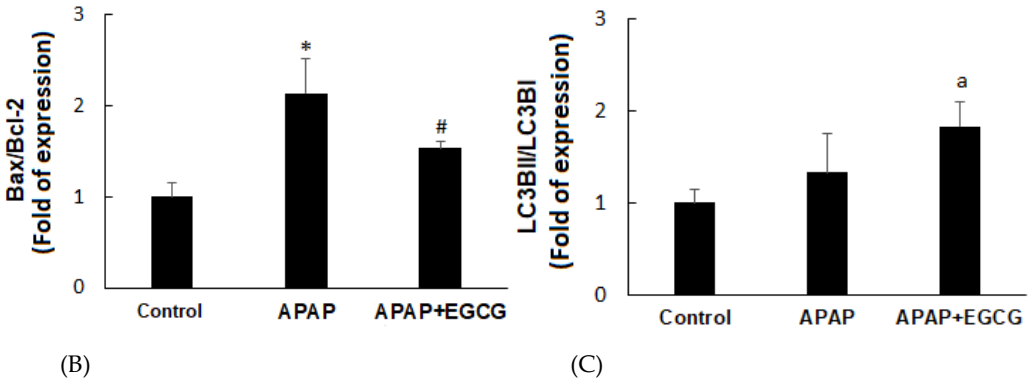
3.2. Apoptosis and autophagy in liver

Apoptosis and autophagy indices in liver were shown in Figure 3A, rats treated with APAP induced apoptosis by increasing Bax protein expression and decreasing Bcl2 protein expression compared with the vehicle-treated control animals ($p<0.05$). A mild increase ($p>0.05$) in LC3BI and LC3BII protein was found in APAP group. EGCG had no effect on Bax expression but significantly increased ($p<0.05$) Bcl2 expression after APAP treatment, resulting in a higher Bax/Bcl2 ratio ($p<0.05$) (Fig 3B). The hepatic LC3BII/LC3BI ratio was not affected ($p>0.05$) by APAP (Figure 3C). However, EGCG mildly increased hepatic LC3BII/LC3BI ratio ($p<0.1$) after APAP treatment.



(A)

Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862



(B) (C)

Figure 3. Effects of EGCG on APAP-induced apoptosis (Bax/Bcl2) and autophagy (LC3B II/ILC3B I) in rat liver. β-Actin served as the loading control. Values are given as the mean ± S.D. (n = 3). *Significantly different from control group, $p< 0.05$. #Significantly different from APAP group, $p< 0.05$. aSignificantly different from APAP group, $p< 0.1$.

3.3. APAP and APAP conjugates in plasma, liver, and urine

At 4 h after intraperitoneal injection of a single dose of APAP, the APAP-glucuronide concentration in plasma was reduced ($p<0.05$) by EGCG treatment (Table 2). APAP, APAP-sulfate, and APAP-GSH concentrations in plasma were not changed by EGCG treatment ($p>0.05$). The APAP-glucuronide content in liver tended to be lowered by EGCG treatment but this change did not reach statistical significance ($p>0.05$). However, APAP-protein adducts was significantly lowered ($p<0.05$) by EGCG treatment. APAP, APAP-sulfate, and APAP-GSH contents in liver were not changed ($p>0.05$) by EGCG treatment. These results suggested that, at first 4h after injection of APAP, a lower CYP-medicated APAP bioactivation could be diminished by EGCG treatment.

At 12 h after APAP injection, the contents of APAP-GSH and APAP-protein adducts in liver were significantly lowered by EGCG treatment ($p<0.05$). Plasma concentrations of APAP, APAP-sulfate, and APAP-glucuronide, however, did not differ significantly with respect to EGCG supplementation. Notably, the urinary excretion of APAP was slightly but significantly higher ($p<0.05$) in EGCG-supplemented rats (APAP group: 3.3 mg/12 h; APAP+EGCG group: 5.4 mg/12 h). However, this mild increase in APAP level in urine by EGCG may not explain the hepatoprotective effect of EGCG. No significant differences ($p>0.05$) in APAP-sulfate or APAP-glucuronide content in urine were noted. There was no significant difference ($p>0.05$) in urine volume after APAP challenge for 12 h (APAP group: 12.5±4.3 mL; APAP+EGCG group: 14.1±1.3 mL).

Table 2. APAP and its metabolites in plasma, liver, and urine after APAP challenge for 12 h

	4h		12h	
	APAP	APAP+ EGCG	APAP	APAP+ EGCG
Plasma				
APAP (μg/mL)	259.4±130.3	187.9±18.1	303.1±61.0	387.5±62.9
APAP-glucuronide (μg/mL)	101.2±5.2	82.2±6.9*	59.5±9.3	51.4±10.9
APAP-sulfate (μg/mL)	224.2±102.9	163.8±23.9	29.1±14.1	19.8±8.7
APAP-GSH (μg/mL)	12.1±9.4	11.9±6.1	32.5±10.9	11.0±9.7*
Liver				
APAP (μg/g liver)	115.7±33.1	128.9±55.6	77.3±31.9	95.9±33.0

Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862

APAP-glucuronide (μg/g liver)	618.1±211.7	450.2±89.1	432.5±181.3	520.3±157.8
APAP-sulfate (μg/g liver)	52.5±15.7	69.4±16.1	49.3±18.0	44.9±14.9
APAP-GSH (μg/g liver)	438.6±88.2	447.8±140.2	645.2±230.6	344.2±122.2*
APAP-protein adducts (mg/g liver)	1.8±0.4	1.1±0.2*	3.5±1.0	2.1±0.4*
Urine				
APAP (mg/12h)			3.3±0.7	5.4±0.7*
APAP-glucuronide (mg/12h)			144.5±61.5	155.7±14.2
APAP-sulfate (mg/12h)			209.2±39.1	189.6±13.5

[a]Results are expressed as the mean±S.D. of four (for 4h) or six (for 12h) rats in each group.
*Significantly different from APAP group, $p<0.05$.

The *in vitro* formation of APAP-GSH in liver microsomes of APAP-treated rats was evaluated. Similar to the results of a previous report [37], the results showed that APAP treatment for 12 h increased ($p<0.05$) the susceptibility of APAP-GSH formation in rat liver microsomes. However, in this study, EGCG supplementation for four weeks had no significant effect ($p>0.05$) on the *in vitro* APAP-GSH formation rate (pmol/min/mg protein) (Control group: 139.6±34.0; APAP group: 268.9±60.0; APAP+EGCG group: 229.2±35.4).

3.4. Drug-metabolizing enzyme activity in APAP-treated rats

The hepatic drug-metabolizing enzyme activity was reduced after APAP treatment (Table 3). Midazolam 1-hydroxylase (CYP3A), nitrophenol 6-hydroxylase (CYP2E1), UGT, NQO1, and GST activities in liver were lower ($p<0.05$) after APAP challenge. EGCG had no significant effects on these enzyme activities ($p>0.05$). No difference on methoxyresorufin *O*-demethylase (CYP1A2) activity was observed among the groups ($p>0.05$). Sulfotransferase activity was not affected by APAP, but EGCG supplementation reduced sulfotransferase activity after APAP treatment ($p<0.05$). Notably, EGCG increased NQO1 activity after APAP treatment ($p<0.05$).

Table 3. Drug-metabolizing enzyme activities in rat liver after APAP challenge for 12 h

	Control	APAP	APAP+ EGCG
Phase I enzymes (pmol/min/mg protein)			
Midazolam 1-hydroxylation (CYP3A)	216.1±60.3	124.3±26.9*	153.6±48.5
Nitrophenol 6-hydroxylase (CYP2E1)	517.6±55.1	388.4±74.8*	436.4±91.3
Methoxyresorufin O-demethylase (CYP1A2)	28.0±2.9	28.0±2.8	26.0±1.9
Phase II enzymes (nmol/min/mg protein)			
UDP-glucurosyltransferase	48.0±6.8	28.9±11.3*	25.9±3.6
Sulfotransferase	0.73±0.16	0.62±0.1	0.44±0.13#
Glutathione S-transferase	146.5±43.5	103.9±15.4*	130.1±30.1
NADPH : quinine oxidoreductase-1	462.4±132.8	279.5±91.3*	383.5±82.4#

Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862

[a]Results are expressed as the mean±S.D. of six rats in each group. *Significantly different from control group; [#] significantly different from APAP group, $p<0.05$.

3.5. Membrane transporters expressions

Immunoblots of liver membrane transporters are shown in Figure 4. Mrp2 and Mrp3 expressions were was mildly increased and OATP 1a1 was mildly decreased by 12 h of APAP treatment ($p>0.05$). EGCG had no significant effect on the protein expressions of p-gp, Mrp2, or Mrp3 ($p>0.05$). However, EGCG significantly reduced ($p<0.05$) OATP 1a1 expression in liver.

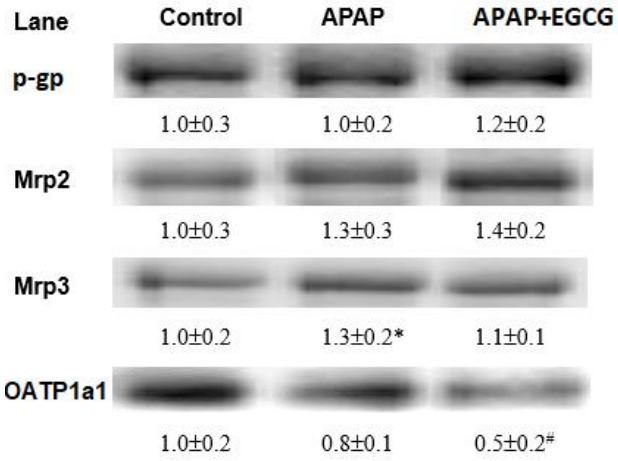


Figure 4. Densitometry quantitation of Mrp2/3, p-gp, and OATP1a1 protein expression from rats fed an EGCG diet for four weeks and then treated with APAP for 12 h. Each lane represents the pooled (n=5-6) liver membrane protein from individual rat. The protein band was quantified by densitometry, and the level of the control was set at 1. *Significantly different from control group, $p< 0.05$. #Significantly different from APAP group, $p< 0.05$.

Discussion

In the present study, the results showed that EGCG supplementation for four weeks significantly reduced oxidative stress and the activities of several drug-metabolizing enzymes in rat liver. After challenge with APAP, EGCG reduced CYP-mediated APAP bioactivation and apoptosis and mildly increased autophagy in liver. In addition, EGCG increased the activities of antioxidant enzymes, including GSH peroxidase and NQO-1, and decreased the expression of the uptake membrane transporter, OATP 1a1, after APAP treatment. These results indicate that dietary EGCG may reduce APAP-induced hepatotoxicity by lowering CYP-mediated APAP bioactivation, increasing antioxidant enzyme activity, and reducing the accumulation of toxic products in the liver.

EGCG shows both antioxidant and pro-oxidant effects in biological systems. EGCG acts as a pro-oxidant compound when it undergoes metabolic processes that produce ROS [9]. At high doses, the oxidized form of EGCG is EGCG o-quinone, which reacts with glutathione to form thiol conjugates, resulting in the accumulation of EGCG o-quinone in hepatocytes and causing liver damage [40]. In our preliminary study, EGCG supplementation in the diet (0.54%, w/w) caused a transient increase in ROS level in liver during the first week of EGCG treatment, but the production of ROS may act as a signal to upregulate GSH synthesis and GSH peroxidase activity in liver. Therefore, EGCG did not cause any hepatotoxicity (Supplementary data 1). In this study, hepatic ROS, GSSG, and TBARS contents were significantly decreased after four weeks of treatment with the same dose of EGCG, indicating that the oxidative stress in liver was diminished. This phenomenon could be partly explained that rats had adapted to the high-dose of EGCG (0.6% in the diet) and maintained high antioxidant capacity in liver after four weeks of EGCG treatment. The other possibility is that EGCG supplementation in the diet may have lowered the intestinal absorption rate of EGCG compared with

Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862

EGCG administered to the animal by intragastric [10] or intraperitoneal injection [11], resulting in a lower concentration of plasma EGCG. Therefore, in this study, EGCG administration for four weeks reduced oxidative stress in liver and caused no hepatotoxicity.

After 12 hours of administration of a single dose of APAP to rats, APAP caused liver damage, which was characterized by an increase in the plasma ALT concentration, a change in hepatocyte morphology, a dramatic decrease in GSH content, and a decrease in liver GSH peroxidase activity (Figure 2). In addition, APAP reduced the Bax/Bcl2 ratio with a little or no change in the LC3B-II/LC3B-I ratio in liver, indicating that APAP induced hepatocyte apoptosis without affecting autophagy. Notably, treatment with EGCG caused a lower Bax/Bcl2 ratio and a higher LC3B II/LC3B I ratio, indicating that EGCG could reduce apoptosis and induce autophagy (Figure 3B, 3C). Activation of autophagy by EGCG has been demonstrated to protect against APAP-induced hepatotoxicity [41]. These results suggest that inhibition of apoptosis and induction of autophagy after EGCG challenge may protect the liver against APAP-induced hepatotoxicity.

In this study, the increased GSH peroxidase activity by EGCG may have reduced ROS production during CYP-mediated APAP metabolism. EGCG has been shown to be proficient at scavenging free radicals [5]. Therefore, reduced oxidative stress by EGCG after APAP challenge may be partially attributed to its direct and/or indirect increase in antioxidant activity or decrease in ROS production in liver, even though the oral bioavailability of EGCG is low (< 1%) [42].

Regarding the drug-metabolizing enzyme activity, a previous study showed that CYP3A, sulfotransferase, and GST enzyme activities in liver were significantly reduced after one week of EGCG feeding (0.54%, w/w) [22]. In this study, hepatic CYP3A, CYP2E1, CYP1A2, UGT and GST activities were suppressed after four weeks of EGCG feeding. This observation is consistent with previous results showing that orally administered EGCG reduces the activities of hepatic drug-metabolizing enzymes [6,22,43]. These results suggest that dietary EGCG may lower the metabolism of drugs or toxic compounds in the liver.

Consistent with previous findings, in this study, APAP treatment for 12 h reduced the activities of drug-metabolizing enzymes and antioxidant enzymes. It is known that APAP-induced liver toxicity is mediated by covalent binding to critical proteins or enzymes with NAPQI [44]. A toxic dose of APAP to animals decreases the catalytic activity of the hepatic enzymes, including CYP enzymes, UGT, GST, and glutathione peroxidase, probably due to covalent binding to these cellular proteins [22,45]. In addition to CYP2E1 and CYP1A2, CYP3A is an important enzyme responsible for the CYP-mediated bioactivation of APAP to generate the electrophile NAPQI in both human and rats, especially when an overdose of APAP is administered [35, 46-48]. In this study, EGCG administration caused lower hepatic CYP3A, CYP2E1, and CYP1A2 activities prior to APAP injection (Table 1). These changes by EGCG may result in a lower CYP-mediated NAPQI production after APAP treatment. Indeed, a lower formation of APAP-GSH and APAP-protein adducts was observed in liver (Table 3). In addition to APAP-protein adducts, APAP- GSH is toxic to the liver because the conjugate can induce mitochondrial impairment, leading to enhanced ROS production [49]. In this study, EGCG increased NQO1 activity after APAP treatment, which may enhance the conversion of NAPQI back to the parent APAP. Therefore, EGCG suppressed CYP enzyme activities prior to APAP injection and had higher NQO1 activity after APAP challenge would result in lower NAPQI production, which might lead to lower formations of APAP-protein adducts and APAP-GSH in liver (Table 3). Therefore, the effect of EGCG to lower APAP-induced hepatotoxicity is likely due to its ability to reduce CYP enzyme activity and enhance NQO1 activity and, thus, lower CYP-mediated APAP bioactivation.

Several studies indicate that the expression of efflux membrane transporters such as Mrp2/3 and p-glycoprotein is increased after APAP intoxication [18,19,50]. In general, APAP-glucuronide and APAP-GSH are mainly excreted into bile, while APAP-sulfate is mainly excreted into urine [51]. These membrane proteins can remove toxic metabolites and oxidative products from liver to urine or bile. Uptake transporters of OATPs in liver, such as Oatp1a1 and Oatp1b2, that mediate the uptake of

Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862

numerous drugs and xenobiotics into cells, were reduced after APAP treatment [18]. Roth et al [52] showed that the expression of Oatp1a1 is inhibited by EGCG. In this study, the protein expression of Mrp2 and Mrp3 was slightly increased and that of OATP1a1 was decreased after 12 h of APAP treatment. EGCG had no effect on Mrp2/3 and p-glycoprotein protein expressions in liver; however, EGCG significantly reduced (-37.5%) OATP1a1 expression. These results suggested that EGCG may reduce hepatic uptake of APAP and its metabolites from circulation into liver. On the other hand, in this study, EGCG supplementation also lowered fecal β-glucuronidase activity, which might diminish de-conjugation of APAP-glucuronide and thus increased fecal APAP-glucuronide excretion. This observation is similar to the result of a previous study [53]. Therefore, reduced hepatic OATP1a1 protein expression and microbial β-glucuronidase activity by EGCG may possibly lead to lower reabsorption of APAP into liver from circulation and intestine, respectively. These actions may lower repeated CYP-mediated APAP bioactivation in liver.

In summary, the results of this study show that EGCG supplementation for four weeks reduced APAP-induced liver damage in rats. The mechanisms contributing to the detoxification of APAP by EGCG may include reduced CYP-mediated APAP bioactivation, oxidative stress, and apoptosis; increased autophagy and lowered accumulation of toxic products in liver.

Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862

Author Contributions: Conceptualization, H-T.Y.; methodology, H-T.Y.; software, C-H.C.; validation, H.T.Y.; formal analysis, C-H.C. and C-C.L.; investigation, C-H.C. and C-C.L.; resources, H-T.Y.; data curation, H-T.Y.; writing-original draft preparation, H-T.Y. and C-C.L.; writing-review and editing, H-T.Y.; visualization, C-H.C.; supervision, H-T.Y.; project administration, H-T.Y.; funding acquisition, H-T.Y. All authors approved the final submitted version.

Funding: This research was financially supported by grant-aid (NSC 102-2313-B-039-007) of the Ministry of Science and Technology, Taiwan.

Conflicts of Interest: The authors have no conflicts of interest to report.

Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862

References

1. Zhang, L.; Wei, Y.; Zhang, J. Novel mechanisms of anticancer activities of green tea component epigallocatechin-3-gallate. Anticancer Agents Med Chem 2014, 14, 779-786. [CrossRef] [PubMed]
2. Chowdhury, A.; Sarkar, J.; Chakraborti, T.; Pramanik, P. K.; Chakraborti, S. Protective role of epigallocatechin-3-gallate in health and disease: A perspective. Biomed Pharmacother 2016, 78, 50-59. [CrossRef] [PubMed]
3. Tipoe, G.L.; Leung, T. M.; Hung, M.W.; Fung, M. L. Green tea polyphenols as an anti-oxidant and anti-inflammatory agent for cardiovascular protection. Cardiovasc Hematol Disord Drug Targets 2007, 7, 135-144. [CrossRef] [PubMed]
4. Muto, S.; Fujita, K.; Yamazaki, Y.; Kamataki, T. Inhibition by green tea catechins of metabolic activation of procarcinogens by human cytochrome P450. Mutat Res 2001, 479, 197-206. [CrossRef] [PubMed]
5. Azman, N.A.; Peiró, S.; Fajarí, L.; Julià, M.P. Radical scavenging of white tea and its flavonoid constituents by electron paramagnetic resonance (EPR) spectroscopy. J Agric Food Chem 2014, 62, 5743-5748. [CrossRef] [PubMed]
6. Yang, C.S.; Pan, E. The effects of green tea polyphenols on drug metabolism. Expert Opin Drug Metab Toxicol 2012, 8, 677-689. [CrossRef] [PubMed]
7. Arffa, M.L.; Zapf, M.A.; Kothari, A.N.; Chang, V.; Gupta, G.N.; Ding, X.; Al-Gayyar, M.M.; Syn, W.; Elsherbiny, N.M.; Kuo, P.C.; Mi, Z. Epigallocatechin-3-gallate upregulates miR-221 to inhibit osteopontin-dependent hepatic fibrosis. PLoS One 2016, 11, e0167435. [CrossRef] [PubMed]
8. Yu, S.J.; Jiang, R.; Mazzu, Y.Z.; Wei, C.B.; Sun, Z.L.; Zhang, Y.Z.; Zhou, L.D.; Zhang, Q.H. Epigallocatechin-3-gallate prevents triptolide-induced hepatic injury by restoring the Th17/T_{reg} balance in mice. Am J Chin Med 2016, 44, 1221-1236. [CrossRef] [PubMed]
9. Lambert, J.D.; Elias, R.J. The antioxidant and pro-oxidant activities of green tea polyphenols: a role in cancer prevention. Arch Biochem Biophys 2010, 501, 65-72. [CrossRef] [PubMed]
10. James, K.D.; Kennett, M.J.; Lambert, J.D. Potential role of the mitochondria as a target for the hepatotoxic effects of (-)-epigallocatechin-3-gallate in mice. Food Chem Toxicol 2018, 111, 302-309. [CrossRef] [PubMed]
11. Wang, D.; Wang Y.; Wan X.; Yang, C.S.; Zhang, J. Green tea polyphenol (-)-epigallocatechin-3-gallate triggered hepatotoxicity in mice: responses of major antioxidant enzymes and the Nrf2 rescue pathway. Toxicol Appl Pharmacol 2015, 283, 65-74. [CrossRef] [PubMed]
12. Ostapowicz, G.; Fontana, R.J.; Schiødt, F.; Larson, V. A.; Davern, T.J.; Han, S.H.; McCashland, T.M.; Shakil, A.O.; Hay, J.E.; Hynan, L.; Crippin, J.S.; Blei, A.T.; Samuel, G.; Reisch, J.; Lee, W.M. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med 2002, 137, 947-954. [CrossRef] [PubMed]
13. Thomas, S.H.L. Paracetamol (acetaminophen) poisoning. Pharmacol Ther 1993, 17260, 91-120. [CrossRef] [PubMed]
14. James, L.P.; Mayeux, P.R.; Hinson, J.A. Acetaminophen-induced hepatotoxicity. Drug Metab Dispos 2003, 31, 1499-1506. [CrossRef] [PubMed]
15. Mossanen, J.C.; Tacke, F. Acetaminophen-induced acute liver injury in mice. Lab Anim 2015, 49, 30-36. [CrossRef] [PubMed]
16. Cao, P.; Sun, J.; Sullivan, M.A.; Huang, X.; Wang, H.; Zhang, Y.; Wang, N.; Wang, K. Angelica sinensis polysaccharide protects against acetaminophen-induced acute liver injury and cell death by suppressing oxidative stress and hepatic apoptosis in vivo and in vitro. Int J Biol Macromol 2018, 111, 1133-1139. [CrossRef] [PubMed]
17. Ni, H.M.; Bockus, A.; Boggess, N.; Jaeschke, H.; Ding, W.X. Activation of autophagy protects against acetaminophen-induced hepatotoxicity. Hepatology 2012, 55, 222-232. [CrossRef] [PubMed]

Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862

18. Keppler, D.; Konig, J. Hepatic secretion of conjugated drugs and endogenous substances. Semin Liver Dis 2000, 20, 265-272. [CrossRef] [PubMed]
19. Slitt, A.L.; Cherrington, N.J.; Maher, J.M.; Klaassen, C.D. Induction of multidrug resistance protein 3 in rat liver is associated with altered vectorial excretion of acetaminophen metabolites. Drug Metab Dispos 2003, 31, 1176-1186. [CrossRef] [PubMed]
20. Aleksunes, L.M.; Augustine, L.M.; Cherrington, N.J.; Manautou, J.E. Influence of acetaminophen vehicle on regulation of transporter gene expression during hepatotoxicity. J Toxicol Environ Health A 2007, 70, 1870-1872. [CrossRef] [PubMed]
21. Oz, H.S.; Chen, T.S. Green-tea polyphenols downregulate cyclooxygenase and Bcl-2 activity in acetaminophen-induced hepatotoxicity. Dig Dis Sci 2008, 53, 2980-2988. [CrossRef] [PubMed]
22. Yao, H.T.; Yang, Y.C.; Chang, C.H.; Yang, H.T.; Yin, M.C. Protective effects of (-)-epigallocatechin-3-gallate against acetaminophen-induced liver injury in rats. Biomedicine 2015, 5, 15-21. [CrossRef]
23. Liu, T.T.; Liang, N.S.; Li, Y.; Yang, F.; Lu, Y.; Meng, Z.Q.; Zhang, L.S. Effects of long-term tea polyphenols consumption on hepatic microsomal drug metabolizing enzymes and liver function in Wistar rats. World J Gastroenterol 2003, 9, 2742-2744. [CrossRef] [PubMed]
24. Carleton, H.M. Carleton's Histological Techniques, 5th ed.; Oxford University Press, London, UK, 1980. [CrossRef]
25. National Research Council. Guide for the Care and Use of Laboratory Animals, 8th ed.; National Academies Press: Washington, DC, USA, 2011. [CrossRef] [PubMed]
26. Yao, H.T.; Hsu, Y.R.; Lii, C.K.; Lin, A.H.; Chang, K.H.; Yang, H.T. Effect of commercially available green and black tea beverages on drug-metabolizing enzymes and oxidative stress in Wistar rats. Food Chem Toxicol 2014, 70, 120-127. [CrossRef] [PubMed]
27. Hanioka, N.; Jinno, H.; Tanaka-Kagawa, T.; Nishimura, T.; Ando, M. Determination of UDP-glucuronosyltransferase UGT1A6 activity in human and rat liver microsomes by HPLC with UV detection. J Pharm Biomed Anal 2001, 25, 65-75. [CrossRef] [PubMed]
28. Dierickx, C.; Vanhoof, H. Massive rotator cuff tears treated by a deltoid muscular inlay flap. Acta Orthopaedica Belgica 1994, 60, 94-100. [CrossRef] [PubMed]
29. Habig, W.H.; Jakoby, W.B. Assays for differentiation of glutathione S-transferases. Methods Enzymol 1981, 77, 398-405. [CrossRef] [PubMed]
30. Tsvetkov, P.; Asher, G.; Reiss, V.; Shaul, Y.; Sachs, L.; Lotem, J. Inhibition of NAD(P)H:quinone oxidoreductase 1 activity and induction of p53 degradation by the natural phenolic compound curcumin. PNAS 2005, 15, 5535-5540. [CrossRef] [PubMed]
31. Guan, X.; Hoffman, B.; Dwivedi, C.; Matthees, D.P. A simultaneous liquid chromatography/mass spectrometric assay of glutathione, cysteine, homocysteine and their disulfides in biological samples. J Pharm Biomed Anal 2003, 31, 251-261. [CrossRef] [PubMed]
32. Mohandas, J.; Marshall, J.J.; Duggin, G.G.; Horvath, J.S.; Tiller, D.J. Low activities of glutathione-related enzymes as factors in the genesis of urinary bladder cancer. Cancer Res 1984, 44, 508-5091. [CrossRef] [PubMed]
33. Uehiyama, M.; Mihara, M. Determination of malonaldehyde precursor in tissue by thiobarbituric acid test. Anal Biochem 1978 86, 271-278. [CrossRef] [PubMed]
34. Ali, S.F.; LeBel, C.P.; Bondy, S.C. Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity. Neurotoxicology 1992, 13, 637-648. [CrossRef] [PubMed]
35. Yao, H.T.; Luo, M.N.; Lii, C.C. Soy isoflavones reduce acetaminophen-induced liver injury by inhibiting cytochrome P-450-mediated bioactivation and glutathione depletion and increasing urinary drug excretion in rats. J Funct Foods 2016, 26, 135-143. [CrossRef]
36. Acharya, M.; Lau Cam, C.A. Comparison of the protective actions of N-acetylcysteine, hypotaurine and taurine against acetaminophen-induced hepatotoxicity in the rat. J Biomed Sci 2010, 17, S35-46. [CrossRef] [PubMed]

Peer-reviewed version available at Nutrients 2019, 11, 1862; doi:10.3390/nu11081862

37. Allameh, A.; Alikhani, N. Acetaminophen-glutathione conjugate formation in a coupled cytochrome P-450-glutathione S-transferase assay system mediated by subcellular preparations from adult and weanling rat tissues. Toxicol In Vitro 2002, 16, 637-641. [CrossRef] [PubMed]
38. Yen, C.C.; Liu, Y.T.; Lin, Y.J.; Yang, Y.C.; Chen, C.C.; Yao, H.T.; Chen, H.W.; Lii, C.K. Bioavailability of the diterpenoid 14-deoxy-11,12-didehydroandrographolide in rats and up-regulation of hepatic drug-metabolizing enzyme and drug transporter expression. Phytomedicine 2019, 61, 152841. [CrossRef] [PubMed]
39. Yao, H.T.; Chiang, M.T. Chitosan shifts the fermentation site toward the distal colon and increases the fecal short-chain fatty acids concentrations in rats. Int J Vitam Nutr Res 2006, 76, 57-64. [CrossRef] [PubMed]
40. Sang, S.; Lambert, J.D.; Hong, J.; Tian, S.; Lee, M.J.; Stark, R.E.; Ho, C.T.; Yang, C.S. Synthesis and structure identification of thiol conjugates of (-)-epigallocatechin gallate and their urinary levels in mice. Chem Res Toxicol 2005, 18, 1762-1769. [CrossRef] [PubMed]
41. Zhou, J.; Farah, B.L.; Sinha, R.A.; Wu, Y.; Singh, B.K.; Bay, B.H.; Yang, C.S.; Yen, P.M. Epigallocatechin-3-gallate (EGCG), a green tea polyphenol, stimulates hepatic autophagy and lipid clearance. PLoS One 2014, 9, e87161. [CrossRef] [PubMed]
42. Xu, J.Z.; Yeung, S.Y.; Chang, Q.; Huang, Y.; Chen, Z.Y. Comparison of antioxidant activity and bioavailability of tea epicatechins with their epimers. Br J Nutr 2004, 91, 873-881. [CrossRef] [PubMed]
43. Choi, J.S.; Burm, J.P. Effects of oral epigallocatechin gallate on the pharmacokinetics of nicardipine in rats. Arch Pharm Res 2009, 32, 1721-1725. [CrossRef] [PubMed]
44. Cohen, S.D.; Pumford, N.R.; Khairallah, E.A.; Boekelheide, K.; Pohl, L.R.; Amouzadeh, H.R.; Hinson, J.A. Selective protein covalent binding and target organ toxicity. Toxicol Appl Pharmacol, 1997, 143, 1-12. [CrossRef] [PubMed]
45. Qiu, Y.; Benet, L.Z.; Burlingame, A.L. Identification of the hepatic protein targets of reactive metabolites of acetaminophen in vivo in mice using two-dimensional gel electrophoresis and mass spectrometry. J Biol Chem 1998, 273, 17940-17953. [CrossRef] [PubMed]
46. Yamaura, K.; Shimada, M.; Nakayama, N.; Ueno, K. Protective effects of goldenseal (Hydrastis canadensis L.) on acetaminophen-induced hepatotoxicity through inhibition of CYP2E1 in rats. Pharmacognosy Res 2011, 3, 250-255. [CrossRef] [PubMed]
47. Li, J.; Kaneko, T.; Wang, Y.; Qin, L.Q.; Wang P.Y.; Sato, A. Troglitazone enhances the hepatotoxicity of acetaminophen by inducing CYP3A in rats. Toxicology 2002, 176, 91-100. [CrossRef] [PubMed]
48. Laine, J.E.; Auriola, S.; Pasanen, M.; Juvonen, R.O. Acetaminophen bioactivation by human cytochrome P450 enzymes and animal microsomes. Xenobiotica 2009, 39, 11-21. [CrossRef] [PubMed]
49. Roušar, T.; Nýdlová, E.; Česla, P.; Staňková, P.; Kučera, O.; Pařík, P.; Červinková, Z. Purified acetaminophen-glutathione conjugate is able to induce oxidative stress in rat liver mitochondria. Physiol Res 2012, 61, S103-S109. [CrossRef] [PubMed]
50. Manov, I.; Bashenko, Y.; Hirsh, M.; Iancu, T.C. Involvement of the multidrug resistance P-glycoprotein in acetaminophen-induced toxicity in hepatoma-derived HepG2 and Hep3B cells. Basic Clin Pharmacol Toxicol 2006, 99, 213-224. [CrossRef] [PubMed]
51. Gregus, Z.; Madhu, C.; Klaassen, C.D. Species variation in toxification and detoxification of acetaminophen in vivo: a comparative study of biliary and urinary excretion of acetaminophen metabolites. J Pharmacol Exp Ther 1988, 244, 91-99. [CrossRef] [PubMed]
52. Roth, M.; Timmermann, B.N.; Hagenbuch, B. Interactions of green tea catechins with organic anion-transporting polypeptides. Drug Metab Dispos 2011, 39, 920-926. [CrossRef] [PubMed]
53. Frejnagel, S.; Juskiewicz, J. Dose-dependent effects of polyphenolic extracts from green tea, blue-berried honeysuckle, and chokeberry on rat caecal fermentation processes. Planta Med 2011, 77, 888-893. [CrossRef] [PubMed]