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Low doses of curcumin protect alcohol-induced liver damage by modulation of the alcohol metabolic pathway, CYP2E1 and AMPK

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ABSTRACT

Aims: This study investigated the hepatoprotective effects of low doses of curcumin against liver damage induced by chronic alcohol intake and a high-fat diet. We also examined several potential underlying mechanisms including action on alcohol metabolism, antioxidant activity, AMPK level and lipid metabolism.
Main method: Alcohol (25% v/v, 5 g/kg body weight) was orally administered once a day for 6 weeks to mice fed a high-fat diet with or without two different doses of curcumin (0.02% and 0.05%, wt/wt).
Key findings: Curcumin significantly decreased the plasma aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and alkaline phosphatase activities ($p < 0.05$) and prevented hepatic steatosis compared with the alcohol control group. Curcumin significantly reversed the alcohol-induced inhibition of the alcohol dehydrogenase, aldehyde dehydrogenase 2 and antioxidant enzyme activities as well as the activation of cytochrome P4502E1 and promotion of lipid peroxidation ($p < 0.05$). Curcumin significantly increased the hepatic total AMPK protein level and concomitantly suppressed the fatty acid synthase and phosphatidate phosphohydrolase activities compared with the alcohol control group ($p < 0.05$). Furthermore, curcumin significantly lowered the plasma leptin, free fatty acids and triglycerides levels and hepatic lipid levels ($p < 0.05$).
Significance: These findings indicate that low doses of curcumin may protect against liver damage caused by chronic alcohol intake and a high-fat diet partly by modulating the alcohol metabolic enzyme activity, the antioxidant activity and the lipid metabolism. Therefore, curcumin may provide a promising natural therapeutic strategy against liver disease.

Introduction

Alcoholic liver disease (ALD) is caused by prolonged high alcohol intake and contributes significantly to the prevalence of liver disease worldwide (Lieber, 2004). ALD is primarily driven by alcohol metabolism byproducts that promote the development of steatosis, which can progress to steatohepatitis, fibrosis, cirrhosis, liver failure and/or hepatocellular carcinoma (Seth et al., 2011). Non-alcoholic fatty liver disease (NAFLD) shares overlapping pathophysiology with ALD, although the initial development of steatosis is primarily due to excessive dietary fat intake (Preiss and Sattar, 2008). Hence, both excessive daily alcohol intake and high dietary fat intake are major risk factors for the development of liver disease.

The excess accumulation of the metabolic end-products of the alcohol metabolism can cause oxidative stress, lipid peroxidation and inflammation and promote fat accumulation through the inhibition of the cellular energy regulator AMP-activated protein kinase (AMPK) (Chen et al., 2010). Oxidative stress and inflammation are major secondary factors that further promote and exacerbate liver damage (Seth et al., 2011; Preiss and Sattar, 2008). At present, there is no effective therapeutics to protect against chronic alcohol and high-fat induced liver damage.

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is the main active component of the Asian spice turmeric (*Curcuma longa*), which is a member of the ginger family (Zingiberaceae). Curcumin is a nutriceutical with wide ranging potential therapeutic actions, including antioxidant, anti-inflammatory, anti-infectious, anti-fibrotic and anticancer activities in cells and animal disease models (Epstein et al., 2010; Aggarwal, 2010). Diet-induced obesity is widely reported to be suppressed by curcumin (Kim and Kim, 2010; Jang et al., 2008; Shao et al., 2012; El-Moselhy et al., 2011; Weisberg et al., 2008; Ejaz et al., 2009). There is also evidence that curcumin treatment may

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help protect against liver injury caused by various factors, including thioacetamide, iron overdose, cholestasis, carbon tetrachloride and ethanol (Rivera-Espinoza and Muriel, 2009). A recent study showed the chow-fed mice that were administered high amounts of ethanol (4 g/kg/day) were protected against oxidative stress by curcumin treatment (Rong et al., 2012). There is also evidence that curcumin reduces inflammation through the inhibition of NF-kB in ethanol-treated rats (Nanji et al., 2003). However, both oxidative stress and inflammation are secondary effects that occur in response to chronic alcohol intoxication due to the accumulation of metabolic byproducts from the alcohol metabolism. To date, the effect of curcumin supplementation on the alcohol metabolism enzyme activity in animals that are intoxicated daily is unknown (Rivera-Espinoza and Muriel, 2009). Furthermore, previous studies have typically used high doses of curcumin, which translate into human doses (>8 g/day). Curcumin is known to trigger apoptosis in cancer cells at high concentrations (Epstein et al., 2010) and may even promote or exacerbate liver damage at high doses in mice (Zhao et al., 2012). However, it is unclear whether lower curcumin doses are sufficient to promote hepatoprotective effects against excessive daily alcohol intake.

In the present study, we assessed whether curcumin supplementation at two different doses can protect against liver damage caused by excessive daily alcohol intake and a high-fat diet.

Materials and methods

Animals and diets

Four-week-old male ICR mice were purchased from Orient Inc. (Seoul, Republic of Korea). The mice were individually housed in polycarbonate cages at 22 ± 2 °C on a 12-h light–dark cycle. All of the mice were fed pelleted commercial chow for 1 week after arrival and then fed a semi-purified high-fat diet based on the AIN-76 diet containing 35% calories from fat (3% corn oil and 14.5% lard, w/w) for the duration of the experiments (American Institute of Nutrition, 1977). The mice were randomly divided into four groups (n = 8). Three groups were orally administered ethanol (25% v/v, 5 g/kg body weight) once a day for 6 weeks to induce a prolonged daily elevation of the blood alcohol levels similarly to that observed in overweight human that regularly binge drink. The normal mice received an equivalent amount of distilled water. Our previous studies found that 0.05% (wt/wt) curcumin exerts a potential hypolipidemic effect in hamster fed in a high-fat diet and that a low dose (0.02%, wt/wt) of curcumin significantly improves hyperglycemia in type 2 diabetic mice. To determine whether curcumin protects against alcohol-induced damage, the mice were supplemented two doses of curcumin (Sigma, St. Louis, MO, USA), which was mixed in the diet at 0.02% or 0.05% wt/wt. These doses of curcumin were equivalent to 19.7 mg/kg/day and 47.5 mg/kg/day, respectively, in each mouse based on their average food intake. Clinical trials of curcumin are currently underway in patients with predominant cancer pathologies using a range of doses from 1 to 8 g/day (Hatcher et al., 2008). We used two relatively low doses of curcumin equivalent to a dose of 112 mg/day or 270 mg/day for a 70 kg human using an allometric scaling factor of 0.081 (Reagan-Shaw et al., 2008); these doses are easily achievable by dietary changes or supplementation. The mice had free access to food and water. Their food consumption was measured daily and weight gain was measured weekly. The normal mice were fed the same amount of food as the alcohol control mice ate the previous day to exclude any effects of reduced food intake due to alcohol. At the end of the experimental period, the mice were fasted for 12 h and then anesthetized with ether. Blood samples were collected from the inferior vena cava for plasma biomarker analysis. The liver and adipose tissue were removed, rinsed with a physiological saline solution and immediately stored at −70 °C. All of the mice were treated in strict accordance with the guidelines for the care and use of laboratory animals of Sunchon National University of the Republic of Korea.

Liver damage biomarkers and histological analysis

To assess the liver damage, the plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities were measured using an automated chemistry analyzer (Fuji Dri-Chem 3500; Fujifilm, Tokyo, Japan). For histological analysis, the liver tissue was fixed in a buffer solution containing 10% formalin. The fixed tissues were paraffin-embedded, and 4-μm sections were prepared and stained with hematoxylin and eosin. The stained areas were viewed using an optical microscope (Olympus, Japan) at 200 × magnification.

Plasma leptin and lipid profile

The plasma leptin levels were determined using a quantitative sandwich enzyme immunoassay kit (R&D systems, MN, USA). The plasma concentrations of total cholesterol, HDL-cholesterol and triglyceride (Asan Diagnostics, Seoul, Korea) were determined using an enzymatic method. The hepatic lipid was extracted as previously described (Do et al., 2011), and the cholesterol and triglyceride concentrations were analyzed using the enzymatic kit that was used in the plasma analysis.

Alcohol metabolism enzyme activities

The alcohol dehydrogenase (ADH) and aldehyde dehydrogenase 2 (ALDH2) activities in the liver were assayed as previously described (Seo et al., 2003). As a measure of ADH activity, the conversion of NAD^+ to NADH was determined by recording the changes in absorbance at 340 nm for 5 min after the initiation of the enzyme reaction. The hepatic microsomal CYP2E1 activity was determined by measuring the formation of 4-nitrocatechol (Choi et al., 2009).

Hepatic lipid metabolism enzyme activities

The fatty acid synthase (FAS) activity was determined with a spectrophotometric assay based on measuring the malonyl-CoA-dependent oxidation of NADPH; one unit of enzyme activity represented the oxidation of 1 nmol of NADPH per minute at 37 °C (Seo et al., 2008). The phosphatidate phosphohydrolase (PAP) activity was determined spectrophotometrically as described previously and the results were expressed as nmol/min/mg protein (Do et al., 2011). The glucose-6-phosphate dehydrogenase (G6PD) activity was determined as described previously; the reaction mixture contained 55 mM Tris–HCl buffer (pH 7.8), 3.3 mM $MgCl_2$, 240 μM $NADP^+$, 4 mM glucose-6-phosphate and the cytosolic enzyme (Do et al., 2011). The activity was determined based on the reduction of 1 mol NADP per min, which was measured using a spectrophotometer at 340 nm.

Antioxidant enzyme activities and lipid peroxidation

The superoxide dismutase (SOD) activity was measured spectrophotometrically based on the inhibition of superoxide-mediated reduction (Seo et al., 2008). One unit was determined to be the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. The catalase (CAT) activity was measured based on the disappearance of hydrogen peroxide, which was monitored spectrophotometrically at 240 nm for 5 min (Seo et al., 2008). The glutathione peroxidase (GSH-Px) activity was also measured spectrophotometrically; the reaction mixture contained 1 mM glutathione, 0.2 mM NADPH and 0.24 units of glutathione reductase in 0.1 M Tris–HCl (pH 7.2) buffer. The reaction was initiated by the addition of 0.25 mM H_2O_2 and the absorbance was measured at 340 nm for 5 min (Seo et al., 2008).

As a marker of the production of lipid peroxidation, the malondialdehyde (MDA) concentration in the liver was measured as described previously (Seo et al., 2008).

Western blotting analyses

The liver was homogenized at 4 °C in lysis buffer and was centrifuged at 12,000 *g* and 4 °C for 20 min. The supernatants were used for the Western blot analyses. The total protein concentrations were determined using the classic Bradford method. The protein samples (50 μg) were separated with 7–10% SDS-PAGE and transferred onto nitrocellulose membranes (Whatman, Dassel, Germany). The membranes were incubated overnight at 4 °C with antibodies against total AMPK (Cell Signaling, Danvers, MA, USA), p-AMPK (Cell Signaling), CYP2E1 (Millipore, Billerica, MA, USA) and β-actin (Sigma, Saint Louis, Missouri, USA). The membranes were then incubated for 2 h with the secondary antibody (Amersham, Buckinghamshire, UK). The protein bands were visualized with the ECL reagent (Santa Cruz Biotechnology, California, USA) followed by a brief exposure using an automated detection system (LAS 4000, Fujifilm, Tokyo, Japan). The amount of protein was quantified by densitometry analysis using the Multi Gauge program (Version 3.0, Fujifilm).

Statistical analysis

All of the data are presented as the means ± S.E. values. The statistically significant differences between groups were determined by one-way analysis of variance using the SPSS software (Chicago, IL, USA). The post-hoc Duncan's multiple-range test was used to assess the significant differences between means. The differences were considered statistically significant when $p < 0.05$.

Results

Effect of curcumin on body weight, fat accumulation and liver damage

The body weight of the mice in the alcohol control group was lower but not significantly different compared to the normal group due to pair feeding. Curcumin supplementation did not affect the overall body weight, food intake and relative liver weight in the alcohol-administered mice (Table 1). The total visceral fat weight was significantly higher in the alcohol control group compared with the normal group. However, both the 0.02% and the 0.05% curcumin treatments significantly lowered the mesenteric fat weight by 34% and 23%, respectively, and total visceral fat weight by 29% and 20%, respectively (Table 1).

Table 1
Effect of curcumin supplementation on the body weights, food intake, and liver weight and visceral fat weights in alcohol-administered mice.[1]

	Normal	Alcohol	Alcohol-curcumin I[2]	Alcohol-curcumin II[3]
Body weight (g)				
Initial	27.21 ± 0.90	27.43 ± 0.76	27.38 ± 0.62	27.79 ± 0.64
Final	37.48 ± 0.74	34.78 ± 0.81	35.28 ± 1.10	36.67 ± 1.40
Food intake (g/day)	3.47 ± 0.05	3.52 ± 0.13	3.47 ± 0.09	3.49 ± 0.19
Liver weight (mg/g BW)	36.50 ± 0.48	37.29 ± 0.62	36.88 ± 0.43	37.13 ± 0.42
Visceral fat weights (mg/g BW)				
Epididymal WAT[4]	22.71 ± 2.16	25.31 ± 3.10	19.13 ± 2.03	20.73 ± 1.80
Perirenal WAT	1.05 ± 0.11	1.33 ± 0.14	1.14 ± 0.23	1.40 ± 0.30
Mesenteric WAT	5.96 ± 0.55[a]	7.58 ± 0.44[b]	4.97 ± 0.47[a]	5.87 ± 0.58[a]
Total WAT[5]	29.26 ± 2.95[a]	34.62 ± 0.98[b]	24.44 ± 0.92[a]	28.06 ± 1.36[a]

[ab]The means in the same row not sharing a common letter are significantly among the groups ($p < 0.05$).
[1] The values are expressed as the means ± S.E.
[2] 0.02% curcumin-supplemented group.
[3] 0.05% curcumin-supplemented group.
[4] WAT: white adipose tissue.
[5] Sum of epididymal, perirenal and mesenteric white adipose tissues.

The liver damage biomarkers, AST, ALT, LDH and ALP, were all significantly elevated by alcohol administration in mice fed high-fat diet. Curcumin supplementation at both doses significantly lowered the AST, ALT, LDH and ALP activities, which indicates reduced liver damage compared to the alcohol control group (Fig. 1A). The morphological analysis showed that alcohol administration promoted steatosis, as indicated by the appearance of lipid droplets. However, curcumin supplementation reduced the steatosis compared to the alcohol control group (Fig. 1B).

Effect of curcumin on alcohol metabolism enzyme activity

To determine whether curcumin acts on the alcohol metabolism pathway to reduce liver damage, we measured the ADH, CYP2E1 and ALDH activities.

The hepatic ADH activity was significantly suppressed in the alcohol control group compared to the normal group. However, supplement with the 0.05% dose of curcumin significantly increased the ADH activity (Fig. 2A). Similarly, the ALDH activity was reduced in the alcohol control group, but both doses of curcumin (0.02% and 0.05%) elevated the ALDH activity compared to the alcohol control mice by 18% and 26%, respectively (Fig. 2B). Chronic alcohol administration resulted in increased CYP2E1 activity and CYP2E1 protein expression in the liver (Fig. 2C and D). Conversely, curcumin supplementation (at both doses) significantly decreased the CYP2E1 activity to normal values (Fig. 2C). Similarly, the CYP2E1 protein expression was also decreased to normal levels by curcumin supplementation (Fig. 2D).

Effect of curcumin on AMPK and lipid metabolism

To determine whether curcumin acts on AMPK and lipid metabolism to reduce steatosis, we measured the AMPK phosphorylation and lipid metabolic enzyme activities.

The Western blot analysis revealed that the administration of alcohol to mice suppressed the level of phosphorylated AMPK and resulted in a concomitant decrease in the total AMPK protein level. However, both doses of curcumin increased the total AMPK and p-AMPK protein levels compared to the alcohol control mice (Fig. 3A). A significant increase in the PAP activity was observed in the alcohol control mice compared to the normal mice, whereas the FAS and G6PD activities were not altered. Nevertheless, curcumin supplementation at both doses lowered the PAP and FAS activities compared with the alcohol control mice (Fig. 3B and C) but did not affect the G6PD activity (Fig. 3D).

Alcohol administration increased the plasma leptin levels compared with the normal mice; however, curcumin supplementation decreased the plasma leptin levels close to those observed in the normal mice (Table 2). Both doses of curcumin significantly lowered the plasma triglyceride and free fatty acid levels compared to the alcohol control group. The plasma total cholesterol concentration was reduced by the 0.02% curcumin dose, whereas the HDL-cholesterol concentration was increased by the 0.05% curcumin dose. Treatment with both doses of curcumin decreased the total cholesterol/HDL-C ratio compared with that obtained in the alcohol control group (Table 2).

The liver triglyceride and cholesterol contents were 66% and 16% higher, respectively, in the alcohol control group compared with the normal group. Both doses of curcumin lowered the hepatic triglyceride and cholesterol levels (Table 2).

Effect of curcumin on antioxidant activity and lipid peroxidation

The hepatic antioxidant enzyme activities and lipid peroxide levels are shown in Fig. 4. The hepatic SOD, CAT and GSH-Px activities were significantly suppressed in the alcohol control group compared to the normal group. However, both doses of curcumin supplementation increased the SOD, CAT and GSH-Px activities. In addition, chronic

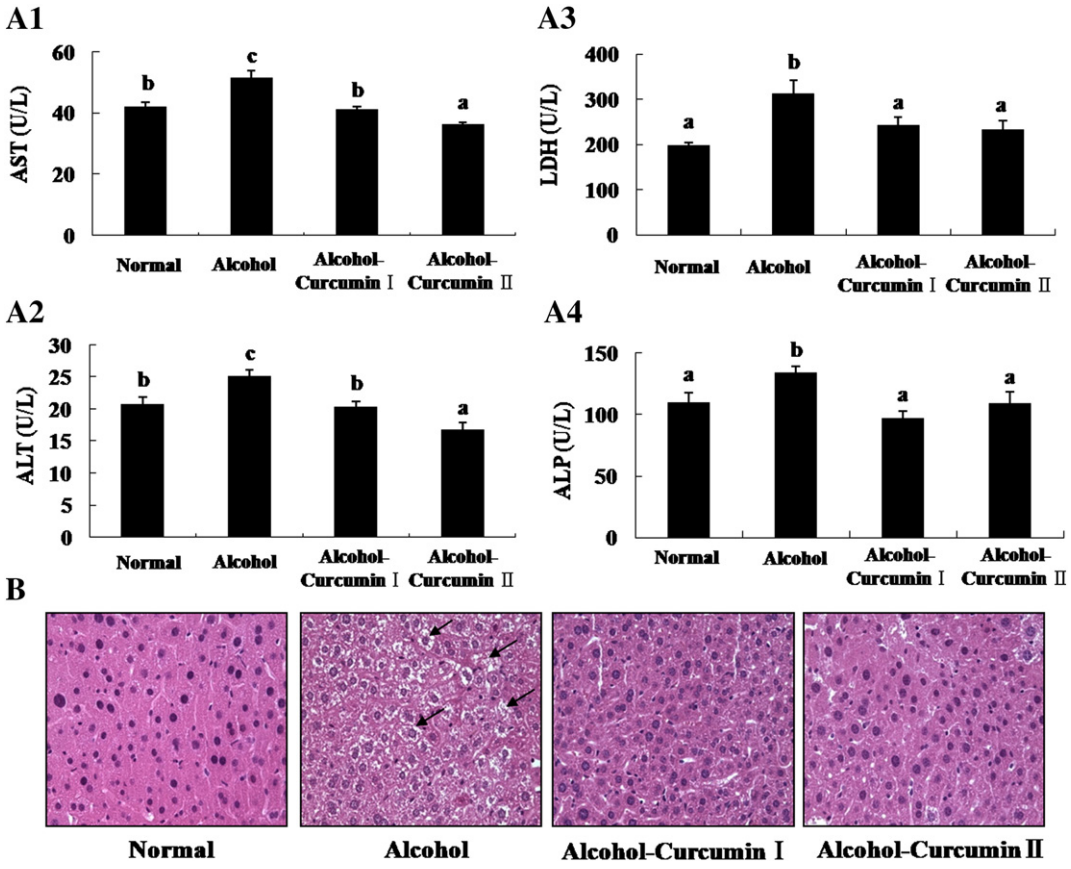

Fig. 1. Effect of curcumin supplementation on the liver damage biomarkers (A1) aspartate aminotransferase, (A2) alanine aminotransferase, (A3) lactate dehydrogenase and (A4) alkaline phosphatase, and (B) the hepatic morphology in alcohol-administered obese mice. The values are expressed as the means ± S.E. [abc]The means not sharing a common letter are significantly different among the groups ($p < 0.05$). The black arrows indicate steatosis. 200 × magnification.

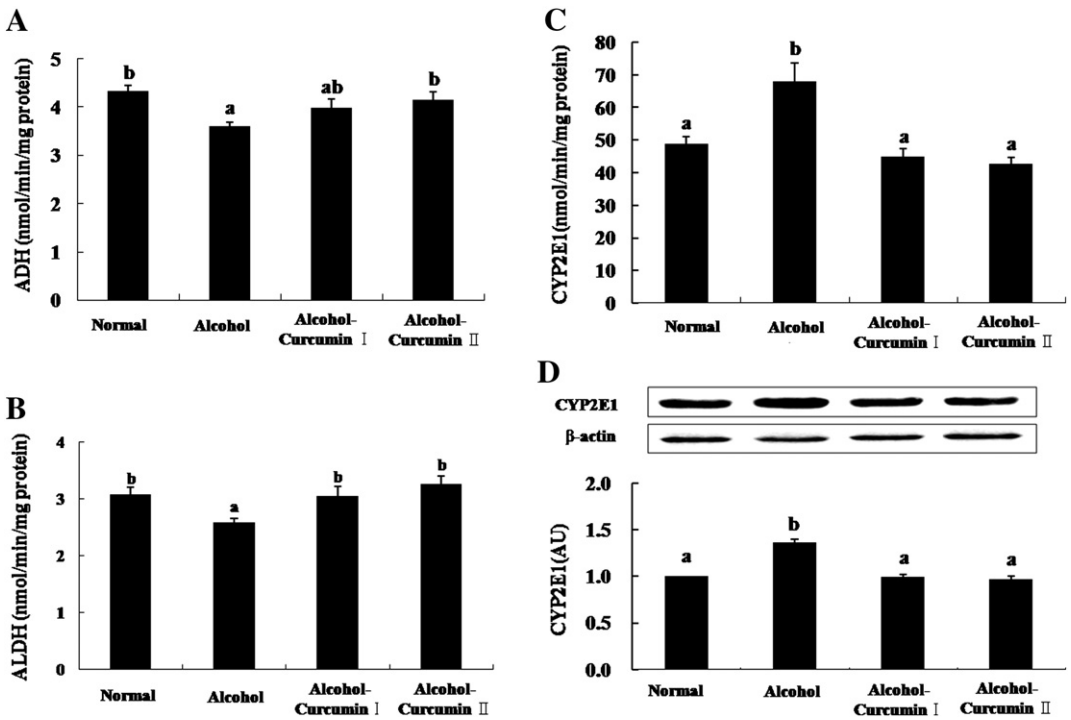

Fig. 2. Effect of curcumin supplementation on (A) hepatic ADH activity, (B) ALDH activity, (C) CYP2E1 activity and (D) CYP2E1 protein expression in alcohol-administered obese mice. The values are expressed as the means ± S.E. The CYP2E1 values are expressed as arbitrary units (AU). The level in each group is related to an assigned value of 1 in the normal group. [ab]The means not sharing a common letter are significantly different among the groups ($p < 0.05$).

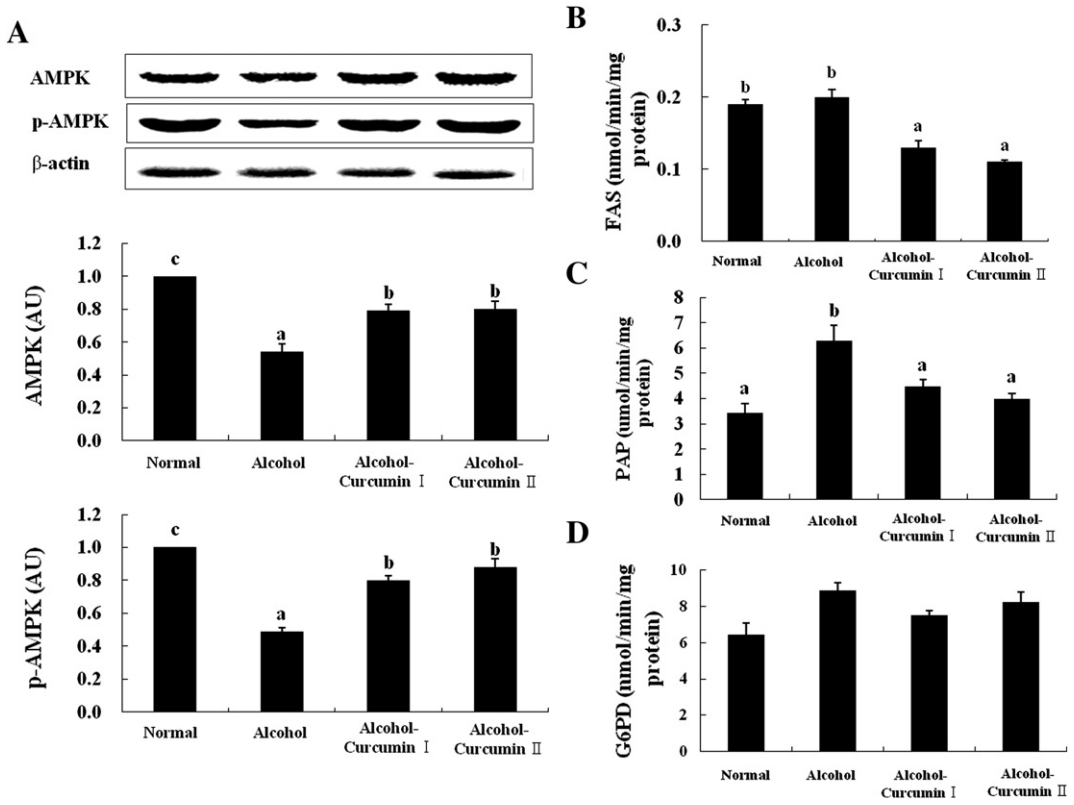

Fig. 3. Effect of curcumin supplementation on hepatic (A) AMPK, (B) fatty acid synthase, (C) PAP and (D) G6PD activities in alcohol-administered obese mice. The values are expressed as the means ± S.E. The AMPK and p-AMPK values are expressed in arbitrary units (AU). The level in each group is related to an assigned value of 1 in the normal group. [abc]The means not sharing a common letter are significantly different among the groups ($p < 0.05$).

alcohol administration resulted in increased lipid peroxidation, as indicated by the increased MDA levels. However, both doses of curcumin significantly lowered the lipid peroxide levels by 33% and 40%, respectively, compared to the levels obtained in the alcohol control group.

Discussion

The present study demonstrated that both doses of curcumin (0.02% and 0.05%, wt/wt) which were tested effectively alleviated the hepatic steatosis induced by chronic alcohol intake and a high-fat diet. Alcohol intake and high-fat diet can independently promote the development of hepatic steatosis, which can progress to fibrosis and cirrhosis (Lieber, 2004). Excess alcohol intake in overweight or obese humans is associated with a significantly higher risk for the development of liver-related disorders (Shen et al., 2010). Therefore, reducing or preventing fat accumulation in the liver may be a major strategy for the prevention of a fatty liver. In the present study, the alcohol-induced increases in the hepatic triglyceride levels were significantly reduced by both doses of curcumin supplementation. In other disease models without alcohol intake, curcumin treatment has also been reported to decrease triglyceride accumulation (Kim and Kim, 2010; Jang et al., 2008; Shao et al., 2012; El-Moselhy et al., 2011; Weisberg et al., 2008; Ejaz et al., 2009). Thus, we found that the relatively low doses of curcumin used in this study improved fat accumulation in alcohol-administered mice fed in a high-fat diet.

There is some evidence to suggest that curcumin exerts a direct effect on the regulators of the lipid metabolism (Aggarwal, 2010) and hence its downstream effectors. AMPK is a well-established cellular energy sensor that switches on catabolic pathways, including fatty acid oxidation and switches off anabolic pathways, including fatty acid synthesis (Hardie, 2011). AMPK has been suggested as a therapeutic target for the treatment of alcohol fatty liver (Cho et al., 2009). We tested whether curcumin can alter the cellular AMPK expression in the liver. Previous studies have indicated that the administration of alcohol significantly decreases both the level of phosphorylated AMPK and the level of total AMPK in the liver compared with the control group (Qin and Tian, 2010; Ajmo et al., 2008), and these findings are consistent with the results of the present study. In this study, we showed that curcumin increases hepatic total AMPK protein expression, which is in turn associated with decreased FAS and PAP activities in mice that are chronically administered alcohol. FAS and PAP are important enzymes in the fatty acid and triglyceride synthesis pathway. Interestingly, we also found that hepatic triglyceride concentration was positively correlated with the FAS and PAP activities. PAP was recently identified as a key regulator of lipid metabolism in several organs, including the liver (Harris and Finck, 2011). G6PD, the rate-limiting enzyme in the pentose phosphate pathway, produces NADPH that is used to reduce glutathione and to support reductive biosynthesis such as fatty acid (Salati and Amir-Ahmady, 2001). The G6PD activity was not significantly modified by curcumin. Taken together, the present findings suggest a role for AMPK in the curcumin-induced decrease in the hepatic triglyceride levels in vivo. Chronic alcohol intake also alters the plasma lipid profiles and lipoprotein metabolism (Lieber, 2004). Dysregulated lipoprotein levels and hyperlipidemia promote atherosclerosis and cardiovascular disease. Long-term curcumin treatment over eighteen weeks has been reported to reduce hyperlipidemia and to suppress atherosclerotic plaque formation and endothelial lipid infiltration (Shin et al., 2011). Although the duration of the present study was much shorter, we showed that curcumin supplementation reduced the plasma free fatty acid, triglyceride and cholesterol levels in the mice that were chronically administered alcohol and fed a high-fat diet.

The liver damage biomarkers, including AST, ALT, LDH and ALP, were all increased by alcohol intake but reduced by curcumin supplementation in high-fat diet fed mice. The present findings are consistent with previous results that showed that curcumin can protect against liver damage in a wide range of disease models (Rivera-Espinoza and Muriel,

Table 2
Effect of curcumin supplementation on the plasma leptin level and lipid profiles in alcohol-administered obese mice.[1]

	Normal	Alcohol	Alcohol-curcumin I[2]	Alcohol-curcumin II[3]
Plasma				
Leptin (ng/mL)	1.44 ± 0.12[a]	2.43 ± 0.12[b]	1.68 ± 0.15[a]	1.48 ± 1.15[a]
Free fatty acids (mmol/L)	0.96 ± 0.02[a]	1.09 ± 0.02[b]	0.99 ± 0.00[a]	0.96 ± 0.04[a]
Triglyceride (mmol/L)	1.01 ± 0.03[a]	1.32 ± 0.12[b]	1.02 ± 0.07[a]	0.96 ± 0.11[a]
Total cholesterol (mmol/L)	3.72 ± 0.14[a]	4.58 ± 0.22[b]	3.63 ± 0.22[a]	4.16 ± 0.20[ab]
HDL-cholesterol (mmol/L)	2.37 ± 0.17[ab]	2.05 ± 0.15[a]	2.22 ± 0.14[a]	2.81 ± 0.15[b]
TC/HDL-C	1.56 ± 0.17[a]	2.23 ± 0.16[b]	1.63 ± 0.13[a]	1.47 ± 0.08[a]
Liver				
Triglyceride (mmol/g)	0.15 ± 0.01[a]	0.25 ± 0.01[b]	0.16 ± 0.01[a]	0.15 ± 0.02[a]
Cholesterol (mmol/g)	0.12 ± 0.01[bc]	0.14 ± 0.01[c]	0.11 ± 0.01[b]	0.08 ± 0.00[a]

[abc]The means in the same row not sharing a common letter are significantly among the groups ($p < 0.05$).
[1] The values are expressed as the means ± S.E.
[2] 0.02% curcumin-supplemented group.
[3] 0.05% curcumin-supplemented group.

2009). The protective role of curcumin against liver damage has been predominantly attributed to antioxidant and anti-inflammatory activities (Rivera-Espinoza and Muriel, 2009). However, both inflammation and oxidative stress are largely secondary effects of liver damage. Therefore, we sought to establish whether curcumin exerted any direct effect on the major enzymes in the alcohol metabolism pathway.

The elimination of alcohol is primarily dependent on the oxidation of alcohol and its metabolic byproducts by ADH, CYP2E1 and ALDH (Lieber, 2004). Low doses of alcohol increase ADH activity, whereas chronic alcohol intake tends to suppress ADH activity. Therefore, higher doses of alcohol are primarily oxidized by CYP2E1, but this also produces additional toxic byproducts, such as hydroxyethyl, superoxide anion and hydroxyl radicals (Lieber, 2004). Recent studies indicated that the knockdown of CYP2E1 protects mice against the early development of alcohol-induced liver disease (Lu et al., 2010). Remarkably, we found that curcumin supplementation was able to reverse the alcohol-induced inhibition of ADH activity, and to suppress CYP2E1 activity and protein levels, which indicates that curcumin supplementation appears to promote alcohol oxidation via ADH. The present findings appear to be consistent with the curcumin-induced suppression of CYP2E1 observed in other liver damage disease models because CYP2E1 is also involved in the metabolism of other toxins in addition to alcohol (Rivera-Espinoza and Muriel, 2009).

The oxidation of ethanol by ADH or CYP2E1 also produces acetaldehyde and NADH (Lieber, 2004). Excess acetaldehyde accumulation is toxic in large quantities, inhibits lipid metabolism regulators and may bind to DNA or hepatic proteins to form adducts, which can promote carcinogenesis and hepatomegaly (Seth et al., 2011). In addition, excess $NADH/NAD^+$ due to alcohol metabolism can upset the redox balance. We found that curcumin also reversed the alcohol-induced inhibition of the ALDH activity. ALDH catalyzes the conversion of acetaldehyde to acetate, which is then predominantly released into the circulation and metabolized in the heart, skeletal muscle or brain. Taken together, these findings reveal a previously unknown hepatoprotective effect of curcumin on the alcohol metabolism enzyme activity in the liver, which promotes alcohol oxidation via ADH and also increases ALDH activity.

Oxidative stress is a well-established factor in the progression of liver damage due to alcohol intake and a high-fat diet. In alcoholic liver disease, the byproducts of dysregulated alcohol and lipid metabolism result in the excessive production of free radicals, which leads to oxidative stress (Lieber, 2004). Alcohol-induced liver damage is more severe in mice lacking specific antioxidant enzymes (Kessova et al., 2003). Chronic alcohol administration typically reduces the antioxidant enzyme activity, as indicated in the present study by the reduced SOD, CAT and GSH-Px activities. Insufficient antioxidant activity allows free radicals to accumulate and bind to unsaturated fatty acids in cell membranes, which causes lipid peroxidation. Kessova et al. (2003) reported

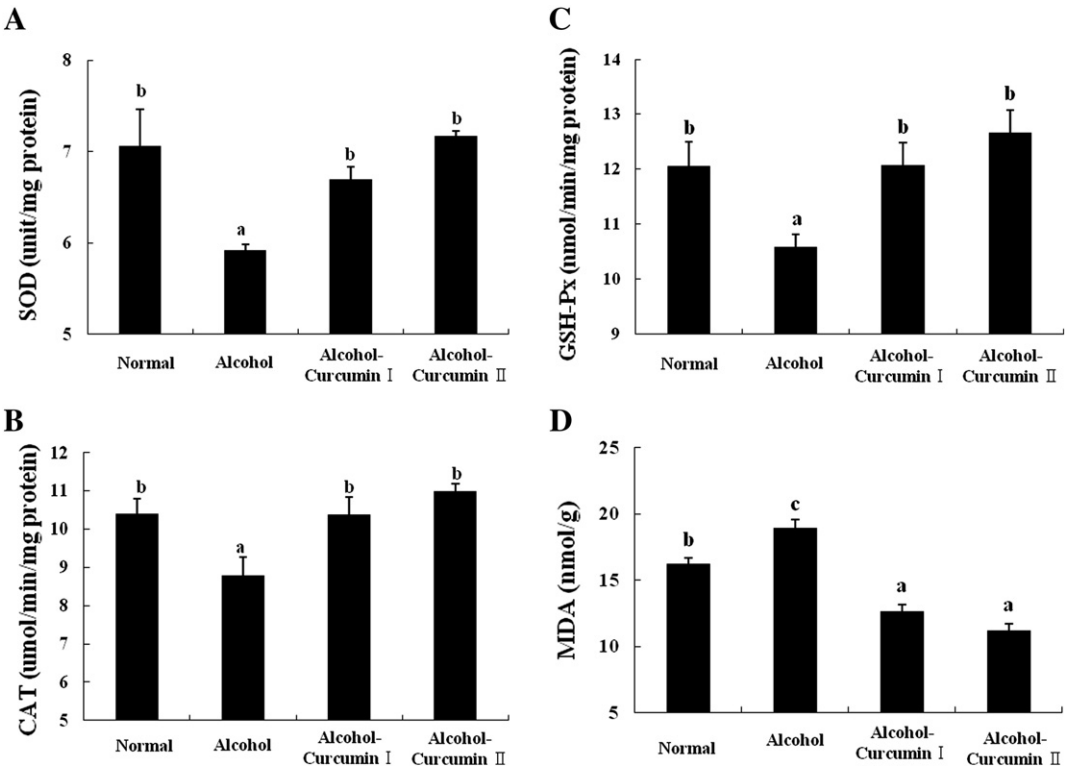

Fig. 4. Effect of curcumin supplementation on hepatic (A) SOD, (B) CAT and (C) GSH-Px activities and (D) lipid peroxidation level in alcohol-administered obese mice. The values are expressed as the means ± S.E. [abc]The means not sharing a common letter are significantly different among the groups ($p < 0.05$).

that the induction of steatosis after chronic alcohol administration is more severe in Cu/Zn SOD-knockout mice. In contrast, curcumin supplementation increased the SOD, CAT and GSH-Px activities as observed in this and in other studies (Rukkumani et al., 2005; Rong et al., 2012), which are decreased in response to a high-fat diet (Jang et al., 2008), diabetes (Seo et al., 2008) and a variety of liver injuries (Rivera-Espinoza and Muriel, 2009). There is also evidence to indicate that curcumin directly reacts with peroxyl radicals (Masuda et al., 2001). In addition, the ability of curcumin to scavenge reactive oxygen species appears to be largely due to its phenolic OH groups (Priyadarsini et al., 2003).

Both doses of curcumin tested induced increases in antioxidant activity and resulted in lower lipid peroxidation, as indicated by reduced MDA levels. A previous study showed a causal link between leptin and lipid peroxidation in the liver (Balasubramaniyan et al., 2003). Mantozoros et al. (1998) also had reported that obesity and alcohol intake are independently and positively associated with the circulating leptin concentration. Previous studies have reported that the leptin levels are also increased in patients with advanced alcoholic cirrhosis due to an increased release of leptin from fat tissue areas and a reduced renal leptin extraction (Henriksen et al., 1999). We also observed significant increased of plasma leptin levels in the mice that were administered with alcohol. However, curcumin supplementation effectively lowered the plasma leptin concentration compared to the alcohol control mice without changes in the body weight and food intakes. In addition, the total visceral fat mass was reduced in the curcumin-supplemented mice. Taken together, the present findings indicate that curcumin may reduce oxidative stress and lipid peroxidation partly through its action on the alcohol metabolism, i.e., by reducing the metabolic byproducts.

Conclusions

These results demonstrated that curcumin doses of 0.02% to 0.05% are sufficient to protect against alcohol-induced hepatotoxicity in diet-induced obese mice. The hepatoprotective effect of curcumin may be mediated by the inhibition of CYP2E1 activity and by increasing the AMPK expression, alcohol metabolism and antioxidant activity. The evidence obtained in this study suggests that curcumin may provide a natural treatment to combat alcoholic fatty liver disease.

Conflict of interest statement

The authors declare no conflict of interest.

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