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Lactobacillus rhamnosus CCFM1107 treatment ameliorates alcohol -induced liver injury in a mouse model of chronic alcohol feeding

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Lactobacillus rhamnosus **CCFM1107 was screened for high antioxidative activity from 55 lactobacilli. The present study attempted to explore the protective properties of *L. rhamnosus* CCFM1107 in alcoholic liver injury. A mouse model was induced by orally feeding alcohol when simultaneously treated with *L. rhamnosus* CCFM1107, the drug Hu-Gan-Pian (HGP), *L. rhamnosus* GG (LGG), and *L. plantarum* CCFM1112 for 3 months. Biochemical analysis was performed for both serum and liver homogenate. Detailed intestinal flora and histological analyses were also carried out. Our results indicated that the administration of *L. rhamnosus* CCFM1107 significantly inhibited the increase in the levels of serum aminotransferase and endotoxin, as well as the levels of triglyceride (TG) and cholesterol (CHO) in the serum and in the liver. Glutathione (GSH), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) were elevated while the levels of malondialdehyde (MDA) were decreased. The enteric dysbiosis caused by alcohol was restored by increasing the numbers of both lactobacilli and bifidobacteria and decreasing the numbers of both enterococci and enterobacter. Histological analysis confirmed the protective effect of *L. rhamnosus* CCFM1107. Compared with the other lactobacilli and to the drug Hu-Gan-Pian, there is a high chance that *L. rhamnosus* CCFM1107 provides protective effects on alcoholic liver injury by reducing oxidative stress and restoring the intestinal flora.**

Keywords: probiotics, alcoholic liver injury, oxidative stress, intestinal flora, endotoxin

Introduction

Alcoholic liver injury results from chronic alcohol abuse or recurrent acute binge drinking. It can range from simple steatosis with minimal injury, to serious liver damage such as fibrosis and cirrhosis (O'Shea *et al.*, 2010). It is reported that alcohol consumption remains a main risk factor for chronic liver disease and represents a major cause of morbidity and mortality worldwide (Gao and Bataller, 2011). The underlying mechanisms of pathogenesis include oxidative stress, production of endotoxins, pro-inflammatory cytokines and specific intracellular pathways (McClain *et al.*, 1997; El-Assal *et al.*, 2004; Dey and Cederbaum, 2006; Albano, 2008; Rishi *et al.*, 2011; Tilg *et al.*, 2011). Early research mainly focused on free radical-mediated oxidative stress. And a lot of experimental and clinical studies have shown that the free radical formation and oxidative damage induced by alcohol contribute in many ways to the pathogenesis of alcohol hepatoxicity (Albano, 2008). Reactive oxygen species (ROS) is induced with the administration of alcohol, which makes the cell more susceptible to oxidative damage (Bailey and Cunningham, 2002). Oxidative stress-mediated interference with mitochondrial damage, protein degradation, inflammatory reaction and lipoprotein secretion are likely involved in the progression of liver injury consequent to alcohol abuse (Donohue, 2002; Hines and Wheeler, 2004; Pan *et al.*, 2004; Fernandez-Checa and Kaplowitz, 2005). Several other studies have demonstrated that addition of different antioxidants and free radical scavengers such as S-adenosyl-L-methionine, vitamin E, resveratrol and GSH precursors could prevent alcohol-induced hepatic injury (Nanji *et al.*, 1996; Iimuro *et al.*, 2000; Fernández-Checa *et al.*, 2002; Ajmo *et al.*, 2008).

Gut-derived endotoxin and other bacterial toxins also play important roles in the pathogenesis of alcoholic liver injury. Endotoxin and bacteria can translocate through the gastrointestinal tract barrier as a result of increased intestinal permeability, which may cause endotoxemia (Yan *et al.*, 2011). Endotoxemia is frequently found in patients with early forms of mild alcohol hepatitis (Parlesak *et al.*, 2000). The plasma endotoxin levels are proven increased during acute or chronic administration of alcohol, which is associated with liver injury (Rao *et al.*, 2004). Lactobacilli constitute an integral part of the normal gastrointestinal microbiota and exhibit probiotic benefits on the maintenance and improvement of intestinal function (Fernandes *et al.*, 1987; Bruzzese *et al.*, 2004). Several studies have reported the beneficial effects of probiotic *lactobacillus* strains on experimental alcohol-induced hepatic injury (Nanji *et al.*, 1994; Mutlu *et al.*, 2009). LGG can survive in the gastrointestinal tract and has been shown to reduce endotoxemia, and to ameliorate alcohol-induced oxidative stress, inflammation and steatohepatitis significantly in a rat model (Forsyth *et al.*, 2009). Moreover, many other lactobacilli also improve hepatocyte damage induced by en-

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dotoxin, D-galactosamine and alcohol (Kasravi *et al.*, 1997; Adawi *et al.*, 2001; Osman *et al.*, 2007; Forsyth *et al.*, 2009).

Thus we hypothesize that probiotics with high antioxidative activity can reduce alcohol-induced oxidative stress and normalize the enteric microbiome and thereby ameliorate liver disease in alcohol fed mice. LGG ATCC 53103 and 55 lactobacilli isolated from traditional fermented milk and pickle were obtained from the Culture Collection of Food Microorganisms (CCFM), Jiangnan University. We investigated the antioxidative effect of these lactobacilli on the scavenging of free radicals and on the inhibition of lipid peroxidation. *L. rhamnosus* CCFM1107 was identified as highly antioxidant (data not shown), while *L. plantarum* CCFM1112 had weak antioxidative activity, and was thus selected as a negative control. The aim of present study was to evaluate the effect of feeding *L. rhamnosus* CCFM1107 on chronic alcoholic liver injury in a mouse model. LGG (Ahotupa *et al.*, 1996; Korpela *et al.*, 1997), *L. plantarum* CCFM1112 and HGP were also compared for their effects on liver injury after feeding supplementation.

Materials and Methods

Microorganisms and media

L. rhamnosus CCFM1107, *L. plantarum* CCFM1112 and LGG were cultured in de Man, Rogosa, and Sharpe (MRS) broth (Hope Bio-Technology Co., Ltd) at 37°C for 18 h, harvested by centrifugation, washed three times with phosphate-buffered saline and then lyophilized (skimmed milk as the protective agent). MC, TPY, VRBDA, and EC were selective media used for culturing intestinal flora and were purchased from Hope Bio-Technology Co., Ltd.

Drugs and reagents

Analytical grade alcohol was purchased from Sinopharm Chemical Reagent Co., Ltd. HGP was purchased from Sunflower Co., Ltd. The assay kits for MDA, GSH, GSH-Px and total superoxide dismutase (T-SOD) were purchased from Jiancheng Bioengineering Institute. The assay kits for alanine aminotransferase (ALT), aspartate aminotransferase(AST) (Cesaratto *et al.*, 2004), γ-glutamyl transferase (γ-GT), TG and total cholesterol (T-CHO) were purchased from Huili Biotechnology Co., Ltd. The ELISA kit for serum endotoxin was the product of Cusabio Biotechnology Co., Ltd. Other reagents were of analytical grade if not mentioned.

Experimental design

Male Kunming mice (19 ± 1 g) were obtained from Shanghai SLRC Laboratory Animal Co., Ltd and housed in an air-conditioned room maintained at 23 ± 2°C at a relative humidity of 50% ± 10%. The room was maintained in an alternating 12-h light and 12-h dark cycle. The animals were allowed free access to food and water and had 1 week to acclimatize to the laboratory environment. All the protocols of the study were approved by the Ethics Committee of Jiangnan University, China. All the procedures were conducted according to European Community guidelines (Directive 2010/63/EU) for the care and use of experimental animals.

The mice were randomly divided into six groups (n = 10 in each): skimmed milk was given to the control group, alcohol to the model group, *L. rhamnosus* CCFM1107 to the target group, HGP to the drug group, LGG to the positive control group and *L. plantarum* CCFM1112 to the negative control group. They were fed by gastric gavage twice a day for 3 months. Mice in the control group were fed skimmed milk throughout the entire experiment. Mice in the other five groups were fed alcohol in the morning. The concentration gradually increased from 20% to 40% in 2 weeks at the increment of 5%, and then maintained at 40% till the end of the experiment. In the afternoon, the model group received skimmed milk while the drug control group was treated with HGP, at a dose dependent on body weight. Mice in the other three groups were administrated different lactobacilli as described above. Recovery of the lyophilized lactobacilli was constructed at 37°C for 30 min in water bath and the concentration was adjusted to approximately 10^9 CFU/ml with skimmed milk. The dose for each mouse was calculated as 10 ml/kg body weight. The mice were sacrificed after 3 months and food was forbidden in the last 24 h before sacrifice.

Measurement of coefficient of hepatic weight and MDA, GSH, GSH-Px, SOD, TG, and CHO in liver homogenate

Liver was removed and weighed at the time of sacrifice. Part of the liver tissue was put in ice-cold saline immediately and tissue homogenate was prepared (10%, w/v) (Sun *et al.*, 2009). Coefficient of hepatic weight was calculated in the formula of hepatic weight divided by body weight. Measurements of MDA, GSH, GSH-PX, SOD, TG, and CHO were performed according to the kits protocol.

Measurement of serum ALT, AST, γ-GT, TG, and CHO

Blood was obtained from the mouse eyeball before sacrifice and rapidly put in an ice-cold tube. The blood was kept still at 4°C for 1 h. Serum was collected after centrifugation at 2,500 × *g* for 15 min. Contents of ALT, AST, γ-GT, TG, and CHO were determined following the instructions supplied with the kits.

Serum endotoxin determination

Determination of endotoxin in serum was carried out using a commercially available ELISA kit (Cusabio Biotechnology Co. Ltd).

Microbiological assays of intestinal flora

Enterococci, enterobacter, lactobacilli, and bifidobacteria were selected for the study of quantitative changes of intestinal flora in fecal samples. CFU (Colony Forming Units) per gram stool were determined by plating different serial dilutions using *E. coli* agar (EC) for enterococci, Violet Red Bile Dextrose Agar (VRBDA) for enterobacter, Modified Chalmers Agar (MC) for lactobacilli and Tryptone phytone yeast extract agar (TPY) for bifidobacteria. Enterococci and enterobacter were aerobically incubated for 48 h at 42°C and 37°C, respectively. Lactobacilli and bifidobacteria were incubated anaerobically at 37°C for 48 h.

Table 1. Levels of body weight, hepatic weight, and coefficient of hepatic weight in different groups

Groups	Body weight (g)	Hepatic weight (g)	Coefficient of hepatic weight
Control	43.32±3.43	1.29±0.22	2.98±0.47
Alcohol	41.98±3.20	1.57±0.27	3.74±0.52[a]
Alcohol + *L. rhamnosus* CCFM1107	44.77±3.32	1.48±0.24	3.31±0.47
Alcohol + HGP	42.95±2.43	1.34±0.22	3.11±0.39[b]
Alcohol + LGG	44.57±2.19	1.37±0.21	3.07±0.39[b]
Alcohol + *L. plantarum* CCFM1112	43.88±3.70	1.57±0.17	3.59±0.36[a]

Results are expressed as mean ± SD (n=10). [a]$p<0.05$ vs Control group; [b]$p<0.05$ vs Alcohol group.

Histological analysis

Part of the liver tissue was fixed in 10% neutral buffered formalin immediately after sacrifice. Hematoxylin and eosin was used for staining. Pictures of the tissue slices were taken under light microscope (Leica DM200, Leica Microsystems Ltd.).

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Differences between groups were evaluated using one way analysis of variance (ANOVA) followed by Tukey test (SPSS for Windows Release 16.0, SPSS Inc.). Probability levels of less than 0.05 were recognized as significant ($p<0.05$).

Results and Discussion

Probiotics are living microorganisms that benefit the host animals when administered in adequate amounts by improving the intestinal microbial balance (Afec, 1989). Probiotics have demonstrated several beneficial properties, including the ability to stimulate intestinal development and mucosal immunity, to improve epithelial barrier function and to ameliorate diarrhea, chronic inflammatory bowel disease (Bruzzese *et al.*, 2004; Ewaschuk and Dieleman, 2006; Versalovic, 2007; Forsyth *et al.*, 2009). Data from trials in animals and in human indicate promising prospects of probiotic treatment in alcoholic liver disease by reducing oxidative stress and inflammation and preserving epithelial barrier function (Nanji *et al.*, 1994; Kirpich *et al.*, 2008; Segawa *et al.*, 2008; Forsyth *et al.*, 2009). Therefore, the effects of *L. rhamnosus*, with its high antioxidative ability (data not shown), on alcohol-induced liver injury in a mouse model were investigated in the present study.

Effects of lactobacilli treatment on body weight, hepatic weight and the coefficient of hepatic weight

As can be seen in Table 1, body weight and hepatic weight were not significantly different among the six groups. Although body weight in the alcohol-fed group was lower (41.98 ± 3.20 g) than that in the control group (43.32 ± 3.43 g), and hepatic weight in the alcohol-fed group was higher (1.57 ± 0.27 g) compared to the control group (1.29 ± 0.22 g), the differences were not significant. However, the coefficient of hepatic weight increased after alcohol-feeding ($p<0.05$) and decreased significantly after administration of HGP or LGG ($p<0.05$).

Effects of lactobacilli treatment on the lipid (TG and CHO) levels of serum and hepatic tissue

Fatty liver, characterized by an accumulation of TG and cholesterol CHO (Yan *et al.*, 2011) in serum and the liver, can be induced by alcohol. It has been reported that approximately 80% of heavy drinkers can develop fatty liver (Song *et al.*, 2006). Both the serum and hepatic levels of TG and CHO in the alcohol group were significantly higher than those in the control group ($p<0.05$, Table 2). After administration of *L. rhamnosus* CCFM1107, HGP and LGG, the levels decreased significantly ($p<0.05$) in comparison to the alcohol group. However, no significant differences in the TG and CHO contents were observed between the *L. plantarum* CCFM1112 and alcohol groups. It is generally believed that oxidative stress is a prerequisite condition of alcohol hepatotoxicity and plays an important role in the progression of alcohol liver disease (Albano, 2008). Several studies have revealed a close link between fatty liver and oxidative stress or lipid peroxidation (Wu and Cederbaum, 2003; Albano, 2006).

Table 2. Lipid levels of serum and hepatic tissue in different groups

Groups	Serum		Hepatic tissue	
	TG (mmol/L)	CHO (mmol/L)	TG (mmol/L)	CHO (mmol/L)
Control	2.24±0.49	2.33±0.51	0.83±0.09	1.34±0.12
Alcohol	3.74±0.65[a]	3.83±0.61[a]	1.28±0.23[a]	2.26±0.27[a]
Alcohol+*L. rhamnosus* CCFM1107	2.32±0.63[b]	2.80±0.58[b]	0.88±0.13[b]	1.80±0.26[a,b]
Alcohol+HGP	2.17±0.45[b]	2.49±0.65[b]	0.99±0.13[b]	1.53±0.21[b]
Alcohol+LGG	2.45±0.69[b]	2.71±0.60[b]	0.87±0.13[b]	1.71±0.19[a,b]
Alcohol+*L. plantarum* CCFM1112	3.37±0.72[a]	3.80±0.72[a]	1.23±0.17[a]	2.25±0.33[a]

Results are expressed as mean ± SD (n=10). [a]$p<0.05$ vs Control group; [b]$p<0.05$ vs Alcohol group.

Effects of lactobacilli treatment on serum aminotransferase (ALT, AST, and γ-GT) levels

ALT and AST are known as indicator enzymes of the presence of liver disease. Injury to liver cells often results in a leak of ALT and AST into the bloodstream and then elevates the serum aminotransferase levels. The enzyme γ-GT is located predominantly in the liver and is known as the cholestatic liver enzyme. It can accumulate and seep out of the liver and into the bloodstream when the bile ducts are blocked (Rishi *et al.*, 2009). Figure 1 shows serum ALT, AST, and γ-GT levels of different groups after 3 months of feeding as described above. Higher levels of AST than ALT were observed in the present study, which was similar to the results of Rishi *et al.* (2009). A small increase of serum ALT levels was noticed in the alcohol group compared to the control group, which was consistent with Palmer's findings for chronic alcohol abuse (Palmer, 2004). ALT levels in the *L. plantarum* CCFM1112 feeding group were significantly higher than that in the control and *L. rhamnosus* CCFM1107 groups, while the result of *L. rhamnosus* CCFM1107 was similar to those of the control and HGP groups, which indicates that *L. rhamnosus* CCFM1107 may be used as a drug for the prevention of increase in serum ALT levels. Similar results were obtained from Fig. 1B and C. There was almost a two-fold increase of the AST and γ-GT levels in the alcohol group when compared with the control group, which suggests that the liver injury model was meaningful. Significant decreases were detected in the *L. rhamnosus* CCFM1107, HGP and LGG feeding groups in comparison to the alcohol group. Furthermore, AST and γ-GT levels dropped to normal levels ($p<0.05$ versus control). However, mice in the *L. plantarum* CCFM1112 supplementing group demonstrated levels of AST and γ-GT as high as the alcohol group. Thus, *L. plantarum* CCFM1112 did not appear to provide a protective effect on alcoholic liver injury, while *L. rhamnosus* CCFM1107 demonstrated a marked benefit for the recovery of liver damage and was comparable with LGG and HGP.

Effects of lactobacilli treatment on lipid peroxidation (MDA) and antioxidants (GSH, GSH-PX, and SOD) levels

MDA is one of the several byproducts of lipid peroxidation and has been shown to be an efficient biomarker for oxidative stress (Nielsen *et al.*, 1997). Elevation of MDA levels in liver results from an imbalance of redox status. As can be seen in Fig. 2A, the levels of hepatic MDA were determined after feeding for 3 months. Content of MDA in the alcohol group was significantly higher than that in the control group, indicating that serious oxidative damage had occurred. However, in the *L. rhamnosus* CCFM1107, HGP, and LGG treated groups, the MDA levels decreased to the normal levels seen

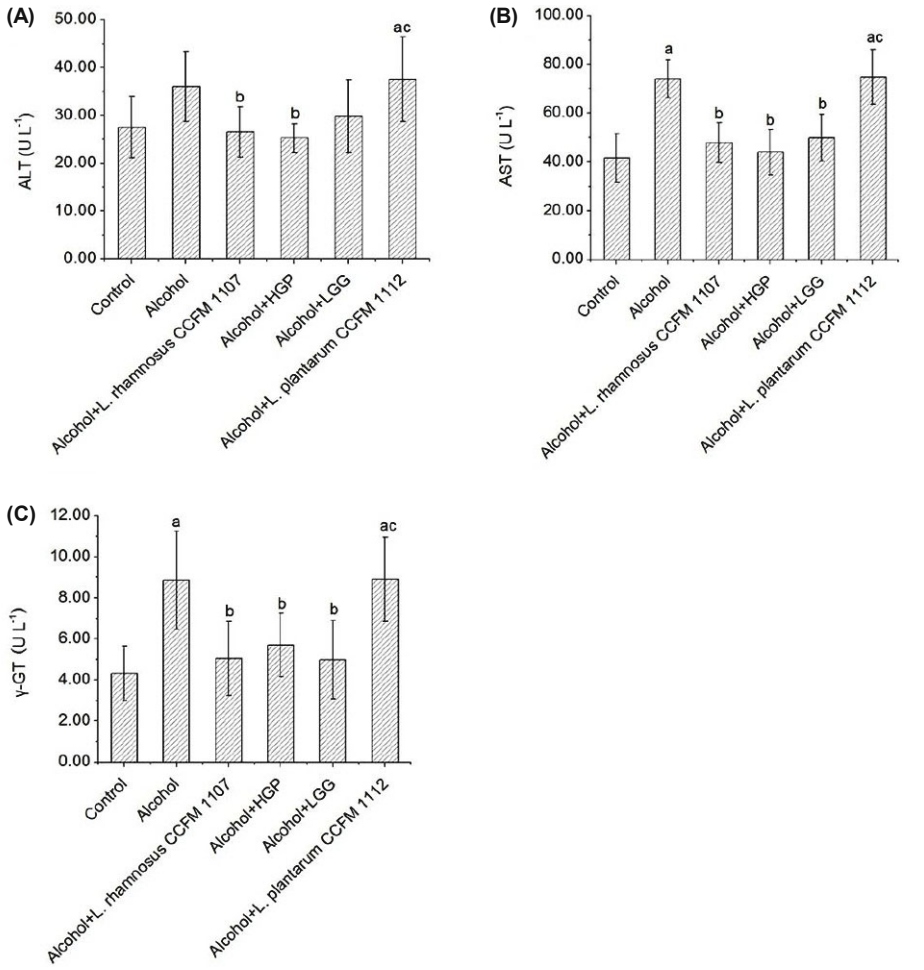

Fig. 1. Estimation of serum ALT, AST and γ-GT levels (Values are expressed as mean ± SD, [a]$p<0.05$ vs Control group; [b]$p<0.05$ vs Alcohol group; [c]$p<0.05$ vs Alcohol + *Lactobacillus rhamnosus* CCFM1107 group).

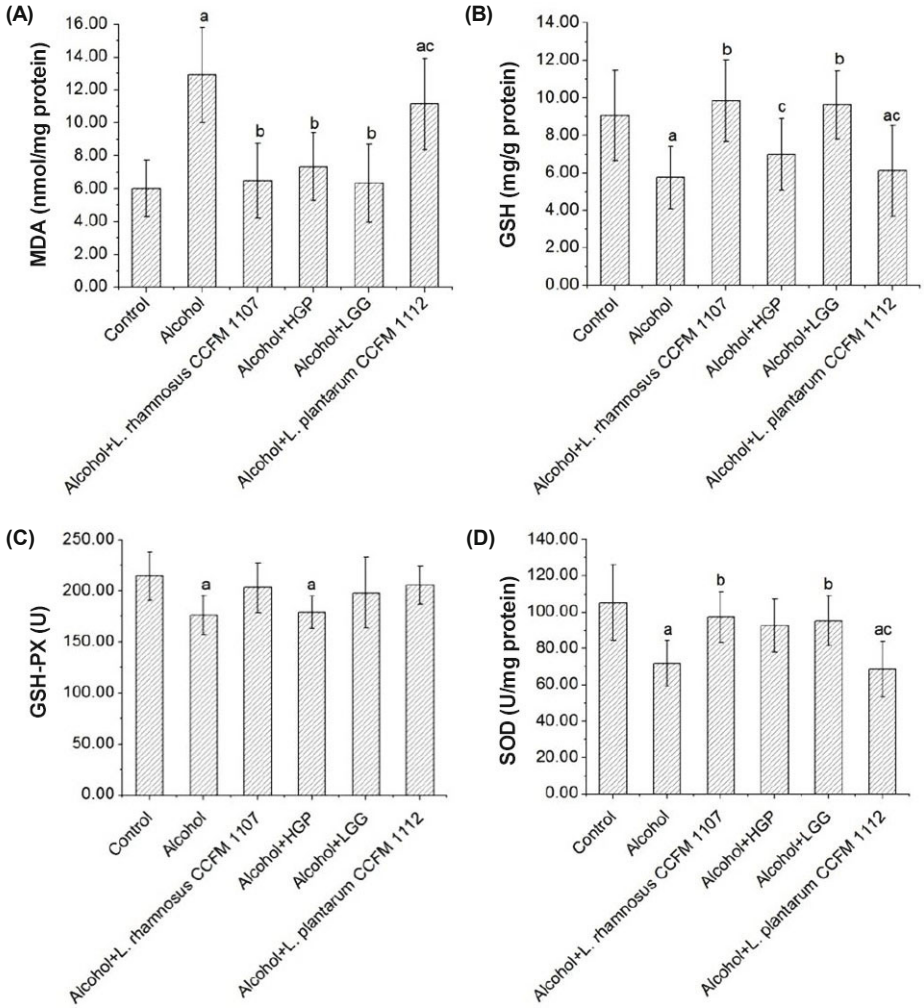

Fig. 2. Estimation of hepatic MDA, GSH, GSH-PX, and SOD levels (Values are expressed as mean ± SD, [a]$p<0.05$ vs Control group; [b]$p<0.05$ vs Alcohol group; [c]$p<0.05$ vs Alcohol + *Lactobacillus rhamnosus* CCFM1107 group).

in the control group. It seemed that recovery of the oxidative damage caused by alcohol was possible with the supplementation of *L. rhamnosus* CCFM1107, HGP, and LGG. These results were also confirmed by measurements of GSH (Fig. 2B), GSH-Px (Fig. 2C) and SOD (Fig. 2D). GSH is the most important redox molecule in the cell and decrease in GSH content elevates the oxidative level, leading to the occurrence of liver injury (Han *et al.*, 2006; Yuan and Kaplowitz, 2009). Moreover, GSH exists in all types of cells and helps neutralize free radicals smoothly. GSH is widely used as a strong antioxidant. GSH-Px and SOD are the two key enzymes in the regulation of balance of redox status in serum (Cesaratto *et al.*, 2004). GSH-Px can catalyze hydrogen peroxide and lipid hydroperoxides to prevent their damage to cells. However, the reaction takes place in the presence of GSH and levels of GSH-Px are closely related to those of GSH. SOD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide and therefore plays a key role in protecting cells from oxidative damage. In the present study, GSH, GSH-Px, and SOD levels fell down after feeding alcohol for 3 months. Yet the GSH and SOD levels in serum returned to those observed in the control group with the administration of *L. rhamnosus* CCFM1107. This was in agreement with Xing's study (Xing *et al.*, 2006) which reported that SOD levels in liver tissue increased with the administration of *Lactobacillus fermentum* and *Bifidobacterium catenulatum*. On the contrary, lower levels of GSH and SOD were noted in the *L. plantarum* CCFM1112 treated group, suggesting that it had little beneficial effect on alcoholic liver injury. This may be attributed to the higher antioxidative activity of *L. rhamnosus* CCFM1107 than *L. plantarum* CCFM1112. *L. rhamnosus* CCFM1107 performed almost the same as LGG in the terms of a protective effect on the recovery of liver injury, and was even superior to HGP to some extent. High antioxidative activity may explain the efficacy of *L. rhamnosus* CCFM1107.

Oxidative stress is one of pathogeneses of alcoholic liver disease. The CYP related enzymes, which are also known as cytochrome P450 super family, are activated by oxygen and result in generation of ROS. Among the cytochrome P450 family, CYP2E1 has been reported as the most relevant enzyme for alcoholic liver disease (Lieber, 1997). CYP2E1 can be induced by alcohol and accelerate the generation of ROS. Previous studies have shown that alcohol toxicity is reduced when CYP2E1 is inhibited or in CYP2E1$^{-/-}$ mice (Bardag-Gorce *et al.*, 2000). Recent work also has shown that *Lactobacillus acidophilus* and *Bifidobacterium bifidum* could down-regulate CYP enzymes in liver and ameliorate liver injury

Table 3. Levels of intestinal flora and serum endotoxins in different groups

Group	Enterococcus log (CFU/g)	Enterobacter log (CFU/g)	Lactobacilli log (CFU/g)	Bifidobacteria log (CFU/g)	Endotoxins (pg/ml)
Control	6.10±0.17	6.13±0.17	8.53±0.20	9.35±0.15	28.29±6.48
Alcohol	6.51±0.23	7.59±0.20[a]	7.90±0.21	8.14±0.26[a]	66.14±12.47[a]
Alcohol+*L. rhamnosus* CCFM1107	4.48±0.26[a,b]	4.52±0.20[a,b]	8.99±0.28[b]	9.89±0.16[a,b]	27.93±12.77[b]
Alcohol+HGP	6.30±0.19	7.03±0.24[a]	8.06±0.27	8.32±0.17[a]	54.35±13.34[a]
Alcohol+LGG	6.14±0.24	4.40±0.29[a,b]	9.56±0.21[a,b]	9.53±0.20[b]	25.96±10.14[b]
Alcohol+*L. plantarum* CCFM1112	5.54±0.20[b]	5.32±0.13[a,b]	8.72±0.22[b]	9.17±0.21[b]	36.28±13.12[b]

Results are expressed as mean ± SD (n=10). [a]$p<0.05$ vs Control group; [b]$p<0.05$ vs Alcohol group.

(Sharan and Kansal, 2011). In addition, there was decline observed in the CYP enzymes expression and in the CYP mRNA levels after orally administration with *Lactobacillus casei* (Matuskova *et al*., 2010). Therefore, the amelioration of alcoholic liver injury in the aspect of oxidative stress by *L. rhamnosus* CCFM1107 might relate to the down-regulation of CYP2E1 and/or other members of cytochrome P450 super family; the mechanism needs to be further demonstrated and elucidated.

Effects of lactobacilli treatment on intestinal flora and serum endotoxin

The effects of different lactobacilli and drugs on intestinal bacterial count of all the six groups were determined and the results were presented in Table 3. Compared to the control group, the mice that had been fed alcohol revealed an increased number of enterococci (6.51 vs 6.10 log CFU/g), enterobacter (7.59 vs 6.13 log CFU/g, $p<0.05$) and a reduced number of lactobacilli (7.90 vs 8.53 log CFU/g) and bifidobacteria (8.14 vs 9.35 log CFU/g, $p<0.05$). After supplementation of different samples, composition of the intestinal floral changed as follows: administration of *L. rhamnosus* CCFM1107, LGG and *L. plantarum* CCFM1112 decreased the levels of enterococci and enterobacter and increased levels of lactobacilli and bifidobacteria to varying degrees. In the *L. rhamnosus* CCFM1107 treated group, enterococci (4.48 vs 6.51 log CFU/g, $p<0.05$) and enterobacter (4.52 vs 7.59 log CFU/g, $p<0.05$) fell down dramatically, while lactobacilli (8.99 vs 7.90 log CFU/g, $p<0.05$) and bifidobacteria (9.89 vs 8.14 log CFU/g, $p<0.05$) significantly rised up in contrast with the alcohol group. Accordingly, the serum endotoxin levels were more than two-fold elevated in the alcohol group compared with the control group (66.14 vs 28.29 pg/ml, $p<0.05$). Endotoxin levels were largely reduced following the administration of lactobacilli and the levels in the *L. rhamnosus* CCFM1107 and LGG groups were even lower than those in the control group. However, no difference was found between the alcohol and the HGP group. There is available evidence that endotoxin plays a crucial role in alcohol-induced liver injury.

In alcoholic liver disease, interaction between gut microbiota and host liver is particularly interesting as alcohol was shown to both change the composition of the microbiota and impair intestinal integrity and barrier function (Szabo and Bala, 2010; Schnabl and Brenner, 2014). Alcohol consumption alters intestinal permeability and microbiota and results in increased levels of gut-derived bacterial endotoxin, which can enter the bloodstream and the liver (Nanji, 2002; Fukui, 2005; Forsyth *et al*., 2009; Yan *et al*., 2011). Recent studies have demonstrated that immune cells in liver are activated by bacterial endotoxin (LPS) through Toll-like receptor 4 to activate the NFκ-B pathway, induce pro-inflammatory cytokines and chemokines and finally result in liver disease (Uesugi *et al*., 2001; Petrasek *et al*., 2010). Among these cytokines and chemokines, TNFα, IL-1β, and IL-22 have shown to increase gut permeability, thereby in turn amplifying the alcohol-induced initial gut leakiness and liver disease process (Yoseph *et al*., 2013). Therefore, gut and liver constitute an interacted cycle which is known as gut-liver axis and this may be the underlying mechanism.

In our study, feeding *L. rhamnosus* CCFM1107 and LGG could rebalance intestinal bacterial composition and particularly increase the numbers of lactobacilli and bifidobacteria, which was consistent with Kirpich's study (Kirpich *et al*., 2008). Restoration of the enteric dysbiosis partly helps the host with liver injury to return to normal.

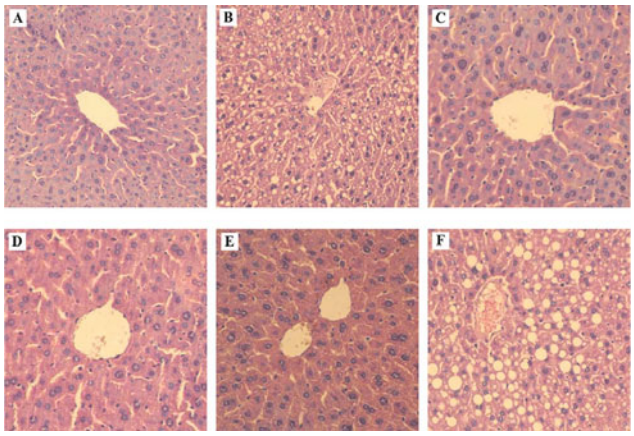

Fig. 3. Histopathological changes of mouse liver (Hematoxylin & Eosin staining at 200×) from the Control group (A), Alcohol group (B), Alcohol + *L. rhamnosus* CCFM1107 group (C), Alcohol + HGP group (D), Alcohol + LGG group (E), and Alcohol+ *L. plantarum* CCFM1112 group (F).

Mouse liver histological changes

Histological analysis of the mice liver was carried out, and photomicrographs of liver samples, which were embedded in paraffin and stained with hematoxylin and eosin, are shown in Fig. 3. The alcohol group demonstrated a dramatic increase in liver cellular lipid deposits as the representative pathologic change (Fig. 3B) compared with the control group (Fig. 3A). Feeding with *L. rhamnosus* CCFM1107 (Fig. 3C), HGP

(Fig. 3D), and LGG (Fig. 3E) could prevent hepatic lipid accumulation caused by alcohol and the overall morphology was almost the same as that of the control group. However, in the *L. plantarum* CCFM1112 treated group, typical steatosis was still observed after 3 months (Fig. 3F). Histological evaluation of liver sections indicated that *L. rhamnosus* CCFM1107 reduced the lipid accumlation and ameliorated the injury induced by long-term alcohol feeding. The histological analysis of liver tissue provides strong evidences to support the results described above. All of these factors contribute to the fact that *L. rhamnosus* CCFM1107 has a protective effect on the alcoholic liver injury.

Conclusion

In summary, the present study demonstrates that the intake of *L. rhamnosus* CCFM1107 might be beneficial for the prevention and improvement of alcohol-induced hepatic steatosis and damage, by decreasing the levels of oxidants and by increasing the levels of antioxidants, along with a decrease in the serum aminotransferase as well as lipid levels in serum and hepatic tissues. The histological assessment of liver tissue slices further confirmed our results. Moreover, the administration of *L. rhamnosus* CCFM1107 could restore and ameliorate the enteric balance, by increasing lactobacilli and bifidobacteria while decreasing enterococci and enterobacter, which would further reduce the levels of serum endotoxin. *L. rhamnosus* CCFM1107 was shown to have almost the same beneficial effects with LGG on alcoholic liver disease and to perform much better than the commercial drug HGP in lessening alcoholic steatohepatitis and improving the enteric microbiome. In contrast, *L. plantarum* CCFM1112 demonstrated few protective effects on liver injury, and the major difference between the two lactobacilli lies in their antioxidative activities. Thus, the underlying protective mechanism may be associated with the high antioxidative abilities and restoration of bowel flora that can be achieved with *L. rhamnosus* CCFM1107. In the future, the clinical trials of *L. rhamnosus* CCFM1107 for the treatment of alcohol-induced liver injury are needed to provide definitive evidence on its therapeutic efficacy.

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