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Effect of Zinc-enriched Yeast FF-10 Strain on the Alcoholic Hepatotoxicity in Alcohol Feeding Rats

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Abstract The possible protective effects of highly zinc-containing yeast *Saccharomyces cerevisiae*, FF-10 strain, isolated from tropical fruit rambutan on acute alcoholic liver injury in rats were evaluated. Zinc concentration in this strain was 30.6 mg%. The activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transpeptidase (γ-GTP) were highly increased when alcohol was treated, relative to the normal rats. Also, a highly significant increase in the blood alcohol and acetaldehyde levels by alcohol treatment was observed. Administration of FF-10 strain markedly prevented alcohol-induced elevation of the activities of serum ALT, AST, and γ-GTP, and the levels of blood alcohol and acetaldehyde, and these reduced levels reached to that of normal rats. As compared with alcohol treated control rats, the FF-10 strain supplementation showed highly decreased the triglyceride concentration in serum. Alcohol treatment induced the marked accumulation of small lipid droplets, hepatocytes necrosis, and inflammation, but FF-10 strain administration attenuated to alcohol-induced accumulation of small lipid droplets and hepatocyte necrosis in the liver. Therefore, the current finding suggests that zinc-enriched yeast FF-10 strain isolated from tropical fruit rambutan may have protective effect against alcohol-induced hepatotoxicity.

Keywords: *Saccharomyces cerevisiae* FF-10 strain, zinc, alcohol, hepatotoxicity

Introduction

Yeast has been utilized in the area of food production such as brewing, wine, and baking. *Saccharomyces cerevisiae* has long been used for producing alcoholic beverages, because this strain has potent ability to produce alcohol dehydrogenase (ADH) (1). Several yeasts contain bioactive components, such as amino acids, peptides, nucleotides, minerals, glutathione, and *S*-adenosylmethionine have been found to protect hepatic injuries which being induced various hepatotoxicants (2,3), and reduce body weight and serum lipid levels in animals (4). The high bioactive components producing yeast strains have been found in some species of yeast, *S. cerevisiae* and *Candida utilis* (5-7).

Several studies have tried to investigate the microorganism effect, especially yeast strain, on hepatic injury animal models induced by hepatotoxicants, such as alcohol, carbon tetrachloride, D-galactosamine, and flutamide (2-4,8). Previous study demonstrated that dietary supplemented with glutathione-enriched *S. cerevisiae* FF-8 strain, which is isolated from traditional Korean rice wine, protected against carbon tetrachloride-induced hepatotoxicity and oxidative stress in rats (9).

The determination of various inorganic elements in yeast is useful in pharmaceutical industry (2) and is useful for biological, nutritional, and toxicological studies (10). *S. cerevisiae* strain contains a number of the essential antioxidant trace elements, mainly selenium, zinc, together with yeast cell well mannans (11) and polysaccharide β-glucan (12). Zinc, an essential trace element, is found in almost all enzyme classes and is required to maintain the biological activities of numerous proteins (13). Zinc is also involved in lipid metabolism, insulin resistance, and body weight loss (14,15). Zinc has shown hepatoprotective effects against hepatotoxins, chlorpyifos, and bromobenzene (16,17). Therefore, we expected that a zinc-accumulating yeast strain would enhance suppressive effects against alcoholic liver injury, because zinc suppresses liver damage.

Rambutan (*Nephelium lappaceum* L.) is one of the native tropical fruit with sweet taste to Southeast Asia (18). A strain of yeast species was isolated from the juice of the rambutan fruit (19). We also isolated zinc-enriched yeast *S. cerevisiae* FF-10 from rambutan. There has not been information on the capacities of highly zinc-containing yeast strain isolated from tropical fruit rambutan to suppress liver injury. Thus, the current study has investigated the possible protective effects of orally administrated zinc-enriched yeast FF-10 strain isolated from rambutan fruit on acute alcohol-induced hepatotoxicity in rats.

Materials and Methods

Yeast strain and culture conditions Highly zinc-containing yeast strain, *S. cerevisiae* FF-10 isolated from the juice of tropical fruit rambutan used in this study. Yeast strain was aerobically cultured in a 5-L flask containing 1 L of the YM medium (glucose 1%, peptone 0.5%, yeast extract 0.3%, and malt extract 0.3%) at 30°C under the agitation at 200 rpm for 48 hr. The culture was centrifuged at 7,000×g for 15 min after the incubation then the supernatant was removed and the yeast cells were washed 3 times with distilled water. The harvested yeast cells were lyophilized

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Table 1. Compositions of experimental diets (%)

Group	Normal	Alcohol	
		Control	FF-10[1)]
Casein	20	20	15
Cornstarch	15	15	15
Sucrose	55	55	55
Cellulose	5	5	5
Corn oil	10	10	10
Mineral mixture[2)]	3.5	3.5	3.5
Vitamin mixture[3)]	1	1	1
Choline bitartrate	0.2	0.2	0.2
DL-Methionine	0.3	0.3	0.3
FF-10 strain	0	0	5

[1)]Yeast strain isolated from tropical fruit rambutan.
[2)]AIN 93 M-MX mineral mix, MP Biomedicals, Illkirch, France.
[3)]AIN 93 VX vitamin mix, MP Biomedicals, , Illkirch, France.

to prepare experimental diet. Zinc concentration in this strain was analyzed 30.6 mg% (per dry weight) by Inductively coupled plasma-atomic emission spectrophotometer (ICP-AES) (20).

Animal and experimental design Seven-week old male Sprague-Dawley rats were obtained from the Hyochang Science Animals Co. (Daegu, Korea). Animal was housed individually in the suspended wire-mesh stainless steel cage under room temperature between 21 to 24°C and lighting between 08:00 and 20:00. Animals were allowed to access freely to semi-purified basal diet for 1 week before the experiment. Animals were then randomly divided into 3 experimental groups based on dietary categories: the normal rats fed with water, the alcohol feeding control rats fed with alcoholic beverage containing ethanol 30%(v/v), the FF-10 strain supplemented rats fed with alcohol and FF-10 strain 5%(w/w). The equal amount of FF-10 strain supplementation was replaced with casein into the alcohol feeding control rats (Table 1). Overall, each group consisted of 6 rats that were fed appropriate diet for a 4-week period. The food consumption and water intake were measured with every day and body weight gain was measured with once a week. Animal care was followed by the National Institute of Health (NIH) guidelines on the care and use of laboratory animals.

Analytical procedure At the end of the experimental period, the rats were sacrificed by withdrawing blood from the abdominal aorta under light diethyl ether anesthesia. Serum was obtained by centrifuging the blood at 1,026×g for 15 min at 4°C. The concentrations of total lipid, triglyceride, total cholesterol, high density lipoprotein (HDL)-cholesterol, and the activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transpeptidase (γ-GTP) in serum were measured by Chemiclinical Chemistry Analyzer in Neodin Medicinal Institute (Seoul, Korea).

Hepatic alcohol metabolizing enzyme activity The ADH activity was assayed using Bergmeyer's method (21). The conversion of nicotinamide adenine dinucleotide (NAD, Sigma-Aldrich, St. Louis, MO, USA) to nicotinamide adenine dinucleotide phosphate hydrogenase (NADH), as a measure of ADH activity, was followed by recording the changes in absorbance at 340 nm for 5 min after the initiation of the enzyme reaction. The acetaldehyde dehydrogenase (ALDH) activity was assayed using the method of Koivula and Koivusalo (22).

Determination of blood alcohol and acetaldehyde concentrations Blood alcohol concentration was determined using a commercial UV-test kit (R-Biopharm Co., Ltd., Darmstadt, Germany). This enzymatic test for alcohol utilizes the coenzyme NAD and ADH. Formation of NADH can then be measured quantitatively by the increase in the absorbance at 378 nm. Blood acetaldehyde concentration was also determined using a commercial kit with ALDH as the enzyme.

ALDH zymogram staining analysis Gel electrophoresis of the hepatic cell-free extract for ALDH activity staining was conducted on nondenaturing polyacrylamide gel electrophoresis (PAGE) with 7.5% polyacrylamide gel at 4°C. The gel was then stained in a zymogram staining solution containing of 50 mM HEPES (pH 7.2), 1.5 mM NAD, 10 mM acetaldehyde, 0.06 mM phenazine methosulfate (PMS), 0.25 mM nitroblue tetrazolium at room temperature for 15 min (23). The active band in the staining appeared as a dark band.

Liver histopathological examination Liver was carefully removed and small fragments fixations for histomorphology were prepared with 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS, pH 7.4). The chemically fixed sample was embedded in paraffin then sliced at an approximate 6 μm thick for standard Hematoxylin & Eosin staining. The morphology of any lesions observed was classified and registered at the Anatomy Laboratory in the College of Medicine, Dong-A University, Busan, Korea.

Statistical analysis The data from animal experiments are presented as the mean±standard error mean (SEM), and were analyzed using one way analysis of variance (ANOVA), with the differences analyzed using the Duncan's new multiple-range test (24). A p value <0.05 was accepted as being a statistical significance of difference.

Results and Discussion

Zinc-enriched *S. cerevisiae* FF-10 yeast strain Highly zinc-containing strain, yeast FF-10 strain was isolated from the juice of tropical fruit rambutan, because zinc shows hepatoprotective effect by reduction in serum AST and ALT activities (25). The zinc concentration of yeast FF-10 strain used in the present study was 30.6 mg%. Zinc content in FF-10 strain was comparatively higher than other results obtained previously that the zinc concentration was active dry yeast by 5.2-6.7 mg% (20), beer brewing dried yeast by 4.0 mg% (4), and yeast *S. cerevisiae* strain by 0.55 mg% (26), and waste brewery yeast was identified as a trace element (27). The Zn concentration in the control diet was 3.01 mg%.

Table 2. Effect of yeast FF-10 strain on the body weight, food intake, and water consumption in alcohol feeding rats

Group	Normal	Alcohol	
		Control	FF-10[1)]
Initial body weight (g)	347.43±4.40[NS2)]	348.54±6.94	341.16±11.64
Final body weight (g)	416.61±6.58[a]	379.13±14.46[b]	357.08±7.29[b]
Food intake (g/day)	20.26±1.65[a]	10.65±1.35[b]	11.61±0.52[b]
Water consumption (mL/day)	29.08±1.25[ab]	25.83±0.83[b]	33.00±5.00[a]

[1)]Zinc-enriched yeast strain isolated from tropical fruit rambutan.
[2)]Values with different letters are significantly different at $p<0.05$ (mean±SEM, n=6); NS, not significant differences.

Table 3. Effect of yeast FF-10 strain on the relative tissues weight (%) in alcohol feeding rats

Group	Normal	Alcohol	
		Control	FF-10[1)]
Liver	2.66±0.09[NS2)]	2.95±0.03	2.79±0.07
Kidney	0.63±0.03[b]	0.71±0.02[a]	0.78±0.03[a]
Heart	0.34±0.01[NS]	0.36±0.00	0.35±0.02
Spleen	0.19±0.01[NS]	0.19±0.01	0.20±0.01
Pancreas	0.17±0.02[NS]	0.16±0.01	0.15±0.01

[1)]Zinc-enriched yeast strain isolated from tropical fruit rambutan.
[2)]Relative each tissue weight means the % of the each tissue weight in the body weight; values with different letters are significantly different at $p<0.05$ (mean±SEM, n=6); NS, not significant differences.

Effects on body weight, food intake, and water consumption Alcohol treatment showed a significant decrease in final body weight and food intake (Table 2). However, FF-10 supplementation in alcohol feeding rats was also decreased the final body weight and food intake, but was significantly increased the water consumption. The physiological effects of Brewer's yeast were observed previously that feeding of a diet containing 5% levels prevented obesity in mice (28). It was also reported that dried yeast dose-dependent reduced in body weight (4).

Effects on relative tissues weight Alcohol treatment rats resulted in a significant increase of the ratio weight between kidney and whole body weight (Table 3). But other tissues weights were not statistically significant different.

Effects on alcohol metabolizing enzyme activities Ethanol is readily absorbed from the gastrointestinal tract, circulated rapidly, and distributed uniformly throughout the body (29). Thereafter, 80-90% of the ethanol absorbed is rapidly oxidized to acetaldehyde by ADH and acetate by ALDH in the liver (30,31). Theoretically, the accumulation of acetaldehyde in the liver after chronic alcohol ingestion is determined by its formation and removal rates as catalyzed by ADH and ALDH, respectively (32,33).

In the current study, alcohol treatment rats resulted in a significant increase in hepatic ADH activity compared to the normal rats (Fig. 1), but it is higher in FF-10 strain supplementation in alcohol feeding rats compared to the alcohol feeding control rats. The ALDH activity was lowed by alcohol treatment control rats, but returned to the normal rat level by FF-10 strain supplementation (Fig. 1). Interestingly, both activities were altered by FF-10 strain supplementation.

The ADH and ALDH enzymes are considered to be essential for the metabolism of alcohol and the hypothesis has been made that these enzymes can be induced by the pharmaceutical action of some natural compounds. Yeast ADH is a tetrameric enzyme containing zinc (33). Yeast ADH activity is directly dependent on the presence of a catalytic zinc, this result confirms the deficiency of the zinc content in the commercial preparation, especially of the catalytic zinc atoms (34). Thus, these results indicating that

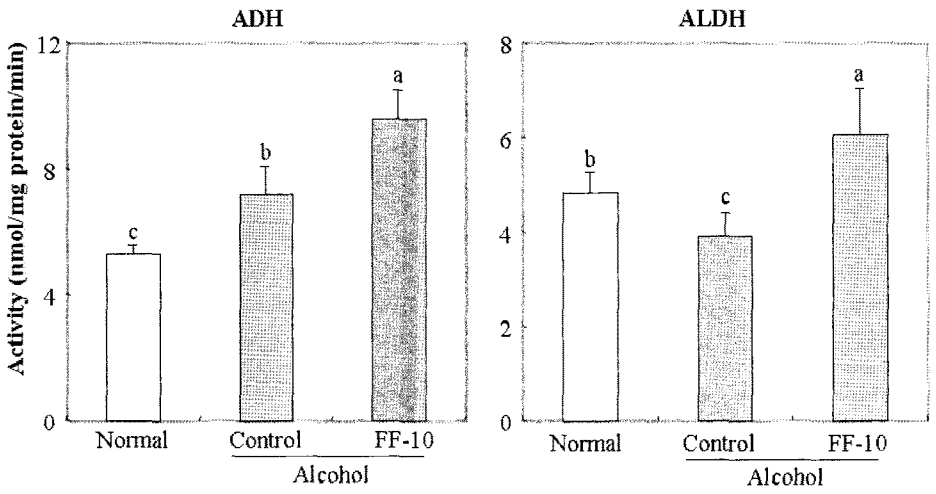

Fig. 1. Effect of yeast FF-10 strain on the hepatic activities of alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) in alcohol feeding rats. Values with different letters are significantly different at $p<0.05$ (mean±SEM, n=6).

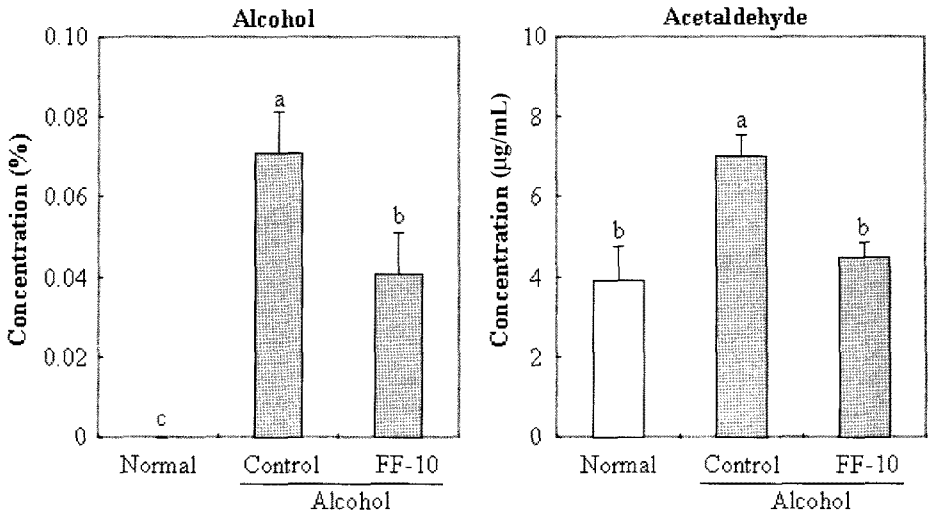

Fig. 2. Effect of yeast FF-10 strain on the serum concentrations of alcohol and acetoaldehyde in alcohol feeding rats. Values with different letters are significantly different at $p<0.05$ (mean±SEM, n=6).

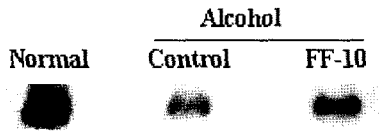

Fig. 3. Effect of yeast FF-10 strain on the acetaldehyde dehydrogenase (ALDH) zymogram active from hepatic cell-free extract in alcohol feeding rats.

supplementation of yeast FF-10 strain containing highly zinc, as catalytic atom of ADH activity was effective stimulating the liver ADH activity.

Effects on blood alcohol and acetaldehyde concentrations No blood alcohol was detected in normal rats (Fig. 2). Alcohol treatment caused a significant increase in blood alcohol concentration. However, the alcohol concentration was markedly lowered after FF-10 strain supplementation in alcohol feeding rats when compared the alcohol alone treatment, seemingly due to the increased ADH and ALDH activities mediated by the FF-10 strain supplementation.

ADH is the major metabolic enzyme for ethanol disposition in the liver (35). Nonetheless, even though ethanol can be efficiently converted into acetaldehyde by high ADH activity, as an ethanol-inducible enzyme, the subsequent conversion of acetaldehyde into acetate can be delayed due to low ALDH activity. However, FF-10 strain supplementation was found to enhance both ADH and ALDH activities, thereby enabling the conversion of ethanol into acetate via acetaldehyde and contributing to a lower blood alcohol concentration.

Effects on ALDH zymogram staining ALDH activity staining is shown in Fig. 3. The electrophoresis of ALDH from rat liver cell-free extract showed a single active band upon zymogram staining analysis and the staining intensity of the active band from rat liver fed with normal diet was stronger than that of alcohol feeding rats, but this was slightly stronger in yeast-feeding rats. The results showed a good correlation between the serum acetaldehyde concentration and the level of ALDH activity staining intensity in rats.

Effects on serum activities of ALT, AST, and γ-GTP ALT and AST levels increased with alcohol intake. These enzymes are well-documented indicators of hepatic dysfunction, with AST and ALT levels reflecting impaired liver function (9). The activities of ALT, AST, and γ-GTP were significantly increased by alcohol treatment (Fig. 4), confirming previous research (33,35). These activities by FF-10 strain supplementation in alcohol feeding rats were significantly decreased in comparison with those in alcohol feeding control rats and similar to those in the normal rats, indicating the hepatoprotective effect of yeast FF-10 strain (Fig. 4). However, AST activity tends to increase in alcohol feeding control rats. Izu *et al.* (3) reported that *sake* yeast feeding by 1% level suppresses ethanol-induced liver injury in mice by ethanol-induced increase in ALT was significantly attenuated. *S*-Adenosylmethionine (SAM)-accumulating *sake* yeast suppressed the ethanol-induced elevation of ALT (36). These results demonstrated that SAM yeast or *sake* yeast suppresses acute alcoholic-induced liver injury in mice. The previous study observed that pretreatment of rats with glutathione-enriched *S. cerevisiae* FF-8 strain had a markedly protective effect against carbon tetrachloride-induced hepatotoxicity, as evidenced by decreased serum ALT (9). Sugiyama and Yamamoto (2) also reported that the treatment with reduced glutathione-enriched extracts from *S. cerevisiae* showed dose-dependent hepatoprotective effects which were associated with decreased in serum AST and ALT activities. FF-10 yeast strain used in this study was also contained the glutathione concentration by 28.9 mg/L in our preliminary experiment (data not shown). The activities of AST and ALT are the most sensitive tests employed in the diagnosis of hepatic diseases (37). These enzyme activities were increase in the result of problem with liver metabolism and loss of liver cell by alcohol intake (37). Mannaa *et al.* (8) reported that Baker's yeast *S. cereviciae* (4.8 mg/kg BW) suppresses the flutamide-induced hepatotoxicity in male rats. Zinc supplementation was significantly reduced AST and ALT activities in crossbred cattle bulls (25). These results suggest the possibility of enriched zinc and glutathione containing yeast FF-10 strain treatment being an excellent

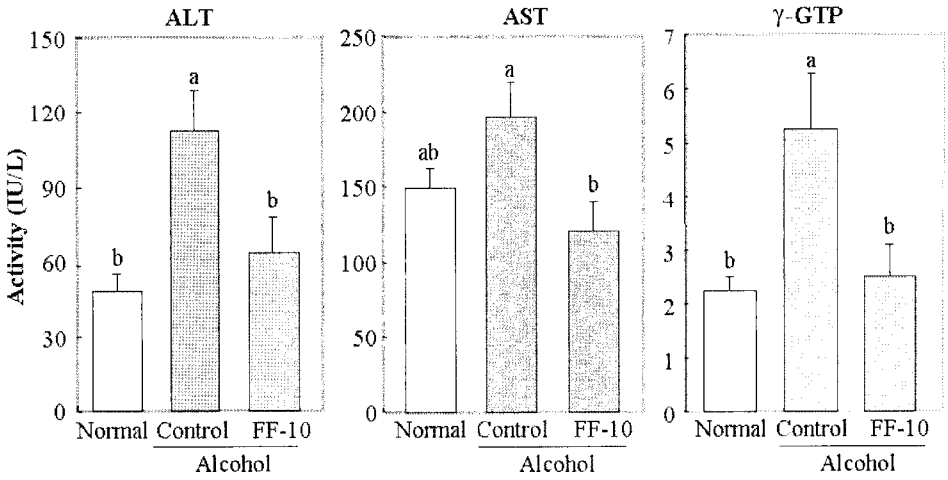

Fig. 4. Effects of yeast FF-10 strain on the serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltranspeptidase (γ-GTP), and in alcohol feeding rats. Values with different letters are significantly different at $p<0.05$. (mean±SEM, n=6).

Table 4. Effects of yeast FF-10 strain on serum lipids concentrations in alcohol feeding rats

Group	Normal	Alcohol	
		Control	FF-10[1)]
Total lipid (mg/dL)	279.98±20.94[ab2)]	234.33±10.56[b]	306.00±13.30[a]
Triglyceride (mg/dL)	54.25±1.33[b]	94.67±9.92[a]	37.00±5.75[b]
Cholesterol (mg/dL)	94.75±4.02[a]	76.33±3.93[b]	92.67±5.79[a]
HDL-cholesterol (mg/dL)	29.63±2.28[NS]	27.17±0.91	28.50±1.23

[1)]Zinc-enriched yeast strain isolated from tropical fruit rambutan.
[2)]Values with different letters are significantly different at $p<0.05$ (mean±SEM, n=6); NS, not significant differences.

candidate to ameliorate the hepatocytes damage induced by alcohol treatment.

Effects on serum lipid concentrations The concentration of serum total lipid showed a tendency to decrease slightly in alcohol treatment control rats, and this was significantly increased by FF-10 strain supplementation in alcohol feeding rats in comparison to normal rats (Table 4). Many reports indicate that alcohol intake significantly increases serum triglyceride level resulting in hypertriglyceridemia (3,38,39). The current study also showed that alcohol treatment significantly increased the concentration of serum triglyceride and this is in agreement with the results in the previous studies (3,38,39). However, the increased serum triglyceride concentration due to the alcohol treatment was significantly decreased by FF-10 strain supplementation and this decrease was reduced to below normal level (Table 4). *Sake* yeast feeding was also suppressed ethanol-induced elevation of serum triglyceride in mice (3). The total cholesterol concentration in the serum was significantly reduced in alcohol treatment control rats compared to normal rats, but FF-10 strain supplementation in alcohol feeding rats was increased the serum total cholesterol concentration. The serum concentration of HDL-cholesterol was not significantly differences in all animal groups. The current study demonstrated a possibility that yeast FF-10 strain would be able to decrease the serum triglyceride concentration by the simultaneous addition of alcohol.

Effects on liver histopathological investigation The current observations demonstrated that FF-10 strain effectively protected liver from the alcohol-induced hepatotoxicity by decreasing those serum ALT, AST, and γ-GTP activities. The evidential histological observations are also represented in Fig. 5. Alcohol treatment induced the marked accumulation of small lipid droplets, the degeneration of hepatocytes (open arrow), as evidenced by the mild inflammation (arrowhead), eosinophilic Mallory bodies (closed arrow), and the dilatation of sinusoids (asterisk) around the central veins (Fig. 5). The lipid droplets in the hepatocytes of alcohol feeding control rats were increased in numbers and volumes as the fatty liver progressions (5) and this was in agreement with the previous reports (33,40). But the current dietary FF-10 strain supplementation was attenuated to the accumulation of small lipid droplets and alcohol-induced mild inflammation and necrosis. However, normal rats revealed clear-cut hepatic lobules with the uniform pattern of the polyhedral hepatocytes radiating towards the periphery from the central vein. Hepatic tissues of yeast FF-10 strain supplementation in alcohol feeding rats, which has morphology similar to that of the normal rats except for the lipid droplets.

In summary, we expected that zinc-enriched yeast FF-10 strain isolated from tropical fruit rambutan would enhance the suppressive effect against alcoholic liver injury.

Acknowledgments

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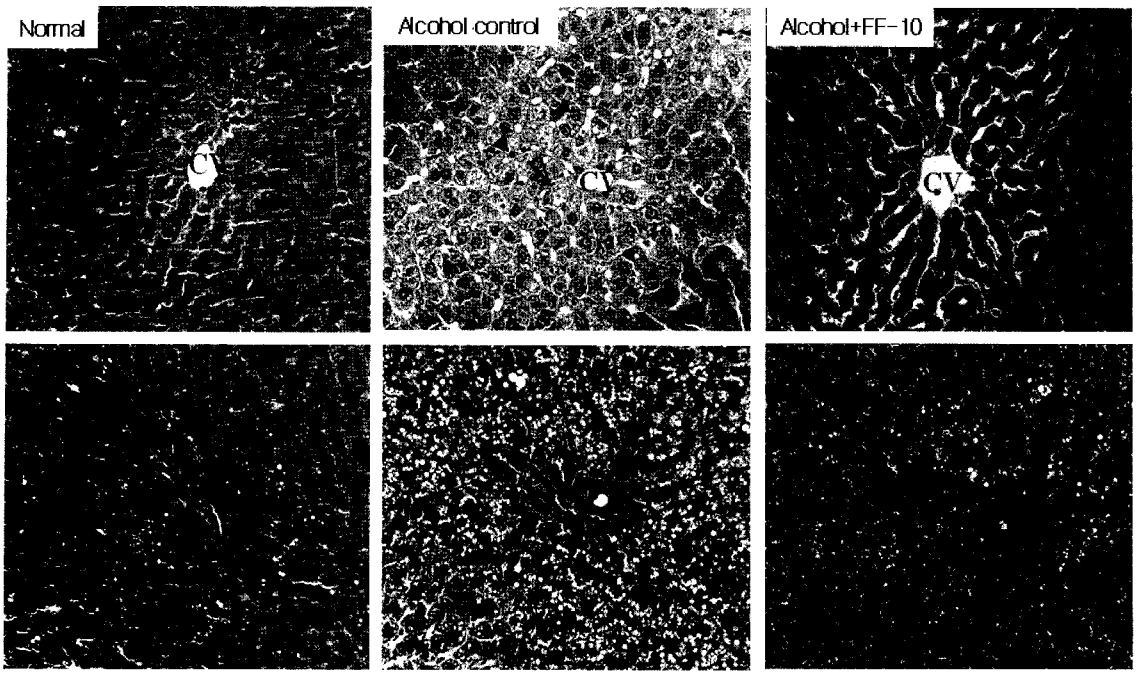

Fig. 5. Hepatic histopathologic changes in the normal, alcohol feeding control, and alcohol+FF-10 feeding rats. Hepatic histopathologic changes in alcohol treated rats (200×). CV, Central vein; asterisk, dilatation of sinusoids; closed arrow, mallory bodies; open arrow, degenerative signs of hepatocytes; arrowhead, inflammatory cell. Hepatocyte staining was carried out with the hematoxylin and eosin staining method.

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