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Alcoholic Hepatotoxicity Suppression in Alcohol Fed Rats by Glutathione-enriched Yeast FF-8 Strain

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Abstract The suppressive effects of glutathione-enriched *Saccharomyces cerevisiae* FF-8 strain (FF-8 GY) on alcohol-induced hepatotoxicity have been studied. FF-8 GY (256 mg/L) from the fermentation at a large scale bioreactor was used. Either of 5% FF-8 GY or 5% commercial glutathione-enriched yeast extract (GYE) with or without 30% alcohol was tested with rats for 4 weeks. FF-8 GY and GYE were found to reduce those alcohol-elevated serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) activities. Blood alcohol and acetaldehyde were also decreased by FF-8 GY and GYE. Interestingly, FF-8 GY drastically increased both hepatic alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) activities in comparison to GYE group, thus FF-8 GY would be more effective in blood alcohol and acetaldehyde reduction. Attenuated lipid droplet accumulation in hepatocytes was observed in both FF-8 GY and GYE when alcohol stimulated the accumulation. Therefore, FF-8 GY may be useful to protect liver from alcohol-induced hepatotoxicity.

Keywords: *Saccharomyces cerevisiae* FF-8 strain, glutathione, alcohol-induced hepatotoxicity, fermentation, rat

Introduction

Bioaccumulation capacities in microorganisms have been studied to obtain certain essential bioactive components for human health (1,2). Some species of yeast, such as *Saccharomyces cerevisiae* (1,2), *Candida utilis* (3), and *Yarrowia lipolytica* (4) have been found to produce high bioactive components. Determinations of those yeast-produced various organic elements are valuable in those studies of biology, nutrition, and toxicology and in pharmaceutical industry (5). Several studies (6-8) have also investigated the effects of yeast strains on hepatic injuries induced by hepatotoxicants, such as alcohol, carbon tetrachloride (CCl_4), acetaminophen, and flutamide. A various hepatotoxicants-induced liver injuries have been protected by a number of the essential bioactive components in *S. cerevisiae* strain which were mainly zinc (2), glutathione (7,9), *S*-adenosylmethionine (6), and amino acids (10). Our previous study with rats, recently, demonstrated that dietary supplementation of glutathione-enriched *S. cerevisiae* FF-8 strain had protective effects on CCl_4-induced hepatotoxicity and oxidative stress and the observation was performed under flask-scale fermentation of 204 mg/L glutathione in optimal YM medium. The protective effect of dietary zinc-enriched *S. cerevisiae* FF-10 strain isolated from tropical fruit rambutan (*Nephelium lappaceum* L.) on alcoholic hepatotoxicity in rats was also found previously. However, whether the massively cultured glutathione-enriched *S. cerevisiae* FF-8 strain, which was fermented in a large scale (100 L) with maximal YM medium, would show such protective effects on alcohol-induced hepatotoxicity in rats is not certain yet. Glutathione, a tripeptide composed of L-glutamate, L-cysteine, and glycine, has widely been used as medicine for the treatment of liver injury and as additives in functional health food (7). Recent studies have well demonstrated that glutathione-containing yeast extract powder showed dose-dependent hepatoprotective effects (7). Therefore, the present study has investigated and compared those protective effects between orally administrated glutathione-enriched *S. cerevisiae* FF-8 and glutathione-enriched yeast extract (GYE) on acute alcohol-induced hepatotoxicity in rats.

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Materials and Methods

Glutathione-containing yeast strain The used high glutathione-containing yeast *S. cerevisiae* FF-8 strain, isolated from Korean traditional rice wine, was obtained by a large scale fermentation (100-L bioreactor) medium containing glucose 3%(w/v), peptone 0.5%, yeast extract 3%, 0.06% KH_2PO_4, and L-cysteine 0.06% and the observed glutathione concentration in the product was 256 mg/L. The initial medium pH was adjusted to 6.0 and agitation speed was set at 200 rpm under inner pressure 0.8 kg_f/cm^2 with aeration rate 2.0 vvm at 30°C (9).

Animal experiments Four-week old male Sprague-Dawley strain rats (Hyochang Science Animals Co., Ltd., Daegu, Korea) were housed individually in the suspended wire-mesh stainless steel cage under controlled temperature (22±2°C) and humidity (50-60%) with 12 hr light/dark

Table 1. Compositions of experimental diets (%)

Group	Normal	Ethanol feeding		
		Alcohol	GYE[1)]	FF-8 GY[2)]
Casein	20	20	15	15
Corn starch	15	15	15	15
Sucrose	55	55	55	55
Cellulose	5	5	5	5
Corn oil	10	10	10	10
Mineral mixture[3)]	3.5	3.5	3.5	3.5
Vitamin mixture[4)]	1	1	1	1
Choline bitartrate	0.2	0.2	0.2	0.2
DL-Methionine	0.3	0.3	0.3	0.3
GYE	0	0	5	0
FF-8 GY	0	0	0	5

[1)]A commercially available glutathione-enriched yeast extract.
[2)]Glutathione-enriched yeast strain isolated from the Korean traditional rice wine as a our laboratory yeast (1).
[3)]AIN 93 M-MX mineral mix, MP Biomedicals, Illkirch, France.
[4)]AIN 93 VX vitamin mix, MP Biomedicals.

cycle condition. After 1 week of adaptation period for normal diet (Table 1), animals were randomly divided into 4 experimental groups which are normal, standard diet only; alcohol, 30% ethanol/water (v/v); GYE, 30% ethanol/water (v/v) plus 5%(w/w) commercially available glutathione-enriched yeast extract (Kohjin Co., Ltd., Tokyo, Japan); FF-8 GY, 30% ethanol/water (v/v) plus 5%(w/w) glutathione-enriched yeast *S. cerevisiae* FF-8 strain. Except those GYE and FF-8 GY treated group, 5% of casein was added more in the diet to equilibrate protein contents (Table. 1). A 4 weeks of experiment was conducted with 6 animals/group and daily feed intake and water consumption were recorded (Table. 2). The change of body weight was measured weekly. All treatments and procedures were conducted in accordance with Dong-A University (Busan) Lab Care Committee protocols for animal use.

Activities of liver marker enzymes The blood sample was collected from the abdominal aorta under light anesthesia with diethyl ether at the end of the experimental period. Serum was obtained with centrifugation at 1,026×g for 15 min at 4°C. The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) in the serum were estimated by using enzymatic methods with commercially obtained assay kits from the Chemiclinical Chemistry analyzer in the Neodin Medicinal Institute (Seoul, Korea).

Activities of hepatic alcohol metabolizing enzymes The liver sample was collected after the blood collection and it was homogenized in ice-cold 0.25 M sucrose solution containing 10 mM Tris (pH 7.4) and 1 mM ethylenediamine tetraacetate (EDTA) using with IKA-Ultra-TURRAX T25 basic homogenizer (Ika-Werke GMBH & Co., KG, Staufen, Germany). The alcohol dehydrogenase (ADH) activity was assayed using the method of Bergmeyer (11). The conversion of nicotinamide adenine dinucleotide (NAD, Sigma-Aldrich) to nicotinamide adenine dinucleotide 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 (12).

Concentrations of blood alcohol and acetaldehyde Serum 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 alcohol dehydrogenase. Formation of NADH can then be measured quantitatively by the increase in the absorbance at 378 nm. Blood acetaldehyde concentration was also enzymatically determined by using a commercial kit with ALDH.

Histopathological examination Liver tissues were 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 by using one-way analysis of variance (ANOVA), with the differences analyzed using the Duncan's new multiple range test (13). A p value <0.05 was accepted as the statistical difference.

Result and Discussion

Glutathione-enriched *S. cerevisiae* FF-8 strain A highly glutathione-enriched *S. cerevisiae* FF-8 strain was isolated from the Korean traditional rice wine and the concentration was 90 mg/L in the basal YM medium (1). The suppressive effects of FF-8 GY with the glutathione concentration at 204 mg/L in the optimal YM medium from flask-scale fermentation on CCl_4-induced hepatotoxicity by have been shown previously (data not shown). The currently observed the glutathione concentration in FF-8 GY culture by using large scale reactor (100-L) with maximal YM medium was 255.8 mg/L. This was much higher concentration than those observations from flask-scale fermentation with basal YM medium and optimal YM medium as 90 and 204 mg/L, respectively (9). The observed glutathione concentration in the current FF-8 strain was also higher than other previous reports that 64.7 mg/L in *S. cerevisiae* (14), 175 mg/L in *Candida* sp. (15), and 119.4 mg/L in yeast (16). Sugiyama and Yamamoto (7) demonstrated that high level of glutathione (i.e., 2% oxidized form plus 10.9% reduced form)-containing yeast extract powder had dose-dependent hepatoprotective effects from alcoholic hepatotoxicity, but low levels of glutathione (only 0.5% oxidized form)-containing bread yeast extract did not show any such effects. However, the currently observed high glutathione-containing yeast supplementation would lead more strong attenuation on alcoholic hepatotoxicity.

Table 2. Effects of FF-8 GY or GYE on the body weight gain, food intake, water consumption, and relative liver weight in alcohol feeding rats

Experimental group	Food intake (g/day)	Water consumption (mL/day)	Total body weight gain (g/4 weeks)	Relative hepatic weight (%, g/100 g BW)
Normal	19.20±2.01[a1)]	27.08±1.09[a]	63.8±3.00[a]	2.81±0.11[NS]
Alcohol	9.70±1.53[b]	15.77±0.75[b]	33.6±2.26[b]	2.59±0.09
Alcohol+GYE	7.72±1.09[b]	13.33±0.71[b]	44.52±11.12[ab]	2.76±0.05
Alcohol+FF-8 GY	8.47±1.27[b]	13.33±1.98[b]	48.52±2.52[ab]	2.62±0.23

[1)]Values with different letters represent the statistical difference at $p<0.05$ (mean±SE, $n=6$); NS, not significant.

Effect on body weight, food intake, water consumption and relative liver weight Changes of body weight gain, food intake, water consumption, and relative liver weight of current animal are shown in Table 2. Alcohol administration showed a significant decrease in body weight gain compared to the normal rats, but FF-8 GY or GYE supplementation tended to increase the body weight gain in alcohol feeding rats. A study somewhere else reported that body weight gain decreased in alcohol-treated rats. Dried yeast was found to reduce body weight dose-dependently (17). Food intake and water consumption were significantly lower in alcohol feeding all rats than those of normal rats. However, the relative liver weights between liver and body weight were not statistically significant differences among the groups. The similar findings in liver weight change were obtained as reported by Levy *et al*. (18) and that increase was suggested as a result of the accumulated lipids in the liver of the alcohol-treated rats. Also, our previous study showed that CCl_4-induced increases of total liver weight and its relative percentage were decreased (5.8%) numerically by FF-8 GY.

Activities of alcohol metabolizing enzymes The absorbed alcohol into bloodstream through the gastro-intestinal tracts (GIT) is circulated rapidly and then it is distributed uniformly throughout body (19). The absorbed alcohol in the liver is rapidly oxidized into acetaldehyde by ADH and then into acetate by ALDH (20,21). Theoretically, the accumulation of acetaldehyde in the liver, after the alcohol absorption, is determined by those rates of its formation and removal due to the catalytic reactions by ADH and ALDH, respectively (22,23). The ADH and ALDH are considered to be essential for the metabolism of alcohol in the liver and the hypothesis has been made that activities of these enzymes could be induced by the pharmaceutical action of some natural compounds. Those activities of hepatic ADH and ALDH in the current study are shown in Fig. 1. The hepatic ADH activity in the alcohol treated rats, as well as GYE and FF-8 GY supplemented animals, was higher than that in the normal rat (Fig. 1). This result agrees to the existing report that the ADH activity becomes increased more in rats with sub-acute alcoholism than rats with no alcohol (21). The hepatic ALDH activity was lower in alcohol treated rats than that in the normal rats (Fig. 1). The ALDH activity in the GYE supplemented rats was not different from that in the normal rats. Interestingly, FF-8 GY supplement drastically increased the hepatic ALDH activity when this was compared with other experimental groups (Fig. 1).

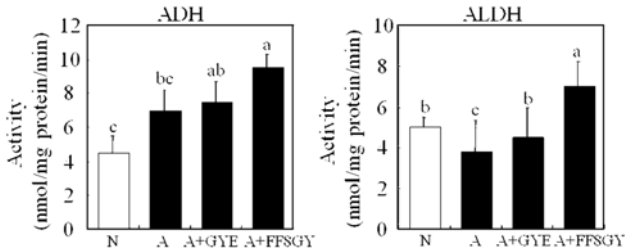

Fig. 1. Effects of FF-8 GY or GYE on the hepatic activities of alcohol dehydrogenase (ADH) and acetaldehyde (ALDH) dehydrogenase in alcohol feeding rats. Values with different letters indicate the significant difference at $p<0.05$ (mean±SE, $n=6$).

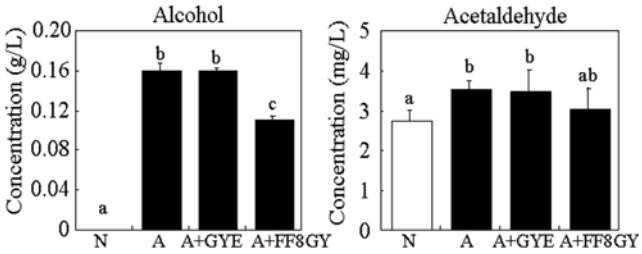

Fig. 2. Effects of FF-8 GY or GYE on the serum concentrations of alcohol and acetaldehyde in alcohol feeding rats. Values with different letters indicate statistical difference at $p<0.05$ (mean±SE, $n=6$).

Concentrations of blood alcohol and acetaldehyde No alcohol was detected in the blood of normal rat (Fig. 2). However, FF-8 GY reduced the level of alcohol-elevated blood alcohol where GYE did not affect the increased level of blood alcohol due to the administration of alcohol. The decrease of blood alcohol level by FF-8 GY supplement might be due to the increased activities of ADH and ALDH, thus FF-8 GY supplementation would be effective in the reduction of blood alcohol level. Because, ADH is a major metabolic enzyme for alcohol disposition in the liver (24) and the subsequent conversion of acetaldehyde into acetate would be delayed due to low ALDH activity, although alcohol can be converted effectively into acetaldehyde by ADH activity. Therefore, the current observation with FF-8 GY supplement that increased both ADH and ALDH activities would lead lower blood alcohol concentration throughout the conversion of ethanol into acetate via acetaldehyde.

Activities of ALT, AST, ALP, and LDH Alcohol-induced hepatic injury is a common model to screen

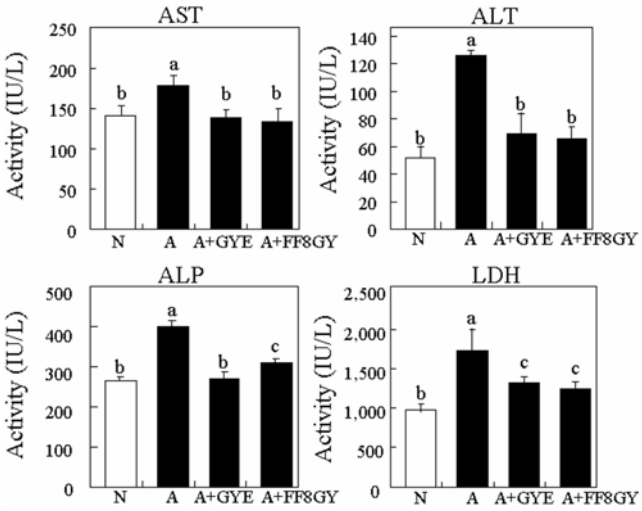

Fig. 3. Effect of FF-8 GY or GYE on the serum activities of AST, ALT, ALP, and LDH in alcohol feeding rats. Values with different letters represent the statistical difference at $p<0.05$ (mean±SE, $n=6$). AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; and LDH, lactate dehydrogenase.

hepatoprotective effects of medicine and functional health foodstuff (24). The activities of AST and ALT are well-documented indicators of hepatic dysfunction (6) because these enzyme activities would be increased when problems with liver metabolism and loss of liver cell by alcohol intake were occurred (25). In the present study, the increased activity of ALT by alcohol intake was observed (Fig. 3) with an agreement to those previous findings (23,24). The FF-8 GY or GYE supplementation significantly decreased the ALT activity in alcohol feeding rat (Fig. 3). Previous studies (26) have shown that several kinds of hepatoprotective components in yeasts suppressed alcoholic liver injury. *Sake* yeast and *S*-adenosylmethionine (SAM)-accumulating yeast have found to suppress ethanol-induced liver injury in mice by the significant attenuation of ethanol-induced ALT activity (6,26). The previous our study in rats observed that pretreatment of glutathione-enriched *S. cerevisiae* FF-8 strain had a marked protective effect that decreased serum ALT activity against CCl_4-induced hepatotoxicity (data not shown). Mannaa *et al*. (8) reported that baker's yeast *S. cerevisiae* (4.8 mg/kg BW) suppresses the flutamide-induced hepatotoxicity in male rats. Sugiyama and Yamamoto (7) reported that the treatment with reduced form of glutathione-enriched extracts from *S. cerevisiae* showed dose-dependent hepatoprotective effects and this was associated with decreased serum AST and ALT activities and recovery of liver glutathione levels from a high intraperitoneal acetaminophen dose-induced acute hepatotoxicity, but the hepatoprotective effect was not observed when low levels of glutathione-containing bread yeast extract was treated. High level of glutathione in yeasts has been introduced as a hepatoprotective agent (8,30,31). FF-8 GY used in this study also contained high concentration of glutathione which was 256 mg/L (15.06 mg/g of dry cell weight) and the observed concentration was similar to the observed glutathione concentration in *sake* yeast extracts (27) that was 13.7 mg/g of dry cell weight. The concentration of mitochondrial glutathione was decreased, however, after ethanol treatment (28). The glutathione consumption from glutathione-enriched yeast probably increased the hepatic glutathione level thus the protective effects against hepatotoxicity were shown after the alcohol treatment. The alcohol treatment tended to decrease the hepatic glutathione level compared with the normal rats with no alcohol in the current study (data not shown), but FF-8 GY feeding in alcohol treatment rats showed significantly high hepatic glutathione level as compared with alcohol fed rats. However, there no significant difference was found in serum AST activity among the experimental groups, except alcohol treated control rats showed a slightly increased tendency of serum AST activity. These observations indicated that the glutathione-enriched *S. cerevisiae* FF-8 GY possibly confer better performance to hepatoprotective effects on alcohol, CCl_4, and acetaminophen-like chemicals-induced acute hepatotoxicity. Several chemicals-induced hepatic injuries were also characterized by the increases of serum LDH and ALP activities (28). Several studies have also indicated the elevation of LDH and ALP levels in the serum by alcohol treatment would accurately be reflected as hepatic injury (12,29). The current study observed significantly increased levels of LDH and ALP activities in response to alcohol treatment, but this was decreased to the levels in normal rat by FF-8 GY supplementation (Fig. 3). Present results suggest that FF-8 GY may be a powerful candidate to ameliorate the hepatocytes damage induced by alcohol treatment.

Effect on liver histopathological investigation Fatty liver is one of the earliest consequences of alcohol abuse (28). The current observations demonstrated that FF-8 GY effectively protected the liver from alcohol-induced hepatotoxicity with those decreased activities of serum ALT, ALP, and LDH. The evidential histological observations are also represented in Fig. 4 and 5. The normal rats revealed the well-structured hepatic lobules with the uniform pattern of the polyhedral hepatocytes radiating towards the periphery from the central vein (Fig. 4). Liver histology of alcohol treatment rats showed pathomorphologic alterations and an increase in numbers and volumes of lipid droplets in the hepatocytes as the fatty liver progressions (Fig. 5), as described earlier (25). Treatment with FF-8 GY reduced the ethanol-induced morphological changes and the microanatomy of the liver was reverted to as well as normal rats and the numbers and volumes of lipid droplets in the hepatocyte were much smaller and hepatic steatosis was attenuated (Fig. 5). The mild fat accumulation by alcohol treatment was also observed in GY fed rats. The activity of FF-8 GY compares well with GYE and seems to be better in the histological abnormalities in the liver.

In summary, alcohol administration caused hepatotoxicity and high glutathione-containing FF-8 GY administration provided hepatoprotective activity by reducing those activities of hepatic biomarker enzymes. These results suggest that a highly glutathione containing *Saccharomyces cerevisiae* FF-8 strain may have positive effects for the protection from alcohol-induced hepatotoxicity.

The current investigation may be concluded that alcohol-induced liver injuries would be prevented or/and recovered

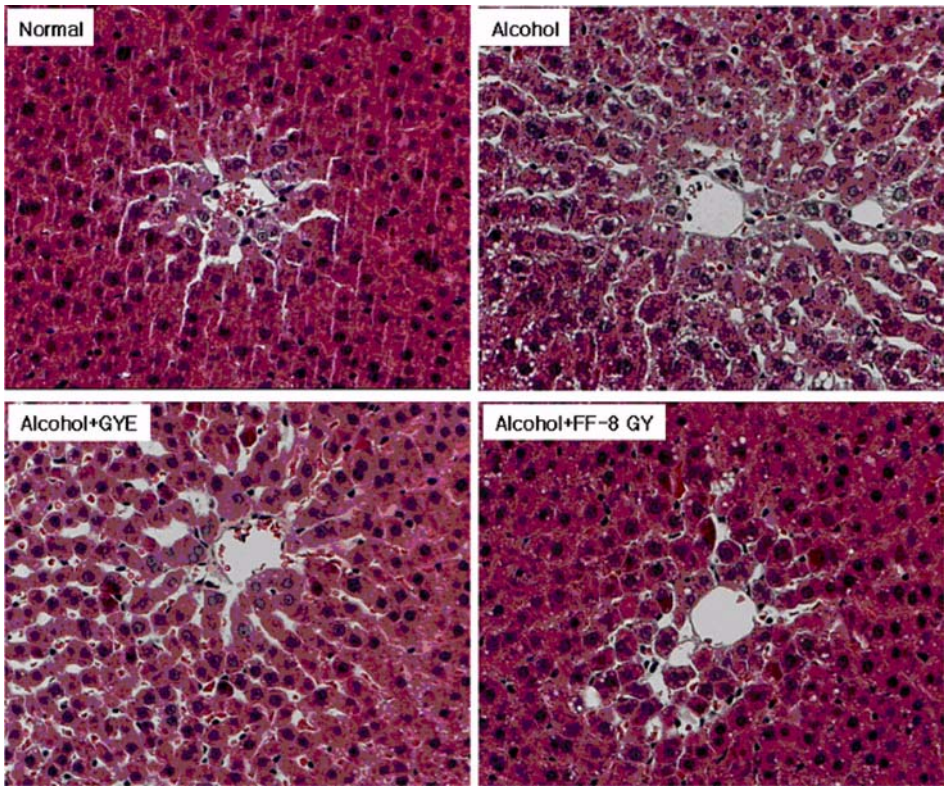

Fig. 4. Histopathologic changes in hepatic central vein by FF-8 GY or GYE in alcohol feeding rats. Magnification 250×.

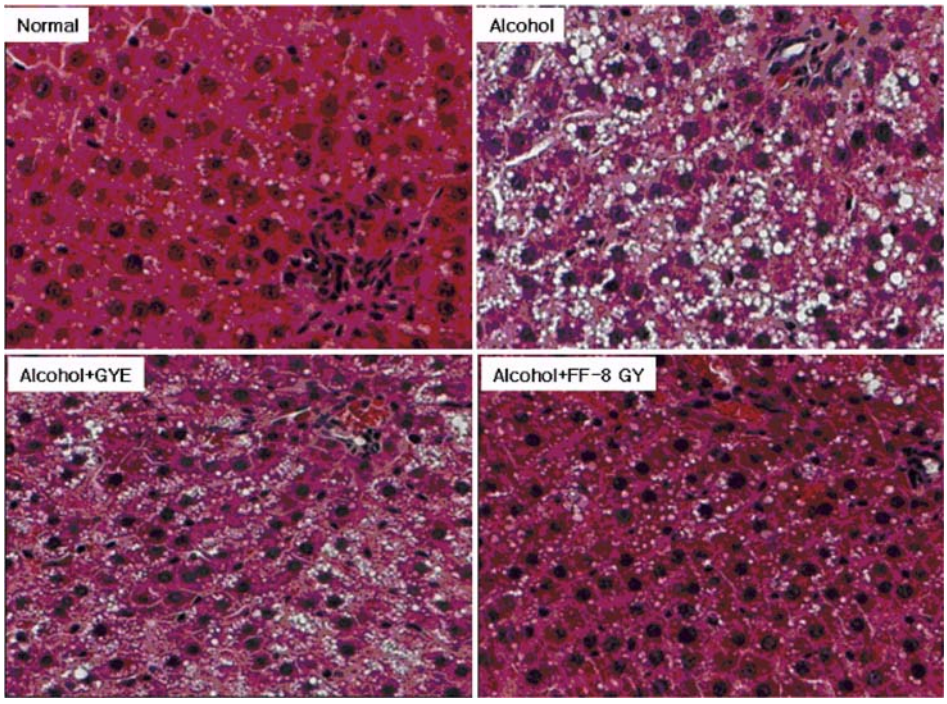

Fig. 5. Histopathologic changes in hepatic portal area by FF-8 GY or GYE in alcohol feeding rats. Magnification 250×.

in response to glutathione-containing microorganisms dosing. The involvements of protective mechanisms are not clear but reaction time regulation for enzymatic responses might be included as well as antioxidative responses. Therefore, chronic investigation would be necessary to see if better resolution could be achieved. However, the observed effects of massively produced glutathione-enriched microorganisms would allow some of positive opportunities for future developments and approaches in the treatment of alcohol-related diseases.

Acknowledgments

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References

1. Park JC, Ok M, Cha JY, Cho YS. Isolation and identification of the high-glutathione producing *Saccharomyces cerevisiae* FF-8 from Korean traditional rice wine and optimal producing conditions. J. Korean Soc. Agric. Chem. Biotechnol. 46: 348-352 (2003)
2. Cha JY, Heo JS, Kim JW, Lee SW, Cho YS. Isolation and identification of zinc-enriched yeast *Saccharomyces cerevisiae* FF-10 from the tropical fruit rambutan. J. Life Sci. 18: 447-457 (2008)
3. Wei G, Li Y, Chen J. Application of a two-stage temperature control strategy for enhanced glutathione production in the batch fermentation by *Candida utilis*. Biotechnol. Lett. 25: 887-890 (2003)
4. Strouhal M, Kizek R, Vacek J, Trnkova L, Nemec M. Electrochemical study of heavy metals and metallothionein in yeast *Yarrowia lipolytica*. Bioelectrochemistry 60: 29-36 (2003)
5. George AZ, Efthymia SR, Demetrius GT, John AS. Determination of mineral content of active dry yeast used in pharmaceutical formulations. J. Pharmaceut. Biomed. 28: 463-473 (2002)
6. Izu H, Shobayashi M, Manabe Y, Goto K, Iefuji H. *Sake* yeast suppresses acute alcohol-induced liver injury in mice. Biosci. Biotech. Bioch. 70: 2982-2989 (2006)
7. Sugiyama Y, Yamamoto K. The protective effect of glutathione-enriched yeast extract on acetaminophen-induced liver damage in rats. J. Jpn. Soc. Nutr. Food 51: 189-193 (1998)
8. Mannaa F, Ahmed HH, Estefan SF, Sharaf HA, Eskander EF. *Saccharomyces cerevisiae* intervention for relieving flutamide-induced hepatotoxicity in male rats. Pharmazie 60: 689-695 (2005)
9. Cha JY, Park SH, Heo JS, Park BK, Lee JW, Cho YS. Culture conditions for glutathione maximum production by *Saccharomyces cerevisiae* FF-8 in bioreactor. J. Life Sci. 18: 620-624 (2008)
10. Yin M, Ikejima K, Arteel GE, Seabra V, Bradford BU, Kono H, Rusyn I. Thurman RG. Glycine accelerates recovery from alcohol-induced liver injury. J. Pharmacol. Exp. Ther. 286: 1014-1019 (1998)
11. Bergmeyer HU. Methods of Enzymatic Analysis. Academic Press, New York, NY, USA. p. 28 (1974)
12. Koivula T, Koivusalo M. Different forms of rat liver aldehyde dehydrogenase and their subcellular distribution. Biochim. Biophys. Acta 397: 9-23 (1975)
13. Duncan DB. Multiple range test for correlated and heteroscedastic means. Biometrics 13: 164-176 (1957)
14. Wei G, Li Y, Chen J. Effect of surfactants on extracellular accumulation of glutathione by *Saccharomyces cerevisiae*. Process Biochem. 38: 1133-1138 (2003)
15. Shin WC, Kim DS, Yu JH, Yu JH, General microbiology, physiology, and metabolism; Isolation, identification, and culture condition of microorganism producing glutathione. Korean J. Appl. Microbiol. Biotechnol. 21: 1-5 (1993)
16. Li Y, Chen J, Zhou N, Fu W, Ruan W, Lun S. The effect of environmental conditions and glucose feeding in shaking flask on glutathione (GSH) production. Chin. J. Biotechnol. 14: 85-91 (1998)
17. Tachiyashiki K, Imaizumi K. Effects of dried yeast on the body weight, serum cholesterol levels, and perirenal and periepididymal adipose tissue cells in rats. J. Jpn. Soc. Nutr. Food Sci. 47: 219-226 (1994)
18. Levy RI, Bonnell M, Ernst ND. Dietary management of hyperlipoproteinemia. J. Am. Diet. Assoc. 58: 406-416 (1971)
19. Godde WH, Agarwal DP. Alcoholism of Medical and Genetics Aspects. Pergam Press, New York, NY, USA. pp. 3-57 (1989)
20. Lieber CS. Metabolism of ethanol, metabolic effects, and pathogenesis of injury. Acta Med. Scand. 703: 11-55 (1985)
21. Gill K, Amit Z, Smith BR. The regulation of alcohol consumption in rats: The role of alcohol-metabolizing enzymes-catalase and aldehyde dehydrogenase. Alcohol 13: 347-355 (1996)
22. Lee HC, Jung HS, Yi SH, Jung SY, Yoon HK, Kim CY. Association between polymorphisms of ethanol-metabolizing enzymes and susceptibility to alcoholic cirrhosis in a Korean male population. J. Korean Med. Sci. 16: 745-750 (2001)
23. Seo HJ, Jeong KS, Lee MK, Park YB, Jung UJ, Kim HJ, Choi MS. Role of naringin supplement in regulation of lipid and ethanol metabolism in rats. Life Sci. 73: 933-946 (2003)
24. Liever CS. Alcoholic liver injury: Pathogenesis and therapy in 2001. Pathol. Biol. 49: 738-752 (2001)
25. Park KJ, Kim HY, Chang BJ, Lee HH. Ameliorative effects of soy 11S protein on liver damage and hyperlipidemia in alcohol-fed rats. Biol. Pharm. Bull. 27: 1636-1641 (2004)
26. Izu H, Shobayashi M, Manabe Y, Goto K, Iefuji H. *Sake* yeast suppresses acute alcohol-induced liver injury in mice. Biosci. Biotech. Bioch. 70: 2488-2493 (2006)
27. Ohtake Y, Okumura Y. Establishing a high glutathione producing yeast species. Biosci. Ind. 50: 29-34 (1992)
28. Nagy LE. Molecular aspects of alcohol metabolism: Transcription factors involved in early ethanol-induced liver injury. Annu. Rev. Nutr. 24: 55-78 (2004)
29. Fernández-Checa JC, Colell A, Garcia-Ruiz C. *S*-Adenosyl-L-methionine and mitochondrial reduced glutathione depletion in alcoholic liver disease. Alcohol 27: 179-183 (2002)