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Alcohol-induced deterioration in primary antioxidant and glutathione family enzymes reversed by exercise training in the liver of old rats

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Abstract

Chronic alcohol consumption causes severe hepatic oxidative damage, particularly to old subjects by decreasing various antioxidant enzymes. In this study, we test the hypothesis that exercise training can protect the aging liver against alcohol-induced oxidative damage. Two different age groups of Wistar albino rats (3 months young, $n = 24$; 18 months old, $n = 24$) were evenly divided into four groups: control (Con), exercise trained (Tr, 23 m/min 30 min/day, 5 days/week for 2 months), ethanol drinking/treated (Et, 2.0 g/kg b.w. orally), and exercise training plus ethanol drinking/treated (Tr + Et). We found significantly ($P < .001$) lowered hepatic antioxidant enzymes including superoxide dismutase, catalase, selenium (Se)-dependent glutathione peroxidase (Se–GSH-Px), Se–non-dependent glutathione peroxidase (non-Se–GSH-Px), glutathione reductase, and glutathione S-transferase activities in aged rats compared with young. Age-related decrease in antioxidant enzyme status was further exacerbated with ethanol drinking, which indicates liver in aged rats is more susceptible to oxidative damage because of decreased free radical scavenging system in aged/old ethanol-drinking rats. However, the decrease in liver antioxidant enzymes status with ethanol consumption was ameliorated by 2 months exercise training in old and young rats. These results demonstrate that age-associated decrease in hepatic free radical scavenging system exacerbated by ethanol drinking. For the first time, we found that this deterioration was significantly reversed by exercise training in aging liver, thus protects against alcohol-induced oxidative damage.

Keywords: Aging liver; Ethanol; ROS; Free radical scavenging enzymes; Exercise; Oxidative stress

Introduction

Alcohol liver disease is known to associate with reduced antioxidant enzyme status (Gupta et al., 2003; Polavarapu et al., 1998). This disease is more prevalent in old alcohol-drinking subjects than young, which might be because of excessive reactive oxygen species (ROS) accumulation (Cahill et al., 2005; Meir and Seitz, 2008). In particular, chronic ethanol feeding leads to a decrease in the major antioxidant enzymes in liver, which includes superoxide dismutase (SOD), catalase (CAT), and glutathione family enzymes (Bailey et al., 2001; Das and Vasudevan, 2005; Jurczuk et al., 2006). In addition, the activities of these antioxidant enzymes are gradually reduced with age (Kumaran et al., 2008; Mallikarjuna et al., 2007). Age-dependent decrease in antioxidant status and changes in alcohol metabolism would affect susceptibility of the liver to alcohol-induced adverse effects (Kim et al., 2003; Seitz et al., 1992). Hence, elder individuals are more prone to alcohol-induced liver damage.

It is well established that regular exercise training enhances antioxidant status in many tissues including liver (Gunduz et al., 2004; Indira and Jhansi, 2001). Previous studies demonstrated that endurance exercise training can improve the reduced hepatic antioxidant enzymes in alcohol-treated rats (Duncan et al., 1997; Husain and Somani, 1997a). Another study showed no significant recovery of antioxidant enzymes in liver against alcohol toxicity by acute exercise (Husain and Somani, 1997b). However, these studies were performed in young animals, and the exact effect of exercise training on depressed hepatic antioxidant enzymes in aged alcohol-drinking rats remains unclear.

In light of the potential role of regular exercise training to cope the liver oxidative damage in young rats, it is important to investigate the impact of exercise training in aged alcohol-treated rats. Therefore, we proposed this study

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to determine the effect of exercise training on the activities of primary antioxidant and glutathione family enzymes in aged rats' liver with chronic alcohol drinking.

Materials and methods

Animal care and maintenance

Forty-eight male Wistar strain albino rats of two different age groups, that is, young (3 months, $n = 24$) weighing 170 ± 10 g and old (18 months, $n = 24$) weighing 240 ± 10 g, were used in the present investigation. This study was approved by the Institutional Animal Ethics Committee (Regd. No. 438/01/a/CP CSEA/dt.17.07.2001; resolution number 9/IAEC/SVU/2001/dt.04.03.2002). The rats in the 3-month age group were considered as "younger" and the 18-month age group were considered as "older" or adult rats as per the life span of Wistar strain (Jang et al., 2001). The rats were housed in clean polypropylene cages, six rats per cage and maintained under hygienic conditions, temperature-controlled room (25 ± 2°C) with a photoperiod of 12-h light and 12-h dark cycle. All the rats were fed with standard laboratory chow (Hindustan Lever Ltd, Mumbai, India) and water ad libitum.

Grouping and treatment

Age-matched rats were divided into four groups of six in each and treated as follows.

Group I—control (Con)

Six rats were put on a six-channel motor-driven treadmill for 5 days/week for a period of 2 months and given 2 m/min exercise for 5 min; the rats also received normal saline (0.9%) orally via an orogastric tube for equivalent handling.

Group II—exercise trained (Tr)

Six rats were given endurance exercise training on a six-channel motor-driven treadmill for 5 days/week for a period of 2 months at the running speed of 23 m/min, 30 min/day. All animals in both age groups completed 2-month period of exercise training protocol. The running program was scheduled between 6.00 and 8.00 a.m. Treadmill was custom-built at University Scientific Instrumentation Center, Sri Venkateswara University Campus.

Group III—ethanol drinking/treated (Et)

This group of rats received 20% of ethanol (vol/vol) at the dose of 2.0 g/kg bodyweight/day via an orogastric tube for a period of 2 months. Before treatment, the rats were subjected to exercise training on a six-channel motor-driven treadmill as described in group I for equivalent handling.

Group IV—exercise plus ethanol (Tr + Et)

In this group, six rats were exercised on a treadmill for 30 min as described in group II, and the same rats received 20% of ethanol as described in group III after exercise training.

All the animals were sacrificed after 24 h of the last treatment, and the liver tissue was excised. The liver tissue obtained from each animal was immediately blotted to remove the excess blood, and then repeatedly washed with ice-cold buffered saline. Because blood contains high levels of antioxidant enzymatic activity, it may interfere with actual liver antioxidant capacity, if adhered blood was not removed completely. Hence, special care has been taken, and the liver tissue was thoroughly rinsed with saline, then immediately immersed in liquid nitrogen and stored at −80°C for further biochemical analyses.

Reagents and chemicals

All the chemicals used in the present study were Analar (AR) grade and obtained from the following scientific companies: Sigma (St. Louis, MO), Fisher (Pittsburg, PA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), and Qualigens (Mumbai, India).

Antioxidant enzyme assays

Hepatic SOD (SOD—extracellular [EC]: 1.15.1.1) activity was determined by the method of Misra and Fridovich (1972). The absorbance was measured at 340 nm for 4 min, and the activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to one unit. CAT (CAT—EC: 1.11.1.6) activity was monitored in the liver homogenates as described by Aebi (1984) with Triton X-100 by measuring the absorbance at 240 nm for 60 s on a UV-Spectrophotometer (Hitachi U-2000). Glutathione family enzymes, selenium (Se)-dependent and Se-non—dependent glutathione peroxidase (GSH-Px—EC: 1.11.1.9) activities were assayed according to the modified versions of Flohe and Gunzler (1984) and Reddy et al. (1981), respectively. Glutathione reductase (GR—EC: 1.6.4.2) activity was measured by the method of Carlberg and Mannervik (1985) by monitoring the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm for 3 min. Another important glutathione family enzyme, glutathione S-transferase (GST—EC: 2.5.1.18) activity was assayed with its conventional substrate, 1-chloro 2, 4-dinitro benzene (CDNB) at 340 nm as described by Habig et al. (1984).

Statistical analysis

Statistical analysis has been carried out by 2^2 factor design analysis of variance using Microsoft Office Excel and GraphPad InStat statistical packages. The data have been analyzed for the significance ($P < .05$) of the main effects, age, and treatments along with their interactions. Tukey's multiple comparison test was used for individual group comparison in both age groups. All the data were expressed as the mean ± standard deviation with six replications.

Results

Alterations in SOD and CAT activities

In this study, SOD, the hepatic primary antioxidant enzyme activity was decreased (43%) as age increased (Fig. 1). In addition, we found that alcohol significantly ($P < .001$) decreased SOD activity in young rats, and this reduction was further continued in old rats. However, exercise training reversed the alcohol adverse effect as shown by increased SOD activity in both young and old alcohol-drinking rats. Notably, this elevation with exercise was higher in old alcohol-drinking rats (33%) than in young alcohol-drinking rats (29%).

Liver CAT activity was significantly ($P < .001$) decreased from young to old age, and also with 2-month ethanol treatment when compared to their respective controls (Fig. 2). The decreased CAT activity with ethanol drinking was more in old rats (44%) than that of young rats (32%). Interestingly, increased CAT activities were noticed with exercise training against alcohol effect in combined treated group. However, the recovered CAT values with exercise combination were lower in old (26%) than the young (44%) aged rats.

Alterations in glutathione family enzyme activities

In our study, changes in Se−GSH-Px activities were similar with that of changes in Se-non−GSH-Px activity (Figs. 3 and 4). Both forms of liver GSH-Px activities were significantly ($P < .001$) lower in older control rats and also with alcohol intoxication. The values of Se−GSH-Px with ethanol feeding were dropped from 0.354 to 0.190 (micromoles of NADPH oxidized/milligrams of protein) in old rats; and from 0.814 to 0.675 (micromoles of NADPH oxidized/milligrams of protein) in young rats. In this study, treadmill exercise training given to ethanol-treated rats exhibited significantly increased both forms of GSH-Px activities. With exercise combination treatment, young rats

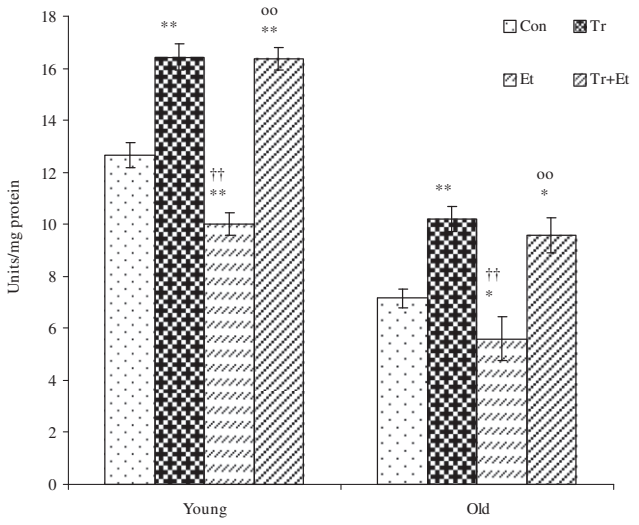

Fig. 1. Effect of exercise training (Tr), ethanol drinking/treatment (Et), and combination of both (Tr + Et) on hepatic superoxide dismutase activity (units/milligrams of protein) in young and old male albino rats. The values are significant compared to the following: control (**$P < .001$; *$P < .01$), exercise ($^{\dagger\dagger}P < .001$), and ethanol ($^{oo}P < .001$). The results are expressed as mean ± standard deviation with six replications. Con = control.

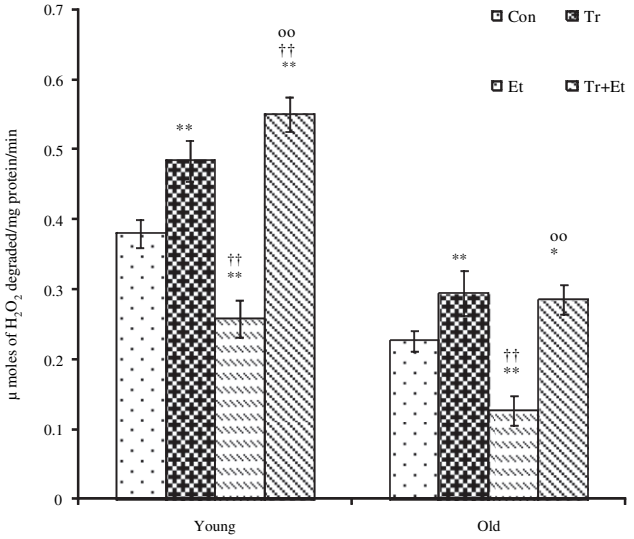

Fig. 2. Effect of exercise training (Tr), ethanol drinking/treatment (Et), and combination of both (Tr + Et) on hepatic catalase activity (micromoles of hydrogen peroxide [H_2O_2] degraded/milligrams of protein/min) in young and old male albino rats. The values are significant compared to the following: control (**$P < .001$; *$P < .01$), exercise ($^{\dagger\dagger}P < .001$), and ethanol ($^{oo}P < .001$). The results are expressed as mean ± standard deviation with six replications. Con = control.

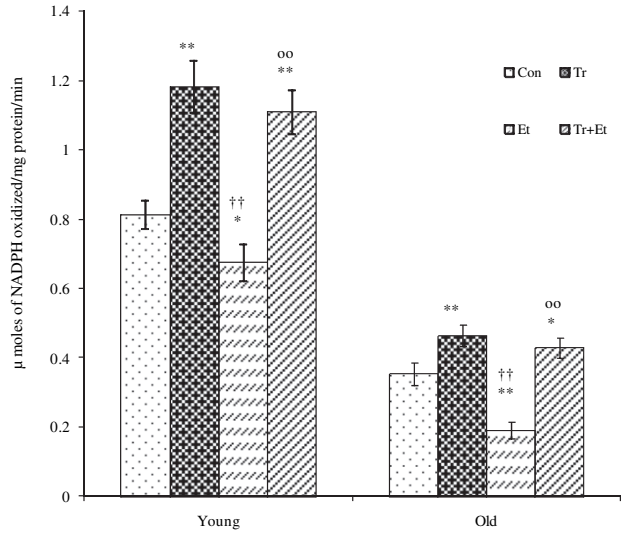

Fig. 3. Effect of exercise training (Tr), ethanol drinking/treatment (Et), and combination of both (Tr + Et) on hepatic selenium-dependent glutathione peroxidase activity (micromoles of nicotinamide adenine dinucleotide phosphate [NADPH] oxidized/milligrams of protein/min) in young and old male albino rats. The values are significant compared to the following: control (**$P < .001$; *$P < .01$), exercise ($^{\dagger\dagger}P < .001$), and ethanol ($^{oo}P < .001$). The results are expressed as mean ± standard deviation with six replications. Con = control.

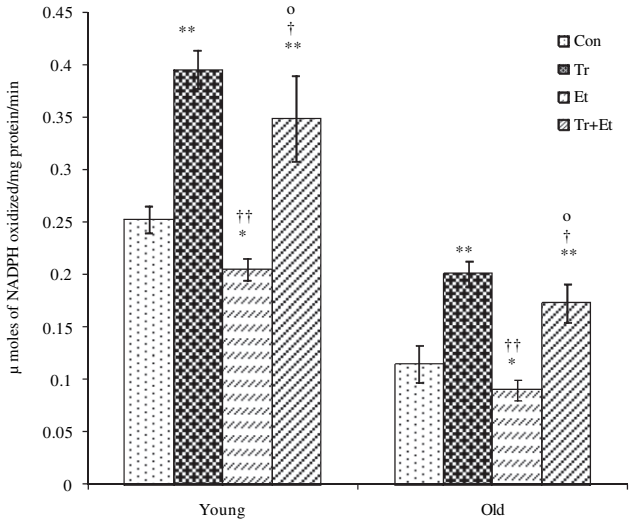

Fig. 4. Effect of exercise training (Tr), ethanol drinking/treatment (Et), and combination of both (Tr + Et) on hepatic selenium-nondependent glutathione peroxidase activity (micromoles of nicotinamide adenine dinucleotide phosphate [NADPH] oxidized/milligrams of protein/min) in young and old male albino rats. The values are significant compared to the following: control (**$P < .001$; *$P < .05$), exercise ($^{\dagger\dagger}P < .001$; $^{\dagger}P < .05$), and ethanol ($^{oo}P < .001$; $^{o}P < .05$). The results are expressed as mean ± standard deviation with six replications. Con = control.

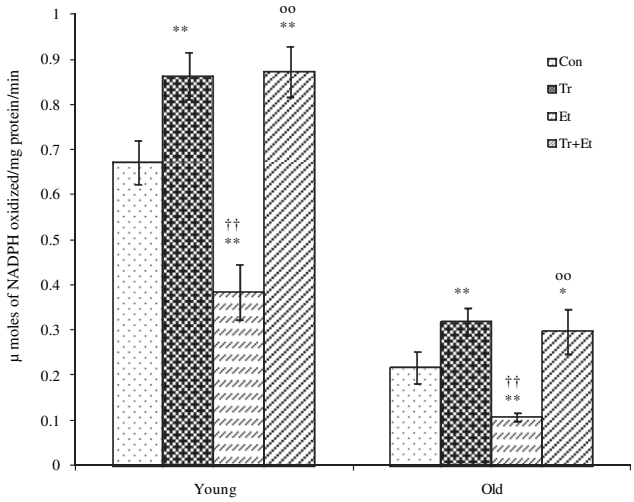

Fig. 5. Effect of exercise training (Tr), ethanol drinking/treatment (Et), and combination of both (Tr + Et) on hepatic glutathione reductase activity (micromoles of nicotinamide adenine dinucleotide phosphate [NADPH] oxidized/milligrams of protein/min) in young and old male albino rats. The values are significant compared to the following: control (**$P < .001$; *$P < .01$), exercise ($^{\dagger\dagger}P < .001$), and ethanol ($^{oo}P < .001$). The results are expressed as mean ± standard deviation with six replications. Con = control.

showed better upturn (36%) than old rats (21%) for Se−GSH-Px activity. The recovery of non-Se−GSH-Px activity was more in old rats (50%) than that of young rats (37%) by exercise plus alcohol combination treatment.

The present data revealed that the sister antioxidant enzyme GR activity was lower in old rats compared with young rats (Fig. 5). In addition, ethanol-induced decrease in GR activity in young rats was mimicked by old rats, and this was more pronounced in old (50%) than young rats (42%). The activity of GR with exercise plus ethanol combination was significantly ($P < .001$) increased to above control levels in both ages, and this was higher in old rats than young rats.

Estimated hepatic GST activity with interactive treatments was represented in Fig. 6. The activity of GST in liver was decreased drastically from young to old (5.59−1.14 μmol CDNB−GSH conjugated/milligrams of protein) in course of advancing age. In contrast to other antioxidant enzymes, we observed increased GST activity with alcohol administration. However, no significant change in GST activity was noticed with exercise plus ethanol interactive treatment when compared with ethanol alone group.

Discussion

The most important finding of the present study was that age-dependent decrease in free radical scavenging system was deteriorated with alcohol drinking, and this was reversed by exercise training. Liver is the major metabolic site for the alcohol metabolism. Chronic ethanol consumption and aging are the two factors leading to decrease in the hepatic antioxidant enzyme activities (Kim et al., 2003; Mallikarjuna et al., 2007). It is generally known that aged animals are more vulnerable to liver oxidative damage with alcohol consumption than young animals, which could be because of profound decrease in antioxidant enzyme activities (Cahill et al., 2005; Meir and Seitz, 2008). Although reports exist on the recovery of antioxidant enzymes by exercise training in young alcohol-drinking rats (Husain and Somani, 1997a), it is unclear in the liver of old alcohol-treated rats. In our study, we used old animals at

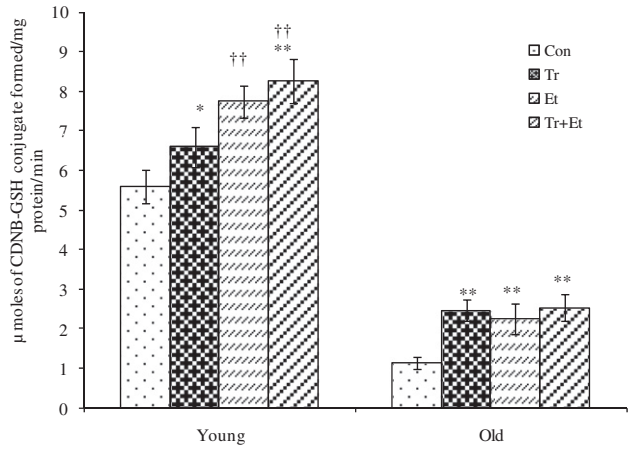

Fig. 6. Effect of exercise training (Tr), ethanol drinking/treatment (Et), and combination of both (Tr + Et) on hepatic glutathione S-transferase activity (micromoles of 1-chloro 2, 4-dinitro benzene glutathione peroxidase [CDNB-GSH] conjugate formed/milligrams of protein/min) in young and old male albino rats. The values are significant compared to the following: control (**$P < .001$; *$P < .01$) and exercise ($^{\dagger\dagger}P < .001$). The results are expressed as mean ± standard deviation with six replications. Con = control.

the age of 18 months and observed a consistent reduction in SOD, CAT, Se–GSH-Px, non-Se–GSH-Px, and GR enzyme activities after chronic alcohol drinking. For the first time, we found that this reduction was significantly reversed by exercise training in aging liver.

In our study, age-related decrease in SOD activity supports the long-standing hypothesis that generation of ROS could be increased with age. This reduction in SOD activity may be because of ROS-induced enzyme degradation or reduced synthesis as age advances (Indira and Jhansi, 2001). Alcohol consumption further decreased the SOD activity in old rat liver. Our earlier findings and other studies also demonstrated that enhanced xanthine oxidase activity during ethanol oxidation in liver may have triggered the superoxide anion production, which eventually affects SOD activity (Kato et al., 1990; Mallikarjuna et al., 2009). Similar decrease in SOD activity was also reported by previous studies in the liver of rats with chronic ethanol feeding (Bindu et al., 2002; Duncan et al., 1997). The drop in SOD activity indicates inefficient scavenging of superoxide radicals (Pigeolet et al., 1990). However, increased SOD activity with moderate exercise training in alcohol-drinking rats avoids the accumulation of superoxide anion radicals. This may be a considerable adaptation to mitigate the ethanol toxicity in old rats. In contrast, Husain and Somani (1997b) reported that combination of acute exercise plus ethanol decreased SOD in liver. This divergent result might be because of the variations in exercise protocol and dosage and/or animal model.

CAT can detoxify the hydrogen peroxide (H_2O_2) to less toxic H_2O and O_2. Aging itself, and exposure of aging organism to life-threatening condition like alcohol, aggravates the antioxidant enzyme homeostasis by diminished CAT activity. In such condition, accumulated H_2O_2 might be converted to highly reactive substance hydroxyl radical ($^{\bullet}OH$), and further caused to form the acetyl and alpha-hydroxyethyl radicals, which are dominant deleterious species that cause oxidative damage (Bindu et al., 2002; Knecht et al., 1993). We reported the increased CAT activity in old alcohol-drinking rats after exercise training, which suggests its active involvement in decomposition of accumulated H_2O_2, thus protecting the old liver cells. Because of CAT participation in ethanol metabolism (Zima et al., 2001), increased CAT activity was expected with exercise training along with increased other metabolic enzymes. Duncan et al. (1997) found that running exercise may prevent an ethanol-induced increase in risk for liver cancer by improving the antioxidant status including CAT activity in rats. A change in the binding characteristics of enzyme to membrane or their release from peroxisomes has been proposed as a possible mechanism for the increased activity of CAT (Somani and Ryback, 1996).

It is well known that both Se-dependent and Se-non–dependent GSH-Px enzymes play a prominent role in removal of toxic hydroperoxides from the cells. In the present study, both forms of GSH-Px activities were significantly decreased with chronic alcohol consumption in young and old rats. Studies performed on rats found that ethanol feeding exacerbates mitochondrial ROS production in hepatocytes and decreases the levels of GSH and GSH-Px activity in the hepatic mitochondrion (Bailey et al., 2001; Hirano et al., 1992). Comparably, the decrease in Se–GSH-Px activity with ethanol feeding was more exaggerated in old liver. In addition to excessive ROS production, alcohol can also reduce the availability of hepatic Se content, which is essential for Se–GSH-Px (Ojeda et al., 2009). Furthermore, Se concentration was gradually decreased with advancing age (Savarino et al., 2001). Because of these reasons, old alcohol-drinking rats exhibit lower Se–GSH-Px activity than young alcohol-drinking rats. Our results support that older animals are more susceptible to alcohol-induced oxidative stress than younger animals. Slower metabolism of alcohol and its byproducts in old rats might be responsible for alcohol-induced hepatic damages. Age and alcohol-induced decrease in GSH-Px activity might be because of the inactivation of enzyme molecules by excessive ROS or depletion in its cosubstrates (GSH and NADPH) or both can occur (Chandra et al., 2000; Kumaran et al., 2008). Interestingly, we reported that both forms of GSH-Px activities were restored with exercise training against ethanol toxicity. We speculate that enhanced ethanol metabolism (El-Sayed et al., 2005; Suter et al., 1992) and increased GSH content (Mallikarjuna et al., 2009) during exercise training may be possible reasons for increased GSH-Px activities in the liver. Increase in both forms of GSH-Px activities in liver of old rats could be a compensatory response to scavenge the hydroperoxides that are generated during ethanol metabolism.

Additionally, alcohol-induced adverse effect on GR, another glutathione family enzyme, was reported as decreased activity with ethanol intoxication, and this decrease was further exacerbated with aging. Similar to our results, Jurczuk et al. (2006) reported decreased GR activity in the liver of rats exposed to 5 g/kg bodyweight of ethanol. Another dose-dependent study showed significant decrease in liver GR activity with increasing ethanol doses (0.8, 1.2, 1.6, 2 g/kg bodyweight) for 4-week treatment in rats (Das and Vasudevan, 2005). The decreased GR activity may lead to alter the crucial GSH/glutathione disulphide ratio and cause oxidative damage to the liver cells. Age-dependent delay in ethanol metabolism and decrease in GSH content in old rats would be responsible for the enhanced toxicities from chronic ethanol consumption (Mallikarjuna et al., 2007; Rikans et al., 1990). Nonetheless, we found increased GR activity with exercise alone and also with ethanol combination in both age groups. It is known that exercise training can enhance the ethanol metabolism via induction of microsomal cytochrome P450 II E1 along with cytosolic alcohol dehydrogenase and also facilitates the elimination and clearance from the body (Ardies et al., 1994; El-Sayed et al., 2005). In addition to these, increased body temperature and GSH content with exercise may also be responsible for increased GR activity in liver (Mallikarjuna et al., 2009;

Suter et al., 1992). Thus, training ameliorates the ethanol-induced oxidative injuries in liver, even in old age rats.

In contrast to other antioxidant enzymes, we observed the increased GST activity with alcohol feeding. It was shown that oxidants may activate gene expression through the antioxidant responsive elements via electrophilic thiol modification (Rushmore et al., 1991), and thus it might be speculated that the overexpression in the enzyme proteins took place during alcoholic stressed condition (Skrzydlewska et al., 2005). The elevated GST activity in liver of ethanol-fed rats could be considered as an adaptive response to protect the tissues against ethanol-induced oxidative stress (Das and Vasudevan, 2005; Oh et al., 1997). In our study, we observed that age-induced reduction in GST activity was reversed by exercise training. Previous studies reported the increased hepatic GST activity with exercise training (Sen et al., 1992) and also with exercise plus ethanol combined treatment (Duncan et al., 1997). These results indicate that the stress of ethanol and exercise, when combined, is sufficient to enhance the GST activity in liver. Exercise-induced higher GST activity in old rats might be helpful to remove the toxic peroxides and may protect the aged liver tissue from damage.

In conclusion, generation of ROS represents an underlying component of cellular aging, and antioxidant enzyme system plays a protective role against this adverse process. Alcohol drinking drastically decreased the enzyme activities in the aged liver of the antioxidant and glutathione family, which means older animals are more prone to alcoholic liver damage. For the first time, here we demonstrated that exercise training significantly reversed this adverse condition and protected the aged liver. The present study provides additional knowledge about the benefits of exercise training against the negative lifestyle factor (alcohol), which is also known to deteriorate the aging process.

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