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Food Chemistry 99 (2006) 305–314
Food Chemistry
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Oxidative damage after chronic ethanol intake in rat tissues: Prophylaxis of *Ginkgo biloba* extract

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Received 14 March 2005; received in revised form 26 July 2005; accepted 26 July 2005

Abstract

The prophylactic effect of *Ginkgo biloba* extract (EGB) on ethanol-induced oxidative stress in various tissues was evaluated by determining an antioxidative parameter and lipid peroxidation in liver, heart, kidney and testes. Chronic ethanol treatment (2.4 g g/kg, orally for 90 days), for male Sprague–Dawley rats, induced a significant depletion of glutathione (GSH), increase in malondialdehyde (MDA) level, and inactivation of antioxidative enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), in all measured tissues, except renal SOD and cardiac CAT. In contrast, low or high EGB pre-administration (48 and 96 mg/kg, respectively), especially a high EGB supplement, partly reversed these alterations resulting from chronic ethanol treatment, although changed parameters could not return completely to the normal range. These results showed that EGB could provide protection against ethanol-induced oxidative stress on various tissues.

Keywords: *Ginkgo Biloba* extract; Oxidative stress; Ethanol; Lipid peroxidation; antioxidant enzymes

1. Introduction

Oxidative damage, inflicted by exceeding ROS, is considered as an important pathophysiological condition, promoting cell injury and death in a broad variety of disorders (Kabuto, Hasuike, Minagawa, & Shishibori, 2003). Normally, the cell is protected by virtue of an intricate antioxidative system, consisting of enzymatic and non-enzymatic systems, to maintain redox status homeostasis. Enzymic superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and non-enzymic reduced glutathione (GSH) play important roles during the process by scavenging reactive oxygen species (ROS) or preventing their formation (Kabuto et al., 2003; Veerappan, Senthil, Rao, Ravikumar, & Pugalendi, 2004). However, when the excessive ROS impairs antioxidant defences or exceeds the scavenging ability of the antioxidant defence system, oxidative stress and injury may be unavoidable.

Alcohol abuse has been linked as a risk factor for various cardiovascular diseases, digestive tract disorder and cancers (Corrao, Bagnardi, Zambon, & La Vecchia, 2004). The most extensively investigated aspect of ethanol on health is alcoholic liver disease (ALD), which is one of major causes of illness and death worldwide. In addition, chronic alcohol binging also induces pathological changes and dysfunction of multi-organs, including myocardium damage, renal inadequacy and spermatiferous

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doi:10.1016/j.foodchem.2005.07.047

disorder (Bekpinar & Tugrul, 1995; Roig, Cascon, Arola, Blade, & Salvado, 2000; Zhang, Lia, Brown, & Ren, 2004; Zima et al., 2001). As an intensive oxidative stressor and hyperosmotic micromolecule possessing both hydrophilicity and lipophilicity, alcohol produces excessive free radicals and ROS during metabolism and results in lipid peroxidation, protein oxidation, DNA damage and adduct formation, by which it induces injury to liver and other organs (Cederbaum, Wu, Mari, & Bai, 2001; Purohit, Russo, & Salin, 2003). However, experiments dealing with the influence of alcohol on some oxidative impairment markers have brought controversial results. More importantly, investigations of prophylactic effects of antioxidants on ethanol-induced oxidative stress in different tissues are still relatively rare.

Ginkgo biloba extract (EGB), a complex mixture extracted from the leaves of *G. biloba*, is standardized at 6% terpenes and 24% flavonol heterosides. Extensive studies have demonstrated that EGB exerts beneficial effects in a multitude of disease states, including cardiovascular and cerebrovascular disease and neurodegenerative disorders. Many of the biological actions have been attributed to its antioxidant properties, either through its reducing capacities per se or through its possible influences on intracellular redox status (Cheng et al., 2003; Williams, Spencer, & Rice-Evans, 2004). Recently, EGB, especially its flavonoids component, has been found to scavenge ethanol-derived free radicals and to induce expression of antioxidative gene, including SOD, γ-glutamyl-cysteinyl synthetase (γ-GCS) and heme oxygenase-1 (HO-1), to defenced cells against oxidative damage on account of lysophosphatidylcholine or ischemia (Chen, Zeng, Chen, Su, & Lai, 2001; Gohil, Moy, Farzin, Maguire, & Packer, 2000; Marfak et al., 2004). However, to our knowledge, nothing is known about the protection effect of EGB on ethanol-induced oxidative damage in different tissues. Accordingly, this study was designed to investigate the effect of chronic ethanol administration on the antioxidant defence system and lipid peroxidation in liver, kidney, heart and testes of rats and to observe whether EGB has a protective effect on the antioxidative defence system.

2. Materials and methods

2.1. Chemicals

Hypoxanthine, xanthine, xanthine oxidase, hydroxylamine, naphthyl ethylenediamine, sulfanilic acid, 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB), 1,1,3,3-tetraethoxypropane, GSH, ammonium molybdate, diethylenetriaminopentaacetic acid (DETAPAC), thiobarbituric acid (TBA) and butylated hydroxy toluene (BHT) were obtained from Sigma-Aldrich Co. Ethyl alcohol (absolute ethanol) was purchased from Zhenxing Chemical Factory (Shanghai, China). The EGB was obtained from Louian Industry Company Ltd. (Shanghai, China) and contained 24% flavonoid and 6% terpenoid. Other chemicals and organic solvents were of analytical grade and purchased from a local reagent retailer.

2.2. Animals

Male Sprague–Dawley rats, each weighing 140–160 g, were obtained from Sino-British Sippr/BK Laboratory Animal Ltd. (Shanghai, China) and acclimatized to the facility for 5 days before experiments. Rodent laboratory chow and tap water was available ad libitum, and rats were maintained on a 12 h light/dark cycle in a temperature-regulated room (20–25 °C) during the experimental procedures. The animals were cared for according to the Guiding Principles in the Care and Use of Animals. The experiments were approved by the Tongji Medical College Council on Animal Care Committee.

2.3. Ethanol ingestion and EGB administration

Rats were divided randomly into five groups of eight animals each and treated for 90 days as follows: (1) normal control group received normal saline 2 times (with a 1 h interval); (2) ethanol group was administered ethanol 2.4 g/kg (30% v/v, 1–0 ml/100 g) orally 1 h after ingestion of normal saline; (3) ethanol plus low EGB group received EGB, 48 mg/kg, 1 h prior to the administration of ethanol, 2.4 g/kg; (4) ethanol plus high EGB group received EGB, 96 mg/kg, 1 h prior to the administration of ethanol 2.4 g/kg; (5) EGB control group received normal saline and EGB, 96 mg/kg, with a 1 h interval. Animals received ethanol, EGB or normal saline via i.g. daily feeding. In the entire treatment period, the rats were weighed once a week, and the consumptions of food and water were monitored every day.

2.4. Tissue homogenate preparation

All animals were sacrificed by decapitation 4 h after final treatments. The liver, kidney, heart and testes samples were quickly dissected, rinsed with ice cold isotonic saline and dried by blotting between two pieces of filter paper, then stored at −80 °C. An accurately weighed piece of each organ was homogenized in ice cold 0.1 M potassium phosphate buffer (pH 7.4) containing 5 mM EDTA, using a Teflon pestle connected to a Braun homogenizer motor, to yield a 10% (w/v) tissue homogenate. The homogenate was centrifuged at 3500*g* for 10 min at 4 °C (Eppendorf centrifuge 5804R, Germany) to remove cell debris and nuclei. The resulting supernatant was stored at −80 °C for various biochemical assays.

2.5. Determination of GSH

The GSH content of homogenate was measured at 412 run by the method of Moron, Depierre, and Mannervik (1979) with slight modification. Briefly, the protein in samples was precipitated with 50% trichloracetic acid (TCA) and then centrifuged at 1000 *g* for 5 min. The reaction mixture contained 0.5 ml of supernatant, 2.0 ml of 0.2 M Tris–EDTA buffer (pH 8.9) and 0.1 ml of 0.01 M DTNB. The solution was kept at room temperature for 5 min, and then read against the blank at 412 nm on the spectrophotometer (Shimadzu LJV 1700, Japan), and the GSH concentration was calculated from a standard curve.

2.6. Determination of SOD

SOD activity was estimated according to the method of Kono (1978) with slight modification. Briefly, the reaction was initiated by mixing an appropriate water-dilution of homogenate supernatant with 0.5 mM hypoxanthine, 0.5 mM hydroxylamine and 0.01 U xanthine oxidase in the buffer, consisting of 104 mM potassium phosphate, 78 mM sodium borate and 0.025 mM EDTA (pH 7.0) at 37 °C for 30 min in a reaction volume of 1.0 ml. The reaction was terminated by adding 2.0 ml of 16% (v/v) acetic acid solution containing 2.6 mM sulfanilic acid and 38.6 μM naphthyl ethylenediamine and the absorbance at 550 nm was recorded for the calculation of SOD activity. Under the conditions, one nitroso unit (Nu) of enzyme activity was calculated as that inhibiting 50% of the oxidation of hydroxylamine without an enzyme source.

2.7. Determination of GPx

GPx activity was estimated by the method of Sazuka, Tanizawa, and Takino (1989) with slight modification. Briefly, homogenate mixed with GSH and H_2O_2 was incubated at 37 °C for 3 min, followed by the addition of 10% TCA. After centrifugation, the supernatant was collected and mixed with disodium hydrogen phosphate and DTNB. The absorbance of the samples was recorded against the blank at 412 nm. The unit of activity was expressed as micromoles GSH oxidation per min per mg protein.

2.8. Determination of hepatic CAT

CAT activity was determined according to the method of Goth with slight modification (Goth, 1991; Milton, 2001). Briefly, a 50 μl sample was mixed with 50 μl substrate (6.5 μmol H_2O_2 in phosphate buffer) for 60 s, then 100 μl of 32.4 mM ammonium molybdate solution were added and absorbance change was measured at 405 nm. One unit of the enzyme was defined as mmol H_2O_2 disproportionated per min per mg protein.

2.9. Determination of lipid peroxidation

Lipid peroxidation (as malondialdehyde, MDA) level was measured by the method of Beuege and Aust (1978). Briefly, the tissue homogenate was mixed with TCA–TBA–HCl solution, BHT and DETAPAC. The mixture was heated for 60 min in a boiling water bath. After centrifugation, the absorbance was recorded at 532 nm by using 1,1,3,3-tetraethoxypropane as standard. The lipid peroxidation was expressed as MDA in nanomoles per milligramme the protein.

2.10. Determination of the protein of homogenate

Protein contents were measured by the method described by Lowry, Rosebrough, Fair, and Randall (1951), using bovine serum albumin (BSA) as standard.

2.11. Statistical analysis

Data were expressed as means ± SEM and subjected to equality of variance *F*-test, if necessary, to variable transformation before one-way analysis of variance (ANOVA), followed by the Student–Newman–Keuls multiple range test (SAS 8.1 software). A probability value of 0.05 was determined to be statistically significant.

3. Results and discussion

3.1. General

The present study was undertaken to assess the response of the antioxidant defence system and the extent of lipid peroxidation in various tissues of rat after chronic ethanol consumption and EGB prophylaxis. Selection of doses of ethanol was based on the research of Husain, Scott, Reddy, and Somani (2001) and our preliminary experiments. The dose over 2 g/kg ethanol, given orally to rats, could yield a plasma ethanol concentration exceeding the threshold level (0.1%) in human beings and have influence on the antioxidant system and lipid peroxidation. The dose exceeding 3.5 g/kg daily might lead to partial deaths with chronic treatment. All the rats survived during the experimental period until sacrifice. There was no statistical significance in weight gain or food and water intake of the observed rats after chronic administration of ethanol and EGB supplement in comparison with normal control, although the food intake and weight gain of ethanol-fed rats were slightly lower (data not shown). This finding is consistent with other reports (Abraham, Wilfred, & Ramakrishna,

2002; Husain et al., 2001; Jurczuk, Brzoska, Moniuszko-Jakoniuk, Galazyn-Sidorczuk, & Kulikowska-Karpinska, 2004). Rajasekaran (2000) reported that pubertal Wistar rats (60–70 g) treated with ethanol (2 g/kg) showed induced reduction only in food intake but not body weight gain. A compensation of calorific deficit by ethanol ingestion may account for the lack of reduction in body weight gain despite decreased food intake in these animals. Of note Kumar, Ponmozhi, Viswanathan, and Nalini (2002) found that exposure to ethanol (9.875 g/kg) resulted in a significant decrease in the net gain in body weight. This apparent discrepancy is mainly attributed to the different doses of ethanol exposure. Alcoholism, with high dose, is associated with increase in metabolic rate and thermogenesis of brown fat but decrease in appetite which, in turn, may be responsible for the decrease in growth of the animals (Gupta & Gill, 2000; Kumar et al., 2002).

3.2. The effect of chronic ethanol administration and EGB prophylaxis on GSH level in rat tissues

As a water-soluble tripeptide, GSH is the most abundant intracellular small thiol molecule and predominant defence against ROS/free radicals in tissues of the body. GSH reacts directly with ROS and electrophilic metabolites, protects essential thiol groups from oxidation, promotes the regeneration of α-tocopherol, and serves as a substrate for GSH-related enzymes, e.g., GPx and glutathione S-transferases (Townsend, Tew, & Tapiero, 2003). A deficiency of GSH puts the cell at risk of oxidative damage. It is not surprising that GSH plays a crucial role in both scavenging ROS and detoxification of drugs, and an imbalance of GSH is observed in a wide range of pathologies, including alcohol-related diseases. Chronic ethanol administration, at a dose of 2.4 g/kg for 90 days, depleted GSH content of rat tissues (Fig. 1). GSH content of ethanol-fed rats decreased by 46.3% in liver ($p < 0.01$), 20.3% in heart ($p < 0.01$), 31.9% in kidney ($p < 0.01$) and 31.7% in testes ($p < 0.01$) as compared with the normal control. Similar results were found in other studies (Balasubramaniyan, Kalaivani, & Nalini, 2003; Calabrese et al., 1998; Pushpakiran, Mahalakshmi, & Anuradha, 2004). The kidney had a lower concentration of GSH in the present study (Fig. 1), which is consistent with other studies (Husain et al., 2001; Kaushik & Kaur, 2003). Husain et al. (2001) found that the GSH level of adult male Fisher-344 rats treated with ethanol (2 g/kg for 6.5 weeks) showed an evident decrease in liver and testes while renal GSH remained unchanged, indicating that renal GSH is not so sensitive as other organs or possessed higher turnover rate. The disparate results might be due to the difference in duration of exposure and animal strain. As the primary organ for metabolism of ethanol, where free radicals/ROS are generated, liver had a higher depletion in GSH than did other organs, which is in agreement with the research of Husain et al. (2001). Importantly, increased cholesterol deposition in the mitochondrial inner membrane, resulting from alcohol-induced liver injury, decreases membrane fluidity and impairs the mitochondrial transport of GSH (Fernandez-Checa & Kaplowitz, 2005). Depletion of mitochondrial GSH is believed to further exacerbate hepatic sensitization to alcohol because mitochondria are the major source of ROS production.

In the present study, GSH depletion, induced by ethanol, is prohibited in part by EGB (Fig. 1). High EGB pre-administration significantly increased GSH content by 25.2% in liver ($p < 0.01$), 17.8% in heart ($p < 0.05$), 16.8% in kidney ($p < 0.05$) and 21.7% in testes ($p < 0.05$) compared with ethanol intake alone.

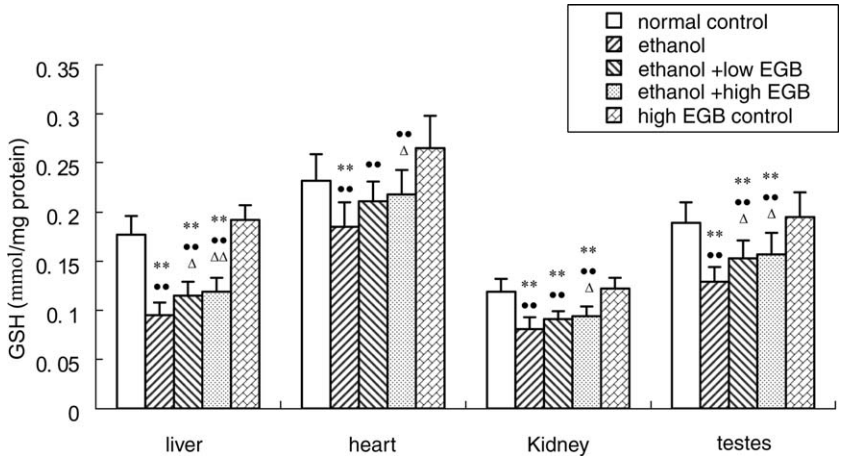

Fig. 1. The effect of chronic ethanol administration and EGB supplement on GSH level in different tissues of rats. The values are expressed as means ± SD ($n = 8$). ✻✻$p < 0.01$ versus normal control; ●●$p < 0.01$ versus high EGB control group (EGB 96 mg/kg); △$p < 0.05$ and △△$p < 0.01$ versus ethanol control group (ethanol 2.4 g/kg).

Low EGB co-administration significantly increased GSH content by 21.0% in liver ($p < 0.05$) and 19.1% in testes ($p < 0.05$) as compared with ethanol-fed rats. Al-Zuhair, Abd el-Fattah, and el-Sayed (1998) also reported that EGB induced an increased GSH level in serum, brain and liver of 24-month-old male rats, which may be explained in the light of its antioxidant properties (Al-Zuhair et al., 1998). EGB medication may partly suppress GSH depletion by scavenging ROS generated during ethanol metabolism. Additionally, γ-GCS, a limiting enzyme for GSH synthesis, can be induced by EGB or its flavonoid ingredient in an ubiquitous way (Gohil et al., 2000; Rimbach et al., 2001). However, no statistical difference in GSH level of examined tissues was obtained between EGB control and normal control, which needs further elucidation.

3.3. The effect of chronic ethanol administration and EGB prophylaxis on SOD activity in rat tissues

SOD is considered as the first line of defence against oxygen toxicity and the central regulators of ROS levels by catalyzing the decomposition of superoxide, the first but most abundant ROS, into hydrogen peroxide and water. Over-expression of SOD reduces oxidative damage and extends life span, while SOD mutation reduces oxidative stress resistance and cell viability with decrease of SOD expression or activity (Landis & Tower, 2005). Prolonged ethanol administration inactivated SOD in measured tissues except kidney (Fig. 2). Ethanol intake resulted in significant decrease on SOD activity by, 45.8% in liver ($p < 0.01$), 25.4% in heart ($p < 0.01$), 28.0% in testes ($p < 0.01$) compared with normal control. This finding is supported by a number of reports on liver, heart or testes (Balasubramaniyan et al., 2003; Calabrese et al., 1998; Pushpakiran et al., 2004; Suresh, Sreeranjit Kumar, Lai, & Indira, 1999). Hepatic SOD was inactivated more than that of organs, which is in agreement with hepatic GSH alteration induced by ethanol. Inhibition of SOD activity in tissues may be a consequence of decrease of de novo synthesis of SOD protein or irreversible inactivation resulting from overproduction of ROS during ethanol metabolism (Santiard, Ribiere, Nordmann, & Houee-Levin, 1995). However, Husain et al. (2001) found that ethanol treatment (2 g/kg for 6.5 weeks) induced hepatic SOD (Husain et al., 2001). SOD can be activated to scavenge excessive superoxide in the presence of moderate oxidative stress with compensation (Landis & Tower, 2005). Hence, biphasic fluxes of SOD activity are common, and an increase or decrease may relate to the presence of excessive superoxide. Renal SOD activity was only decreased slightly (no statistical significance) following ethanol treatment in the present study, indicating that it is not as susceptible as that of other organs. Rodrigo, Rivera, Orellana, Araya, and Bosco (2002) also found similar results. However, some other studies found that renal SOD was inactivated after ethanol administration (Balasubramaniyan et al., 2003; Pushpakiran et al., 2004; Suresh et al., 1999). The overdose of ethanol (exceeding 6.0 g/kg) in these studies may contribute to the disparate result.

EGB prophylactic medication suppressed SOD inactivation in liver, heart and testes induced by ethanol but had no evident impact on renal SOD (Fig. 2). Low EGB supplement only increased SOD activity in liver by 17.0% versus the ethanol group ($p < 0.05$). High EGB supplement significantly increased it by 21.7% in liver ($p < 0.05$), 16.5% in heart ($p < 0.05$) and 19.1% in testes ($p < 0.05$). Naidu, Kumar, Mohan, Sundaram, and Singh (2002) discovered that EGB significantly protected mice from myocardial damage by normalizing

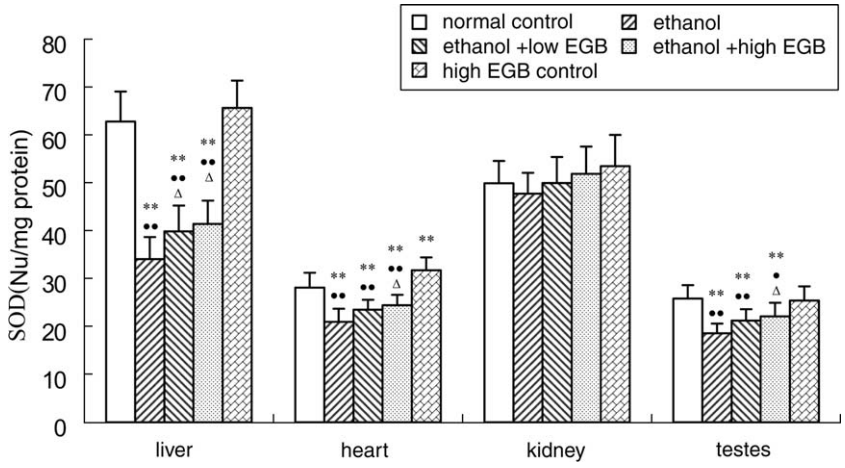

Fig. 2. The effect of chronic ethanol administration and EGB supplement on SOD activity in different tissues of rats. The values are expressed as means ± SD ($n = 8$). **$p < 0.01$ versus normal control; ●$p < 0.05$ and ••$p < 0.01$ versus high EGB control group (EGB 96 mg/kg); △$p < 0.05$ versus ethanol control group (ethanol 2.4 g/kg).

of SOD activity decreased by doxorubicin treatment (Naidu et al., 2002). Lin and Chang (1997) found that EGB with 50% diluted alcohol not only locally induced SOD activity in epidermis after topical application, but also systemically increase the activity in the liver, heart and kidney of Sprague–Dawley rats. Gohil et al. (2000) reported that EGB directly induced SOD expression in a human bladder cancer cell line by a genechip technique. However, no significant difference was obtained between EGB control and normal control, indicating that EGB alleviates the SOD inactivation of ethanol-fed rats owing to its antioxidant capacity.

3.4. The effect of chronic ethanol administration and EGB prophylaxis on GPx activity in rat tissues

GPx is a selenium-containing antioxidant enzyme, located in mitochondria and cytosol, which effectively reduces hydrogen peroxide and lipid peroxides to water and lipid alcohols, respectively. In the absence of adequate GPx activity or glutathione levels, hydrogen peroxide and lipid peroxides are not detoxified and may be converted to hydroxyl radicals and lipid peroxyl radicals, respectively, by transition metals (e.g., Fe^{2+}). The GPx/glutathione system is considered to be a major defence in low-level oxidative stress (Wassmann, Wassmann, & Nickenig, 2004). Chronic ethanol administration inactivated GPx activity in various tissues (Fig. 3). Ethanol significantly decreased GPx activity, by 31.8% in liver ($p < 0.01$), 21.8% in heart ($p < 0.01$), 26.2% in kidney ($p < 0.01$) and 27.8% in testes ($p < 0.01$). This finding is supported by extensive studies (Balasubramaniyan et al., 2003; Molina, Sanchez-Reus, Iglesias, & Benedi, 2003; Pushpakiran et al., 2004). The decreased activity of GPx of ethanol-treated rats indicates a reduced capacity to scavenge produced hydrogen peroxide or lipid peroxides. However, Husain et al. (2001) reported that testicular GPx activity decreased slightly (no statistical significance), although hepatic and renal GPx decreased after chromic ethanol administration (2 g/kg for 6.5 weeks). The difference in duration of ethanol exposure may have contributed to the disparate results. Meanwhile, no alteration in GPx shows enhanced tolerance or low susceptibility in testes in response to the chronic ethanol stress.

EGB pretreatment alleviated GPx inactivation induced by ethanol in various tissues (Fig. 3). Low EGB supplement significantly increased GPx activity by 16.8% ($p < 0.05$) in kidney and testes as compared with ethanol-fed rats. High EGB supplement significantly increased GPx activity, by 21.3% in liver ($p < 0.05$), 16.1% in heart ($p < 0.05$), 30.8% in kidney ($p < 0.01$) and 18.8% in testes ($p < 0.05$). Similar research showed that EGB normalized GPx activity decreased by doxorubicin and protected mice from myocardial damage (Naidu et al., 2002). Ahmad et al. (2005) also reported that EGB dose-dependently (50–150 mg/kg) restored the activities of glutathione-dependent enzymes and exerted an anti-Parkinsonian etfect. The restoration of GPx in part indicates the enhanced resistance to hydrogen peroxide and lipid peroxides resulting from ethanol metabolism. Similar to SOD, EGB had no direct induction on GPx because of nearly the same GPx activity in various tissues between EGB control and normal control. Its restoration by EGB may relate to the antioxidant effect of EGB.

3.5. The effect of chronic ethanol administration and EGB prophylaxis on CAT activity in rat tissues

CAT is an intracellular antioxidant enzyme that is mainly located in cellular peroxisomes, mitochondria and, to some extent, in cytosol, which catalyzes the reaction of hydrogen peroxide to water and molecular oxygen. By removing hydrogen peroxide, it indirectly detoxifies superoxide radicals, which are turned into

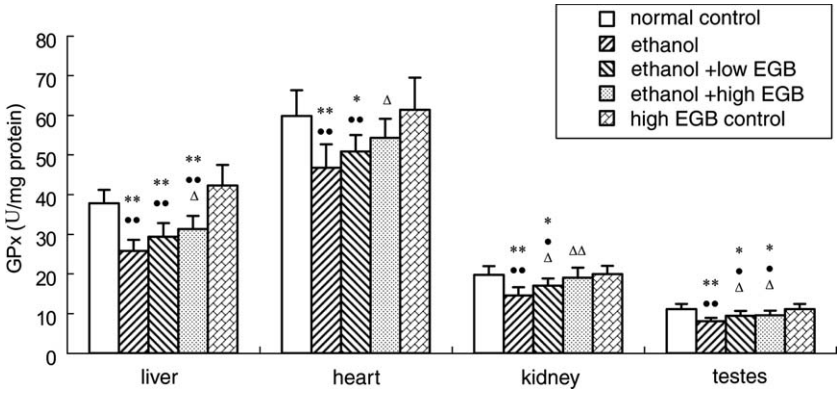

Fig. 3. The effect of chronic ethanol administration and EGB supplement on GPx activity in different tissues of rats. The values are expressed as means ± SD ($n = 8$). ✻$p < 0.05$ and ✻✻$p < 0.01$ versus normal control; ●$p < 0.05$ and ●●$p < 0.01$ versus high EGB control group (EGB 96 mg/kg); △$p < 0.05$ and △△$p < 0.01$ versus ethanol control group (ethanol 2.4 g/kg).

hydrogen peroxide by SOD. The enzyme also has peroxidase activity and reacts with organic peroxides and hydrogen donors to water and organic alcohols (Wassmann et al., 2004). CAT is very effective in high-level oxidative stress and protects cells from hydrogen peroxide produced within the cell. The enzyme is especially important in the case of limited glutathione content or reduced GPx activity and plays a significant role in the development of tolerance to oxidative stress in the adaptive response of cells by compensating for the inability of GPx, although it has lower affinity for hydrogen peroxide than GPx (Wassmann et al., 2004). CAT also has a secondary role in the metabolism of ethanol (Husain et al., 2001). In the present study, CAT activity was inactivated in tissues measured, except heart, after long-term ethanol treatment (Fig. 4). Chronic ethanol intakes significantly decreased CAT activity, by 33.0% in liver ($p < 0.01$), 16.6% in kidney ($p < 0.05$) and 38.1% in testes ($p < 0.01$) compared with normal control. The finding is consistent with other reports (Balasubramaniyan et al., 2003; Jurczuk et al., 2004; Kumar, Manickam, Periyasamy, & Namasivayam, 2003). The decreased activity of CAT, as well as GPx, shows the highly reduced capacity to scavenge hydrogen peroxide produced; meanwhile, no alteration in cardiac CAT demonstrates enhanced tolerance or a compensation effect of GPx in particular tissus, in response to chronic ethanol stress.

Of note, different from GSH and other antioxidant enzymes, EGB supplement seemed to have a small role on reversion of inactivated CAT in measured tissues except that significant increase was found in liver with high EGB pre-administration ($p < 0.05$, Fig. 4). However, EGB, by topical application, not only reinforced CAT activity in epidermis, but also systemically increased it in the liver, heart and kidney of Sprague–Dawley rats (Lin & Chang, 1997). Different medication routes and different models (UV irradiation versus alcoholic oxidative stress) may have diverse impacts on CAT.

3.6. The effect of chronic ethanol administration and EGB prophylaxis on MDA content in rat tissues

Lipid peroxidation is used as an index for measuring the damage that occurs in membranes of tissues as a result of free radical infliction (Husain et al., 2001). MDA, as a marker of lipid peroxidation, was significantly increased in tested tissues after chronic ingestion of ethanol in the present study. As shown in Fig. 5, MDA level increased significantly, by 68.9% in liver ($p < 0.01$), 34.1% in heart ($p < 0.01$), 35.8% in kidney ($p < 0.01$) and 44.5% in testes ($p < 0.01$). Increased MDA equivalents, resulting from ethanol-treatment, have been found in extensive research (Balasubramaniyan et al., 2003; Molina et al., 2003; Pushpakiran et al., 2004; Suresh et al., 1999). The higher levels of hepatic lipid peroxidation may be linked to the fact that the majority of ethanol metabolism occurs in the liver, as discussed above. In addition, it is known that liver has relatively high concentrations of easily peroxidizable fatty acids and is highly enriched in iron, a metal that, in its free form, is catalytically involved in production of damaging oxygen free radical species (Meng & Zhang, 2003). MDA levels in tested tissues of ethanol-fed rats decreased significantly after low or high EGB prophylaxis (Fig. 5). Low prophylaxis decreased MDA level, by 18.7% in liver ($p < 0.01$), 12.2% in heart ($p < 0.05$), 11.0% in kidney ($p < 0.01$) and 13.7% in testes ($p < 0.01$), as compared with ethanol-fed rats. High prophylaxis decreased MDA level, by 24.5% in liver ($p < 0.01$), 16.9% in heart ($p < 0.05$), 16.2% in kidney ($p < 0.01$) and 16.0% in testes ($p < 0.01$). The decreased MDA content in measured tissues indicates alleviated oxidative stress owing to chronic ethanol administration after EGB pretreatment. Bahçecioglu et al. (1999) also found that EGB is effective in arresting the development of liver fibrosis and fatty degeneration in oxidative liver damage after CCl_4 treatment, by reducing lipid peroxidation.

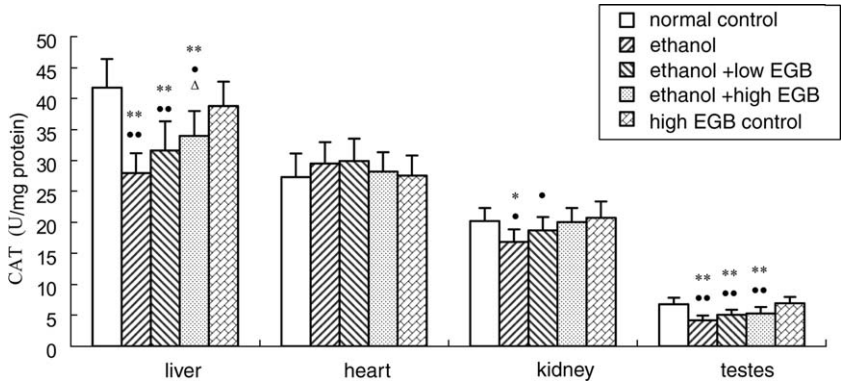

Fig. 4. The effect of chronic ethanol administration and EGB supplement on CAT activity in different tissues of rats. The values are expressed as means ± SD ($n = 8$). ✻$p < 0.05$ and ✻✻$p < 0.01$ versus normal control; ●$p < 0.05$ and ●●$p < 0.01$ versus high EGB control group (EGB 96 mg/kg); △$p < 0.05$ versus ethanol control group (ethanol 2.4 g/kg).

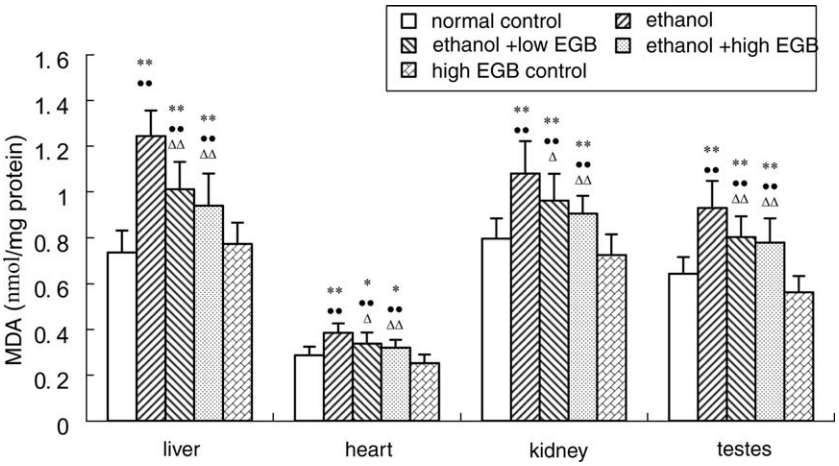

Fig. 5. The effect of chronic ethanol administration and EGB supplement on MDA level in different tissues of rats. The values are expressed as means ± SD ($n = 8$). ✻$p < 0.05$ and ✻✻$p < 0.01$ versus normal control; ●●$p < 0.01$ versus high EGB control group (EGB 96 mg/kg); △$p < 0.05$ and △△$p < 0.01$ versus ethanol control group (ethanol 2.4 g/kg).

Despite divergent opinions on details, the basic mechanism in the pathogenesis of alcohol-related diseases is the induction of oxidative stress. Ethanol is principally metabolized to acetaldehyde in the liver and seldom in other tissues by alcohol dehydrogenase, as well as CAT. Acetaldehyde is further oxidized into acetate by acetaldehyde dehydrogenase/oxidase, leading to the generation of ROS/free radical. Ethanol is also oxidized by a microsomal ethanol oxidizing system (CYP 2E1 mainly) to acetaldehyde and 1-hydroxyethyl radical, especially following chronic ethanol consumption, by which CYP 2E1 is induced (Husain et al., 2001; Lieber, 2005). Excessive alcohol intake results in disequilibrium in iron homeostasis and iron overload, which further enhance oxidative stress by catalyzing the formation of more noxious hydroxy free radical. Hence, induction of CYP2E1 and iron overload by ethanol are critical pathways by which ethanol generates a state of oxidative stress in hepatocytes (Cederbaum et al., 2001; Purohit et al., 2003). In addition, immune reactions at the cellular and humoral level also contribute to the negative consequences of alcohol consumption, which, at the end, increases the oxidative stress in multi-tissues (Dai et al., 2000). Produced free radicals and ROS have an intensively destructive effect on tissues. Therefore, chronic alcohol binging provokes disorder in multi-tissues, ad hoc in liver (Bekpinar & Tugrul, 1995; Roig et al., 2000; Zhang et al., 2004; Zima et al., 2001).

Numerous studies have demonstrated that EGB exerts potent antioxidant activity by acting as scavenger of free radicals, e.g., superoxide anions, hydroxyl radicals and nitric oxide, to protect antioxidant defence system (Bridi, Crossetti, Steffen, & Henriques, 2001; Kwon et al., 2004; Mahady, 2002). However, there were no significant differences in measured antioxidant enzymes, on GSH and MDA levels, between EGB control and normal control, although EGB has been found to have an inductive effect on SOD, CAT and γ-GCS in other studies (Gohil et al., 2000; Rimbach et al., 2001). Hence, protective effects of EGB on the antioxidant system may be linked, not with its inductive effect on SOD, CAT and γ-GCS, but with its direct scavenging activity in the present study. EGB reinforces its antioxidant capacity by up-regulating HO-1. HO-1 is a stress protein and its induction represents an adaptive response to various injuries and enhanced resistance to various oxidative stresses (Hangaishi et al., 2000; Sharma, Das, & Verdouw, 1999). Through the use of primary neuronal cultures, Zhuang, Pin, Christen, and Dore (2002) demonstrated that EGB induced HO-1 in a dose-dependent and time-dependent manner (Zhuang et al., 2002). Our results also showed similar induction on HO-1 in the EGB control as compared with the normal control, and in low or high prophylaxis group as compared with the ethanol-treated group (data not shown). Chen et al. (2001) found that induction of HO-1 gene expression and increased activity by EGB pretreatment partially contributed to cellular defence against oxidative damage. Therefore, the prophylactic role of EGB on the antioxidant system may be associated with its induction of HO-1.

4. Conclusions

The present results suggest that chronic ethanol intake caused oxidative stress on various tissues of rats; on the other hand, EGB supplement could partially contribute to tissue defence against oxidative stress. The detoxification action of EGB was clearly beneficial for ethanol-treated rats; however, the detoxification mechanism at the pharmacological and biochemical level still needs to be elucidated.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 30271130) and Program for New Century Excellent Talents in University of China (NCET).

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