JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 3191–3196 3191
DOI:10.1021/jf904407x

Effect of Anthocyanin-Rich Extract from Black Rice (*Oryza sativa* L. *Japonica*) on Chronically Alcohol-Induced Liver Damage in Rats

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The study evaluated the protective effect of anthocyanin-rich extract from black rice (AEBR) on chronic ethanol-induced biochemical changes in male Wistar rats. Administration of ethanol (3.7 g/kg/day) to Wistar rats for 45 days induced liver damage with a significant increase ($P < 0.05$) of aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyl transferase (GGT) in the serum and the hepatic malondialdehyde (MDA) level. In contrast, administration of AEBR (500 mg/kg) along with alcohol significantly ($P < 0.01$) decreased the activities of liver enzymes (AST, ALT and GGT) in serum, the MDA levels and the concentrations of serum and hepatic triglyceride (TG) and total cholesterol (TCH). Rats treated with AEBR showed a better profile of the antioxidant system with normal glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and glutathione *S*-transferase (GST) activities. All these results were accompanied by histological observations in liver. The results demonstrate that AEBR has a beneficial effect in reducing the adverse effect of alcohol.

KEYWORDS: Black rice; anthocyanin-rich extract; liver damage; rats; ethanol

INTRODUCTION

Anthocyanins are a group of plant pigments that are widely distributed in flowers, fruits and cereals, and contribute to the bright colors (green, red and blue) of these plant components (*1*). As a group of flavonoid pigments, anthocyanins have not only their colorant potential but also significant health implications, because of their nontoxicity and nonmutagenic, antioxidant activity (*2*). As a result, anthocyanins have been widely used in the food industry as well as in human health (*1*).

The "health-promoting" activity of anthocyanins has drawn attention in recent years (*3*, *4*). As an abundant component in the human diet (fresh fruit, juices, wine, cereals), anthocyanins have demonstrated anti-inflammatory (*5*) and antioxidant activities (*6*). Systemic antioxidative effects by circulating anthocyanins in body fluids are expected to reduce the body's load of oxidants and ultimately the risk for developing of diseases (*7*).

Black rice (*Oryza sativa* L. *Japonica*), as a special anthocyanin-rich cultivar of rice, has been regarded as a health-promoting food and widely consumed in Eastern Asia. Previous studies showed that the supplementation of black rice pigment fraction markedly reduced oxidative stress, improved lipid profile and modulated atherosclerotic lesions in two different animal models (*8*).

The antioxidant nature of anthocyanin-rich extract from black rice might be helpful to alleviate the pathological changes caused by alcohol in liver. The liver is subject to acute and potentially lethal injury by several substances including phalloidin, carbon tetrachloride (CCl_4), galactosamine, ethanol, and other compounds (*9*). Long-term ethanol consumption induces oxidative stress in the liver due to the imbalance between the prooxidant and antioxidant systems (*10*). The susceptibility of liver to ethanol toxicity can promote alcoholic liver disease (*11*), while the efficacy of any protective drug essentially depends on its capacity of either reducing the harmful effects or in maintaining the normal physiology of cells and tissues. In recent years, a wide variety of antioxidants and diverse diets have been tested to alleviate the oxidative stress induced by ethanol abuse (*12*, *13*). The aim of the present study was to investigate the effects of anthocyanin-rich extract from black rice on ethanol-induced liver damage in rats.

MATERIALS AND METHODS

Animals. Male Wistar rats (150 g ± 20 g) were used in the experiment. The animals were kept under the standard conditions of animal house with 12 h light−dark cycle (light 7:00−19:00) at a temperature 22 °C ± 2 °C and humidity 70% ± 4%, and had free access to food and water. The experiment was performed following the European Community Guidelines for the Use of Experimental Animals and approved by the Peking University Committee on Animal Care and Use.

Preparation of Anthocyanin-Rich Extract from Black Rice (AEBR). Black rice was purchased from a local market in Jilin province, China. The procedure for preparing AEBR was described previously (*14*). In brief, whole black rice was ground with a laboratory mill and passed through a 60 mesh screen sieve. Black rice powder was extracted twice with ethanol/water/hydrochloric acid (50:50:0.5, v/v/v) of solid−liquid ratio 1:10 for 2 h at 50 °C. The filtrates were combined and subjected to vacuum evaporation (Rotavapor R 210; Büchi, Flawil, Sweden) to remove ethanol. The concentrated extracts were loaded onto an AB-8 resin. The AB-8 resin was washed with distilled water, and subsequently the absorbed anthocyanins were eluted with 80% ethanol. The ethanol eluent was sprayed to yield anthocyanin-rich powders.

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Published on Web 02/09/2010

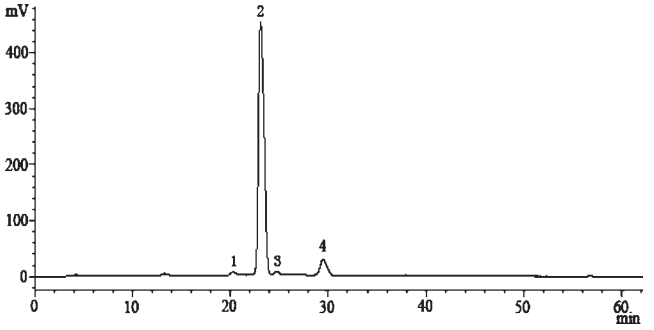

Figure 1. Anthocyanin profile in black rice extract analyzed using HPLC. Detection was performed at 520 nm. Peak 1, cyanidin-3,5-diglucoside; peak 2, cyanidin-3-glucoside; peak 3, cyanidin-3-rutinoside; peak 4, peonidin-3-glucoside.

Identification and Quantification of Anthocyanins. Anthocyanins in AEBR were identified using HPLC-MS method by comparing their retention time and MS data with standards and published data. Cyanidin-3-glucoside (HPLC grade) was obtained from Polyphenols Laboratories (Sandnes, Norway). Electrospray mass spectrometry was performed with an Esquire-LC mass spectrometer (MS) (Bruker Daltoniks, Billerica, MA), an ion trap instrument equipment with an electrospray interface. Spectra were recorded in positive ion mode between m/z 50 and 1500. Major MS parameters were as follows: capillary exit, 4000 V; capillary offset, 500 V; skim 1, 38.3 V; nebulizer gas, nitrogen, 40 psi; dry gas, nitrogen, 12 L/min; dry temperature, 300 °C. The experimental conditions were described previously (*2*, *15*).

Four anthocyanins were identified as cyanidin-3-glucoside (91.01%), peonidin-3-glucoside (7.13%), cyanidin-3, 5-diglucoside (0.92%), cyanidin-3-rutinoside (0.94%) (**Figure 1**). Analyses were performed on a Shimadzu LC-20A HPLC. **Figure 1** shows the HPLC anthocyanin profile of black rice. Quantification of anthocyanin of AEBR were performed on an Shimadzu LC-20A HPLC and expressed as cyanidin-3-glucoside. The total anthocyanin content is up to 22.5% of AEBR.

In addition to anthocyanins, AEBR also contained moisture (6.96%), crude protein (8.78%), crude fat (4.35%), total carbohydrate and other ingredients (57.41%).

Experimental Design. The experimental animals were randomly divided into five groups with eight rats in each group. Alcohol and AEBR dissolved in water (*16*) was administered using an intragastric tube for 45 days, according to preliminary experiments and previous literature (*16*, *17*). The experimental design was as follows. Group 1: Control rats treated with distilled water (3.7 g/kg body weight). Group 2: Normal rats orally received ethanol (3.7 g/kg BW) (*17*). Group 3: Normal rats orally received ethanol (3.7 g/kg BW) with AEBR (500 mg/kg BW). Group 4: Normal rats orally received ethanol (3.7 g/kg BW) with AEBR (250 mg/kg BW). Group 5: Normal rats orally received ethanol (3.7 g/kg BW) with AEBR (125 mg/kg BW). At the end of the experimental period, animals were sacrificed by decapitation. Livers were excised immediately, washed with ice-cold physiologic saline solution (0.9%), blotted dry, and weighed.

Preparation of Subcellular Fractionation and Blood. Blood was taken from the neck vessels and stood for 30 min at room temperature. Serum was obtained by centrifugation at 3000*g* for 10 min and stored at −80 °C in aliquots until the analysis.

Hepatic subcellular fractions (microsomes, cytosol, and mitochondria) were isolated as previously described (*18*, *19*). A portion of each liver sample was homogenized in 0.1 M Tris-HCl, 0.25 M sucrose, 0.1 mM ethylenediaminetetraacetic acid (EDTA, pH7.4), and centrifuged at 2000*g* for 15 min at 4 °C, to obtain the postnuclear supernatant. The postnuclear supernatant was further centrifuged at 10000*g* for 30 min at 4 °C; the pellet was mitochondria. The postmitochondrial supernatant was then centrifuged at 100000*g* for 1 h at 4 °C to obtain microsomes (pellet) and cytosol (supernatant). Both mitochondrial and microsomal pellets were suspended in 0.1 M Tris-HCl (pH7.4) containing 10% glycerol, 0.1 mM EDTA and were quickly frozen in liquid nitrogen.

To prepare a 10% liver homogenate, liver tissue was homogenized with an ice-cold 0.9% solution of NaCl (1:10).

Table 1. Effects of AEBR on Body Weight and Liver Index in Ethanol-Treated Rats[a]

	body wt (g)			
parameters	initial	final	liver wt (g)	liver/wt (%)
control	157.92 ± 8.24	348.75 ± 15.56	8.26 ± 0.81	2.48 ± 0.12
ethanol	159.11 ± 6.22	316.70 ± 12.41**	8.63 ± 0.59	2.74 ± 0.19**
ethanol + AEBR				
125 mg/kg	155.72 ± 10.6	321.44 ± 16.41	8.64 ± 0.73	2.58 ± 0.06$^{\#}$
250 mg/kg	158.82 ± 7.02	335.75 ± 19.26	8.42 ± 0.58	2.55 ± 0.07$^{\#\#}$
500 mg/kg	153.73 ± 5.37	343.57 ± 23.86$^{\#}$	8.53 ± 0.97	2.52 ± 0.11$^{\#\#}$

[a] Values are mean ± dev for 8 rats in each group. Compared with control group: *P < 0.05, **P < 0.01. Compared with ethanol group: $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01.

Biochemical Parameters of Liver Function. Activities of aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyltransferase (GGT), and glutathione *S*-transferase (GST) in serum were measured by using kits [Nanjing Jiancheng Bioengineering Institute (NJBI), China].

Measurement of TC, TG and MDA in Liver Homogenate and Serum. Total cholesterol (TC), triglycerides (TG) and malondialdehyde (MDA) were measured by colorimetric method. MDA was assayed by the measurement of thiobarbituric acid-reactive substance (TBARS) levels spectrophotometrically at 532 nm. The results were expressed as nmol/mg protein (*20*).

Biochemical Assays. Superoxide peroxidation (SOD) in microsomal and mitochondrial fractions, glutathione *S*-transferase (GST) in postmitochondrial fraction, GSH-peroxidase in serum and mitochondrial fraction were determined by kits obtained from NJBI (China).

Histopathology. The caudal portion of the left lobe of the liver was removed and fixed in 10% neutral-buffered formaldehyde solution. Fixed tissues were embedded in paraffin, cut into 5−6 μm thick sections and placed on microscope slides. Slides were stained with hematoxylin and eosin (H&E), which mounted in neutral distyrene-dibutylphthalate-xylene (DPX) medium for microscopic observations.

Statistical Analysis. All data are expressed as the mean ± standard deviation. Data were analyzed statistically by one-way analysis of variance (ANOVA), using SPSS Statistical program (version 13.0 software, SPSS Inc. Chicago, USA). A value of $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Growth Performance and Liver Index. Table 1 shows the initial and final body weights in control and experimental animals. The ethanol-treated rats gained significantly less weight than controls ($P < 0.01$). Liver index in the ethanol-fed group increased significantly ($P < 0.01$) compared with the control group, while it increased insignificantly in the AEBR (500 mg/kg BW) group. The rats treated with ethanol showed a significantly smaller body weight (**Table 1**), in comparison with the control group, which is similar to the previous report (*21*); the reason may be due to food intake loss and malabsorption (*21*).

Effect of AEBR on AST, ALT and GGT Activities. Ethanol also induced pathologic changes in the liver including hepatomegaly and serologic changes along with the increase of the activities of aspartate transaminase (AST), alanine transaminase (ALT) and gamma glutamyl transferase (GGT). **Table 2** shows the activities of serum AST, ALT and GGT in control and experimental rats. Ethanol administration significantly ($P < 0.05$) increased the activities of AST, ALT and GGT. Administration of AEBR (500 mg/kg) along with alcohol significantly ($P < 0.01$) reversed these functional markers toward to near normal. AEBR at a dose of 500 mg/kg body weight was more effective when compared with two other doses (250 and 125 mg/kg body weight).

It is well-known that ethanol ingestion causes liver damage with the leakage of cellular enzymes into plasma being a sign of hepatic injury (*22*). Alcohol-induced oxidative stress in the liver cells plays a major role in the development of alcoholic liver disease. ALT and AST are the reliable makers for liver function.

Table 2. Effect of AEBR on Hepatic Markers in the Serum of Control and Ethanol-Administered Rats[a]

parameters	AST (IU/L)	ALT (IU/L)	GGT (IU/L)
control	48.58 ± 19.88	20.93 ± 3.99	4.65 ± 2.28
ethanol	75.79 ± 8.33**	28.76 ± 2.03**	6.99 ± 1.57*
ethanol + AEBR			
125 mg/kg	61.19 ± 11.16	25.83 ± 7.08	5.10 ± 1.66
250 mg/kg	49.71 ± 9.15##	23.71 ± 5.97	4.77 ± 1.12#
500 mg/kg	47.51 ± 11.94##	20.85 ± 3.34##	4.21 ± 1.52##

[a] Values are mean ± dev for 8 rats in each group. Compared with control group: *$P < 0.05$, **$P < 0.01$. Compared with ethanol group: #$P < 0.05$, ##$P < 0.01$.

Table 3. Effect of AEBR on Hepatic and Serum TCH and TG[a]

	liver		serum	
parameters	TCH (mmol/L)	TG (mmol/L)	TCH (mmol/L)	TG (mmol/L)
control	1.24 ± 0.12	1.45 ± 0.11	2.92 ± 0.09	1.48 ± 0.19
ethanol	1.54 ± 0.16**	1.73 ± 0.22**	3.26 ± 0.62	1.76 ± 0.08*
ethanol + AEBR				
125 mg/kg	1.43 ± 0.14	1.59 ± 0.06	2.71 ± 0.57#	1.58 ± 0.21
250 mg/kg	1.43 ± 0.08	1.57 ± 0.099#	2.61 ± 0.33#	1.46 ± 0.31#
500 mg/kg	1.34 ± 0.10##	1.51 ± 0.10##	2.48 ± 0.39##	1.57 ± 0.09

[a] Values are mean ± dev for 8 rats in each group. Compared with control group: *$P < 0.05$, **$P < 0.01$. Compared with ethanol group: #$P < 0.05$, ##$P < 0.01$.

The increased levels of serum enzyme such as AST and ALT indicate the increased permeability and damage and/or necrosis of hepatocytes (*23*). The membrane bound enzyme GGT is released into the bloodstream depending on the pathological phenomenon (*24*). In our study, chronic ethanol consumption caused a significant increase in the activities of AST, ALT and GGT (**Table 2**), which could cause severe damage to tissue membrane. Obi et al. (*25*) examined that anthocyanin obtained from the petals of *Hibiscus rosainensis* significantly decreased the levels of serum aspartate and alanine aminotransferase activities (*25*). In this study, the decreased activities of these enzymes on AEBR administrated rats indicate the hepatoprotective effect.

Effect of AEBR on TCH and TG in Liver and Serum. Levels of triglycerides (TG) and total cholesterol (TCH) in 10% liver homogenate and serum are presented in **Table 3**. Ethanol intake led to the increase of serum and hepatic TG and TCH levels ($P < 0.05$). AEBR administration improved these adverse effects. Compared with the ethanol group, the serum and hepatic TG levels decreased significantly in ABER group.

Hepatic steatosis has been defined as either more than 5% of hepatocytes containing fat droplets or total lipid exceeding 5% of liver weight (*26*). Accumulation of fat is the earliest and most common response to heavy alcohol intake. Alcoholic fatty liver is usually characterized by the enlargement of the liver, the increase of the serum and hepatic TG levels, together with a lot of fat droplets in the liver sections (*27*). In the current study, ethanol administration resulted in the considerable increase of liver index, the elevation of the serum and hepatic TG and TCH levels (**Table 3**), suggesting that ethanol administration induced typical fatty liver. Parallel to these changes, histological examination showed a lot of droplets in ethanol-treated rat livers.

Effect of AEBR on GSH and MDA Concentrations and SOD, GST and GSH-Px Activities. The levels of MDA and GSH in serum and liver of control and experimental rats are shown in **Table 4**. Ethanol administration caused a severe increase of serum and liver MDA concentrations ($P < 0.01$). Pretreatment of rats with AEBR reduced the formation of MDA of serum and liver. **Table 4** represents the levels of nonenzymatic antioxidant (GSH) in tissues. The levels of GSH were significantly ($P < 0.05$) reduced

Table 4. Effect of AEBR on Hepatic and Serum MDA and GSH Concentrations[a]

	MDA		GSH	
parameters	serum (nmol/L)	liver (nmol/mg protein)	serum (mgl/L)	liver (mg/g protein)
control	5.18 ± 0.29	3.61 ± 0.69	9.15 ± 0.48	5.31 ± 0.63
ethanol	5.92 ± 0.34**	4.79 ± 0.79**	7.92 ± 0.95*	3.67 ± 0.42**
ethanol + AEBR				
125 mg/kg	5.43 ± 0.55	4.19 ± 0.65	8.55 ± 0.55	4.32 ± 0.66#
250 mg/kg	5.18 ± 0.36##	3.83 ± 0.57#	8.69 ± 1.01	4.45 ± 0.32#
500 mg/kg	5.13 ± 0.63##	3.85 ± 0.79#	9.48 ± 0.93##	4.47 ± 0.43#

[a] Values are mean ± dev for 8 rats in each group. Compared with control group: *$P < 0.05$, **$P < 0.01$. Compared with ethanol group: #$P < 0.05$, ##$P < 0.01$.

in alcohol-treated rats when compared with control rats. Administration of AEBR (500 mg/kg) significantly ($P < 0.05$) restored the levels of nonenzymatic antioxidants in tissues.

Ethanol-induced liver injury associated with increased oxidative stress and free radical-mediated tissue damage is widely demonstrated in rats and humans (*11*, *28*, *29*). Free radicals or reactive oxygen species (ROS) are responsible for ethanol induced oxidative stress (*10*, *30*). Free radicals formed from the ethanol-mediated process have a great potential to react rapidly with lipids, which in turn leads to lipid peroxidation (LPO). The level of malondialdehyde (MDA) has been widely used as a biomarker of LPO for many years (*31*).

Chiang et al. (*32*) reported that black rice anthocyanin extract (cyanidin-3-glucoside and peonidin-3-glucoside) may attenuate oxidative stress by reducing ROS and increasing antioxidant enzyme activities both in vitro and in vivo(*32*). Tsuda et al. (1999) found that cyanidin-3-glucoside (C3G), a potent antioxidant in vivo, can lower the serum thiobarbituric acid-reactive substance (TBARS) concentration and increase the oxidation resistance of serum to lipid peroxidation in rats (*1*). In the current study, AEBR was found dramatically inhibiting the elevation of MDA levels caused by ethanol, indicating that the protective effects of AEBR may be associated with antioxidant activities.

In antioxidant system, nonenzymatic antioxidant such as GSH plays an important role in protecting the cell from lipid peroxidation in biological system. Supplementation of AEBR to alcohol-treated rats restored the nonenzymatic antioxidants levels in liver and serum. We observed near-normal levels of these antioxidants in alcohol-administered rats (**Table 4**). AEBR acts as an oxygen-radical scavenger and chain-breaking antioxidant, which minimizes the consumption of endogenous antioxidants and improves the levels of those nonenzymic antioxidant in circulation of alcohol-treated rats.

The activities of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxide (GPx) and glutathione *S*-transferase (GST) are given in **Table 5**. A significant increase in the activities of enzymes was observed in alcohol-treated rats. Administration of AEBR (500 mg/kg) to alcohol-treated rats significantly ($P < 0.01$) decreased the activities.

Long-term ethanol treatment resulted in hepatic oxidative stress by exacerbated lipid peroxidation and decreased antioxidant enzyme activity. Free radical scavenging enzymes such as SOD, GST and GPx are the first line of defense against oxidative injury. The inhibition of antioxidant system may cause the accumulation of ROS or products of its decomposition (*32*, *33*).

The GSTs are a multigene family of isozymes that catalyze the conjugation of GSH to a variety of electrophilic compounds, and thereby exert a critical role in cellular protection against

Table 5. Effect of AEBR on Activities of SOD, GST and GSH-Px[a]

	GST		SOD		GSH-Px		
parameters	serum (U/mL)	postmitochondria (U/mg prot)	serum (U/mL)	mitochondria (U/mg prot)	serum (U/mL)	microsome (IU/L)	mitochondria (IU/L)
control	32.31 ± 2.56	42.26 ± 2.61	144.75 ± 10.86	291.89 ± 44.32	1650.13 ± 156.59	62.44 ± 8.30	60.65 ± 6.48
ethanol	27.68 ± 2.88**	37.29 ± 2.63*	128.33 ± 13.14*	210.18 ± 48.23**	1343.69 ± 120.66**	45.18 ± 5.85**	53.31 ± 11.47*
ethanol + AEBR							
125 mg/kg	28.49 ± 2.29	38.94 ± 6.36	139.39 ± 16.11	246.65 ± 23.43	1345.88 ± 95.20	46.57 ± 3.61	63.11 ± 7.04
250 mg/kg	29.34 ± 1.73	40.19 ± 5.38	142.79 ± 11.18	260.25 ± 41.99#	1393.06 ± 168.26	49.95 ± 6.62	64.65 ± 7.31#
500 mg/kg	31.48 ± 2.99#	46.60 ± 1.96##	175.76 ± 12.33##	280.13 ± 29.51##	1539.19 ± 137.17#	52.99 ± 7.12#	69.82 ± 9.75##

[a] Values are mean ± dev for 8 rats in each group. Compared with control group: *$P < 0.05$, **$P < 0.01$. Compared with ethanol group: #$P < 0.05$, ##$P < 0.01$.

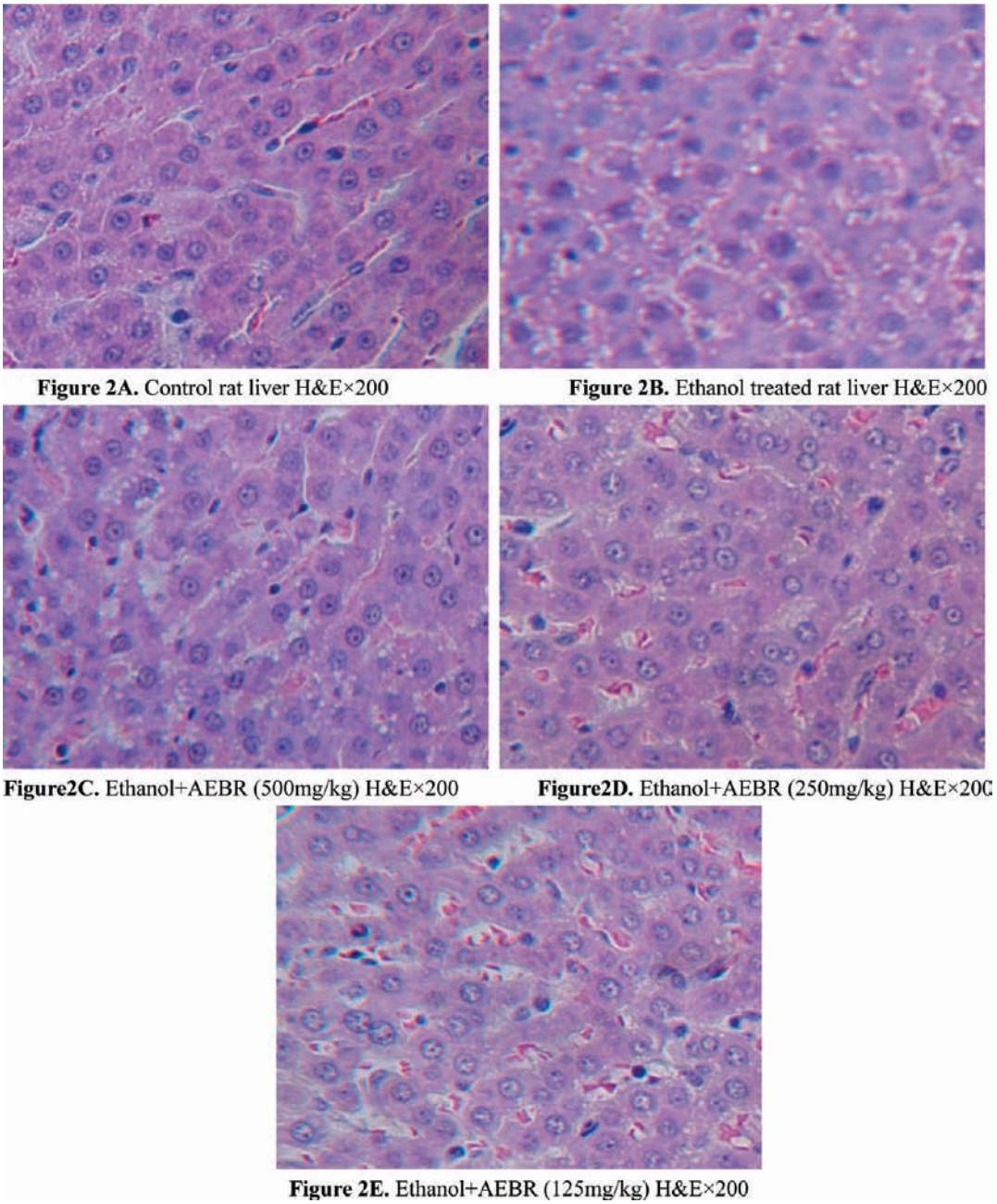

Figure 2. Representative photomicrographs of livers in control and experiment rats. The liver section of each rat from different groups was stained by hematoxylin and eosin staining, and the images were examined by Olympus BX50 light microscope.

ROS (*34*, *35*). Ethanol or its metabolic products might specifically target GST isoenzymes, and the reduction in enzyme activity or expression may contribute to ethanol hepatoxicity (*36*). In consistent with these reports, our results showed that oral supplementation of AEBR to rats treated with ethanol chronically restored the activities of GSH-Px, SOD and GST in liver (**Table 5**).

Histopathological Examination of Rat Liver. Ethanol can induce severe liver damage. The liver samples of alcohol-treated rats showed the focal hepatocytes' damage and degeneration (**Figure 2B**). It was found that administration of AEBR reversed this liver damage. AEBR at a dose of 500 mg/kg body weight (**Figure 2C**) was more effective when compared with two other doses (250 and 125 mg/kg body weight) (**Figure 2D**,**E**). The administration of ethanol along with AEBR (500 mg/kg body weight) showed near normal appearance (**Figure 2A**). The liver was almost normal in appearance with mild changes in hepatocytes of rats treated with AEBR (500 mg/kg BW) group. The results of histological observations suggest that alcohol leads to serious changes in histology of liver (**Figure 2A**). The present study suggests that the focal hepatocytic necrosis with inflammatory cell infiltration in alcohol-treated rats might be due to the accumulation of lipids and its content in tissues, which could also increase the LPO, as a basis for cellular damage. Administration of AEBR (500 mg/kg) (**Figure 2A**) remarkably reduced the histological alterations caused by alcohol, which may be due to attenuation of the ethanol-mediated oxidative threat and reduction of the pathological changes with restoration of normal physiological function.

AEBR contained four anthocyanins (cyanidin-3-glucoside, peonidin-3-glucoside, cyanidin-3,5-diglucoside, and cyanidin-3-rutinoside), and the anthocyanin structures were different. The structure of anthocyanin modulated functionality of anthocyanins; absorption and metabolism of anthocyanins were different in vivo. Delphinidin-3-glucoside was metabolized to 4′-methyl delphinidin-3-glucoside (*37*), whereas cyanidin-3-glucoside produced both 3′- and 4′-methyl cyanidin-3-glucoside (*38*). However, synergistic effects of anthocyanins are not clear; it is worth being researched.

In conclusion, our data indicate that AEBR has a protective action against alcohol-induced toxicity as evidenced by the lowered tissue lipid peroxidation and elevated levels of the enzymic and nonenzymatic antioxidants in liver, which is probably due to its antioxidant properties, scavenging ethanol-induced free radicals. AEBR plays a beneficial role in the treatment of alcohol induced tissue damage, which indicates the therapeutic values of black rice.

ABBREVIATIONS USED

AEBR, anthocyanin-rich extract from black rice; ALT, alanine transaminase; AST, aspartate transaminase; BW, body weight; GGT, gamma glutamyl transferase; GST, glutathione *S*-transferase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase; TCH, total cholesterol; TG, triglyceride; ROS, reactive oxygen species.

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Received for review December 14, 2009. Revised manuscript received January 26, 2010. Accepted January 26, 2010. The present study was supported by the Institute Fund (No. 2060302-15) from The Ministry of Sciences and Technology, People's Republic of China, and the Public Service (Agriculture) Special Fund for Research (No.200803056) from Ministry of Agriculture, People's Republic of China.