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Protective effects of the *Alisma orientalis* extract on the experimental nonalcoholic fatty liver disease

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Abstract

The aim of this investigation was to evaluate the efficacy of *Alisma orientalis* methanolic extract (AOME) on the experimental nonalcoholic fatty liver disease (NAFLD) induced by high-fat diet. Rats were fed with high-fat diet for six weeks and then gavaged the AOME for another six weeks. Typical pathological symptoms of NAFLD occurred in the high-fat diet rats. Administration with the AOME (150, 300 and 600 mg kg^{-1}) markedly decreased the serum and liver lipids; the high level of fasting serum glucose was reduced and insulin resistance was improved. The AOME treatment was also helpful in preventing the oxidative stress by lessening lipid peroxidation and activating antioxidant enzymes. Markers of the liver injury, aminotransferase abnormalities and hepatomegaly were improved and morphological changes, such as liver steatosis, mixed inflammation and collagen deposition, were lessened in rats treated with the AOME. These results suggested that the AOME showed hepatoprotective effects on NAFLD and may be a potential clinical application for treatment of this chronic liver disease.

Introduction

In recent years, nonalcoholic fatty liver disease (NAFLD) has been suggested to be the most common cause of chronic liver disease throughout the world, with a suggested incidence of 10–24% in the general population in the USA and similar figures in Europe and Japan (Clark et al 2002, 2003; Jimba et al 2005). At least 20% of individuals with NAFLD develop cirrhosis and half of those with cirrhosis die within a decade of diagnosis, making this liver disease the second leading cause of death in this population (Matteoni et al 1999).

The pathogenesis of NAFLD has remained poorly understood since the earliest description of this disease. One popular and accepted mechanism is the two-hit theory, in which the first hit is the accumulation of fatty acids in the liver and insulin resistance plays the promoting role (Day & James 1998). As a result of the first hit, the liver becomes steatotic and is vulnerable to secondary insults, including oxidative stress, adipocytokines, endotoxins and so on. The second hit usually leads to hepatocellular inflammation, liver fibrosis and even cirrhosis (Chitturi et al 2002).

No effective pharmacological therapy is currently available for patients with NAFLD (Adams et al 2005). There is a strong association between NAFLD and conditions related to the metabolic syndromes. Hence, drugs that have been used in hyperlipidaemia, type II diabetes mellitus and some antioxidants now are receiving great attention in prevention of NAFLD. However, some of the drugs have been found to be potentially hepatotoxic in clinical trials (Laurin et al 1996; Caldwell et al 2001; Marchesini et al 2001a,b), and developing effective and low-hepatotoxic drugs for treating NAFLD is a big challenge for us.

Herbal medicine has been used in China for thousands of years. In the past decade, it has attracted the interest of modern scientific communities as alternative medicine. *Alisma orientalis* Juzep is a traditional Chinese medicinal herb. The chief effective components from *Alisma orientalis* are terpenoids, mainly including a series of triterpenoids and sesquiterpenoids (Peng et al 2002). *Alisma orientalis* Juzep has been prescribed for diuretic and anti-inflammatory purposes in traditional Chinese medicine. In local folk prescriptions, the *Alisma orientalis* extract is also used as a modulator for a variety of disorders, such as renal lithiasis, hypertension, chronic nephritis and chronic kidney failure (Yin & Wu 2001; Feng et al 2003; Cao et al 2005).

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There was no hepatotoxicity or nephrotoxicity of the *Alisma orientalis* extract reported, even at high dosage. Recently, a number of animal studies (Yang & Huang 1998; Yang et al 2004) have shown that the *Alisma orientalis* extract possesses potent effects of lowering high levels of serum lipids and improving insulin resistance, which are usually presented in the patients and animals with NAFLD. Accordingly, the main purpose of our study is to test the efficacy of the *Alisma orientalis* methanolic extract (AOME) as a treatment for the rat model of NAFLD induced by high-fat diet.

Materials and Methods

Plant extract

The *Alisma orientalis* methanolic extract (AOME) was provided by Xi'an Sanjiang Bio-engineering, Co. Ltd (China). The rhizome of *Alisma orientalis* was collected from Jiangxi Province (China) and botanically authenticated by the technicians of Xi'an Sanjiang Bio-engineering, Co., Ltd (China). Herb-to-product ratio was 10:1. Further analysis in our laboratory showed that the content of the triterpenoid alcoholic ketones in the extract was 8–12% detected by colorimetric method according to Wang's instructions (Wang et al 2003), containing 4.27% alisol-B-23-acetate and 2.06% alisol-C-23-acetate detected by HPLC according to Wang's method (Wang et al 2002). The extract was dissolved in the ultrapure water before use.

Animals and experimental protocol

Male Sprague-Dawley rats, 180–200 g, were obtained from the Laboratory Animal Institute of Zhejiang Academy of Medical Science (China). Rats were kept in an environmentally controlled breeding room (temperature 23±3°C, humidity 60±5%, 12-h dark–light cycle). All rats were housed in stainless steel wire-bottom cages under the institutional guidelines of Zhejiang University for the humane treatment of laboratory animals. Rats were randomly divided into five groups of 12 rats in each group. The rats of group I had free access to normal diet and tap water. The rats of the other four groups (group II, group III, group IV, group V) were fed with a high-fat diet consisting of 2% cholesterol, 0.25% cholic acid, 2.5% glucose and 8% hog fat added to normal chow. Six weeks later, Group II (HFD), rats were fed with high-fat diet continuously and gavaged the ultrapure water daily; Group III (l-AOME) rats were fed with high-fat diet continuously and gavaged a low dose of the AOME (150 mg kg^{-1}) daily, Group IV (m-AOME) rats were fed with high-fat diet continuously and gavaged a medium dose of the AOME (300 mg kg^{-1}) daily; Group V (h-AOME) rats were fed with high-fat diet continuously and gavaged a high dose of the AOME (600 mg kg^{-1}) daily. Body weight was measured and recorded every week for the duration of this study. The pharmacological agents were regulated according to the body weights.

Preparation the tissues and serum samples

At the end of the experiment, all rats were anaesthetized and decapitated. Blood samples were collected immediately. Serum was separated and stored at −76°C for analysis of the biochemical parameters. Livers and epididymal fat pads were dissected out and weighed. One portion of the liver tissue was fixed in 10% neutral buffered formalin for histological analysis, and the remaining was snapped in liquid nitrogen and stored at −76°C for estimation of the other various parameters. All the experimental protocols were approved by the Institutional Animal Ethics Committee of Zhejiang University.

Biochemical assays

All the test principle methods used in the biochemical assays are based on the recommendations of the International Federation for Clinical Chemistry (IFCC).

Lipid profile

A known weight and fixed section of the liver samples were homogenized in cold isopropanol (10% m/v: 1 g tissue in 10 mL isopropanol) using a homogenizer. The homogenates were centrifuged at 1500 g min^{-1} for 15 min. The clear supernatants were used for the estimation of TC (total cholesterol) and TG (triglyceride) levels enzymatically using commercial kits (Olympus, Japan) with auto biochemical analysis apparatus (Olympus, Japan). Serum TC and TG levels were estimated in the same methods.

Pro-oxidants and antioxidants

Oxidative stress was assessed by the concentrations of superoxide dismutase (SOD) and malondialdehyde (MDA). Serum SOD and MDA levels were detected by the use of diagnostic kits (Jiancheng, China) with a UV-visible spectrophotometer (Shimadzu, Japan) according to the supplier's instructions.

Liver function

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were detected by the use of biochemical kits (Olympus, Japan) with auto biochemical analysis apparatus (Olympus, Japan) according to the manufacturer's instructions.

Determination of the fasting serum insulin level and fasting serum glucose level

Serum insulin level was detected by the use of insulin radioimmunoassay kit (Beijing Atom High Tech Co. Ltd, China) with Full Automatic γ RIA-Immunity analyzer (Xi'an nuclear instrument factory, China) according to the manufacturer's instructions. Fasting serum glucose level was detected by the use of biochemical kit (Zhongsheng, China) with auto biochemical analysis apparatus (Olympus, Japan). The insulin sensitivity index (ISI) and the insulin resistance index (IRI) were calculated according to Li's methods (Li et al 1998).

Morphological studies

All the liver specimens were fixed in 10% neutral buffered formalin for 18–24 h and embedded in paraffin. Sections (4 μm) were stained with haematoxylin and eosin (HE) stain and Masson's trichrome (M3T) stain. Each section was

scored by two independent observers for the extent of steatosis, inflammation and fibrosis. At least three different sections were examined per group.

Statistical analysis

Results were expressed as mean ± s.d. for 12 rats in each group. Differences between groups were assessed by analysis of variance using the SPSS software package for Windows. Post-hoc testing was performed for inter-group comparisons using the least significance difference test (LSD). $P<0.05$ was considered significant.

Results

Body weights and epididymal fat weights

Body weights, epididymal fat weights and the epididymal fat weight/body weight ratio of rats are summarized in Table 1. High-fat diet significantly increased the body weights ($P<0.01$), epididymal fat weights ($P<0.001$) and the epididymal fat weight/body weight ratio ($P<0.01$) as compared with normal diet rats. Administration of the AOME (150,300 and 600 mg kg^{-1}) daily had little effect on the body weights, as it was not significant when compared with the high-fat diet rats ($P>0.05$, $P>0.05$ and $P>0.05$, respectively). Administration of the AOME (150, 300 and 600 mg kg^{-1}) daily markedly reduced the epididymal fat weight ($P<0.01$, $P<0.01$ and $P<0.001$, respectively), and the epididymal fat weight/body weight ratio ($P<0.01$, $P<0.01$ and $P<0.001$, respectively), when compared with the high-fat diet rats.

Table 1 Effect of AOME on body weight, epididymal fat weight and the epididymal fat weight/body weight ratio of rats

Group	BW (g)	EFW (g)	EFW/BW (%)
ND	410.13 ± 26.87	3.42 ± 0.57	0.83 ± 0.15
HFD	441.17 ± 12.39##	5.12 ± 1.25###	1.16 ± 0.30##
l-AOME	450.38 ± 28.24	3.79 ± 0.91**	0.85 ± 0.24**
m-AOME	440.08 ± 15.85	3.64 ± 0.84**	0.83 ± 0.18**
h-AOME	447.03 ± 35.66	3.22 ± 0.68***	0.72 ± 0.14***

BW, body weight; EFW, epididymal fat weight; EFW/BW, the epididymal fat weight/body weight ratio; ND, normal diet group; HFD, high-fat diet group; l-AOME, high-fat diet plus low dose of the AOME group; m-AOME, high-fat diet plus medium dose of the AOME group; h-AOME, high-fat diet plus high dose of the AOME group. Data are presented as mean ± s.d., 12 rats per group. **$P<0.01$ and ***$P<0.001$ represent the significant difference from HFD rats; ##$P<0.01$ and ###$P<0.01$ represent the significant difference from ND rats.

Liver weights and ALT and AST levels

Liver weights and serum ALT and AST levels are summarized in Table 2. High-fat diet significantly increased the liver weights ($P<0.001$) and the liver weight/body weight ratio ($P<0.001$) when compared with the normal diet rats. Administration of the AOME (300, 600 mg kg^{-1}) daily reduced the liver weights ($P<0.05$ and $P<0.01$, respectively) and the liver weight/body weight ratio ($P<0.05$ and $P<0.01$, respectively) when compared with the high-fat diet rats. Administration of the AOME (150 mg kg^{-1}) daily reduced the liver weights and the liver weight/body weight ratio, but not significantly ($P>0.05$ and $P>0.05$, respectively) when compared with the high-fat diet rats.

High-fat diet significantly increased serum AST and ALT levels ($P<0.01$ and $P<0.001$, respectively) as compared with the normal diet rats. Administration of the AOME (150, 300, 600 mg kg^{-1}) daily reduced serum AST levels ($P<0.05$, $P<0.05$ and $P<0.01$, respectively) and serum ALT levels ($P<0.01$, $P<0.01$ and $P<0.001$, respectively)), when compared with the high-fat diet rats.

Lipid profile

Serum and hepatic TC and TG levels of all groups of rats are shown in Figure 1. Compared with the normal diet rats, high-fat diet increased serum TC and TG levels significantly ($P<0.001$ and $P<0.01$, respectively). Administration of the AOME (150, 300 and 600 mg kg^{-1}) markedly reduced the serum TC level ($P<0.001$, $P<0.05$ and $P<0.01$, respectively) when compared with the high-fat diet rats. Administration of the AOME (300 and 600 mg kg^{-1}) markedly reduced the serum TG level ($P<0.05$ and $P<0.01$, respectively) when compared with the high-fat diet group rats. The AOME (150 mg kg^{-1}) reduced the serum TG level, but this was not significant ($P>0.05$), when compared with the high-fat diet rats (Figure 1A).

Table 2 Effect of AOME on liver weight, the liver/body weight ratio and serum ALT and AST levels in rats

Group	LW (g)	LW/BW (%)	AST (U L^{-1})	ALT (U L^{-1})
ND	10.43 ± 0.98	2.53 ± 0.23	271.56 ± 54.25	70.92 ± 17.72
HFD	18.03 ± 1.73###	4.07 ± 0.34###	336.94 ± 42.14##	104.92 ± 18.18###
l-AOME	17.27 ± 1.87	3.84 ± 0.33	274.22 ± 73.52*	86.06 ± 22.49**
m-AOME	16.31 ± 1.78*	3.72 ± 0.45*	273.72 ± 75.97*	87.19 ± 21.38**
h-AOME	15.88 ± 1.74**	3.57 ± 0.47**	246.39 ± 80.30**	75.91 ± 13.05***

BW, body weight; LW, liver weight; LW/BW, liver weight/body weight ratio; ND, normal diet group; HFD, high-fat diet group; l-AOME, high-fat diet plus low dose of the AOME group; m-AOME, high-fat diet plus medium dose of the AOME group; h-AOME, high-fat diet plus high dose of the AOME group. Data are presented as mean ± s.d., 12 rats per group. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ represent the significant difference from HFD rats; ##$P<0.01$ and ###$P<0.001$ represent the significant difference from ND rats.

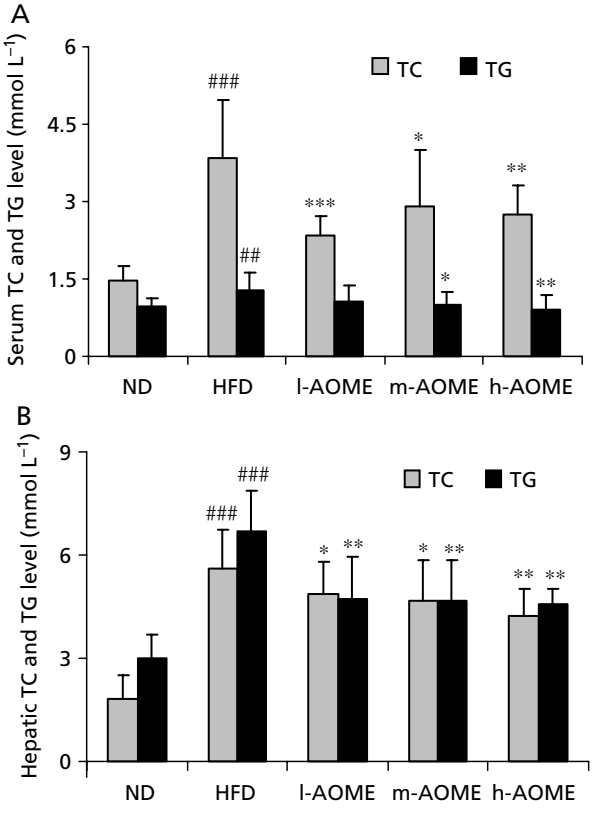

Figure 1 Effect of the AOME on serum (A) and hepatic (B) triglyceride (TG) and total cholesterol (TC) levels in rats. ND, normal diet group; HFD, high-fat diet group; l-AOME, high-fat diet plus low dose of the AOME group; m-AOME, high-fat diet plus medium dose of the AOME group; h-AOME, high-fat diet plus high dose of the AOME group. Data are presented as mean ± s.d., 12 rats per group. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ represent the significant difference from HFD rats; ##$P<0.01$ and ### $P<0.001$ represent the significant difference from ND rats.

Compared with the normal diet rats, high-fat diet increased hepatic TC and TG levels significantly ($P<0.001$ and $P<0.001$, respectively). Administration of the AOME (150, 300, 600 mg kg^{-1}) markedly reduced hepatic TC level ($P<0.05$, $P<0.05$ and $P<0.01$, respectively) and hepatic TG level ($P<0.01$, $P<0.01$ and $P<0.01$, respectively) when compared with the high-fat diet rats (Figure 1B).

Serum insulin level, fasting serum glucose level, the ISI and the IRI

Fasting serum glucose level (FSG), fasting serum insulin level (FSI), the insulin resistance index (IRI) and the insulin sensitivity index (ISI) of all groups of rats are presented in Table 3. Compared with the normal diet rats, high-fat diet caused a marked elevation of fasting serum glucose level ($P<0.001$) and fasting serum insulin level ($P<0.01$). The IRI was increased significantly ($P<0.001$) while the ISI was decreased ($P<0.001$). Treatment with the AOME (150, 300 and 600 mg kg^{-1}) significantly reduced fasting serum glucose level ($P<0.01$, $P<0.01$ and $P<0.001$, respectively) and serum insulin level ($P<0.05$, $P<0.05$ and $P<0.01$, respectively); the ISI ($P<0.01$, $P<0.05$ and $P<0.001$, respectively) and the IRI improved ($P<0.01$, $P<0.01$ and $P<0.001$, respectively) as compared with the high-fat diet rats (Table 3).

Serum MDA and SOD levels

Table 4 shows serum MDA and SOD levels of all rats. High-fat diet increased serum MDA level ($P<0.01$) and decreased serum SOD activity ($P<0.05$) when compared with the normal diet rats. Administration of daily gavage with the AOME (150, 300 and 600 mg kg^{-1}) significantly reduced serum MDA level($P<0.01$, $P<0.01$ and $P<0.001$, respectively) when compared with the high-fat diet rats. Administration of daily gavage with the AOME (300 and 600 mg kg^{-1}) significantly increased serum SOD activity ($P<0.05$ and $P<0.01$, respectively) as compared with the high-fat diet rats. However, treatment with AOME (150 mg kg^{-1}) increased the SOD activity, but it was not significant as compared with the high-fat diet rats.

Histological evaluation of the liver

Photomicrographs of liver samples stained with haematoxylin and eosin are shown in Figure 2 and Masson's trichrome (M3T) stain are shown in Figure 3. Livers from the rats fed with ordinary diet for 12 weeks are normal without any pathological symptoms (Figure 2A). In the hepatocytes of the high-fat diet rats, a lot of large vacuoles within many lipid droplets, mononuclear cell infiltration, picnotic nuclei, and the rupturing in the endothelium of some central veins were presented (Figure 2B). The degenerative changes of such

Table 3 Effect of AOME on the fasting serum glucose level, fasting serum insulin level, insulin sensitivity index and insulin resistance index in rats

Group	FSG (mmol L^{-1})	FSI (IU L^{-1})	IRI	ISI
ND	4.02 ± 1.13	19.89 ± 4.24	3.74 ± 1.86	–4.32 ± 0.51
HFD	8.16 ± 0.91###	28.13 ± 10.01##	10.14 ± 3.52###	–5.38 ± 0.35###
l-AOME	5.88 ± 1.66**	21.26 ± 4.84*	5.63 ± 2.24**	–4.76 ± 0.46**
m-AOME	5.98 ± 1.39**	23.00 ± 4.01*	6.04 ± 1.52*	–4.88 ± 0.25**
h-AOME	4.11 ± 1.33***	20.25 ± 4.22**	3.76 ± 1.95***	–4.32 ± 0.52***

FSG, Fasting serum glucose; FSI, fasting serum insulin; ISI, insulin sensitivity index; IRI, insulin resistance index; ND, normal diet group; HFD, high-fat diet group; l-AOME, high-fat diet plus low dose of the AOME group; m-AOME, high-fat diet plus medium dose of the AOME group; h-AOME, high-fat diet plus high dose of the AOME group. Data are presented as mean ± s.d., 12 rats per group. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ represent the significant difference from HFD rats; ##$P<0.01$ and ###$P<0.001$ represent the significant difference from ND rats.

Table 4 Effect of AOME on serum MDA and SOD levels in rats

Group	MDA (nmol L^{-1})	SOD (U mL^{-1})
ND	4.33 ± 1.39	424.25 ± 13.95
HFD	7.79 ± 3.34##	396.94 ± 14.83#
l-AOME	4.11 ± 1.06**	417.75 ± 19.14
m-AOME	3.76 ± 1.09**	428.64 ± 23.04*
h-AOME	3.65 ± 0.74***	432.95 ± 49.13**

Serum MDA and SOD levels from different experimental groups of rats. ND, normal diet group; HFD, high-fat diet group; l-AOME, high-fat diet plus low dose of the AOME group; m-AOME, high-fat diet plus medium dose of the AOME group; h-AOME, high-fat diet plus high dose of the AOME group. Data are presented as mean ± s.d., 12 rats per group. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ represent the significant difference from HFD rats; #$P < 0.05$, ##$P < 0.01$ represent the significant difference from ND rats.

hepatic lesions of NAFLD were observed in the AOME-treated rats (600, 300 and 150 mg kg^{-1}) (Figure 2C–E). No fibrosis was found in the periportal areas in the livers of the normal diet rats (Figure 3A). Livers from the rats fed with high-fat diet showed collagen deposition, which was most prominent in the zone near the central veins (CV) of the hepatic lobule and the portal tracts (PT) (Figure 3B). The AOME treatment (600, 300 and 150 mg kg^{-1}) attenuated the hepatic steatosis and the collagen deposition in the liver (Figure 3C–E).

Discussion

Feeding animals with high-fat diet usually resulted in NAFLD, as shown by a marked increase in the serum and hepatic lipids, insulin resistance, lipid peroxidation and aminotransferase abnormalities. On histological examination, hepatic lesions of NAFLD, such as hepatomegaly, liver steatosis, mixed inflammation and collagen deposition were observed (Matteoni et al 1999; Lieber et al 2004). In our study, typical pathological changes of NAFLD were reproduced in the rats fed with high-fat diet, suggesting it is a useful and reliable model for NAFLD.

At present, there is no pharmacological agent that is known to prevent or reverse NAFLD, and weight reduction remains the mainstay of non-pharmacological intervention available for NAFLD patients (Dixon et al 2004). Some trials found that reduction of the visceral fat could reverse the liver steatosis and insulin resistance in NAFLD animals (Hansen et al 1997; Neuschwander-tetri 2005). In our study, administration of the AOME markedly reduced the epididymal fat weight and improved the liver tests in NAFLD rats, indicating that visceral fat reduction might be a potential mechanism of the AOME in curing NAFLD.

There is a strong association between NAFLD and dyslipidaemia (Matteoni et al 1999; Marchesini et al 2001a), and there has been some recent evidence that lipid-lowering agents improve hepatic steatosis in NAFLD patients (Angulo et al 2001). However, other researchers found that some of the lipid-lowering agents succeeded in improving hyperlipidaemia, but resulted in fat accumulation in the liver, and aggravated hepatic steatosis (Zeng 2001). In our study, administration of AOME improved dyslipidaemia and lessened hepatic steatosis in NAFLD rats. However, the mechanisms of these effects are unclear, and maybe the AOME not only regulates lipid transport between the peripheral adipose tissue and the liver, but also regulates the lipid metabolism in the liver, and these were proved to be hepatoprotective.

Insulin resistance and oxidative stress contribute to the transition of simple fatty liver to steatohepatitis and liver fibrosis. The severity of insulin resistance has been shown to parallel the severity of fatty liver disease and it is the most specific finding in human NAFLD (Choudhury et al 2004). Insulin resistance provoked hepatic fat accumulation in the liver and accelerated the steatosis formed, and in turn, fat accumulation in the liver aggravates the insulin resistance by affecting the insulin signalling cascade (Samuel et al 2004). Insulin resistance also indirectly provokes hepatotoxicity by regulating some cytokines. These cytokines induce perisinuous fibrosis, impair microvascular hepatic blood flow and produce damaging reactive oxygen species (ROS), further exacerbating liver damage (Paradis et al 2001). Our study found that high-fat diet provoked insulin resistance. Administration of the AOME lessened the liver steatosis and improved insulin resistance in the NAFLD rats. It caused a significant decrease in the fasting serum glucose and serum insulin levels; meanwhile, the insulin sensitivity index (ISI) was increased while the insulin resistance index (IRI) was decreased significantly. However, what interested us is how the AOME improved the insulin resistance, and whether its beneficial effects on insulin resistance simply reflects its ability to constrain dyslipidaemia or whether the AOME directly regulates some genetic factors that participated in the pathogenesis of insulin secretion and insulin action to reverse insulin resistance, or both actions had produced a marked effect. Further study need to be done.

Once the presence of hepatic steatosis is established, the liver becomes susceptible to the so-called second hit, especially oxidative stress (Seki et al 2002). Oxidative stress results from an imbalance between pro-oxidant and antioxidant chemical species, which usually leads to oxidative damage of cellular macromolecules. The predominant pro-oxidant chemicals in the fatty liver are collectively referred to as ROS (Robertson et al 2001). ROS initiate lipid peroxidation within the cell, producing a series of aldehyde by-products such as MDA and *trans*-4-hydroxy-2-nonenal (4-HNE) (Gardner 1989). Previous studies showed that MDA depleted the natural antioxidant enzymes, increased the production of the pro-inflammatory cytokines, promoted the influx of inflammatory cells into the liver, and it is believed to play important roles in the transition from steatosis to steatohepatitis and liver fibrosis (Clark et al 2003; Cockell et al 2005).

Oxidative stress is usually accompanied by degenerative changes in the enzymatic antioxidant defence systems. SOD is important for cellular defence against ROS toxicity and is usually sensitive to inactivation by ROS. Low levels of SOD and increased levels of MDA were usually found in the animal model of NAFLD (Polavarapu et al 1998; Kumagai et al 2000). In our study, the AOME treatment increased the activity of SOD and decreased the level of MDA in blood, and these were in association with the degenerative changes of collagen deposition

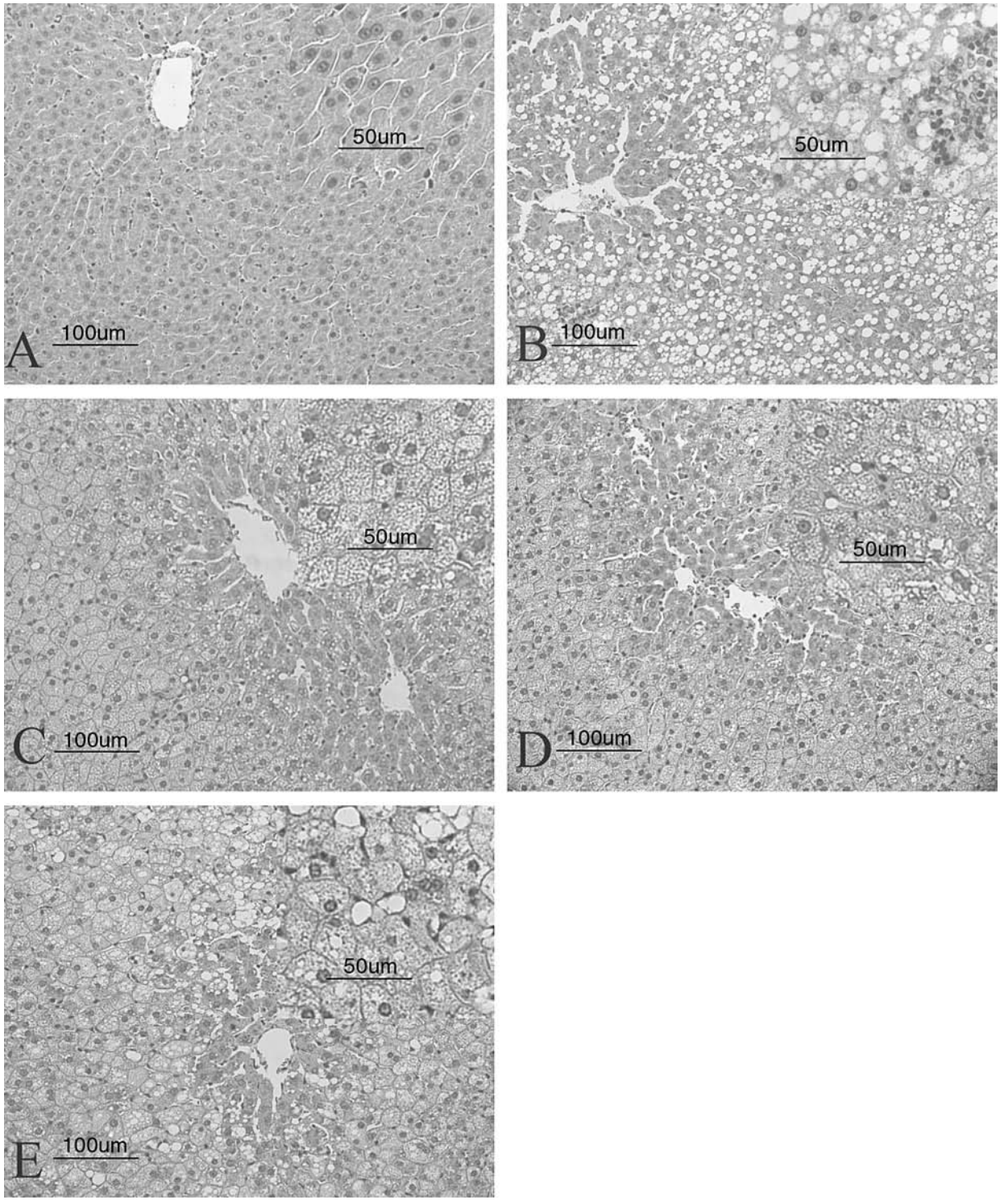

Figure 2 Rats fed ordinary diet for 12 weeks are normal without any pathological symptoms (A). Significant steatosis and inflammatory cells appeared in the liver of the rats fed high-fat diet (B). The degenerative changes of hepatic lesions of NAFLD were observed in the AOME-treated rats (600, 300 and 150 mg kg^{-1}) (C, D and E, respectively).

in the liver. The chief effective components from the *Alisma orientalis* are terpenoids, mainly including a series of triterpenoids and sesquiterpenoids (Peng et al 2002). In fact, researchers found that the naturally occurring pentacyclic triterpenes that come from plants have been reported to possess hypoglycaemic, antihyperlipedaemic and antioxidant activity (Sunithan et al 2001).

Liver biopsy remains the gold standard for the diagnosis of NAFLD, but the aminotransferase abnormalities and

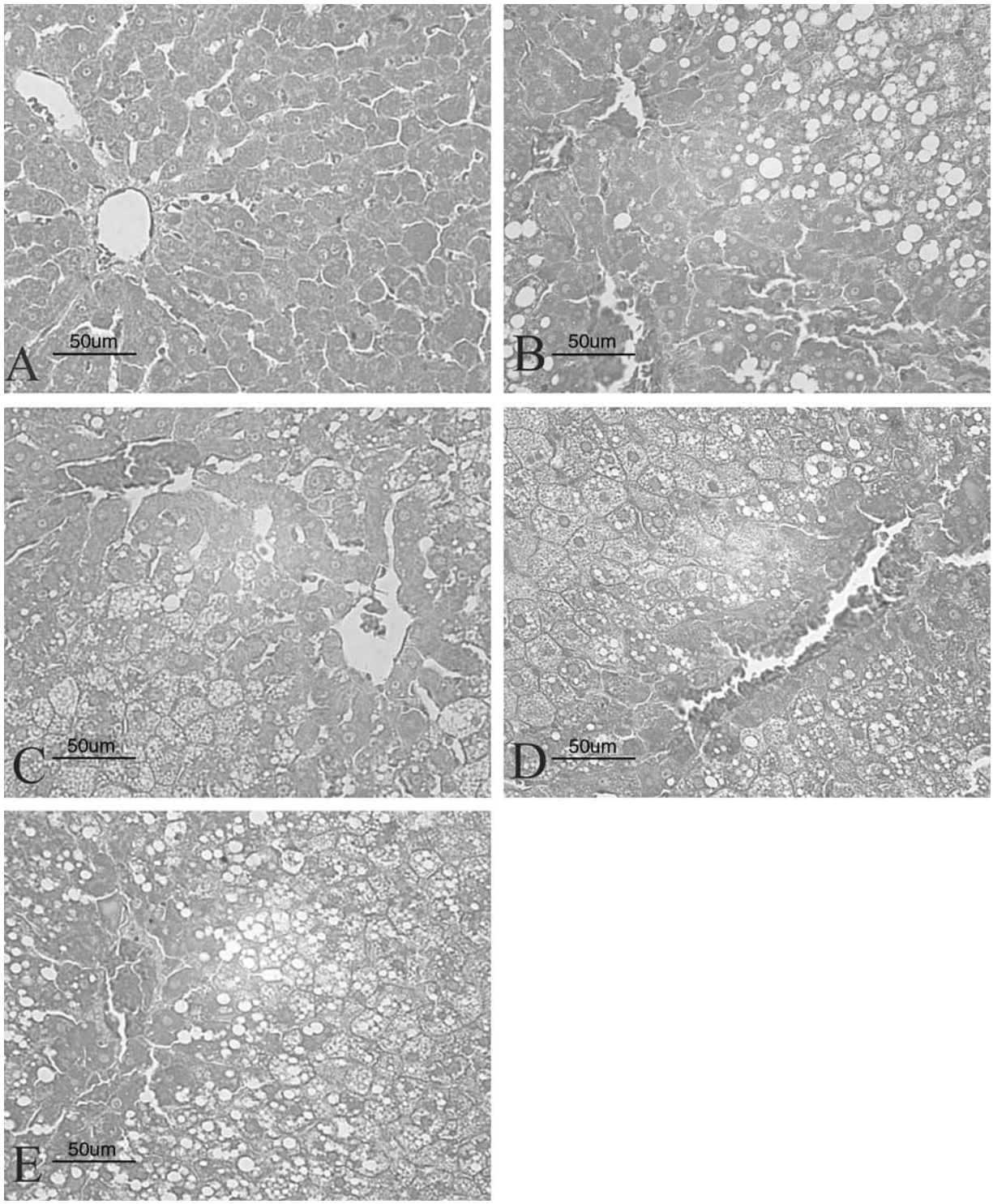

Figure 3 Rats fed ordinary diet for 12 weeks are normal (A). Typically collagen deposition was found in the rats fed high-fat diet (B). The AOME treatment (600, 300 and 150 mg kg^{-1}) attenuated the hepatic steatosis and collagen deposition in the liver (C, D and E, respectively).

hepatomegaly are also reliable clinical features of NAFLD (Lieber et al 2004; Giannini et al 2005). Interestingly, aminotransferase abnormalities and hepatomegaly were also observed in the patients who had been treated with the hypolipidaemic drugs and these are thought to be the side effects of such drugs (Laurin et al 1996; Kaplowitz 2005). Administration of AOME remarkably prevented the elevation of serum aminotransferases levels and improved the hepatomegaly without affecting the body weights of rats, suggesting that the AOME treatment was hepatoprotective in the NAFLD rats.

Conclusion

In this study, all the biochemical data and morphological results suggested that oral administration with the AOME showed significant protective effects in the NAFLD rats. The AOME treatment markedly improved the liver-test results but displayed no conspicuous hepatotoxicity and may be a potential clinical application in the treatment of this chronic liver disease. Following investigations are focused on elucidating the molecular mechanisms of these beneficial effects.

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