Research Article

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Hepatoprotective potential of *Aloe vera* polysaccharides against chronic alcohol-induced hepatotoxicity in mice

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Abstract

BACKGROUND: *Aloe vera* polysaccharides are reported to exhibit multiple biological effects, including anti-oxidation, anti-inflammation and immune enhancement. However, their influence on alcoholic liver disease (ALD) remains unclear. This study was designed to determine the protective effect of extracted *A. vera* polysaccharides (AVGP) against ALD in a chronic alcohol-feeding mouse model and investigate the possible underlying mechanisms.

RESULTS: Supplementation of AVGP significantly attenuated the levels of serum aminotransferases, lipids and hepatic TG and ameliorated histopathological alterations in the model of ALD. Interestingly, AVGP markedly up-regulated hepatic expression of lipolytic genes (AMPK-α2 and PPAR-α) but had no effect on lipogenic gene expression. AVGP diminished alcohol-dependent oxidative stress partly through a decrease in MDA and increase in GSH and SOD. Alcohol-induced inflammation was also mitigated by AVGP treatment via significant reduction in LPS and TNF-α, down-regulation of TLR-4 and MyD88 and up-regulation of IκB-α.

CONCLUSION: This study clearly showed that AVGP exerts a potent protective effect against chronic alcohol-induced liver injury. Its hepatoprotective effect appears to be associated with its antioxidant capacity and its ability to accelerate lipolysis and inhibit inflammatory response. The results indicate that AVGP could be considered as a potent food supplement in the prevention of ALD.

Keywords: *Aloe vera* polysaccharides; chronic alcoholic liver injury; hepatoprotective; lipid accumulation; oxidative stress; inflammation

INTRODUCTION

Alcoholic liver disease (ALD) is considered a major health and economic problem worldwide. Owing to the increased frequency of drinking and changes in diet formulation, the incidence of ALD has increased quickly in China, becoming an important risk factor for morbidity and mortality in addition to viral hepatitis.[1] ALD is a group of diseases associated with a spectrum of liver injury ranging from steatosis and steatohepatitis to fibrosis/cirrhosis.[2,3] Many pathways are thought to be associated with ALD, involving an increase in NADH/NAD^+ ratio, causing lipid accumulation, and up-regulation of cytochrome P4502E1 (CYP2E1), thus leading to oxidative stress and inflammatory injury.[4–6] In the past several decades, many drugs, including bicyclol, tiopronin and bifendate, have been reported to have a hepatoprotective effect on ALD,[7–10] but no satisfactory therapy for ALD exists at present. Recently, agents developed from natural products and traditional medicinal plants that possess hepatoprotective effects have become increasingly attractive in the prevention and therapy of ALD.[11–13]

Aloe barbadensis Miller (*Aloe vera*), a rich source of polysaccharides, has been used as a traditional food and medicine around the world for its curative and therapeutic properties for several thousand years.[14] In traditional Chinese medicine, *A. vera* is commonly used to treat constipation, verminosis, indigestion and diseases of the liver and spleen. Recently, it was reported that *A. vera* exerted a potential hepatoprotective effect against experimental liver injury induced by various chemical toxins, including carbon tetrachloride, petroleum products, b enzene hexachloride and paracetamol.[15–18] In addition, it also showed a protective effect on acute alcohol-induced liver damage.[19,20] However, the active principle(s) responsible for its hepatoprotective action and the underlying mechanisms are still poorly understood.

Polysaccharides, the main components of *A. vera* gel, are responsible for many of the health benefits of *A. vera*.[21] In our previous studies, the extracted polysaccharides (AVGP) from concentrated lyophilized *A. vera* gel powder (200:1X) showed significant protective effects against acute alcohol-induced liver injury and effectively alleviated hangover and decreased plasmatic alcohol levels in mice.[22] However, the physiological functions and

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the related mechanisms in chronic alcohol-induced hepatotoxicity are still unclear. To extend our previous findings with respect to the hepatoprotection of AVGP, this study attempted to assess the potential protective effect of AVGP against ALD in a chronic alcohol-feeding mouse model. The mechanisms underlying its protective effects were also investigated.

MATERIALS AND METHODS

Materials and chemicals

The lyophilized *A. vera* gel powder (200:1X) used in this study was prepared on an industrial scale by Ever Green (Kunming, China). Compound Biejiaruangan Troche (CBT) as a commercially available hepatoprotective drug was produced by Inner Mongolia Furui Medical Science Co., Ltd (Inner Mongolia, China). Triglyceride (TG), superoxide dismutase (SOD), reduced glutathione (GSH), nitric oxide (NO) and malondialdehyde (MDA) diagnostic kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Lipopolysaccharide (LPS), interleukin-1β (IL-1β), interleukin-10 (IL-10) and tumor necrosis factor-α (TNF-α) enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D Systems (Minneapolis, MN, USA). All other chemicals used were of analytical grade.

Preparation of *Aloe vera* polysaccharides

Aloe vera polysaccharides were extracted from the lyophilized *A. vera* gel powder according to the method described by Wu *et al.*,[23] with minor modifications. Initially, the lyophilized *A. vera* gel powder was completely dissolved in distilled water. After extraction for 2 h, the filtrate of the obtained extract was condensed in a rotary evaporator, to which 4 volumes of cold anhydrous alcohol was added. The crude polysaccharide part was precipitated from the alcoholic liquor during its subsequent standing at 4 °C overnight. The resulting precipitate was filtered and then washed successively with 80% alcohol, anhydrous alcohol, acetone and anhydrous diethyl ether to remove protein and lipid. After deproteinization by the Sevag method,[24] the extract was precipitated with 4 volumes of anhydrous alcohol followed by concentration (2658 $\times$ *g* (5000 rpm), 10 min). Finally, polysaccharide (AVGP) powder was obtained by freeze-drying.

Animals and treatments

Male Kunming mice weighing 18–22 g were obtained from Shanghai Laboratory Animal Center (SLAC, Shanghai, China). The mice were housed under standard conditions with a 12/12 h light/dark cycle at a temperature of 23 $\pm$ 2 °C and a humidity of 60 $\pm$ 5%. All animals were fed commercial standard chow (SLAC) and water *ad libitum*. Animal protocols were developed according to the institution's guidelines for the care and use of laboratory animals and were approved by the local Animal Care and Use Committee.

After acclimatization for 7 days, 60 mice were randomly divided into five groups of 12 mice each: (1) control group; (2) alcohol group; (3) alcohol + CBT group (500 mg kg^{-1}, in normal saline, positive control, PC); (4) alcohol + AVGP low-dose group (10 mg kg^{-1}, in normal saline, AAL); (5) alcohol + AVGP high-dose group (30 mg kg^{-1}, in normal saline, AAH). The control group served as a vehicle-treated control without alcohol administration. In the four alcohol-intoxicated groups, mice were administered intragastrically with alcohol in water (50% v/v) twice a day for 11 weeks. The amount of 50% alcohol administered to the animals was gradually increased every week from 10 to 16 mL kg^{-1} day^{-1} according to animal tolerance (5, 6 and 7 mL kg^{-1} twice daily for the first 3 weeks respectively and 8 mL kg^{-1} day^{-1} twice daily for the following 8 weeks; the corresponding doses of alcohol were 4.0, 4.7 and 5.5 g kg^{-1} day^{-1} for the first 3 weeks respectively and 6.3 g kg^{-1} day^{-1} for the following 8 weeks). After every alcohol administration, the mice of experimental groups orally received the corresponding test substance according to the dose stated above. All intragastric administrations were performed without anesthesia.

At the end of the 11 weeks, the mice were sacrificed after 12 h of fasting. Blood samples were collected and the serum was separated for assays. Liver samples were excised immediately and weighted. Two small pieces of liver section from the same lobe of liver in each animal were fixed properly for histopathological examination. The remaining liver tissues were frozen in liquid nitrogen and transferred to a refrigerator at −80 °C for biochemical assays, ELISA and RNA isolation.

Assay of biochemical parameters in serum

The serum was separated after clot formation by centrifugation at 4 °C. The levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), TG, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were measured using an automatic biochemical analyzer (Cobas C501, Roche, Basel, Switzerland).

Hepatic TG content determination

For the determination of total hepatic TG content, liver tissue was homogenized in 2 volumes of physiological saline. The hepatic lipid was extracted with methanol/chloroform (1:2 v/v). After centrifugation at 1200 $\times$ *g* for 10 min, the lower organic layer was separated, dried and resuspended in anhydrous alcohol. Colorimetric assessment of TG levels was conducted using a commercially available enzyme assay kit (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's protocol.

Evaluation of hepatic MDA, GSH and SOD levels

For the determination of hepatic lipid peroxidation and antioxidant capacity, the weighed liver tissues were homogenized in 9 volumes of physiological saline to obtain 10% (w/v) homogenates. The homogenates were then centrifuged at 664 $\times$ *g* (2500 rpm) for 10 min at 4 °C and the supernatants were used for the determination of MDA, GSH and SOD. The corresponding kits (Nanjing Jiancheng Bioengineering Institute) were used according to the manufacturer's instructions. The levels of MDA, GSH and SOD were normalized with the corresponding protein content.

Determination of NO and cytokines

The 10% (w/v) liver homogenate of each sample was obtained. After centrifugation, the supernatant was isolated and kept at −80 °C during analysis. Colorimetric assessment of hepatic NO level was conducted using a commercially available kit (Nanjing Jiancheng Bioengineering Institute). Cytokines (IL-1β, IL-10 and TNF-α) in the liver were determined with commercially available ELISA kits (R&D Systems). The corresponding protein content was determined by the method of Bradford[25] using bovine serum albumin as a standard.

Table 1. Primers used for quantitative real-time PCR

Target gene	Forward/reverse primers
GADPH	Forward: 5′-CTTTGGCATTGTGGAAGGGCTC-3′
	Reverse: 5′-GCAGGGATGATGTTCTGGGCAG-3′
AMPK-α2	Forward: 5′-GCTACCTATTTCCTGAAGACCCCTC-3′
	Reverse: 5′-CTTGGTTCATTATTCTCCGATTGTC-3′
PPAR-α	Forward: 5′-ATTCTTACCTGTGAACACGACCTG-3′
	Reverse: 5′-GGGTTGTTGCTGGTCTTTCC-3′
SREBP-1c	Forward: 5′-TAGAGCGAGCGTTGAACTGTATTG-3′
	Reverse: 5′-CCATGCTGGAGCTGACAGAGAA-3′
TLR-4	Forward: 5′-CTGTATTCCCTCAGCACTCTTGATT-3′
	Reverse: 5′-TGCTTCTGTTCCTTGACCCACT-3′
MyD88	Forward: 5′-ATGGTGGTGGTTGTTTCTGACG-3′
	Reverse: 5′-GTCGCATATAGTGATGAACCGCA-3′
IκB-α	Forward: 5′-AATCCTGACCTGGTTTCGCTCTT-3′
	Reverse: 5′-ATCCTCGCTCTCGGGTAGCAT-3′

Endotoxin assay

Endotoxin (LPS) in serum was assayed using a commercial ELISA kit (R&D Systems) according to the manufacturer's protocol.

Quantitative analysis of gene expression in liver

Total RNA was isolated from the stored frozen livers with Trizol reagent using a commercial RNA kit (Shanghai Generay Biotech Co., Ltd, Shanghai, China) according to the manufacturer's instructions. Reverse transcription was performed with 1 μg of total RNA using RevertAid™ M-MuLV Reverse Transcriptase (Thermo Scientific, Rockford, IL, USA) in accordance with the manufacturer's instructions. The specific polymerase chain reaction (PCR) primers for target genes, including sterol-regulatory element-binding protein-1c (SREBP-1c), AMP-activated protein kinase-α2 (AMPK-α2), peroxisome proliferator-activated receptor-α (PPAR-α), toll-like receptor-4 (TLR-4), myeloid differentiation primary response gene 88 (MyD88) and inhibitor κB-α (IκB-α), are listed in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used as an internal reference in all reactions. For each target mRNA, 2 μL of required cDNA was mixed with SYBR Green® PCR Premix (TaKaRa, Dalian, China). Primers were added to a final concentration of 400 nmol L^{-1}. PCR amplification was performed using an ABI 7900 Fast Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA) with an initial hold step (95 °C for 30s) and 40 cycles of two-step PCR (95 °C for 3 s, 60 °C for 30 s). The levels of target genes were determined by the comparative *Ct* method by normalizing to GAPDH and relative to a calibrator ($2^{-\Delta\Delta Ct}$). The purity of PCR products was verified by melting curves.

Histopathological analysis

A portion of fresh liver tissue was fixed immediately in 100 mL L^{-1} neutral formalin and then embedded in paraffin. Tissue sections (5 μm) were cut and stained with hematoxylin and eosin (H&E).

Statistical analysis

All data are presented as mean ± standard deviation (SD). Data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using the Statistical Package for Social Studies (SPSS) IBM, Armonk, USA. A difference of $P < 0.05$ was regarded as statistically significant.

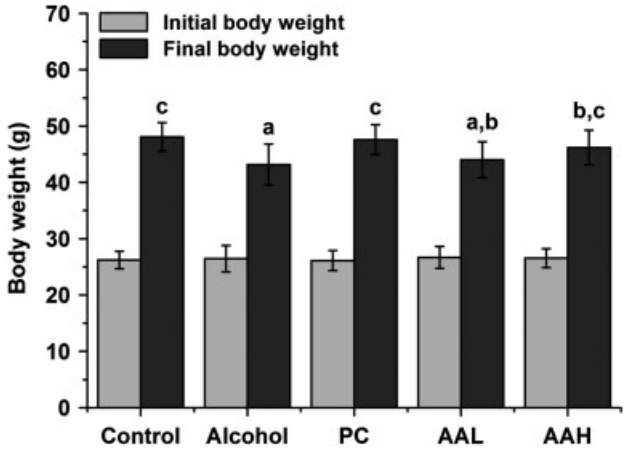

Figure 1. Body weight of experimental animals. Data are expressed as mean ± SD ($n = 12$). Values with different letters for each test parameter are significantly different from each other ($P < 0.05$).

RESULTS

Effect of AVGP on body weight

The body weight of animals (Fig. 1) was recorded on days 1 and 77 of the experiment. At the end of the 11 week experimental period, the alcohol group had lower body weight than the control group ($P < 0.01$). However, co-administration of high-dose AVGP (AAH, $P < 0.05$) or CBT (PC, $P < 0.01$) with alcohol significantly inhibited its reduction as compared with the alcohol group. No mortality was observed during the entire experiment.

Effect of AVGP on chronic alcohol-induced liver injury

The activities of serum ALT and AST as markers for liver injury were measured to assess chronic alcohol-induced liver damage. The alcohol group revealed abnormally higher levels of serum ALT and AST than the control group ($P < 0.05$), indicating the sustained liver damage caused by chronic alcohol treatment for 11 weeks (Table 2). Interestingly, the levels of these marker enzymes were significantly decreased to near-normal levels in alcohol-treated mice supplemented with AVGP or CBT ($P < 0.05$).

Alcohol caused significantly higher serum TC, TG and LDL levels than those found in the control group ($P < 0.01$, $P < 0.01$ and $P < 0.05$ respectively) (Table 2). Co-administration of AVGP or CBT with alcohol markedly suppressed the elevation of serum TC (AAL, $P < 0.05$; AAH, $P < 0.01$; PC, $P < 0.05$), TG (AAL, $P < 0.01$; AAH, $P < 0.01$; PC, $P < 0.01$) and LDL (AAH, $P < 0.05$; PC, $P < 0.05$) and almost completely restored them to values similar to those of the control group (Table 2). However, the levels of serum HDL were not affected regardless of treatment. Simultaneously, the hepatic TG content was also determined after 11 weeks of treatment. As shown in Fig. 2(A), the alcohol group exhibited a significant increase in hepatic TG level ($P < 0.001$) compared with the control group, as expected. After administration of AVGP or CBT for 11 weeks, levels of hepatic TG were significantly decreased as compared with the alcohol group (AAL, $P < 0.05$; AAH, $P < 0.01$; PC, $P < 0.05$).

To determine the effect of AVGP on chronic alcohol-induced hepatic steatosis and physiological changes, H&E staining was conducted in mouse liver samples. The histopathological observations are shown in Fig. 2B. Consistent with the results of biochemical analysis described above, alcohol treatment resulted in an enlargement of the hepatocytes, higher steatosis (fat

Table 2. Effect of AVGP on serum marker enzymes and lipid outputs

Parameter	Control	Alcohol	PC	AAL	AAH
ALT (IU L^{-1})	41.59 ± 7.79a	56.49 ± 16.48b	44.51 ± 5.00a	41.28 ± 8.47a	42.23 ± 9.85a
AST (IU L^{-1})	140.29 ± 18.29a	180.40 ± 33.95b	147.64 ± 10.02a	143.98 ± 33.69a	141.65 ± 30.41a
TC (mmol L^{-1})	2.64 ± 0.29a	3.17 ± 0.50b	2.74 ± 0.46a	2.81 ± 0.35a	2.64 ± 0.15a
TG (mmol L^{-1})	1.05 ± 0.24a	1.48 ± 0.29b	1.09 ± 0.30a	1.12 ± 0.22a	1.08 ± 0.32a
LDL (mmol L^{-1})	0.12 ± 0.04a	0.19 ± 0.08b	0.13 ± 0.05a	0.15 ± 0.03a,b	0.13 ± 0.05a
HDL (mmol L^{-1})	2.36 ± 0.27	2.45 ± 0.25	2.28 ± 0.29	2.27 ± 0.16	2.29 ± 0.21

Data are expressed as mean ± SD ($n = 12$). Values with different letters for each test parameter are significantly different from each other ($P < 0.05$).

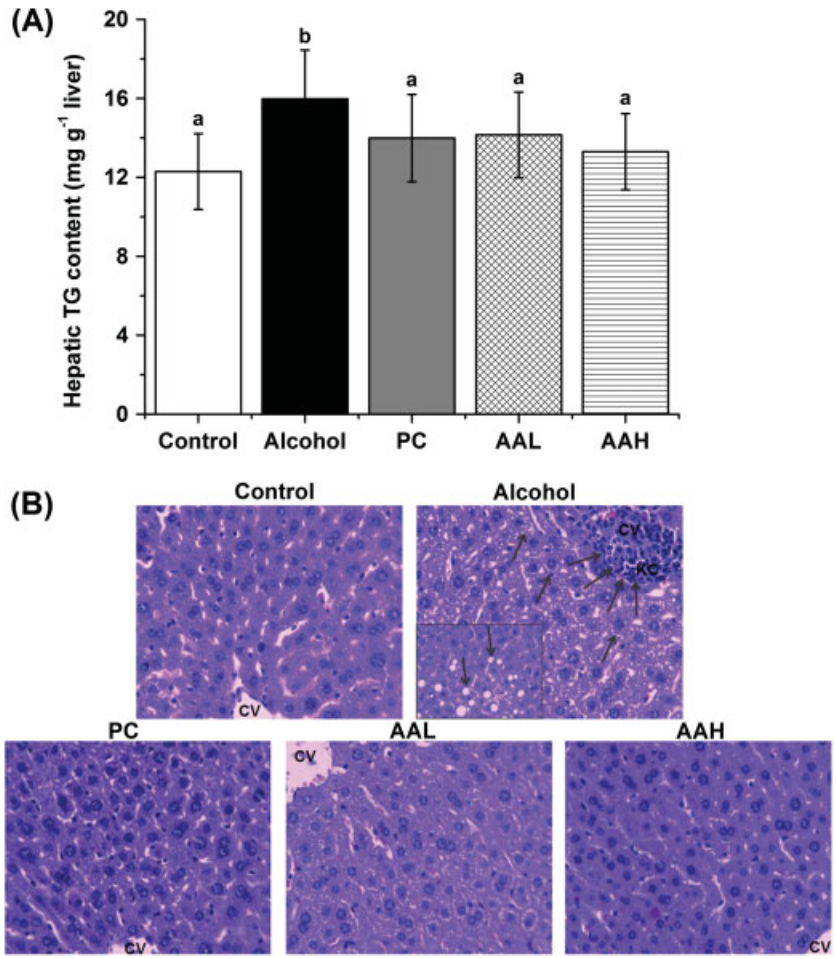

Figure 2. (A) Effect of AVGP on hepatic TG content. Data are expressed as mean ± SD ($n = 12$). Values with different letters for each test parameter are significantly different from each other ($P < 0.05$). (B) Representative photomicrographs of H&E staining of liver sections from control, alcohol, AAL and AAH groups (magnification × 400). Alcohol treatment induced activation of Kupffer cells (KC) and enlargement of hepatocytes (arrows); CV, central vein. Inset: alcohol treatment induced macro- and microvesicular steatosis (arrows).

accumulation) and inflammatory injury (Kupffer cell activation). Surprisingly, these chronic alcohol-induced hepatic pathological changes were clearly inhibited by the supplementation of AVGP or CBT.

Effect of AVGP on hepatic MDA and antioxidant status

As shown in Table 3, the hepatic MDA content was significantly elevated by 143.6% after chronic alcohol feeding compared with the control group ($P < 0.001$). Interestingly, this elevation was dramatically reduced with AVGP intake ($P < 0.05$). The hepatic GSH level showed a marked depletion in the alcohol group ($P < 0.05$), while the SOD activity decreased by 11.6%. After administration of AVGP for 11 weeks, both GSH and SOD activities were significantly increased with high dose as compared with the alcohol group ($P < 0.05$ and $P < 0.001$ respectively). However, CBT showed no significant effect on MDA, GSH and SOD levels when compared with the alcohol group.

Effect of AVGP on inflammatory response

The levels of hepatic NO and TNF-α were significantly increased in the alcohol-treated mice ($P < 0.05$) whereas the IL-10 level in liver was significantly decreased ($P < 0.01$) when compared with

Table 3. Effect of AVGP on lipid peroxidation, antioxidant status and inflammatory response

Parameter	Control	Alcohol	PC	AAL	AAH
MDA (nmol mg^{-1} protein)	2.34 ± 1.09a	5.70 ± 1.97c	4.45 ± 2.03bc	4.00 ± 1.56b	3.81 ± 1.58ab
GSH (nmol mg^{-1} protein)	2.60 ± 1.06b	1.91 ± 0.21a	2.19 ± 0.24ab	2.43 ± 0.48ab	2.55 ± 0.66b
SOD (U mg^{-1} protein)	2.93 ± 0.72a	2.59 ± 0.70a	2.83 ± 0.53a	3.15 ± 1.12a	4.38 ± 1.40b
NO (nmol mg^{-1} protein)	0.28 ± 0.11a	0.41 ± 0.10b	0.37 ± 0.07ab	0.32 ± 0.07ab	0.32 ± 0.13ab
TNF-α (pg mg^{-1} protein)	48.69 ± 3.86a	52.44 ± 3.01b	47.51 ± 1.91a	48.89 ± 3.89a	45.78 ± 4.35a
IL-1β (pg mg^{-1} protein)	13.82 ± 1.28	14.57 ± 1.98	13.98 ± 1.15	13.87 ± 1.59	13.55 ± 0.98
IL-10 (pg mg^{-1} protein)	89.24 ± 14.35b	71.76 ± 11.74a	83.45 ± 13.32ab	75.65 ± 11.25a	75.19 ± 12.75a
LPS (U L^{-1})	9.02 ± 1.61a	12.01 ± 1.73b	9.21 ± 1.09a	9.32 ± 1.60a	8.44 ± 1.49a

Data are expressed as mean ± SD ($n = 12$). Values with different letters for each test parameter are significantly different from each other ($P < 0.05$).

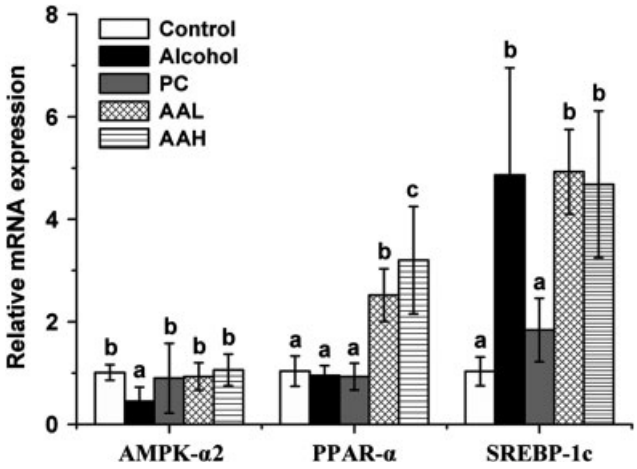

Figure 3. Effect of AVGP on expression of hepatic AMPK-α2, PPAR-α and SREBP-1c. Data are expressed as mean ± SD ($n = 10–12$). Values with different letters for each test parameter are significantly different from each other ($P < 0.05$).

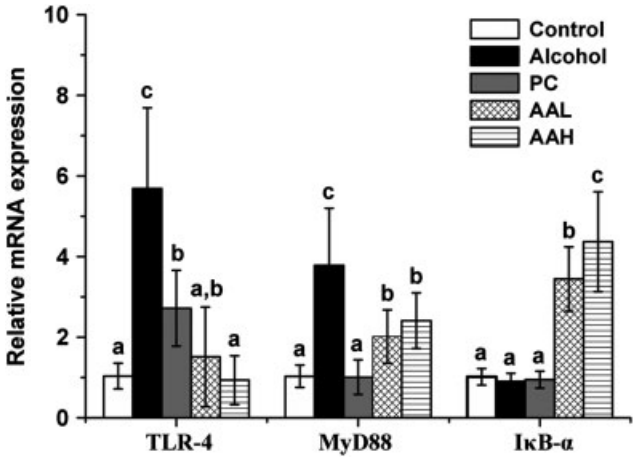

Figure 4. Effect of AVGP on mRNA levels of TLR-4, MyD88 and IκB-α in mouse liver. Data are expressed as mean ± SD ($n = 10–12$). Values with different letters for each test parameter are significantly different from each other ($P < 0.05$).

the control mice (Table 3). After treatment with AVGP or CBT for 11 weeks, hepatic TNF-α was markedly decreased (AAL, $P < 0.05$; AAH, $P < 0.001$; PC, $P < 0.01$) when compared with the alcohol-treated mice. However, no significant changes were observed in NO and IL-10 levels among the alcohol- and AVGP- or CBT-treated groups. Simultaneously, endotoxin (LPS) was also determined after 11 weeks of treatment. It was found that the serum LPS level was markedly elevated in the alcohol-treated mice ($P < 0.001$) relative to the control value. The elevation of serum LPS was markedly inhibited by 22.4% ($P < 0.001$) and 29.7% ($P < 0.001$) with low and high doses of AVGP treatment respectively, while the LPS level was significantly decreased by 23.3% with CBT treatment ($P < 0.001$).

Effect of AVGP on expression of hepatic lipid metabolism-related genes

To identify the mechanism of the protective effect of AVGP against chronic alcohol-induced steatosis in mice, we profiled the expression of a group of lipid metabolism-related genes. As shown in Fig. 3, alcohol administration significantly decreased the mRNA level of AMPK-α2 when compared with the control group ($P < 0.01$). The down-regulation of AMPK-α2 expression in alcoholic liver was markedly restored to a normal level with AVGP treatment ($P < 0.05$). In addition, PPAR-α gene expression was dramatically up-regulated by 162.5% ($P < 0.001$) and 233.3% ($P < 0.001$) with low and high doses of AVGP treatment respectively. However, AVGP supplementation had no significant effect on the over-expression of SREBP-1c caused by chronic alcohol ingestion. CBT markedly reversed alcohol-derived down-regulation of AMPK-α2 ($P < 0.05$) and over-expression of SREBP-1c ($P < 0.001$) but had no significant effect on up-regulation of PPAR-α as compared with the alcohol group.

Effect of AVGP on hepatic TLR-4, MyD88 and IκB-α expression

To determine the mechanism underlying the protective effect of AVGP on alcohol-dependent induction of inflammatory response, relevant genes including TLR-4, MyD88 and IκB-α were quantified. Alcohol consumption significantly elevated the hepatic expression of TLR-4 ($P < 0.001$) and MyD88 ($P < 0.001$) when compared with the control group (Fig. 4). Supplementation of AVGP or CBT significantly down-regulated them in the alcohol-treated mice and even normalized them ($P < 0.001$). Moreover, AVGP treatment also markedly increased IκB-α gene expression compared with the alcohol group ($P < 0.001$).

DISCUSSION

Aloe vera has been drawing experimental and clinical attention for many centuries owing to its curative and therapeutic properties. An increasing number of experimental studies as well as clinical trials have provided supporting data for the hepatoprotective effects of

A. vera.[15,19,26] *Aloe vera* gel, one of the most commonly used aloe products, has been demonstrated to show a potential protective effect against acute alcohol-induced hepatotoxicity.[19,20] However, the active principle(s) responsible for this action and the underlying mechanisms are still poorly understood. In addition, as far as our literature survey could ascertain, no studies have so far been reported on its hepatoprotective role in chronic alcohol-induced liver injury. In the present study, we extracted and obtained AVGP from concentrated lyophilized *A. vera* gel powder (200:1X) and investigated the protective effects and underlying mechanisms of AVGP against ALD in a chronic alcohol-feeding mouse model.

The results of this study demonstrated that chronic alcohol ingestion resulted in a decrease in body weight, which is in line with earlier findings,[12] and caused liver damage, as evidenced by elevation of serum ALT and AST activities, serum TC, TG and LDL levels, hepatic TG content, steatosis (lipid accumulation) and inflammatory response, which reflected early biochemical and pathological changes in ALD. AVGP or CBT administration offered significant protection to chronic alcohol-intoxicated mice by restoring body weight gain and reversing the levels of these serum marker enzymes and lipids, indicating the stabilization of plasma membrane and the repair of hepatic tissue. In addition, the hepatic histopathological changes induced by alcohol were also markedly improved by AVGP or CBT treatment. Our results indicated that AVGP was able to alleviate the hepatotoxicity of chronic alcohol ingestion and that the hepatoprotective effect was comparable to that of conventionally reputed CBT.

Numerous studies have demonstrated that fatty liver is a most common feature of alcohol hepatotoxicity and plays an important role in the development of ALD.[27] Chronic alcohol consumption increases the hepatic NADH/NAD^+ ratio, which in turn suppresses mitochondrial β-oxidation of fatty acids and stimulates *de novo* lipogenesis, thus causing lipid accumulation in hepatocytes.[28,29] AMPK, a key regulator of lipid metabolism in liver, is responsible for the inactivation of acetyl-CoA carboxylase (ACC), which results in increased fatty acid oxidation in liver,[4,30] while SREBP-1c particularly regulates fatty acid synthesis.[31] Our results showed that alcohol consumption decreased AMPK-α2 expression and elevated SREBP-1c levels, which was consistent with other published research.[32–34] Supplementation of AVGP significantly restored the decreased AMPK-α2 gene expression to a near-normal level, which could explain the lower serum lipid levels and hepatic TG content. However, AVGP had no significant effect on the alcohol-induced over-expression of SREBP-1c. Additionally, AVGP treatment markedly increased the hepatic PPAR-α expression in alcohol-fed mice, which resulted in higher β-oxidation in liver, thus increasing lipid expenditure and decreasing the development of alcoholic fatty liver. Our results also illustrated that administration of AVGP greatly suppressed the steatosis in alcohol-fed mice via a histological examination. Based on the above results, it is speculated that AVGP can suppress alcohol-dependent lipid accumulation mainly through its ability to accelerate fatty acid oxidation, which may be mediated at least in part by AMPK-α2 activation and up-regulation of PPAR-α expression.

Oxidative stress plays an important role in the development of ALD.[35] A large number of studies have demonstrated that ALD is associated with increased lipid peroxidation, damage to the mitochondria, free radical generation and a decrease in hepatic antioxidant defense, providing the most convincing evidence for the pathogenic role of oxidative stress.[34,36,37] Animal studies have shown that preventing lipid peroxidation with antioxidants reduces focal necrosis and inflammation.[38] As we know, polysaccharides isolated from *A. vera* have been found to possess high antioxidant efficiency.[14,39,40] Thus the obtained AVGP might be suggested as a therapeutic agent in experimental models of ALD. As expected, we observed here that the administration of alcohol-treated mice with AVGP was associated with a decrease in the oxidative stress marker MDA and an increase in the hepatic non-enzymatic antioxidant GSH and enzymatic antioxidant SOD. In combination with previous reports, it could be concluded that the attenuation of chronic alcohol-induced oxidative stress by AVGP was partly due to its ability to alleviate lipid peroxidation and scavenge free radicals. Also, these antioxidant capacities could be partly attributed to the acetyl groups and the reductive nature of the monosaccharides in AVGP molecules.[23,40]

In addition to lipid accumulation and oxidative stress, inflammatory injury is also a major feature of ALD. The most favored current hypothesis suggests that alcohol-dependent inflammatory responses are a consequence of alcohol-induced elevation of the translocation of gut-derived LPS to portal blood, which in turn leads to the induction of TLR-4 expression and activates nuclear factor-κB (NF-κB), triggering the release of pro-inflammatory cytokines (TNF-α and IL-1β) and NO, and ultimately results in hepatocelluar necrosis.[4,41,42] Both our results and other published studies[14,43,44] showed that the polysaccharides obtained from *A. vera* have significant anti-inflammatory properties. Supplementation of AVGP not only significantly suppressed blood LPS but also markedly inhibited the overproduction of TNF-α in alcohol-fed mice. To further investigate the underlying mechanisms of AVGP that attenuated alcohol-induced inflammation, the mRNA expression of TLR-4, MyD88 and IκB-α in mouse liver was determined. Consistent with previous reports,[4] the results of the present study showed a significant up-regulation of TLR-4 and MyD88 genes in the liver during chronic alcohol administration. However, treatment with AVGP clearly alleviated the alcohol-dependent induction of TLR-4 and MyD88 expression, suggesting that AVGP might block the alcohol-induced activation of hepatic Kupffer cells. The significant up-regulation of IκB-α gene in AVGP-treated mice indicated the higher suppression of NF-κB activation, thus alleviating the alcohol-induced inflammatory responses, which could be associated with the lower TNF-α and NO levels. Taken together, these results suggest that the protective effect of AVGP under the conditions of our experiments may result from an inhibition of the alcohol-induced elevation of blood LPS, which may involve the prevention of gut adsorption of LPS, in turn lead to the reduction in the subsequent expression of TLR-4 and MyD88, as well as up-regulate IκB-α expression, resulting in lower stimulation of hepatic inflammation and less oxidative stress.[4,41,45,46] However, the mechanisms by which AVGP decreases endotoxin levels and blunts the deleterious effects of alcohol are still not established at present and will require further studies.

CONCLUSIONS

This study demonstrated that AVGP (the main bioactive component in *A. vera*) prevents alcoholic liver injury, decreasing steatosis, oxidative stress and inflammation caused by chronic alcohol ingestion. Our results revealed that AVGP lowered alcohol-dependent lipid accumulation by up-regulating the hepatic expression of AMPK-α2 and PPAR-α genes. AVGP supplementation attenuated alcohol-induced oxidative stress and led to recovery of antioxidant status. Furthermore, AVGP also suppressed the inflammatory response by blocking the LPS/TLR-4 signal pathway and up-regulating IκB-α gene expression. To the best of our knowledge,

this is the first observation of the hepatoprotective effect of AVGP against ALD in a chronic alcohol-feeding mouse model. Although further detailed studies are required to establish its clinical application, our results suggest that AVGP could be a good candidate to treat and prevent liver injury caused by alcohol administration, and the further investigation of AVGP might be crucial to develop dietary supplements for enhancing liver function.

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