We have previously reported that Aloe vera gel had hypoglycemic activity and anti-obesity effects, although the effect on alcoholic fatty liver was unclear. We examined in this present study the effect of an Aloe vera gel extract (AVGE) on hepatic lipid metabolism by using an ethanol-induced transient fatty liver mouse model. Ethanol (3 g/kg of mouse weight) was orally administered to induce an accumulation of triglyceride (TG) and increase the mRNA expression of such lipogenic genes as sterol regulatory element-binding protein-1 (SREBP-1) and fatty acid synthase (FASN) in the liver. Although ethanol ingestion caused a 5.4-fold increase in liver TG, pre-treating with AVGE (1 mg/kg/d) for 1 week significantly suppressed this elevation of the ethanol-induced liver TG level. The expression of lipogenic genes was also lower in the AVGE pre-treatment group than in the control group. This inhibitory effect on the ethanol-induced accumulation of TG was attributed to a reduction in the expression of lipogenic genes that were increased by ethanol.

**Key words:** hepatic lipid metabolism; Aloe vera; alcohol; fatty liver; sterol regulatory element-binding protein-1 (SREBP-1)

Hepatic metabolism plays a critical role in regulating the whole-body energy status. Fatty liver has recently been focused on as a complicating factor of liver disease. Fatty liver is the initial stage of liver disease, as is the case of hepatitis and fibrosis, and may lead to liver cirrhosis. Fatty liver is characterized by an accumulation of lipid droplets, triglyceride (TG), and cholesterol in the liver.

Broader speaking, there are two types of fatty liver in humans: one develops due to an excessive intake of alcohol, while the other develops in non-drinkers, namely, non-alcoholic fatty liver disease (NAFLD). It is known that acute and chronic alcohol consumption induces an accumulation of lipid and advances fatty liver in rodent and human liver. These events are attributed to alcohol consumption increasing the biosynthesis of fatty acids and TG and inhibiting lipid oxidation, as well as mobilizing fatty acids from adipose tissue. Alcohol-induced hepatic lipid accumulation has also been reported to be due to increased intestinal translocation of bacterial endotoxin. Although the cause of NAFLD is unclear, it is thought to be very common and in many cases is linked to being obese or overweight. Therapy for improving fatty liver is still limited to exercise and dietary regulation, so that the development of a useful method for preventing hepatic lipid accumulation on a daily basis is needed.

Aloe barbadensis MILLER (Aloe vera) is a species of succulent plant that belongs to the lily family, Liliaceae. Aloe vera is one of the most well-known herbs, and has been widely used for centuries. Aloe is commonly known for its topical use to treat wounds and burns. A clinical trial has demonstrated the usefulness of Aloe vera for the prophylaxis of radiation-induced dermatitis. Polysaccharides isolated from gel of the Aloe species have various biological activities, previous studies having reported anti-diabetic, anti-inflammatory, anti-oxidative, and anti-tumor effects.

We have reported in previous studies that Aloe vera gel had hypoglycemic activity and identified the anti-diabetic components. We isolated five minor plant sterols, Aloe-sterols, namely, lophenol, 24-methyllophenol, 24-ethyl-lophenol, cycloartenol, and 24-methylene-cycloartenol, as the active ingredients. We also found that the administration of these Aloe-sterols improved hyperglycemia and glucose metabolism, and reduced intra-abdominal fat accumulation in Zucker diabetic fatty (ZDF; ZDF/Crl-Lepr<sup>fa/fa</sup>) rats. We performed supercritical fluid extraction to enrich the anti-diabetic ingredients from dried Aloe vera gel powder, and the Aloe vera gel extract (AVGE) was obtained. We have also reported that AVGE had the potential to ameliorate serum alanine aminotransferase (ALT; also called glutamate pyruvate transaminase, GPT) and γ glutamic pyruvic transaminase (γ-GPT) in humans. However, the effect of AVGE on fatty liver has not yet been understood.

Acute and chronic types of alcohol-induced liver damage (e.g., lipid accumulation) seem to share a similar mechanism; animal models of acute alcohol exposure can be used to mimic the very early effects of ethanol in the development of alcohol-induced liver disease. For instance, it has previously been

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**Abbreviations:** TG, triglyceride; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; PPARα, peroxisome proliferator-activated receptor α; SREBP-1, sterol regulatory element-binding protein-1; FASN, fatty acid synthase; DGAT2, diacylglycerol acyltransferase 2
Materials and Methods

Preparation of the Aloe vera gel extract (AVGE). Leaf skins of Aloe vera were peeled, and the mesophyll parts were collected. These mesophyll parts were dried, and an Aloe vera gel dry powder was prepared. To obtain the extract, including hydrophobic Aloe-sterols, we studied a technique for producing an Aloe vera gel extract (AVGE) without using an organic solvent. Supercritical fluid extraction was carried out, using CO₂ as a solvent, at 34 MPa and 60°C (under supercritical conditions). Approx. 1 mg of AVGE was obtained from 50 g of Aloe vera gel.

AVGE obtained was homogeneously suspended in propylene glycol (PG; Wako Pure Chemicals, Osaka, Japan) to prepare an AVGE suspension for the treatment sample. The concentration was adjusted to 0.1 mg/mL with distilled water, the final PG concentration being adjusted to 1%.

Animals and treatment. Eight-week-old male C57BL/6j mice were purchased from Charles River Japan (Yokohama, Japan). They were housed 3–4 per cage with a controlled temperature (20–22°C), humidity (55–65%), and lighting (on at 8 a.m. and off at 8 p.m.). They were fed a normal laboratory diet (AIN93G; Oriental Yeast, Tokyo, Japan) for 1 week to stabilize their metabolic conditions. After this adaptation period, the animals were assigned to 4 groups (control-ethanol (CE) group, AVGE-ethanol (AE) group, control-glucose (CG) group, and AVGE-glucose (AG) group). Each solution was orally administered once a day with a sonde (1 mg/kg/d) for the AE group, or a vehicle (1% PG solution in water) (PG; Wako Pure Chemicals, Osaka, Japan) to prepare an AVGE suspension for the treatment sample. The concentration was adjusted to 0.1 mg/mL with distilled water, the final PG concentration being adjusted to 1%.

Food was then removed 16 h after the last administration of the AVGE pre-treatment and the mice were fasted. After 2 h of fasting, the mice in the CE and AE groups were intragastrically administered with ethanol (Wako) in water (40% v/v), and with isocaloric glucose (Wako) in water (40% w/w), as a control, in the CG and AG groups. Ethanol was administered at a dose of 3 g/kg of body weight. The mice were humanely killed 6 h after the ethanol administration under fasting conditions; they were laparotomized under sevoflurane anesthesia, blood samples were obtained by cardiac puncture, and serum was prepared by centrifuging the blood at 1000 x g for 10 min at 4°C. A part of the liver was isolated and immediately frozen in liquid nitrogen. All animal experiments were conducted with the approval of Morinaga Milk Industry Committee on Animal Research.

Hepatic triglyceride determination and serum parameter measurement. Liver TG was extracted from the tissue samples with chloroform/methanol (Wako) by Folch’s method. The organic solvent was evaporated under nitrogen gas, and the dried liquid extract was resuspended in 1% Triton X-100 (Nacalai Tesque, Tokyo, Japan) in 2-propanol (Wako). The TG concentration of the supernatant was measured with a TG E-test kit (Wako).

Serum TG and the nonesterified fatty acid (NEFA) were measured with an equipment (NEFA-C test (Wako) and NEFA C-test (Wako) kits according to the manufacturer’s protocol. The levels were determined by using a microplate reader. Serum glutamic oxaloacetic transaminase (GOT) and GPT were measured by using a transaminase CII-test kit (Wako).

Oil red O staining. Frozen sections of the liver were stained with Oil red O and counterstained with hematoxylin to determine the hepatic lipid accumulation. An image acquisition and analysis system (Olympus, Tokyo, Japan), incorporating an Olympus microscope, was used to capture and analyze the Oil red O-stained tissue samples at 200 x magnification.

Results

Effect of AVGE on the blood-ethanol concentration. We examined whether or not AVGE would have an effect on the change of blood-ethanol concentration at several time points. The blood-ethanol concentration reached a peak 30 min after ethanol administration and then decreased with time for the CE group and AE group (Fig. 1). There was no difference between the CE group and the AE group in terms of the blood-ethanol concentration (Fig. 1).
Aloe vera Gel Extract and Alcoholic Fatty Liver

Table 1. Effect of Pre-Treating with AVGE for 1 Week on the Liver Weight and Serum Analysis of Mice

<table>
<thead>
<tr>
<th></th>
<th>CG</th>
<th>CE</th>
<th>AG</th>
<th>AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver weight (g)</td>
<td>0.91 ± 0.02</td>
<td>0.94 ± 0.01</td>
<td>0.98 ± 0.01</td>
<td>0.91 ± 0.01</td>
</tr>
<tr>
<td>Serum TG (mg/dL)</td>
<td>50.3 ± 8.1</td>
<td>114.2 ± 4.34*</td>
<td>53.5 ± 19.7</td>
<td>111.2 ± 19.2*</td>
</tr>
<tr>
<td>Serum NEFA (mEq/dL)</td>
<td>1.90 ± 0.14</td>
<td>2.39 ± 0.16*</td>
<td>1.82 ± 0.11</td>
<td>2.57 ± 0.17*</td>
</tr>
<tr>
<td>Serum GOT (IU/L)</td>
<td>44.6 ± 2.8</td>
<td>77.1 ± 13.2*</td>
<td>56.3 ± 8.0</td>
<td>64.2 ± 6.5</td>
</tr>
<tr>
<td>Serum GPT (IU/L)</td>
<td>3.2 ± 0.3</td>
<td>4.6 ± 0.8</td>
<td>2.6 ± 0.3</td>
<td>3.7 ± 1.0</td>
</tr>
</tbody>
</table>

Ethanol (3 g/kg) was administered orally, and 6 h later, the mice were killed (CG, control-glucose; CE, control-ethanol; AG, AVGE-glucose; AE, AVGE-ethanol). Data are presented as the mean ± SEM, n = 6. *p < 0.001 CG group vs. CE group, **p < 0.001 AG group vs. AE group, ***p < 0.05 CG group vs. CE group.

Fig. 1. Effect of AVGE on the Blood-Ethanol Concentration 30 min, 1 h, 2 h, and 4 h after Acute Ethanol Ingestion by Mice.

Fig. 2. Effect of AVGE on Hepatic Lipid Accumulation 6 h after Acute Ethanol Ingestion by Mice.

Fig. 3. Oil Red O-Stained Tissue Samples at 200× Magnification.

After pre-treating with AVGE or the vehicle for 1 week, ethanol administration induced significant serum elevation of the TG level and NFCA level; however, there was no statistically significant difference between the CG and AG groups or between the CE and AE groups (Table 1). No significant difference was apparent in the serum GOT and GPT levels between the CG and AG groups. Acute ethanol ingestion increased the GOT and GPT levels in the vehicle-treated group. The ethanol-induced elevation in GOT and GPT was lower in the AVGE-treated group than in the vehicle-treated group (Table 1). However, statistical analyses revealed no significant difference in the serum GOT and GPT levels between the CE and AE groups (Table 1).

AVGE had no effect on the ethanol-induced decrease of lipid oxidation-related gene expression in mouse liver.

We examined by real-time PCR the mRNA expression levels of hepatic genes 6 h after ethanol administration to elucidate the mechanism by which AVGE ameliorated ethanol-induced transient fatty liver.

In the absence of ethanol, the hepatic mRNA expression level of PPARα in the AG group mice was significantly higher by 1.3-fold than in the CG group (Fig. 4A). However, the mRNA expression levels of CPT-1 and MCAD, which are PPARα target genes and lipid oxidation enzymes, did not differ between the CG and AG groups (Fig. 4B and C).

Figure 4A and C show that the mRNA expression levels of PPARα and MCAD fell under the treatment with ethanol. The mRNA expression level of PPARα in...
the CE group was no different from that in the AE group (Fig. 4A). Similarly, the mRNA expression levels of CPT-1 and MCAD were not significantly different between the CE and AE groups under the ethanol treatment (Fig. 4B and C).

**AVGE suppressed the ethanol-induced increase of lipogenesis-related gene expression in mouse liver**

SREBP-1 encodes a transcription factor that regulates fatty acid and TG synthetic enzyme genes. To examine whether the AVGE effect of preventing liver TG accumulation occurred via regulation of the lipogenesis-related genes, we investigated the hepatic mRNA expression levels of SREBP-1, FASN, and DGAT2 by quantitative RT-PCR.

In the absence of ethanol, there was no difference in the mRNA expression levels of SREBP-1, FASN, and DGAT2 between the CG and AG groups (Fig. 5A, B, and C).

Acute ethanol administration induced a 1.7-fold increase in the mRNA expression level of SREBP-1 after pre-treating mouse liver with the vehicle (Fig. 5A). However, pre-treating mouse liver with AVGE resulted in no change to the SREBP-1 mRNA level (Fig. 5A). As well as SREBP-1, the mRNA expression level of FASN in the CE group was 5.5-fold higher than that in the CG group (Fig. 5B), while the expression of FASN mRNA in the AE group was 5.1-fold higher than that in the AG group (Fig. 5B). However, the mRNA expression level in the AE group was lower than that in the CE group, the pre-treatment with AVGE prior to ethanol administration significantly blunting ($p < 0.001$) the increase in the mRNA expression level of FASN by about 34% (Fig. 5B). In addition, the mRNA expression level of DGAT2 in the CE group tended to be higher than that in the AE group (Fig. 5C).

**Discussion**

Therapy for treating the initial stage of fatty liver remains restricted. However, many remedies for ameliorating liver damage have been studied since ancient times throughout the world; for example, *Curcuma longa* (commonly known as turmeric), fish oil, *Pueraria lobata*, and cinnamon have been reported to be effective therapeutic agents for treating liver damage. Some dietary compounds can affect ethanol absorption and metabolism; for example, a soymilk product has been found to inhibit ethanol absorption and facilitate ethanol metabolism. These effects can be expected to ameliorate alcoholic liver damage. To investigate the possibility of AVGE affecting ethanol absorption, we measured the time-dependent blood-ethanol concentration. There was no significant difference in the change of blood-ethanol concentration between the CE and AE groups (Fig. 1). This result suggests that AVGE had little or no effect on ethanol absorption.
A previous study has described that Aloe vera gel had a hepatotherapeutic effect on alcohol-induced liver damage. However, the protective effect of AVGE on alcoholic fatty liver was uncertain. We have demonstrated in this study that an AVGE pre-treatment significantly suppressed the accumulation of hepatic lipids induced by ethanol administration (Fig. 2).

Ethanol administration also caused acute liver damage in mice, and the serum GOT and GPT levels were increased. This study has also demonstrated that ethanol administration evoked a significant elevation of GOT and tended to increase GPT (Table 1), these results being consistent with another report. It is possible from the results of this study that AVGE prevented acute ethanol-induced liver damage, as demonstrated by its suppression of the elevation of GPT level (Table 1). The inflammatory reaction is one of the major reasons for these marker levels increasing. With the assumption that AVGE had an anti-inflammatory effect and inhibited GPT elevation, we therefore investigated the expression of such pro-inflammatory cytokines as IL-1, IL-6, and MCP-1. The results show that the mRNA expression of these cytokines was elevated after ethanol administration. However, no significant difference was found between the CE and AE groups (data not shown). This was contrary to our expectations and required a modified approach. Oxidative stress also seems to aggravate ethanol-induced fatty liver and elevates the GOT and GPT levels.

It has been reported that Aloe vera has an anti-oxidative effect as well as an anti-inflammatory effect. A previous study has shown that Aloe vera preserved such metabolizing enzymes as glutathione, glucose-6-phosphatase and microsomal aniline hydroxylase, and exhibited anti-oxidative activity through these enzymes. We therefore speculate that AVGE also had an anti-oxidative effect, although this needs to be explored further.

There are multiple mechanisms underlying the development of ethanol-induced fatty liver, driving de novo fatty acid synthesis and inhibiting fatty acid lipoxidation. Acute alcohol exposure has decreased the mRNA expression of PPARα and PPARγ target genes involved in fatty acid catabolism such as CPT-1, ACO, and MCAD. It is considered that PPARα has an important role against alcoholic steatosis. PPARα-null mice have actually been found to be more sensitive than the wild type to alcohol damage and more likely to be affected by the development of hepatic steatopathy. We have previously reported that AVGE and some Aloe-sterols isolated from Aloe vera gel had ligand activity of PPARα. We therefore hypothesized that AVGE would have the potential to activate the transcriptional activity of PPARα and to increase the expression of PPARα-regulating β-oxidation genes which would prevent ethanol-induced TG accumulation. The present results show that, under the ethanol treatment, the mRNA expression levels of PPARα, CPT-1, and MCAD did not differ among the groups (Fig. 4). These data suggest that the protective effect of AVGE against ethanol-induced fatty liver was not attributable to the activation of PPARα. Accordingly, an increase in the expression of PPARα mRNA by pre-treating with AVGE was not sufficient to prevent ethanol-induced fatty liver.

Another reason for ethanol-induced TG accumulation is that ethanol has increased the mRNA expression level of such lipogenic genes as SREBP-1 and FASN, stearoyl-CoA desaturase 1 (SCD-1), and DGAT2 in mouse liver. We examined in this study the mRNA expression level of these lipogenic genes in mouse liver under an ethanol treatment. The results show that AVGE inhibited the increase of SREBP-1 and its target genes that promoted de novo fatty acid synthesis under the ethanol treatment (Fig. 5). However, in the absence of ethanol, there was no difference in the expression of lipogenic genes between the CG and AG groups (Fig. 5). These results suggest that, when the mice were administered with ethanol, AVGE demonstrated an inhibitory effect on the increase in mRNA expression of lipogenic genes and suppressed the lipid accumulation in the liver.

Fatty liver in humans results from the accumulation of cholesters and TG; accordingly, we examined the content of hepatic cholesters 6 h after administering ethanol in this study. The total cholesterol content was 1.8-fold higher in the liver of the CE group than of the CG group. The cholesterol content of the AE group tended to be higher than that of the AG group (1.2-fold); however, there was no significant difference between the AG group and the AE group in this respect (Supplementary Fig. 1, see Biosci. Biotechnol. Biochem. Web site). We therefore hypothesize that, similarly to SREBP-1, the expression of SREBP-2 and other cholesterol synthesis enzyme genes was increased by the ethanol administration. However, the mRNA levels of these genes were unchanged in liver 6 h after the ethanol administration, irrespective of the pretreatment with AVGE (Supplemental Table 1). These results suggest that AVGE blunted the ethanol-induced accumulation of cholesterol in the mouse liver, although the mechanism for this is unclear. We should conduct further studies on the relationship between AVGE and cholesterol metabolism.

This study has shown that, without an ethanol treatment, the mRNA expression level of PPARα was significantly higher in the AG group than in the CG group (Fig. 4). Our previous study has demonstrated that, when diet-induced obese (DIO) mice ingested Aloe-sterol for 12 weeks, their liver TG level was lower than that of mice fed only on a high-fat diet (HFD). These results indicate that AVGE, containing Aloe-sterols, prevented HFD-induced lipid accumulation in the liver due to an increase in the mRNA expression of PPARα and in the activation of lipid β-oxidation-related genes. It can therefore be expected that AVGE would prevent lipid accumulation in the liver by two different pathways, namely, one induced by ethanol and the other by HFD.

In conclusion, we found that AVGE prevented ethanol-induced fatty liver by suppressing the mRNA expression of lipogenic genes in the liver. The results of our study suggest that AVGE may be a promising agent for preventing alcoholic fatty liver. However, future studies will be needed to identify the compounds responsible for the preventive effect against ethanol-induced accumulation of lipids in the liver.

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References