Saeng-Maek-San, a Medicinal Herb Complex, Protects Liver Cell Damage Induced by Alcohol

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The effect of treatment with Saeng-Maek-San (SMS) Complex (SMS1 or SMS2) upon rat hepatocytes exposed to alcohol was investigated. We compared the serum biochemistry and liver histology of rats administered both alcohol and SMS to control rats treated with alcohol alone. SMS treatment resulted in a significant reduction in the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and triglycerides (TG) compared to the control rats. In contrast, expression levels of alcohol dehydrogenase (ADH) were increased. Electron microscopy indicated that administration of SMS preserved the structure of organelles, including the nucleus and mitochondria. In addition, lipid droplets and secondary lysosomes were observed in the control rats. These data suggest that SMS represents an excellent candidate for protection of rat hepatocytes from alcohol-mediated damage.

Key words Saeng-Maek-San; rat liver cell; alcohol

Much effort has been made to develop a reproducible and robust rodent model of alcohol-related liver disease in order to facilitate the study of the various factors involved in the initiation and progression of alcohol hepatotoxicity.[1,2] Excessive intake of alcohol may severely damage such organs as liver and heart, resulting in dysfunction including derangement of blood pressure and triglyceride levels.[3] There have been numerous attempts to develop clinically useful compounds to ameliorate or cure alcohol-related disorders.[4,5] However, it is well documented that these compounds may exhibit severe cytotoxicity, reproductive toxicity and other important side effects. Therefore, in order to find an alternative to the traditional cure, studies have increasingly focused on the development of therapeutic agents based on natural products and medicinal herbs.

In this study, we investigated whether Saeng-Maek-San (SMS), a medicinal herb complex, protects rat hepatocytes from alcohol-induced damage, thereby resulting in protection from hangovers, cardiovascular symptoms and alcohol-induced hepatitis.[6] SMS is composed of Korean such medicinal herbs as *Panax ginseng* C. A. MEYER, *Liriope platyphylla* WANG *et* TANG, *Schizandra chinensis* BAILLON, *Astragalus membranaceus* BUNGE, and *Cucurbita moschata* DUCHESNE.[7] It is well known that the spectrum of alcoholic liver disease can be reproduced in a rat model utilizing an intragastric infusion of ethanol.[8,9] SMS was administered to rats treated with alcohol. The protective effect of SMS was examined by measuring the blood levels of the enzymes AST (asparate aminotransferase) and ALT (alanine aminotransferase) before and after SMS administration in alcohol-treated rats. Serum levels of triglyceride and total cholesterol, important causes of hyperlipidemia and arteriosclerosis, were also measured. The major finding of this paper is that SMS is hepatoprotective and ameliorates alcohol-mediated damage and alcohol-induced liver symptoms whilst concomitantly improving lipid metabolism. Additionally, we performed histopathological and hematological studies to investigate the protective effects of SMS.

MATERIALS AND METHODS

+Preparation and Treatment of Saeng-Maek-San 1 (SMS1) and Saeng-Maek-San 2 (SMS2) Production of SMS1 and SMS2 was based on a recipe derived from Korean traditional medicine books and the recommendations of Korean traditional medical doctors. SMS1 and SMS2 are traditional Korean prescriptions containing a mixture of four (SMS1) or five (SMS2) herbs. SMS1 was made from the Korean medicinal herbs *Panax ginseng* C. A. MEYER, *Liriope platyphylla* WANG *et* TANG, *Schizandra chinensis* BAILLON and *Astragalus membranaceus* BUNGE, with the relative amounts of each herb in the preparation being 2 (24 g), 1 (12 g), 1 (12 g), and 2 (24 g), respectively. SMS2 was composed of SMS1 plus *Cucurbita moschata* DUCHESNE made up in a ratio of 2 (24 g), 1 (12 g), 1 (12 g), 2 (24 g), and 1 (12 g), respectively. Boiling water extracts of SMS1 and SMS2 were prepared from the dried herbs. Each volume of mixed herbs was added to 1100 ml of sterilized water and boiled for 150 min using a herbal and medicinal boiling pot (Daewoong Co., Ltd., Seoul, Korea). Aqueous extracts from each sample were filtered through 3 mm filter papers (Whatman, England), and the final volume was adjusted to around 300 ml in order to prepare an appropriate volume for administration (10 ml/kg body weight). The extracts were administered *p.o.* daily at a dose of clinical use for 5 consecutive weeks.

Animal Models Young adult male Sprague Dawley rats, initial weight 200±10 g, were obtained from Daehan Biolink Co., Ltd. (Seoul, Korea). Animals were housed in individual cages under conditions of constant temperature (22±2 °C) and humidity (55±5 °C). They were kept on a 12 h light/dark cycle and acclimatized to the housing situation for four weeks prior to the experiments. Rats were divided into five groups ($n=6$) as follows: (i) normal control rats administered water, (ii) rats administered ethanol/water, (iii) rats administered ethanol/water and a commercially available hangover cure solution (Condition: Cheil-je-dang Co., Ltd., Seoul) (iv) rats administered ethanol/SMS1 and (v) rats administered

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Table 1. Experimental Groups

Experimental group	Number of rats	Treatment
(i) Normal control	6	Distilled water
(ii) Disease control	6	Alcohol+distilled water
(iii) HRS	6	Alcohol+HRS[a)]
(iv) SMS1	6	Alcohol+SMS1
(v) SMS2	6	Alcohol+SMS2

a) HRS: Hangover release solution (Condition; Cheil-Je-Dang, Seoul, Korea) commercially available in Korea.

ethanol/SMS2. Rats were treated with these various regimens for the same time period (Table 1). Rats administered ethanol consumed a 40% ethanol solution and an intake of 5 g ethanol/kg/d was achieved. The body weight and general condition of the animals were monitored every two days.

Biochemical Analysis Blood was collected and allowed to clot for half an hour before separation of the serum by centrifugation at 3000 ***g*** for 15 min. Serum AST or ALT activity was determined using the AST kit (Boehringer Mannheim, Germany) or ALT kit (Boehringer Mannheim). Serum triglyceride levels were measured using the TG kit (Boehringer Mannheim) while the enzymatic colorimetric test for cholesterol content was performed using the Total Cholesterol kit (Boehringer Mannheim).

Determination of ADH Rats were killed by decapitation and bled. Liver tissue was transferred to 0.25 M sucrose solution and homogenized to facilitate the measurement of ADH. The homogenized solution was centrifuged at 14000 ***g*** for 15 min and the supernatant was filtered using a 0.45 μm membrane filter (Millipore, France) and stored at 4 °C.

Liver Histology Liver tissue (1 mm^3) was removed and pre-fixed for 2 h at 4 °C in 4% paraformaldehyde solution with 0.1 M phosphate buffer (pH 7.3) and 2.5% glutaraldehyde. The tissue was then rinsed in the same buffer and post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.3). Post-fixation was followed by dehydration in ethanol, embedding in Epon 812 and polymerization. Tissues were cut using an LKB 2088 ultramicrotome, stained with 1% uranyl acetate and lead citrate, and examined with a transmission electron microscope (JEM-2000 EXII, 80 KV, Japan).

Statistical Analysis All results are shown as mean±standard deviation. Statistical evaluation of data was performed by Duncan's multi-range test to make comparisons between groups.

RESULTS

Weight Gain and Ratio of Liver Weight to Body Weight The daily intake of ethanol plus SMS1 or SMS2 did not affect body weight gain during the study, as shown in Table 2. The SMS2 group exhibited a slightly increased weight gain (61.50±7.40 g) compared to the control groups while the SMS1 group exhibited a weight gain of 52.5±6.25 g, which was the lowest value of the tested groups ($p<0.05$). The groups administered SMS also exhibited a slightly decreased ratio (%) of liver weight to body weight compared to other groups. The disease group administered ethanol alone exhibited the significantly highest ratio.

Activities of AST, ALT and ADH Normal untreated control rats exhibited AST levels of 60.33±4.97 U/l (Table 3). Treatment of rats with ethanol resulted in a significant increase in serum AST levels to 117±20.02 U/l. Rats treated with both alcohol and the commercially available hangover release medicine exhibited lower AST levels of 95.33±4.46 U/l with the SMS1 group being comparable at 96.17±10.57 U/l. The amount of AST increase of the SMS1 group was significantly ($p<0.05$) inhibited as shown in Table 3. Normal untreated control rats exhibited ALT levels of 19.17±2.14 U/l, while administration of ethanol resulted in a significant increase in the serum ALT level to 45.83±7.14 U/l ($p<0.05$). Rats treated with both alcohol and hangover release medicine exhibited significantly elevated serum ALT levels of 43.50±8.62 U/l. Interestingly, the SMS1 and SMS2 groups exhibited significantly reduced levels of ALT with near-normal ALT levels of 25.17±2.4 U/l and 29.00±1.55 U/l, respectively. Table 5 lists the hepatic ADH activity of the different experimental groups. The ADH activity of rats treated with alcohol was elevated at 1.05±0.40 U/mg tissue compared to normal untreated rats (0.61±0.52 U/mg tissue). The SMS2 group exhibited a marked elevation in ADH activity, at 2.02±0.4 U/mg tissue, which represents an almost four-fold increase to that evident in normal untreated rats. Similarly, the ADH activity of the SMS1 group was also significantly increased at 1.67±0.50 U/mg tissue.

Table 2. Total Body Weight Gain and Liver and Kidney Weights

Group	Total body weight gain (g) Mean±S.D.	Liver (% of body weight) Mean±S.D.	Kidney (% of body weight) Mean±S.D.
(i) Normal control	59.83±5.49	3.98±0.40	0.78±0.02
(ii) Disease control	58.50±12.49	4.06±0.24	1.11±0.0
(iii) HRS	56.17±11.50	4.33±0.13	1.14±0.02
(iv) SMS1	52.50±6.25	3.98±0.33	1.05±0.04
(v) SMS2	61.50±7.40	3.77±0.31	1.02±0.06

Each value represents the mean body weight±S.D. ($n=6$).

Table 3. Serum AST and ALT Levels

Group	AST (U/l) Mean±S.D.	ALT (U/l) Mean±S.D.
(i) Normal control	60.33±4.97	19.17±2.14
(ii) Disease control	117.00±20.02**	45.83±7.14**
(iii) HRS	95.33±4.46**	43.50±8.62**
(iv) SMS1	96.17±10.57**	25.17±2.04**
(v) SMS2	112.50±7.61**	29.00±1.55**

Each value represents the mean±S.D. ($n=6$). Statistical evaluation of data was performed by Wilcoxon rank sum test to compare between groups $*p<0.05$, $**p<0.01$.

Serum Triglycerides and Total Cholesterol Levels Serum triglyceride levels were determined in order to examine the effects of SMS upon the lipid metabolism of animals exposed to hepatotoxic levels of alcohol. As shown in Table 4, the SMS-treated groups are quite distinct from the other diseased groups. Triglyceride levels in normal untreated rats were 14.17±6.80 mg/dl while the levels found in rats administered alcohol and rats treated with both alcohol and hangover release medicine were markedly elevated at 71.33±44.27 mg/dl and 76.33±23.57 mg/dl, respectively. In contrast, the triglyceride levels in SMS1 and SMS2 treated groups were only mildly elevated at 18.50±7.77 mg/dl and

21.83±5.08 mg/dl, respectively. Table 4 demonstrates that SMS1 and SMS2 had significantly lesser effects upon serum cholesterol levels. These data indicate that SMS1 and SMS2 clearly reduce serum triglyceride levels, although there is a weak effect of SMS treatment upon cholesterol levels compared to the disease control.

Histological Studies The hepatocytes of rats administered alcohol and rats treated with both alcohol and hangover release medicine exhibited a diffuse accumulation of variously sized lipid droplets compared with the SMS1 and SMS2 treatment groups. Otherwise, the hepatocytes of the SMS1 treated group were comparable to those of normal untreated control rats (Fig. 1). We examined morphological cell changes using transmission electron microscopy. Figure 2A depicts the normal morphology of hepatocytes. The rough endoplasmic reticulum (RER) is well developed and the mitochondria exhibit a normal morphology. The hepatocytes of rats administered alcohol exhibit various degenerative changes. The mitochondria were swollen and demonstrated significant degeneration and destruction of the cristae. In addition, lysosomes were increased and myelin-like figures were also frequently observed in the cytoplasm. Biliary canaliculi between the contact membrane of hepatocytes were generally dilated and luminal microvilli were markedly depleted. Hepatocytes exhibited a diffuse accumulation of variously sized lipid droplets (Figs. 2B, C). In contrast, the hepatocytes of rats treated with both alcohol and hangover

Table 4. Serum Lipid Levels

Group	TG (mg/dl)	Cholesterol (mg/dl)
	Mean±S.D.	Mean±S.D.
(i) Normal control	14.17±6.20	42.83±12.77
(ii) Disease control	71.33±40.4**	70.50±5.65*
(iii) HRS	76.17±21.54**	81.17±6.39**
(iv) SMS1	18.50±7.09	66.50±6.75*
(v) SMS2	21.83±4.63	75.00±5.7**

Each value represents the mean±S.D. (n=6). Statistical evaluation of data was performed by Wilcoxon rank sum test to compare between groups $*p<0.05$, $**p<0.01$.

Table 5. Alcohol Dehydrogenase Activities

Group	ADH (U/mg) Mean±S.D.
(i) Normal control	0.61±0.52
(ii) Disease control	1.05±0.40
(iii) HRS	1.31±0.55*
(iv) SMS1	1.67±0.50*
(v) SMS2	2.02±0.4*

Each value represents the mean±S.D. (n=6). Statistical evaluation of data was performed by Wilcoxon rank sum test to compare between groups $*p=0.05$.

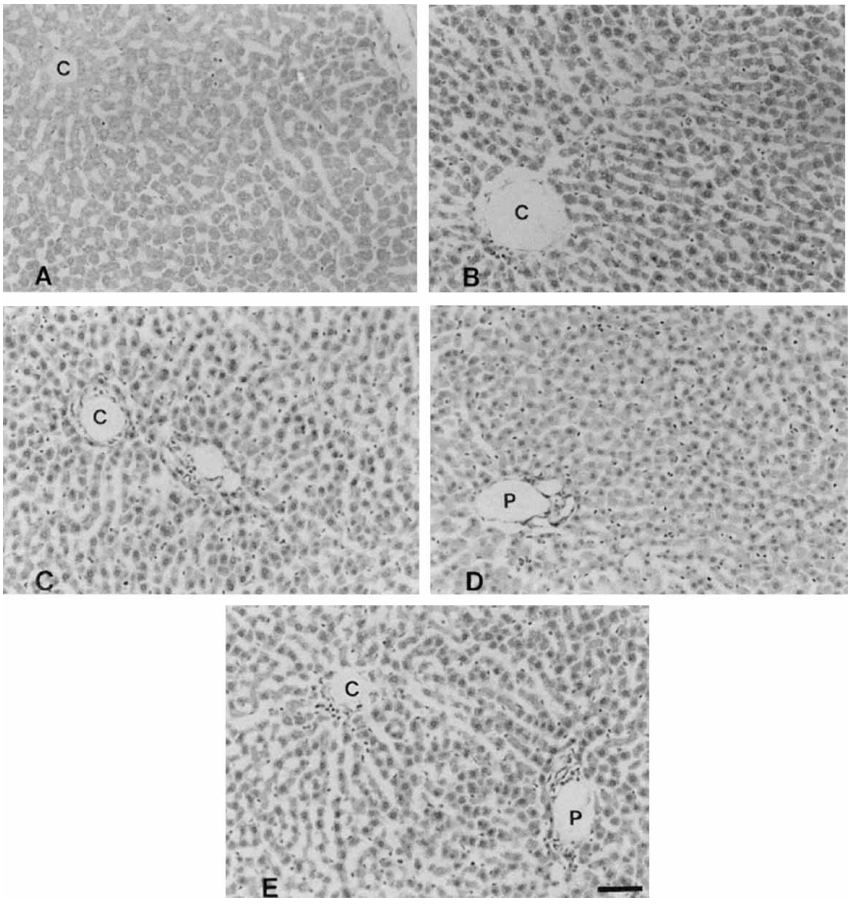

Fig. 1. Light Micrographs of Hepatic Tissue

(A) Hepatic tissue from normal control rat exhibiting normal morphology. (B) Hepatic tissue from rat treated with ethanol alone. Cytoplasmic lipid droplets are evident. (C) Hepatic tissue from rat treated with both ethanol and hangover release medicine. (D) Hepatic tissue from rat treated with ethanol and SMS-1. (E) Hepatic tissue from rat treated with ethanol and SMS-2. (C-Central vein, P-Portal vein) Scale bar represents 50 μm. Cell staining was carried out by the H&E staining method.

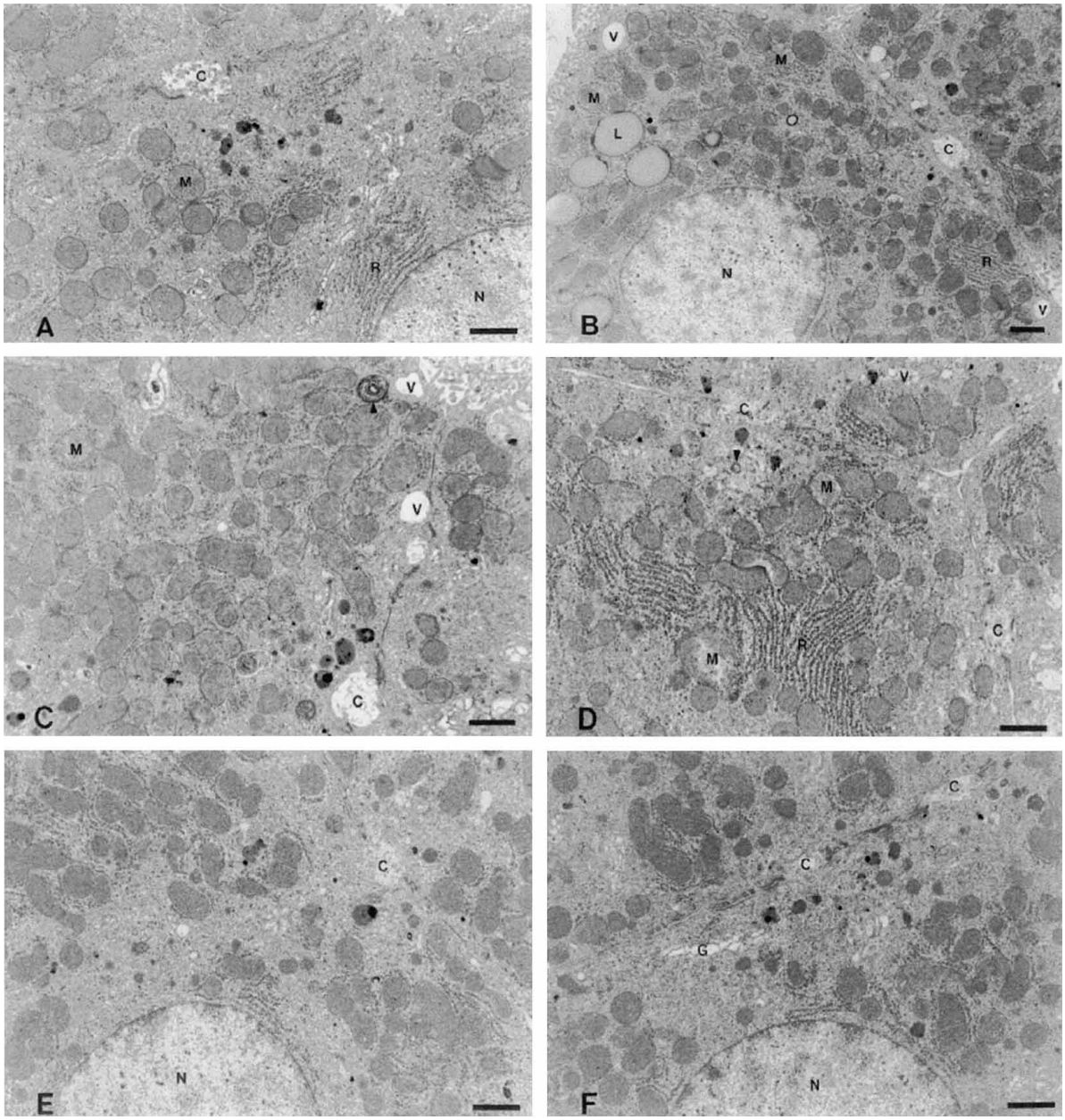

Fig. 2. Electron Micrographs of Hepatocytes from Rat Treated with Ethanol and SMS1 or 2

(A) Hepatocytes from normal control rat. (B) Hepatocytes from rat treated with ethanol alone. B. Swollen mitochondria with destroyed cristae are observed. Lipid droplets and variably sized vesicles are increased in the cytoplasm. C. Bile canaliculi between the adjacent hepatocytes are usually enlarged and microvilli in the lumen are severely depleted. Myelin figures, swollen mitochondria and vesicles are also observed in the cytoplasm. (C) Hepatocytes from rats treated with both ethanol and hangover release medicine. Degenerate mitochondria, myelin-like figures and small vesicles are still observed, but these signs of degeneration are less marked compared to the disease group. (D) Hepatocytes from rat treated with ethanol and SMS1. General ultrastructural appearance is similar to that of normal untreated control rat. (E) Hepatocytes from rat treated with ethanol and SMS2. General ultrastructural appearance is similar to that of normal untreated control rat except for the slight dilatation of the Golgi cisterns. C, bile canaliculus; G, Golgi cisterns; L, lipid droplet; M, mitochondria; N, nucleus; R, rough endoplasmic reticulum; V, vesicle; Arrow head, myelin-like figure. Scale bar=1 μm.

release medicine exhibited decreased mitochondrial swelling and moderately dilated biliary canaliculi with short microvilli. A small number of cytoplasmic myelin-like structures were still evident (Fig. 2D). In the SMS1 and SMS2 treated groups, the hepatocytes were more comparable to those of normal untreated rats (Figs. 2E, F). In rats treated with SMS2, the golgi cisterns of hepatocytes were slightly dilated (Fig. 2F). These data indicate that SMS1 and SMS2 exhibit cytoprotection from the toxic effects of alcohol upon the liver in rats.

DISCUSSION

The aim of this study was to investigate the hepatoprotective effects of SMS1 and SMS2 in alcohol-treated rats. The effect of daily intake of ethanol plus SMS1 or SMS2 was clearly powerful, as seen from the weight gain during 5 weeks of treatment (Table 2). In contrast with the great weight gain of the SMS2, which was applied with alcohol only, showed a low rate of increase in weight gain. The same features were found in the liver weight change as reported by Levy *et al.*,[10] suggesting that the liver weight increase is due to accumulated lipids in the liver of alcohol-treated rats. AST

and ALT levels increased with increased alcohol intake. These enzymes are well-documented indicators of hepatic dysfunction, with increased AST and ALT levels reflecting impaired liver function.[11,12] However, rats administered SMS1 and SMS2 exhibited diminished levels of these enzymes, with effects upon ALT levels being most marked, although the AST level for SMS2 appeared comparable to the disease control. Furthermore, since a component of SMS is presumed to be hepatoprotective, it is of significant interest that reports indicate that *schizandrae fructus* exhibits a protective effect on the liver.[13,14]

Many reports indicate that alcohol intake significantly increases both serum and hepatic TG levels resulting in hypertriglyceridemia and fatty liver.[15—18] The development of fatty liver may be augmented by the decreased food intake associated with chronic alcoholism, with reduced intake of protein, methionine, choline, vitamin E and selenium being particularly relevant.[19] Data summarized in Table 4 indicates that the administration of SMS has markedly beneficial effects upon serum lipid levels. Indeed, the data suggests that TG levels may be reduced to almost normal with regular administration of SMS.

Based on reports[20,21] indicating that an elevated blood cholesterol level is one of the main causes of vascular disease in the heart and circulatory system, a number of drugs have been developed to lower plasma cholesterol concentrations, such as cholestyramine, probucol and statins. However, little work has been done in developing natural materials to prevent hyperlipidemia. In this context, this report suggests that Saeng-maek-san may represent an alternative therapeutic agent to assist in the prevention and treatment of hyperlipidemia. ADH oxidizes ethanol and the activity of the enzyme is altered by the duration of ethanol administration.[22] In animal tests, ADH activity was increased by the chronic administration of alcohol.[23] It is widely known that alcohol-induced cell damage is closely related to alcohol metabolism.[23] Organic tissue damage and hangovers are caused by the acetaldehyde generated during the metabolic process. In this study, SMS increased the activity of ADH. The results indicate that the long-term ingestion of alcohol may lead to an accumulation of acetaldehyde and that the administration of SMS promotes alcohol metabolism. This suggests that SMS can enhance alcohol metabolism, discourage the accumulation of acetaldehyde and prevent alcohol poisoning.

In anatomical observations, many lipid droplets appeared in the rats administered alcohol (see Figs. 2B, C), which is consistent with a report that the alcohol-damaged liver can accumulate fat.[24] The present study also showed that lipid particles expand from the central vein (CV) through the portal vein to the hepatic lobule. This is also supported by the results of studies of the fatty liver of milking cows, which indicate that fat accumulation begins from the CV and steadily spreads to the portal vein.[25,26] Deposition of lipid droplets in the SMS groups (Figs. 2E, F) was markedly limited compared to other disease groups and this may be the combined effect of several functional elements in the SMS.

The SMS treated groups (Figs. 2E, F) exhibited little change in the microstructure of the cytoplasm, with an appearance similar to that of the normal control group. SMS has been found to have excellent effects in protecting the liver and limiting hepatic damage. The rough ER influences protein synthesis and the appearance of a number of large lipid droplets in alcohol-treated rats is consistent with a report that the liver accumulates fat following prolonged damage by ethanol.[24]

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