INTRODUCTION

Chronic ethanol consumption has fatal effects on the main organs of the body, in particular by causing dysfunction of hepatocytes, which are responsible for key metabolic functions. Ethanol intake may also cause alcoholic hepatitis, fatty liver, and liver cirrhosis.1) Therefore, in those who consume large amounts of alcohol for extended periods, it is important to devise a proper regimen to help prevent alcohol-induced syndromes. Recently, studies have utilized the acute toxicity model, whereby CCl₄ or ethanol is administered at excessive dosages to induce fatty liver or hepatitis in animal models. However, in view of the differences between animals and humans in relation to alcoholic fatty liver or hepatitis, in the present study we chose to utilize a chronic alcoholic hepatotoxicity model to induce fatty liver.

Furthermore, various investigations on natural substances have been performed to determine whether they protect the liver from alcohol-induced hepatotoxicity. Of these, several natural extracts have been found to have noticeable protective effects on the liver and hepatic lipid levels, indicating the onset of alcoholic fatty liver. Greater increases in alanine transaminase (ALT) and alkaline phosphatase (ALP) activities in serum were observed in the groups fed alcohol-containing diets compared with those in the ND group. Treatment with ED + VST, ED + VGS, ED + VGT, and ED + VAP decreased the levels of triglycerides, free fatty acids, and total cholesterol in the serum and liver, with a concomitant reduction in the activity of serum ALT and ALP. These data suggest that the plant extracts examined in this study can be utilized as a health functional food or new drug candidates for the treatment of fatty liver and hepatotoxicity induced by chronic alcohol consumption.

Key words —— *Vitis vinifera, Schisandra chinensis, Taraxacum officinale, Gardenia jasminoides, Angelica acutiloba, Paeonia japonica*

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Several types of procyanidins are present in grapeseed extracts (including catechin at up to 25%); the main procyanidin is (+)-catechin. It has been reported that the procyanidins in grapeseed extracts have antioxidant effects, protective effects against cardiovascular disease, antiinflammatory effects, antiviral effects, antiallergy effects, and artery — relaxant properties.

Omiza (S) is a tree that belongs to the Magnolia family and has a hepatoprotective effect. It is well known that the administration of Omiza to normal rats results in reduced levels of neutral fatty acids in the serum, lipids in the liver, and the stimulation of drug — metabolizing enzyme activities. In addition, it has also been reported to have antioxidant effects. Hikino et al. found that Omiza had protective effects on the liver and Lee and Lee found that it neutralized the poisonous effects of alcohol.

Pogongyung (T) is a perennial herb that belongs to the Asteraceae family, and the herb is known to have beneficial effects in geriatric diseases, such as hypertension, heart disease, liver disease, and others.

G is either a tree that belongs to the Rubiacceae family or a mature fruit obtained from other plants within the same family. The pharmacologic effects of gardenia were confirmed in an experiment showing that it suppresses serum cholesterol level increases, reduces lipid levels in the serum and in liver, reduces the secretion of bile acid, and inhibits hepatocyte dysfunction in white rats fed a high-carbohydrate diet.

Danggi (A. gigas) is a perennial herb belonging to the Umbelliferous family and it has been widely studied for its effects on immunity, antioxidative ability, antimutagenic activity, and anticancer activity.

Jakyak (P) is a perennial herb belonging to the Buttercup family. Based on in vitro studies on its physiologic activities, it has noticeable antioxidative activity, and it is reported that several solvent extracts from the roots of Jakyak are effective in lowering serum levels of cholesterol when levels are increased by a high cholesterol-containing diet.

Therefore this study was designed to determine the hepatoprotective effects in experimental rats chronically fed a Lieber-DeCarli ethanol diet. Mixtures of several natural materials such as grapeseed, Omiza, Pogongyung, and Gardenia, all of which have been reported to have noticeable effects against alcohol toxicity, were administered to Sprague-Dawley SD rats. Effects on lipid metabolism in the serum were observed, and the livers of rats were examined to compare the hepatoprotective effects of various mixtures.

MATERIALS AND METHODS

Sample Preparation —— Dried S, T, and G were purchased from the Kyung-Dong (Seoul, Korea) market and distilled water was added at 10-fold the volume to each. G was boiled at 60°C for 5 hr, while the other natural materials were extracted twice at 90°C for 4 hr. They were filtered, concentrated, and then freeze-dried. The yield of S and T was 40%, G 29%, A 54%, and P 34%. Grapeseed (Vitis vinifera, catechine 25%, V) extracts were purchased from Herb Valley Co., Ltd.

Experimental Animals and Experimental Diets —— Male SD white rats, 5 weeks old, weighing 140–150 g were purchased from Orient Inc. (Seoul, Korea) and used as an experimental animal model. The rats were adapted to solid feed for 1 week in the laboratory environment where the temperature was kept constant at 24.2°C and the humidity at 60% using a climate — control system. The 6 experimental groups (n = 8) each were fed an experimental diet for 6 weeks. The group with hepatotoxicity was orally administered prepared samples, and the control group was fed a diet without alcohol while simultaneously receiving the equivalent amount of distilled water. The experimental groups were designated the normal diet (ND), ethanol diet (ED), ED + V 100 + S 150 + T 150 mg/kg/day (ED + VST), ED + V 100 + G 150 + S 150 mg/kg/day (ED + VGS), ED + V 100 + G 150 + T 150 mg/kg/day (ED + VGT), ED + V 100 + A 150 + P 150 mg/kg/day (ED + VAP) groups.

The experimental diet was prepared according to the Lieber-DeCarli rat liquid diet method, and was prepared fresh every day prior to use. Table 1 shows the compositions of the diets, which were designed to provide 1 kcal/ml. The ED consisted of 35% fatty acids, 11% carbohydrate, 18% protein, and 36% ethanol, while the ND consisted of 35% fatty acids, 47% carbohydrate, and 18% protein. In the ED group, 36% of total calories was obtained from ethanol, while maltose dextrin corresponding to the amount of the above calories replaced ethanol in the ND group. The 5 ED groups were adapted to increasing amounts of ethanol for the first 4 days and then received ethanol (36% of total calories) for the
We studied the effects of administering herbal extract mixtures (HEM) for 6 weeks on rat body and liver weights (Table 2). Body weights were significantly lower in the ED group than in the ND group. HEM-treated groups did not significantly differ in liver weights (Table 2). Body weights were significantly different from ethanol diet (ED) group at $^*p<0.05$. Significantly different from ethanol diet (ED) group at $^\dagger p<0.05$. a) Liver index = liver/body weight.

**RESULTS**

**Change in Body and Liver Weights**

We studied the effects of administering herbal extract mixtures (HEM) for 6 weeks on rat body and liver weights (Table 2). Body weights were significantly lower in the ED group than in the ND group. HEM-treated groups did not significantly differ in liver and body weight from those in the ND group. But HEM-treated groups showed slower body weight.
gain than the ED group. It is considered that the reasons for this decrease in body weight gain are a result of the increased oxygen uptake due to alcohol consumption, an increase in metabolic rates, and a reduction in the production of ATP through alcohol oxidation in microsomes.38)

The ED group showed an 18% increase in the liver index compared with that in the ND group. HEM-treated groups had reduced liver weights compared with the ED group. The ED + VST, ED + VGS and ED + VGT groups had values similar to that of the ND group because their liver weights were significantly reduced by alcohol.

Levels of Triglycerides and Free Fatty Acids in Serum

Levels of TG and FFA are shown in Table 3. The results were obtained after feeding alcohol in diet for 6 weeks. The levels of TG and FFA in serum were markedly affected by the experimental diets. TG values in the ED group were about two-fold higher than those in the ND group. This confirmed that toxicity was induced by the ingestion of ethanol. Compared with the ED group, there was a decrease of 48.7% in the ED + VST group, of 45.6% in the ED + VGS group, of 49.1% in the ED + VGT groups, and of 31.9% in the ED + VAP group. All HEM-treated groups had significantly decreases in their TG values compared with those in the ED group. In particular, values in the ED + VST and ED + VGT groups decreased to the level in the ND group. FFA levels showed a similar tendency to TG levels, where the ED group showed a 29% increase compared to that in the ND group. HEM-treated groups had lower levels compared with those in the ED group, whereas the ED + VST group had significantly decreased FFA levels.

TC and HDL-C in Serum

Levels of TC and HDL-C in each group are shown in Table 3. The ED groups showed a 47% increase in TC and HDL-C levels compared with the ND group. Pikaar et al. reported that the long-term consumption of alcohol results in an increase in serum.39) HEM-treated groups showed a tendency toward lower total TC levels compared with the ED groups, especially the ED + VST and ED + VGS groups, which showed significant decreases versus the ED group. No significant differences were observed between groups in HDL-C levels, although the ED + VST and ED + VAP groups tended to have higher levels than the ED group. Based on these results, HEM reduced levels of TG, FFA, and TC and enhanced HDL-C levels in the serum of rats given alcohol long term.

Changes in Liver Tissue Lipids

Changes in liver tissue lipids after receiving an alcohol diet for 6 weeks are shown in Table 4. The ED groups had about a 79% increase in TG levels compared with the ND group. HEM-treated groups showed a tendency toward lower total TC levels compared with the ED groups, especially the ED + VST and ED + VGS groups, which showed significant decreases versus the ED group. No significant differences were observed between groups in HDL-C levels, although the ED + VST and ED + VAP groups tended to have higher levels than the ED group. Based on these results, HEM reduced levels of TG, FFA, and TC and enhanced HDL-C levels in the serum of rats given alcohol long term.

ALT and ALP Activity in Serum

The effects of HEMs on the alcohol-induced elevation of ALT and ALP activities are presented in Table 5. ALT values in the ED group were significantly higher than in the ND group. Among the
decreased food intake and malabsorption.1) Pikaar et al. explained that fatty liver develops as a result of alcohol consumption in patients is mainly attributable to a loss in body fat, a reduction in the amount of diet consumed, and sharp increase in energy consumption.39) HEMs used in this study restored animals to normal weight after weight loss due to the administration of an ethanol-containing diet.

It is considered that the liver weights per 100 g of body weight in the experimental groups increased significantly due to the increased volumes of cells caused by the accumulation of fats, proteins, and water in alcohol-induced cirrhosis of the liver.40) The administration of VST, VGS, and VGT HEMs significantly reduced the liver index.

As a result of our investigation of changes in lipid metabolism in livers injured by long-term alcohol ingestion, we found that groups fed alcohol-containing diets had increased levels of TG and FFA in serum compared with the ND group. This is concurs with the findings of studies that showed the development of hyperlipidemia through the long-term intake of alcohol.41,42)

The administration of several HEMs led to a markedly lower overall concentration of TG which was increased by feeding the alcohol-containing diet. The concentration of FFA was also significantly decreased by HEMs.

In addition, the concentration of TC in the alcohol-fed groups was higher than in the ND group, which agreed with a report by Pikaar et al.39) that increased levels of TC in serum are directly linked to chronic alcohol intake. The present study confirms that the administration of VST and VGS lowers elevated TC concentrations due to chronic alcohol intake.

Increased levels of TG, FFA, and TC in the liver were observed in groups fed alcohol-containing diets as compared with the ND group. Dutta et al.41) explained that fatty liver develops as a result of chronic alcohol intake, and that this is the result of malnutrition due to a reduction in food consumption and in particular to a lack of anti-fatty liver fac-

### DISCUSSION

The rats fed an alcohol-containing diet for 6 weeks showed significant weight loss versus the ND group, which concurs with a report that weight loss occurs in chronic alcohol abusers because of decreased food intake and malabsorption.1) Pikaar et al. reported that weight loss due to alcohol consumption in patients is mainly attributable to a loss in body fat, a reduction in the amount of diet consumed, and sharp increase in energy consumption.39) HEMs used in this study restored animals to normal weight after weight loss due to the administration of an ethanol-containing diet.

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### DISCUSSION

Table 4. Reducing Effects of Herbal Extract Mixtures on Liver Triglyceride, Free Fatty Acid, and Total Cholesterol Levels in Ethanol-Administered Rats

<table>
<thead>
<tr>
<th></th>
<th>TG (mg/g liver)</th>
<th>FFA (µEq/g liver)</th>
<th>TC (mg/g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>2.97 ± 0.58</td>
<td>0.94 ± 0.14</td>
<td>11.69 ± 1.06</td>
</tr>
<tr>
<td>ED</td>
<td>5.32 ± 1.90*</td>
<td>1.34 ± 0.25*</td>
<td>11.86 ± 3.50</td>
</tr>
<tr>
<td>ED + VST</td>
<td>3.17 ± 1.33†</td>
<td>0.81 ± 0.22†</td>
<td>10.33 ± 1.81</td>
</tr>
<tr>
<td>ED + VGS</td>
<td>3.46 ± 1.64†</td>
<td>0.88 ± 0.27†</td>
<td>11.01 ± 0.55</td>
</tr>
<tr>
<td>ED + VGT</td>
<td>4.28 ± 0.55</td>
<td>0.81 ± 0.16†</td>
<td>11.47 ± 0.22</td>
</tr>
<tr>
<td>ED + VAP</td>
<td>5.45 ± 0.90*</td>
<td>0.64 ± 0.10‡</td>
<td>12.22 ± 2.89</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. Significantly different from normal diet (ND) group at *p < 0.05. Significantly different from ethanol diet (ED) group at †p < 0.05 and ‡p < 0.01, respectively.

Table 5. Protective Effects of Herbal Extract Mixtures on Serum ALT and ALP Values in Ethanol-Administered Rats

<table>
<thead>
<tr>
<th></th>
<th>ALT (Karmen/ml)</th>
<th>ALP (20K-A)</th>
</tr>
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<tbody>
<tr>
<td>ND</td>
<td>31.03 ± 4.62</td>
<td>22.79 ± 4.19</td>
</tr>
<tr>
<td>ED</td>
<td>39.84 ± 6.58*</td>
<td>27.59 ± 7.18</td>
</tr>
<tr>
<td>ED + VST</td>
<td>31.60 ± 2.43†</td>
<td>19.16 ± 2.64†</td>
</tr>
<tr>
<td>ED + VGS</td>
<td>25.38 ± 3.29†</td>
<td>22.21 ± 3.93</td>
</tr>
<tr>
<td>ED + VGT</td>
<td>25.90 ± 1.71‡</td>
<td>22.49 ± 3.41</td>
</tr>
<tr>
<td>ED + VAP</td>
<td>37.99 ± 3.12*</td>
<td>23.95 ± 6.76</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. Significantly different from normal diet (ND) group at *p < 0.05. Significantly different from ethanol diet (ED) group at †p < 0.05.
tors, such as proteins, methionine, choline, vitamin E, Se, and others. Situnayake et al. reported that ADH and ALAH are NAD-dependent enzymes, which participate in the oxidation of alcohol. When alcohol is consumed on a chronic basis, NAD is converted into NADH, which may induce changes in the redox state of the liver and cause the accumulation of TG in liver. Suter et al. reported that the oxidation of lipids was greatly reduced by alcohol intake. In the present study, several HEMs markedly reduced the concentrations of TG, FFA, and TC in the liver, especially in the VST and VGS groups. The above results show that the HEMs used in this study prevent the accumulation of lipids due to chronic alcohol consumption.

Increased activities of serum ALT and ALP may signal the onset of hepatic dysfunction. All activities of ALT and ALP in the groups fed ethanol-containing diets increased compared with those in the ND group, which resulted from liver injury caused by chronic alcohol consumption. The present study shows that the administration of HEMs effectively improved liver function by lowering the activities of ALT and ALP.

Our study showed that the ED + VST, ED + VGS, and ED + VGT groups were effectively protected against liver toxicity from chronic alcohol exposure and lipid metabolism in both the liver and serum was protected compared with the ED + VAP group. This result agrees with those from previous comparative studies on herbal materials. We found that the ED + VST group was the most effectively protected.

We confirmed that the HEMs examined in this study are effective in improving liver functions and treating liver dysfunction and lipid metabolism disorders induced by chronic alcohol consumption in an experimental rat model, which has been confirmed to reflect alcohol-induced liver disease in humans. In addition, the HEMs play a role in preventing or reducing alcohol-induced hepatotoxicity. Further studies on the effects of these HEMs on alcohol and lipid metabolism are required.

REFERENCES


