Study on the Anti-Hyperlipidemia and Liver Cell Protection of Korean Medicinal Herb Complex of Alcohol fed Rats

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Abstract – This study sought to investigate the ameliorating effects of a Korean medicinal herb (KMH) complex on the impacts of alcohol consumption in rat hepatocytes and in reducing the total cholesterol levels and the total lipid levels in the serum. We compared the body weight gain and ratio of the liver, the kidney to body weight, and also the serum biochemistry of the rats administered with both the alcohol and the KMH complex to the control rats treated with alcohol alone. The clinically important enzyme markers (Aspartate Aminotransferase, AST, and Alanine Aminotransferase, ALT) of rats, administered with both the alcohol and the KMH complex treatments, were compared with those in the control group. The treatment regimen (KMH complex) significantly reduced the serum AST and ALT levels, indicating the hepato-protective effects of the KMH complex. Furthermore, total cholesterol and total lipid levels were significantly reduced. These results indicate that the KMH complex may positively mediate the effects of alcohol on hepatocytes and the general liver functions.

Key words : Korean medicinal herb (KMH), rat hepatocytes, alcohol

INTRODUCTION

Alcoholism is a social and economic problem that is global in scope. Alcohol is the most frequently abused drug throughout the world and has a long history of use. Alcoholism can also be considered as one of the costliest diseases of the modern era in terms of life years lost (Murray and Lopez 1996), even costlier than tobacco use. Alcohol-laden blood then travels to the liver via the veins and capillaries of the digestive tract, which affects nearly every liver cell. The liver cells are the only cells in the body that can produce sufficient amounts of the enzyme alcohol dehydrogenase to oxidize alcohol at an appreciable rate (Maher 1997). The impairment of bodily functions and the damage caused by the consumption of alcohol works mainly in two ways: 1) indirectly, by interfering with digestive processing of food, thereby causing malnutrition; and 2) through direct toxic effects caused organ pathology, the effects of which focus particularly upon the liver (Lieber 1995). The liver is the body's largest internal organ. The functions of the liver (filtration of circulating blood, removal and breakdown of toxic substances) are essential to life and play a critical role in the metabolic processes (Diehl 1993). Ameliorating effects of alcohol consumption chronically has long been a focus for many researchers and clinicians. There have been various efforts to develop compounds to ameliorate or to treat alcohol-related pathology (Potter 1995; Kovanen and Scheider 1999; Park *et al.* 2002). However, the chemical compounds can have harmful and unforeseen side effects. Therefore there has been a focus on natural or herbal treatments for alcohol-induced diseases. There are a number of studies that have researched the nutri-

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tional and physiologic benefits of natural products and medicinal plants (Tsukamoto *et al.* 1986; Tsukamoto *et al.* 1990).

We investigated whether KMH, a Korean medicinal herb complex, protects rat hepatocytes from alcohol-induced damage, thereby resulting in a protection from hangovers, cardiovascular symptoms and alcohol-induced hepatitis (Lieber 2001) in this study. KMH is composed of Korean medicinal herbs such as *Cornus officinalis* SIEB. *et* ZUCC., *Panax ginseng* C. A. Meyer, *Artemisia capillaris* Thunb. and *Astragalus membranaceus* Bunge (Huh 1981). It is well known that the spectrum of alcoholic liver disease can be reproduced in a rat model utilizing an intragastric infusion of ethanol (Tsukamoto *et al.* 1986; Tsukamoto *et al.* 1990). KMH was administered to rats treated with alcohol. The protective effect of KMH was examined by measuring the blood levels of the enzymes AST (aspartate aminotransferase) and ALT (alanine aminotransferase) before and after KMH administration in the alcohol-treated rats. Serum levels of triglyceride and the total cholesterol, a mainly causes of hyperlipidemia and arteriosclerosis, were also measured. The major finding of this paper is that KMH is hepatoprotective and ameliorates alcohol-mediated damage and alcohol-induced liver symptoms whilst concomitantly improving the lipid metabolism.

MATERIALS AND METHODS

1. Animal models

Young adult male Sprague Dawley rats, initial weight 100 ± 10 g, were obtained from Daehan Biolink Co., Ltd. (Seoul, Korea). Animals were housed in individual cages under conditions of a constant temperature $(22 \pm 2°C)$ and humidity $(55 \pm 5\%)$. 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 six groups (n = 7) as follows: No. 1) normal control rats administered with water, No. 2) negative control rats administered with ethanol/water, No. 3) positive control rats administered with ethanol/water and a commercially available hangover cure solution (HCS, Condition: Cheil -je-dang Co., Ltd., Seoul), No.4) experimental group rats

Table 1. Composition of Groups

Groups	No. of exam.	Treatment
No. 1 (Normal control)	7	None-alcohol
No. 2 (Negative control)	7	Alcohol+Water
No. 3 (Positive control)	7	Alcohol+HCS*
No. 4 (Test group 1-T 1)	7	Alcohol+KMH*

HCS* : Hangover cure solution (Condition: Cheil-je-dang Co., Ltd., Seoul). KMH* : Korean medicinal herb complex.

administered with the ethanol/Korean medicinal herb (KMH) complex (Table 1). Rats were treated with these various regimens for the same time period. Rats administered with ethanol consumed a 40% ethanol solution and an intake of 5 g kg^{-1} day^{-1} was achieved. The body weight and general condition of the animals were monitored every two days.

2. Preparation and treatment of KMH complex

Production of KMH was based on a recipe derived from Korean traditional medicine books and the recommendations of Korean traditional medical doctors (Huh 1981). KMH is a traditional Korean prescriptions containing a mixture of four herbs, *Panax ginseng* C. A. Meyer, *Cornus officinalis* SIEB. *et* ZUCC., *Artemisia capillaris* Thunb., *Astragalus membranaceus* Bunge, with the relative amount of each herb in the preparation being 1 (25 g), 1 (25 g), 1 (25 g), and 1 (25 g), respectively. Boiling water extracts of KMH were prepared from the dried herbs. Each 25 g of the mixed herbs was added to 1,000 mL of sterilized water and boiled for 150 min using a herbal and medicinal boiling pot (Daewoong Co., Ltd., Seoul, Korea). After centrifugation at $6,000 \times g$ for 15 min, aqueous extracts from the sample were filtered through 3 mm filter papers (Whatman, England), and the final volume was adjusted to 400 mL in order to prepare an appropriate volume for administration (about 1.6 g kg^{-1} body weight day^{-1}).

3. Dissection and biochemical analysis

After fasting for 16 h at the last day of housing, rats were dissected under an anasesthetic state and 3-4 mL blood was collected using an injector. Liver and kidney were removed and rinsed with cold 0.1 M phosphate buffer (pH 7.3). Collected blood was allowed to clot for

Table 2. Total body weight gains and the weight ratio of liver and kidney

Groups	Total body weight gains (g)	Liver (% of body weight)	Kidney (% of body weight)
	Mean±S.D.	Mean±S.D.	Mean±S.D.
No. 1 (Normal control)	41.22±2.46*[1]	2.66±0.062	0.629±0.037*
No. 2 (Negative control)	35.62±5.02	3.02±0.081	0.651±0.038
No. 3 (Positive control)	38.21±1.72*	2.62±0.101*	0.601±0.032
No. 4 (Test group 1–T 1)	42.10±7.52*	2.62±0.111*	0.611±0.037*

[1]Each value represents the mean±S.D. of 7 rats. Means with different superscript asterisks within a column and significantly different from each other at P<0.5 (*) as determined by Student's T-test.

half an hour before separation of the serum by centrifugation at 3,000 × g for 15 min. Serum AST and ALT activity was determined using the AST kit and ALT kit, respectively (Boehringer Mannheim, Germany). Serum triglyceride levels were measured using the TG kit (Boehringer Mannheim, Germany) while the enzymatic colorimetric test for the cholesterol content was performed using the total cholesterol kit (Boehringer Mannheim, Germany).

4. Statistical analysis

All the results are shown as a mean±standard deviation. Statistical evaluation of the data was performed at p<0.5 by the student's t-test to make comparisons between the groups.

RESULTS AND DISCUSSION

1. Weight gain and ratio of the liver weight to body weight

Pirola and Lieber (1975) reported that the body weight gain decreased in alcohol-treated rats and that the body weight decreased by 50% with an alcohol ingestion instead of sugar in the total energy source of a man. These results suggested that the oxygen consumption and metabolic rate were increased, ATP production was decreased in microsome by an excessive alcohol ingestion (Gruchow et al. 1985). This study also represented similar results as seen in Table 2. The effect of a daily intake of the No. 4 group (ethanol plus KMH) was clearly powerful, as seen from the weight gain during 4 weeks of treatment. In contrast with the great weight gain of the KMH complex, that was applied with alcohol only which showed the lowest rate of increase in the weight

gain. The same features were found in the liver weight change as reported by Levy et al. (1976) suggesting that the increase of liver weight is due to the accumulated lipids in the liver of the alcohol-treated rats. The negative control group (No. 2) administered with ethanol alone exhibited significantly the highest ratio. But the groups administered with KMH (No. 4) exhibited a similar level to the normal control group. The ratio (%) of the kidney weight to body weight in the No. 4 group showed a remarkable decrease compared with the ratio of negative control group.

2. Activities of AST and ALT

AST and ALT levels increased with an increased alcohol intake. These enzymes are well-documented indicators of hepatic dysfunction, with increased AST and ALT levels reflecting on impaired liver function (Kien and Ganther 1983; Thompson and Scott 1970). In this study, normal untreated control rats exhibited AST levels of 84.43±47.88 U L⁻¹ (Table 3). Treatment of rats with ethanol resulted in a significant increase in the serum AST levels to 257.57±412.20 U L⁻¹. Rats treated with both alcohol and the commercially available hangover release medicine exhibited lower AST levels of 70.21± 11.29 U L⁻¹ with the test group (No. 4) being comparable at 66.54±3.12 U L⁻¹ . The amount of AST increase of the KMH group was significantly (p<0.5) inhibited as shown in Table 3. Normal control rats exhibited ALT levels of 44.14±9.21 U L⁻¹, while an administration of ethanol resulted in a significant increase in the serum ALT level to 215.41±412.31 U L⁻¹. Interestingly, the test group (No. 4) exhibited significantly reduced levels of ALT with lower normal ALT levels of 34.32±4.12 U L⁻¹ (p<0.05). Since a component of the KMH complex is presumed to be hepatoprotective, it is of significant int-

Table 3. Enzyme activity of AST and ALT in plasma

Groups	AST(U L^{-1})	ALT(U L^{-1})
	Mean±S.D .	Mean±S.D.
No. 1 (Normal Control)	84.43±47.88[*1)]	44.14±9.21*
No. 2 (Negative Control)	257.57±412.20	215.41±412.31
No. 3 (Positive Control)	70.21±11.29	37.21±9.03*
No. 4 (Test group1-T1)	66.54±3.12*	34.32±4.12**

[1)]Each value represents the mean±S.D. of 7 rats. Means with different superscript asterisks within a column and significantly different from each other at P<0.05 (**) and P<0.5 (*) as determined by Student's t-test.

Table 4. Concentration of TG and TC in plasma

Groups	TG (mg dL^{-1})	TC (mg dL^{-1})
	Mean±S.D.	Mean±S.D.
No. 1 (Normal Control)	39.87±8.61[*1)]	95.70±6.26*
No. 2 (Negative Control)	75.71±51.21	114.28±36.10
No. 3 (Positive Control)	30.16±6.71*	90.42±6.21**
No. 4 (Test group 1-T 1)	26.16±3.42***	86.12±10.82*

[1)]Each value represents the mean±S.D. of 7 rats. Means with different superscript asterisks within a column and significantly different from each other at P<0.5 (*) , P<0.05 (**) and P<0.01 (***) as determined by Student's t-test.

erest that the reports indicate *Panax ginseng* C. A. Meyer and *Cornus officinalis* SIEB. *et* ZUCC. Which exhibit a protective effect on the liver (Bode 1974; Liu 1989).

3. Serum triglycerides and total cholesterol levels

Many reports indicate that alcohol intake significantly increases both the serum and hepatic triglyceride levels resulting in hypertriglyceridemia and a fatty liver (Baraona and Lieber 1970; Glueck *et al.* 1980; Karsentry *et al.* 1985). The development of a fatty liver may be augmented by the decreased food intake associated with chronic alcoholism, with a reduced intake of protein, methionine, choline, vitamin E and selenium being particularly relevant (Gutta *et al.* 1983). Data summarized in Table 4 indicates that the administrtion of KMH has markedly beneficial effects upon the serum lipid levels. Indeed, the data suggests that the TG levels may be reduced to lower than normal with a regular administration of KMH. As shown in Table 4, the KMH complex treated group is quite distinct from the negative control group. Triglyceride levels in the normal untreated rats were 39.87±8.61 mg dL^{-1} while the levels found in the rats administered with alcohol were markedly elevated at 75.71±51.21 mg dL^{-1}. In contrast, the triglyceride level in the test group (No. 4) was exhibited as significantly reduced with lower than normal levels of 26.16±3.42 mg dL^{-1} (p<0.01). Also Table 4 demonstrates that the KMH complex had significantly lesser effects upon the serum cholesterol levels. Especially, the test group (No. 4) exhibited strongly reduced ALT levels of 86.12±10.82 U L^{-1} (p<0.5) compared with the negative control group. Many reports (Rahimtoola 1985; Castelli *et al.* 1990) have indicated that an elevated blood cholesterol level is one of the main causes of vas-

cular disease in the heart and circulatory system, a number of drugs have been developed to lower the 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 the KMH complex may repre-sent an alternative therapeutic agent to assist in the prevention and treatment of hyperlipidemia.

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