Pine Needle Oil and Korean Medicinal Herb Complex Protect Hyperlipidemia and Liver Cell Damage Induced by Alcohol

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Abstract - The effect of treatment with pine needle oil complex (complex of pine needle oil and Korean medicinal herbs) upon rat hepatocytes exposed to alcohol was investigated. We compared body weight gain and ratios of liver and kidney to body weight and the serum biochemistry of rats administered both alcohol and Pine needle oil complex to control rats treated with alcohol alone. Pine needle oil complex treatment resulted in a significant reduction in the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and triglycerides (TG) compared to the control rats. These data suggest that Pine needle oil complex represents an excellent candidate for protection of rat hepatocytes from alcohol-mediated damage.

Key words : Pine needle oil complex, rat liver cell, alcohol

INTRODUCTION

Pines of all kinds have been used medicinally in many countries from the earliest times (Bown, 1995). The young branches of black spruce (*Pinus nigra*) are the source material for "essence of spruce", and the essential oil distilled from the leaves of the dwarf pine (*P. umilio*) is the source material for "oil of pine" (Grieve 1979). The topical anti-eczematic and rubefacient over-the-counter drug Pine Tar USP (syn. *Pix liquida*) is obtained from the distillation of the wood of longleaf pine (*P. palustris* Mill.) or other species of pine (Budavari 1996; Taber 1962). The essential oil distilled from the fresh leaves of *P. pinea* and/or *P. sylvestris* is used in northern India as a component of a compound preparation (oil of pine, magnesii carbonas levis, distilled water) for inhalation to treat chronic laryngitis (NadKarni 1976). The steam-distilled essential oil from the balsam of *P. densiflora* is official in the oriental pharmacopeias. *Song-jie* (its Chinese name) was first mentioned in Chinese medical literature ca. 500 C.E. as an antiarthritic and analgesic drug. Currently, it is used in the traditional medicine of Korea, China, and Japan, administered as a topical paint to treat rheumatism (But *et al.* 1997).

Pine needle oil is distilled from the finest pines using only the needles. Pine needle oil differs from pine oil, which may utilize all or any part of the tree when distilled. Pine needle oil promotes healthy immune, nervous, musculoskeletal and urinary systems. Pine needle oil was used by the ancient Romans and Greeks to treat respiratory problems and muscular aches. Distilled in Austria from the finest pines, pine needle can be diffused to help strengthen the respiratory tract and maintain sinus passages. When massaged into the skin, pine needle supports healthy circulation and soothes the dis-

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comfort of sore joints and muscles. A true disinfectant, a strong germ killer, excellent for viral infections and for muscular aches, rheumatism and arthritis. In the bath pine needle improves the circulation and relieves muscular pains.

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 (Sherlock 1993). Excessive intake of alcohol may severely damage such organs as liver and heart, resulting in dysfunction including derangement of blood pressure and triglyceride levels (Nakanishi *et al.* 2001). There have been numerous attempts to develop clinically useful compounds to ameliorate or cure alcohol-related disorders (Kono *et al.* 2001a, b). 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 pine needle oil complex (complex of Pine needle oil and Korean Medicinal Herbs) protects rat hepatocytes from alcohol-induced damage, thereby resulting in protection from hangovers, cardiovascular symptoms and alcohol-induced hepatitis (Lieber 2001). 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, 1990). Pine needle oil was administered to rats treated with alcohol. The protective effect of pine needle oil complex was examined by measuring the blood levels of the enzymes AST (asparate aminotransferase) and ALT (alanine aminotransferase) before and after extract of pine needle oil complex 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 pine needle oil complex is hepatoprotective and ameliorates alcohol-mediated damage and alcohol-induced liver symptoms whilst concomitantly improving lipid metabolism.

Table 1. Composition of Groups

Group	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 group1-T1)	7	Alcohol+pine needle oil (pine needle oil +Korean medicinal herbs*)

HCS* : Hangover cure solution (Condition: Cheil-je-dang Co., Ltd., Seoul) commercially available in Korea. Korean medicinal herb* : *Cornus officinalis* SIEB. *et.* ZUCC.+*Panax ginseng* CA Meyer+*Artemisia capillaris* Thunb.+*Astragalus membranaceus* BUNGE

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 constant temperature (22±2°C) and humidity (55±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 four groups (n = 7) as follows: No. 1 normal control rats administered water, No. 2 rats administered ethanol/water, No. 3 rats administered ethanol/water and a commercially available hangover cure solution (HCS, Condition: Cheil-je-dang Co., Ltd., Seoul), No. 4 rats administered ethanol/Pine needle oil complex (complex of Pine needle oil and Korean Medicinal Herbs). 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 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 pine needle oil complex

Pine needle oil is the steam-distilled essential oil extracted from the fresh needles, branch tips or from the combined fresh branches with needles and branch tips of *P. densiflora* or other essential oil-containing species of Pinus. Production of Korean medicinal herb

(KMH) complex was based on a recipe derived from Korean traditional medicine books and the recommendations of Korean traditional medical doctors. KMH are 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 amounts 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. Total 100 g of 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 6000 g for 15 min., aqueous extracts from sample were filtered through 3 mm filter papers (Whatman, England), and the final volume was adjusted to around 400 ml order to prepare an appropriate volume for administration (about 6.7 ml kg^{-1} body weight day^{-1}). Pine needle oil were administered at a dose of about 30 μl kg^{-1} day^{-1} for 30 consecutive days.

3. Dissection and biochemical analysis

After fasting for 16 hours at 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). A part of blood was transferred to EDTA -tube and the rest blood was allowed to clot for half an hour before separation of the serum by centrifugation at 3,000 g for 15 min. AST and ALT activity was determined using the AST kit (Boehringer Mannheim, Germany) and ALT kit (Boehringer Mannheim). 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).

4. Statistical analysis

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

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)
No. 1 None-alcohol	41.20±2.48*[1)]	2.67±0.062	0.629±0.027*
No. 2 Alcohol	35.50±5.02	2.91±0.089	0.652±0.028
No. 3 Alcohol+HCS	38.20±1.72*	2.61±0.116*	0.618±0.032
No. 4 T1	40.17±10.29*	2.76±0.052**	0.641±0.030

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

RESULTS AND DISCUSSION

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

It was reported that body weight gain decreased in alcohol-treated rats (Piola and Lieber 1975) and body weight decreased by 50% alcohol ingestion instead of sugar in total energy source of man (Piola and Lieber 1972), suggesting that oxygen consumption and metabolic rate were increased, ATP production was decreased in microsome by excessive alcohol ingestion (Gruchow *et al.* 1985). In this study, similar results were represented as shown in Table 2. The effect of daily intake of ethanol plus pine needle oil complex was clearly powerful, as seen from the weight gain during 4 weeks of treatment (Table 2). In contrast with the great weight gain of the pine needle oil complex, the negative control group (No. 2) exhibited the significantly low body weight. The same features were found in the liver weight change as reported by Levy *et al.* (1976) suggesting that the liver weight increase is due to accumulated lipids in the liver of alcohol-treated rats. The negative control group (No. 2) administered ethanol alone exhibited the significantly highest ratio. But the groups administered pine needle oil complex (No.4) exhibited a similar level to normal control group, and also significantly decreased ratio (%) of liver weight to body weight compared to other groups ($p<0.01$). The ratio (%) of kidney weight to body weight in No. 4 group showed slightly decrease compare to negative control.

Table 3. Enzyme activity of AST and ALT in plasma

Groups	AST (U L^{-1})	ALT (U L^{-1})
No. 1 None-alcohol	84.43±47.88*[1)]	44.00±9.04*
No. 2 Alcohol	254.57±463.20	215.43±428.93
No. 3 Alcohol+HCS	70.29±12.60	37.29±9.03*
No. 4 T1	60.43±5.37*	32.71±4.68**

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

Table 4. Concentration of plasmid lipid in rat

Groups	TG (mg dL^{-1})	Cholesterol (mg dL^{-1})
No. 1 None-alcohol	39.57±8.62*[1)]	95.71±6.86*
No. 2 Alcohol	73.71±61.20	113.80±38.19
No. 3 Alcohol+HCS	30.14±6.73*	91.57±6.30**
No. 4 T1	27.86±5.17**	88.86±9.95*

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

2. Activities of AST and ALT

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 (Thompson 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 serum AST levels to 254.57±463.20 U L^{-1}. Rats treated with both alcohol and the commercially available hangover release medicine exhibited lower AST levels of 70.29±12.60 U L^{-1} with the test group (T1) being comparable at 60.43±5.37. Normal untreated control rats exhibited ALT levels of 44.00±9.04 U L^{-1}, while administration of ethanol resulted in a significant increase in the serum ALT level to 215.43±428.93 U L^{-1}. Interestingly, the test group (T1) exhibited significantly reduced levels of ALT with lower normal ALT levels of 32.71±4.68 U L^{-1} ($p<0.05$). Furthermore, since a component of Pine needle oil is presumed to be hepatoprotective, it is of significant interest that reports indicate that *Schizandrae Fructus* exhibits a protective effect on the liver (Thompson 1970; Liu 1989).

3. Triglycerides and total cholesterol levels

Many reports indicate that alcohol intake significantly increases both serum and hepatic triglyceride (TG) levels resulting in hypertriglyceridemia and fatty liver (Baraona 1970; Bode 1974; Glueck *et al.* 1980; Karsentry *et al.* 1985). 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 (Cutta *et al.* 1983). Data summarized in Table 4 indicates that the administration of pine needle oil complex have markedly beneficial effects upon lipid levels. Indeed, the data suggests that TG levels may be reduced to lower normal with regular administration of pine needle oil complex. As shown in Table 4, the pine needle oil complex-treated groups are quite distinct from the other negative control group. Triglyceride levels in normal untreated rats were 39.57 ±8.62 mg dl^{-1} while the levels found in rats administered alcohol were markedly elevated at 73.71±61.20 mg dl^{-1}. In contrast, the triglyceride levels in T1 group was exhibited significantly reduced with lower normal level of 27.86±5.17 mg dl^{-1} ($p<0.05$). Also, table 4 demonstrates that pine needle oil complex had significantly lesser effects upon cholesterol levels. Previously it was reported indicating that an elevated blood cholesterol level is one of the main causes of vascular disease in the heart and circulatory system (Rahimtoola 1985; Castelli *et al.* 1990). 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 pine needle oil complex may represent an alternative therapeutic agent to assist in the prevention and treatment of hyperlipidemia.

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