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Ameliorative Effect of Pine Needle Oil on Liver Protection and Lipid Metabolism of Alcohol Fed Rats

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Abstract The effect of treatment with pine needle oil upon rat hepatocytes exposed to alcohol was investigated. The body weight gain, ratio of liver and kidney to body weight, and serum biochemistry of rats administered both alcohol and pine needle oil were compared to control rats treated with alcohol alone. Normal untreated control rats, negative control rats with ethanol treatment, positive control rats with both alcohol and the commercially available hangover cure solution (HCS) treatment and the test group with both alcohol and pine needle oil treatment exhibited aspartate aminotransferase (AST) levels of 84.43±47.88 U/L, 254.57±463.20 U/L, 70.29±12.60 U/L and 67.00±5.06 U/L, respectively, and cholesterol levels of 95.71 ±6.86 mg/dl, 113.80±38.19 mg/dl, 91.57±6.30 mg/dl and 82.29±4.98 mg/dl, respectively. Alanine aminotransferase (ALT) levels were 44.00±9.04 U/L in normal untreated control rats, increased to 215.43±428.93 U/L with the administration of ethanol, but interestingly were significantly reduced to 37.83±6.57 U/L in the test group ($p<0.05$). Triglyceride (TG) levels were 39.57±8.62 mg/dl in normal untreated rats, increased to 73.71±61.20 mg/dl in rats administered alcohol, but were reduced to 26.14±4.82 mg/dl in the test group ($p<0.01$). The pine needle oil treatment significantly reduced the levels of AST, ALT and TG compared to the control rats. These results indicate that pine needle oil can positively mediate the effects of alcohol on hepatocytes and general liver functions.

Keywords: Pine needle oil, alcohol, AST, ALT, Triglyceride, cholesterol

Introduction

Pines of all kinds have been used medicinally in many countries from the earliest times (1, 2). 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. pumilio*) is the source material for "oil of pine" (3). The topical antiñeczematic 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 (4, 5). 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 (6). The steam-distilled essential oil from the balsam of *P. densiflora* is officially recognized 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. Today, it is used as a traditional medicine in Korea, China, and Japan, and is administered as a topical paint to treat rheumatism (7).

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 (8, 9). Excessive intake of alcohol may severely damage such organs as the liver and heart, resulting in dysfunction including derangement of blood pressure and triglyceride (TG) levels (10). There have been numerous attempts to develop clinically useful chemical compounds to ameliorate or cure alcohol-related disorders (11, 12). However, it is well documented that these chemical compounds may exhibit severe cytotoxicity, reproductive toxicity and other important side effects. Therefore, in order to find an alternative to traditional cures, studies have increasingly focused on the development of therapeutic agents based on natural products and medicinal herbs.

In this study, pine needle oil was administered to rats treated with alcohol. The protective effect of pine needle oil was examined by measuring the blood levels of the enzymes AST (aspartate aminotransferase) and ALT (alanine aminotransferase) before and after pine needle oil administration in alcohol-treated rats. These two enzymes are indicators of hepatic dysfunction. Serum levels of TG and total cholesterol, which are important causes of hyperlipidemia and arteriosclerosis, were also measured. The major finding of this paper is that pine needle oil is hepatoprotective and ameliorates alcohol-mediated damage and alcohol-induced liver symptoms while concomitantly improving lipid metabolism

Materials and Methods

Animal models Young adult male Sprague Dawley rats, initial weight 100±10 g, were obtained from Daehan Biolink Co., Ltd. (Seoul, Korea). Rats were fed a diet (Samyang Assorted Diet produced by Samyang Co., Ltd., Korea) and were allowed free access to drinking water (distilled). 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 the following four groups (n=7). No.1: normal control rats administered with only water. No. 2: negative control rats administered

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with both ethanol and water. No. 3: positive control rats administered with both ethanol and HCS (hangover cure solution which is commercially available, Condition: Cheil-je-dang Co., Ltd., Seoul) (13). No. 4: experimental group rats administered with both ethanol and pine needle oil. Rats in all four groups were treated for 30 days. Rats administered with ethanol consumed a 40% ethanol solution to give a total intake of 5 g/kg/day. The body weight and general condition of the animals were monitored every two days.

Preparation and treatment of pine needle oil and Korean medicinal herb 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*. Soxhlet apparatus was used for oil extraction and chloroform was used as a solvent. The filtrated pine needle oil was administered orally at a dose of 0.023 g/kg/day for 30 consecutive days using a syringe.

Dissection and biochemical analysis After fasting for 16 hours on the last day of housing, rats were dissected under an anesthetic state and 3-4ml blood was collected using an injector. The liver and kidney were removed and rinsed with a cold 0.1 M phosphate buffer (pH 7.3). The blood collected was allowed to clot for half an hour before separation of the serum by centrifugation at 3,000 g for 15 min. Serum AST and ALT activities were determined using the AST (Boehringer Mannheim, Germany) and the ALT kits (Boehringer Mannheim), respectively. Serum TG 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).

Statistical analysis All results are shown as mean± standard deviation. Statistical evaluation of data was performed at a level of significance of $p<0.05$ by Student's t-test to make comparisons between groups.

Results and Discussion

Weight gain and ratio of liver weight to body weight The effect of daily intake of ethanol plus pine needle oil (No.4) was clearly powerful, as seen from the weight gain during 30 days of treatment (42.50±14.92, Table 1), compared to that in the alcohol only group which showed the lowest rate of weight gain (35.50±5.02). The ratio (%) of liver weight to body weight in No. 2 group, administered ethanol alone, exhibited the significantly highest ratio (2.91±0.089). However, the group administered pine needle oil (No.4) exhibited a low level (2.62±0.112) which was similar to that of the normal control group (2.67± 0.062). The ratio (%) of kidney weight to body weight in No. 4 group was decreased compared to negative control. Pirola and Lieber (14) reported that body weight gain decreased in alcohol-treated rats and that body weight decreased by 50% alcohol ingestion instead of sugar in the total energy source of humans (15). These results suggested that oxygen consumption and metabolic rate were increased. ATP production was decreased in microsomes by excessive alcohol ingestion (16) and similar results were found in this study (Table 1). The features found for liver weight change were the same as those reported by Levy *et al.* (17), suggesting that the liver weight increase is due to accumulated lipids in the liver of alcohol-treated rats.

Table 1. Total body weight gain and 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 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	42.50±14.92**	2.62±0.112*	0.645±0.036

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

Activities of AST and ALT AST levels were 84.43± 47.88 U/L in normal untreated control rats (Table 2), significantly increased to 254.57±463.20 U/L in rats treated with ethanol, and were reduced to 70.29±12.60 U/ L in rats treated with both alcohol and HCS and to 67.00±5.06 in the test group (No.4). ALT levels were 44.00±9.04 U/ L in normal untreated control rats, significantly increased to 215.43±428.93 U/L with the administration of ethanol, but were significantly reduced to 37.83±6.57 U/L in the test group (No.4) ($p<0.05$).

AST and ALT levels both increased with increased alcohol intake. These enzymes are well-documented indicators of hepatic dysfunction, with increased AST and ALT levels reflecting impaired liver function (18). However, rats administered pine needle oil exhibited diminished levels of these enzymes, with effects upon ALT levels being most marked, although the AST level for pine needle oil appeared comparable to the disease control.

Table 2. Enzyme activity of AST and ALT in plasma

Groups	AST(U/L)	ALT(U/L)
	Mean±S.D .	Mean±S.D.
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	67.00±5.06*	37.83±6.57*

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

Table 3. Concentration of plasmid lipid in rats

Groups	TG(mg/dL)	Cholesterol(mg/dL)
	Mean±S.D.	Mean±S.D.
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	26.14±4.82[***]	82.29±4.98[****]

[1)]Each value represents the mean±SD of 7 rats. Means with different superscript asterisks within a column are significantly different from each other at P<0.05(*), P<0.01(**), P<0.005 (***) and P< 0.001(****), as determined by Student's t-test.

Furthermore, since a component of pine needle oil is presumed to be hepatoprotective, it is of significant interest that reports indicate that essential oil distilled from the fresh leaves of *P. pinea* and/or *P. sylvestris* exhibits a protective effect on the liver (1, 6, 7).

In this study, No.4 group exhibited significantly reduced AST and ALT levels compared with No.2 group. These data suggest the possibility of pine needle oil being an excellent candidate to ameliorate the effect of hepatocytes and anti-hyperlipidemia from alcohol-mediated damage in the rat.

Serum triglycerides and total cholesterol levels The data suggest that TG levels may be reduced to below normal levels with regular administration of pine needle oil. As shown in Table 3, the pine needle oil-treated group was quite distinct from the negative control group. TG levels in normal untreated rats were 39.57±8.62 mg/dl, were markedly elevated to 73.71±61.20 mg/dl in rats administered alcohol, but were significantly reduced to 26.14±4.82 mg/dl in the test group ($p<0.01$). In addition, Table 3 demonstrates that pine needle oil had significantly lesser effects upon serum cholesterol levels. Many reports indicate that alcohol intake significantly increases both serum and hepatic TG levels, thereby resulting in hypertriglyceridemia and fatty liver (19). The development of a 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 (20). Data summarized in Table 3 indicate that the administration of pine needle oil has markedly beneficial effects upon serum lipid levels. Based on reports (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, the results of this report suggest that pine needle oil may represent an alternative therapeutic agent to assist in the prevention and treatment of hyperlipidemia.

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