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Biological Effects of Korean Puerariae Radix Catechins on the Liver Function in Rats Administered with Ethanol

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Abstract

Crude catechin extracts were prepared using ethyl acetate from Korean Puerariae Radix(PR) and their biological effects on the alcoholic liver damage were investigated in male Sprague-Dawley rats(6 weeks old). Ethanol(5g ethanol/kg body weight/day) administration was performed for 8 weeks and after ethanol consuming rats were treated with one or two% catechin extracts of diet for 8 weeks. At the end of experimental period, lipid hydroperoxides in liver were analyzed using a chemiluminescence-high performance liquid chromatography(CL-HPLC) method. Compared with control animals, ethanol consumed rats showed lighter body weights, lower ratios of liver/body weight, higher activities of GOT and GPT, and increased lipid hydroperoxide amount in liver. With one or two% catechin extracts treatment, GOT and GPT activites returned to normal ranges. Lipid hydroperoxide contents in liver of one or two% PR treated rats lowered to 20% or 25% respectively, compared with the levels of those ethanol consumed animals without catechin extracts treatment. Therefore, we concluded that one or two% PR crude catechins treatment could be effective for alcoholic liver damage caused by lipid peroxidation.

Key words: Puerariae Radix crude catechin extracts, lipid peroxidation, liver function, alcohol consumption

INTRODUCTION

One of the most serious consequences of chronic alcohol abuse is alcoholic liver damages, leading causes of death in Asian countries. Recent decades, many studies have been performed to investigate the mechanisms concerning alcoholic liver damages. It has been possibly suggested that chronic ethanol consumption causes an excess oxidative stress with increased free radical(1,2), therefore, enzyme-mediated antioxidant defence systems(glutathione peroxidase, superoxide dismutase and catalase) and non-enzyme related antioxidants(tocopherol, beta-carotene, zinc, selenium and so on) are depleted(3-6). The formation of lipid peroxides are stimulated by exposing to alcohol in liver(7). However, several contradictory results have been reported. That is chronic ethanol ingestion did not result in any changes of liver lipid peroxidation(4) and in any alteration in glutathione peroxidase activity of the liver(8). Several factors such as analytical methods for the lipid peroxides and the enzyme activities, the amount of the alcohol consumption, and the degree of liver damages may cause the different results above.

Recently, antioxidant treatment attracts an attention of researchers due to their preventive effects on many diseases such as atherosclerosis, viral infection, immune deficiency, cancers and even alcoholic liver damages. Vitamin E supplement improved the enzyme-mediated antioxidant system resulting in the recovery from liver disease(9). Other exogeneous antioxidants have shown to be beneficial to chronic alcohol consumption related liver damages(10,11). Catechins, polyphenolic compounds(12) are one of the most strong antioxidants(13) in nature and very interesting substances having many additional functions, an anti-atherosclerosis(14), an antiviral(15) and an anticarcinogenesis(16). Not only green tea but also many edible plants such as cereal grains, legume seeds, forage and browses(17) contain catechins. Catechins are also important components in Puerariae Radix extracts(PR), which has been used in the chinese medicine for crapulence and asthma treatment(18).

In the present study, we extracted catechins from Puerariae Radix(PR), and elucidated that catechin extracts contained a couple of unknown catechin derivatives. And the effects of PR catechin extracts on alcoholic liver damages were investigated in rats. In order to estimate the liver function, amounts of lipid hydroperoxides in the liver were measured using recently developed, very sensitive, and rapid chemiluminescence-high performance liquid chromatography(CL-HPLC) method(19,20).

MATERIALS AND METHODS

Preparation of catechin extracts from PR

Total catechins were extracted from Korean PR using ethyl acetate(21), and the yield of crude catechins was 7%. The contents of catechins in catechin extracts were measured by a colorimetric method using a ferrous tartrate as previously described(22), and the purity of crude catechins was 70%. HPLC analysis was performed to determine catechin components of PR catechin extracts as described(23) by using a Waters 600E multi-solvent delivery system(Waters Associates, Milford, MA).

Animals and diets

Six week old male Sprague-Dawley rats were obtained from Charles River Breeding Laboratory(Japan). Animals were maintained in individual screen-bottom wire mesh cages in a temperature(23±1°C) and humidity (50±5%) controlled room with a 12-h light: dark cycle (08 : 00 to 20 : 00). A standard chow diet(Jeil Feed Co. Seoul, Korea) and water were provided *ad libitum*. Food consumption was measured everyday and body weight was measured once a week.

Animals were randomized to four groups, each group with eight animals. Rats in three groups were orally by tube administered with the solution of the 25% ethanol contents(5g ethanol/kg B.W.) everyday for 8 weeks. Then, two ethanol-treated groups(ETL) of rats received 1%(ETL+1%PRC) and 2%(ETL+1%PRC) of PR crude catechin extract after the solution of 25% ethanol contents(5g ethanol/kg B.W.) for 8 weeks. For the control groups(CON), water was given in place of ethanol and PR catechin extract.

Blood and tissue collection

At the end of 8 week experimental period, overnight fasting rats were anesthetized with diethyl ether. Blood was withdrawn from abdominal aorta, collected into sodium heparin coated glass tubes, and centrifuged at 1800g for 20mins at 4°C. Heart, liver, kidney, spleen, lung and testis were removed, washed with saline and weighed. Plasma was stored at 4°C and all tissues at -20°C for further analysis.

Biochemical assays of plasma

Plasma triglyceride(TG) and total cholesterol(TCHO) concentrations were determined on Auto analyzer(JCA-VX1000 clinalyzer, Jeol Co) using enzymatic reagents. The activities of glutamic oxaloacetic transaminase(GOT) and glutamic pyruvic transaminase(GPT) were measured by UV rate method. Biuret method was used to determine the concentration of albumin(ALB) and total protein(TP). Blood urea nitrogen(BUN) was assayed by Urease-UV method and blood glucose(GLU) by the glucose oxidase method.

Preparation of lipid extracts from liver

Liver was perfused in situ with ice-cold 0.15M saline solution and total lipid was extracted from 200mg liver by the method of Folch et al.(24). Butylated hydroxytoluene(BHT, 0.002%) was added in liver homogenizing buffer for prevention of lipid oxidation and 5ml chloroform/methanol(2 : 1, v/v) was used for lipid extraction. After lipid extraction, the dry total liver lipid was suspended with 200μl of chloroform/methanol(2 : 1, v/v) and a 20μl portion was subjected to hydroperoxide assay by CL-HPLC.

Determination of hydroperoxide in the liver lipids using CL-HPLC

Phosphatidylcholine hydroperoxide(PCOOH) in the total lipid fraction of liver was determined by the CL-HPLC method(19,20) using normal-phase HPLC with a JASCO Finepak SIL column(5μm, 250×4.6cm) and a hydroperoxide specific chemiluminescence detector (JASCO 825-CL detector). The column mobile phase was chloroform : methanol(1 : 9, v/v) and the flow rate was controlled to 1.0ml/min using a FASCP 880-PV pump. The chemiluminescence was generated by dissolving 10μg/ml of cytochrome C and 1μg/ml of luminol in 50mM alkaline borate buffer. The concentration of hydroperoxide in liver lipid extracts was calculated using standard curve and expressed as nmol of hydroperoxide. A standard curve was made using authentic PCOOH isolated from a silica column chromatography after photo-oxidation of egg yolk phosphatidyl choline (PC).

Statistics

The effects of catechin extracts administration on each biochemical parameter of chronic etanol administered rats were evaluated using Student's t-test and one-way analysis of variance with the test for the im-

proved Scheffe's least significant difference(25). Differences were considered to be statistically significant at $p<0.05$.

RESULTS

Composition of the catechin extracts

The content of catechins in PR catechin extracts was 70%. The percentage composition of the catechin extracts analyzed by HPLC is shown in Table 1. Major catechin in PR was (-)-epicatechin(EC) appeared to 60% of total catechins. 25% of (-)-epigallocatechin gallate(EGCg), (-)-epigallocatechin(EGC) and 2% of (-)-epicatechin gallate(ECG) were minor components in PR catechin extracts. A couple of unknown catechins were found in a HPLC chromatogram of catechin extracts(Fig. 1).

Food consumption, body weight and tissue weight

During the experimental period for 8 weeks, ethanol treated rats tended to consume less amount of food compared with control. However, food consumption was not significantly different between groups of rats. Body and tissue weights of animals at the end of experimental period are shown in Table 2. The average body weight gains in ETL of PR catechin extracts consumed animals were less than those of control. The ratio of liver weight to body weight was 22% lower in ethanol treated rats than that of control rats. However, groups of rats with 1%(ETL+1%PRC) and 2%(ETL+2%PRC) showed heavier liver/body weight ratio. Other tissues were not significantly different in their weights between groups of rats.

Table 1. Percentage composition of catechins in Korean Puerariae Radix catechin extracts[1)]

Catechins	Percentage(%)
(-)-Epigallocatechin gallate(EGCg)	25
(-)-Epigallocatechin(EGC)	0
(-)-Epicatechin gallate(ECg)	2
(-)-Epicatechin(EC)	60
Other catechins	13

[1)]Catechin components were analyzed by HPLC using catechin standards(Funagoshi, Co. Japan) and a Waters 600E multi-solvent delivery system(Waters Associates, Milford, MA.)

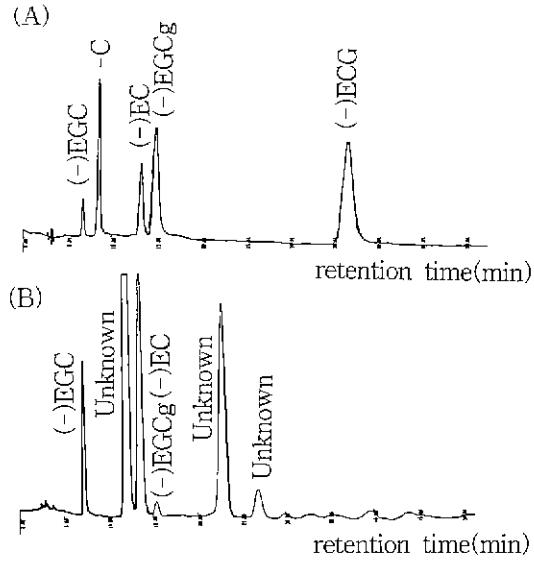

Fig. 1. Isolation of catechin and catechin derivatives by HPLC using catechin standards(Funagoshi, Co. Japan) and a Waters 600E multi-solvent delivery system(Waters Associates, Milford, MA.).
(A) Catechin standards(Funagoshi, Co. Japan)
(B) Catechin extracts of Korean Puerariae Radix

Plasma biochemical parameters

The effects of the catechin extracts on the plasma biochemical parameters are shown in Table 3. Ethanol consumption resulted in a decrease of total plasma cho-

Table 2. Body and tissue weights of experimental animals after ethanol and catechin extract consumption[1)]

	CON	ETL	ETL+1%PRC	ETL+2%PRC
Body weight(g)	257.2±22.3	225.0±23.7[2)]	220.0±29.2[3)]	234.0±6.9[3)]
		(Tissue weight g/100g Body weight)		
Heart	37.0±0.05	37.0±0.04	39.0±0.07	40.0±0.04
Liver	3.6±0.4	2.8±0.3[2)]	3.9±0.8[4)]	3.8±0.3[4)]
Kidney	2.0±0.04	1.6±0.3	1.8±0.3	1.8±0.3
Spleen	0 8±0.1	0.5±0.1	0.5±0.3	0.4±0.1
Lung	0 9±0.1	1.0±0.6	0.8±0.1	0.8±0.04
Testis	1.0±2.5	1.3±0.1	1.2±0.1	1.2±0.1

[1)]Values are mean±SEM for 8 rats/group
Significantly different from CON: [2)]$p<0.05$, [3)]$p<0.01$
Significantly different from ETL: [4)]$p<0.01$

Table 3. Effects of Puerariae Radix catechins on the plasma biochemical parameters in experimental rats[1)]

	CON	ETL	ETL+1%PRC	ETL+2%PRC
TG(mmol/L)	2.2±0.4	2.2±0.3	1.4±0.3[2,3)]	1.2±0.2[2,3)]
TCHOL(mmol/L)	2.1±0.2	1.6±0.2[4)]	0.7±0.1[2,3)]	0.6±0.1[2,3)]
GPT(IU/L)	43.1±6.7	58.0±14.0[4)]	31.7±7.3[2,3)]	36.1±5.0[2,3)]
GOT(IU/L)	149.2±22.4	178±31.4[4)]	144.6±22.7[3)]	144.8±15.5[3)]
Albumin(mmol/L)	0.7±0.03	0.7±0.05	0.5±0.03[2,3)]	0.5±0.05[2,3)]
TPROT(mmol/L)	0 5±0.03	0.5±0.03	0 4±0.02[2,3)]	0.4±0.03[2,3)]
BUN(mMOL/L)	20.3±2.2	18.3±0.1[4)]	18.2±2.8[2)]	14.0±2.4[2,3,5)]
Glucose(mmol/L)	11.7±1.4	11.2±1.6	12.1±1.8	11.8±1.0

[1)]Values are means±SEM for 8rats/group
[4)]Significantly different from CON: [2)]$p<0.01$, [4)]$p<0.05$
Significantly different from ETL. [3)]$p<0.01$
Significantly different from ETL+1%PRC: [5)]$p<0.01$

lesterol($p<0.05$) but not triglyceride contents. 1%(ETL +1%PRC) and 2%(ETL+2%PRC) induced a further decrease in total cholesterol($p<0.01$) compared by ethanol consumed animal, and it was associated with the decrease of triglyceride concentration($p<0.01$). The activities of GOT and GPT were significantly higher in ethanol consumed rats compared to those of control($p<0.01$). The treatment with PR catechin extracts showed normal range of the activities of GOT and GPT. The concentration of albumin and total proteins in blood were not changed with ethanol consumption but were lower in animal treated with PR catechin extracts than control. BUN concentrations were lower in ethanol consumed rat than control, and they were lower in PRC treated rat than control and ethanol. No differences in all animals were observed in blood glucose.

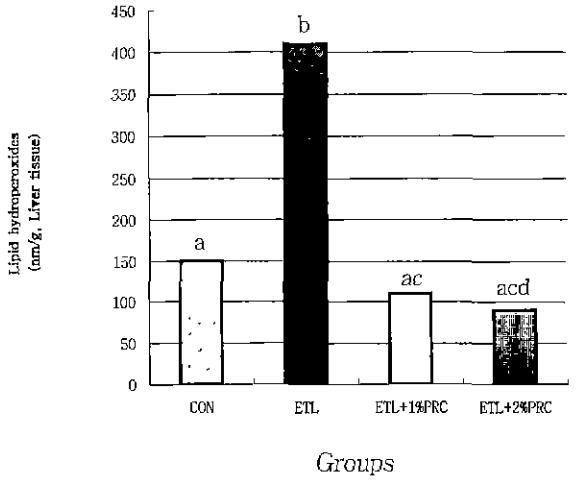

Fig. 2. Lipid peroxide contents in the liver of experimental animals.
CL-HPLC were performed to determine lipid peroxides.
CON: Control
ETL: Ethanol treated rats
ETL+1%PRC: 1% Puerariae Radix catechin extracts treatment to the ETL.
ETL+2%PRC: 2% Puerariae Radix catechin extracts treatment to the ETL.
The different letters surmounted on the bars are significantly different at the $p<0.05$ level.

Lipids peroxidation in rat liver

The amounts of lipid hydroperoxide in the liver are shown in Fig. 2. The contents of lipid hydroperoxides in the liver of ethanol consumed rats were higher than those of control. The groups of 1%(ETL+1%PRC) and 2%(ETL+2%PRC) resulted in 3.5 or 5 times, respectively reduction of lipid hydroperoxide levels in ethanol consumed rats.

DISCUSSION

Present study conformed the previous reports(1,2,5) that chronic ethanol consumption induced the formation of active oxygen free radicals accumulating lipid hydroperoxides in the liver. In addition, the amounts of lipid hydroperoxides in the liver were lower in animals with PR catechin extract administration than those in ethanol consumed animals without any treatment. These results suggest that the administration of PR catechin extracts into the ethanol consumed rats enhance the liver function. In addition, catechins are well known component in green tea and a strong antioxidant to inhibit the lipid peroxidation(26). Catechin extracts may be localized near membrane to scavenge aqueous oxygen radicals and consequently they prevent the decrease of lipophilic antioxidants such as tocopherol in membrane(27). Catechins extracted from Korean Puerariae Radix also show strong preventative effects of lipid per-

oxidation in this study.

The increased GOT and GPT levels indicated the impairment of liver function under chronic alcohol ingestion. The decreases in body weights and the ratios of liver weight to body weight in alcohol consumed rats may represent the metabolic disorders due to the liver damages, or to the less food intake during the period of chronic ethanol consumption(13).

The other important observation in the present study is that the administration of PR catechin extracts in ethanol consumed rats results in a increase in the ratio of liver/body weight and a decrease of GOT and GPT activities. Our results indicate that one of the major causes of alcoholic liver damage is the lipid peroxidation in the membrane, since lipid hydroperoxides were measured in phosphatidyl choline, a major structural component on tissue membrane. The decreases of liver transaminase activities and the increases in body and liver weight to the normal in PR catechin treated rats may be resulted from a decrease of lipid hydroperoxides.

Several studies(14-16) reported the biological activity of tea catechin extracts(TCE) and their catechin components. The percentage of the main catechin components in crude catechin mixture prepared Indian green tea were EGCg(58%), EGC(17%), ECg(15%), EC(10%) (25). While, PRC crude catechin consists of two main catechin components which are regarded as catechin derivatives: (-)-epicatechin and (-)-epigallocatechin gallate. EC is the predominating catechin, accounting for more than the half of the total catechin content. Namiki reported that antioxidant activities is ordered EGCg>EGC>ECg>EC from molar concentration in the food system(28). Accordingly, the concentration of EC 50ppm has similar antioxidant activities with the 20ppm EGCg. EGCg is a major catechin components in tea and has the most strong antioxidant activities, while EC is quantitatively a major in PR. A HPLC chromatogram shows the existence of unknown and unique catechins in PR. Although the antioxidant activity of EC was more lower than that of EGCg. It may play an important and beneficial role with the synergistic effect of EGCg(25%) and these unknown catechin derivatives (12%) which improve the liver function disorder.

Other important role of tea catechins has been considered to be as a hypocholesterolemic and hypolipidemic factor(14). The addition of tea catechins to the lard-cholesterol diet in CCl_4 induced liver injured rats decreased plasma total cholesterol and triglycerides(29). The results of an experiment carried out by Japanese scientists showed that the 0.5~1% EGCg supplemented diet decreased significantly the level of total cholesterol, free cholesterol, LDL cholesterol and triglyceride in plasma(30). It has increased the level of HDL cholesterol simultaneously. In addition, the excretion of lipids and cholesterol in feces are increased. Although PR catechin extracts contained different catechin components from tea catechin extracts, it showed very potent hypocholesterolemic and hypolipidemic effects in this study. Simultaneous CL-HPLC used in this study has been reported to be very sensitive and selective.

In conclusion, the treatment of PR catechin extracts to rats consumed ethanol may be beneficial to the liver loaded with active oxygen free radicals and excess lipid hydroperoxides. The biological function of each catechin component in PR needs further investigation.

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