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Effect of Medicinal Plant Extracts on Alcohol Metabolism in Rat Liver

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ABSTRACT : The experiment was conducted to evaluate the effects of medicinal plants on ethanol-metabolism. Sprague Dawley rats divided into 6 groups (n=8), fed with 10% ethanol and diets supplemented with each 1% of four plant extracts, α-tocopherol (as positive control) and fiber (as negative control) for 4 weeks. Group supplemented with plant extract of *Ulmus davidiana* showed the most high value (322 nM NADH/min/㎎ protein) in alcohol dehydrogenase (ADH) activity among the experimented groups (144~312 nM NADH/min/㎎ protein) at $p<0.05$. Groups fed with *Lagerstroemia indica* and *Zelkova serrata* extract-supplemented diets indicated high activity in aldehyde dehydrogenase (ALDH, 16.7 & 12.3 M NADH/min/㎎ protein), which were comparatively lower than 20.1 M NADH/min/㎎ protein of α-tocopherol fed group. All of the groups fed with plant extracts indicated very low GPT activities (13.9~17.3 IU/ℓ) compared to those (146.1 & 128.6 IU/ℓ) fed with α-tocopherol and fiber at $p<0.05$. From these results, it is suggested that *Lagerstroemia indica* have a potent ethanol-metabolizing activity.

Key words : alcohol dehydrogenase, aldehyde dehydrogenase, microsomal ethanol-oxidizing system, glutamate-pyruvate transferase, medicinal plant

INTRODUCTION

Intake of alcohol by human beings have been continued for a long time, but the side effects of alcohol also have been reported in liver and extra-hepatic tissues of animal or man (Rintala *et al.*, 2000; Hill *et al.*, 1998).

The primary metabolism of alcohol is taken place in liver by alcohol dehydrogenase (ADH) producing acetaldehyde and NADH (Mezey & Tobon, 1971). Acetaldehyde is rapidly oxidized to acetate by aldehyde dehydrogenase (ALDH) (Gill *et al.*, 1996). The other enzymes including catalase and microsomal ethanol-oxidizing system (MEOS) also contribute in ethanol metabolism (Koechkln & Amit, 1992; Petersen *et al.*, 1981).

Although acetaldehyde is used for energy resource in extrahepatic tissues, it can also cause fatty liver (Keshavarzian *et al.*, 1994). Free radicals including superoxide anion produced from ethanol-metabolism were also the reason for tissue injuries (Lieber, 1997; Altura & Gebrewold; 1996). It has been reported that increases of carcinogenesis, malnutrition, antioxidant reduction, and lipid peroxidation of tissue originated from chronic alcohol consumption(Wright *et al.*, 1999; Ward & Peters, 1992; Devi *et al.*, 1996; Coudray *et al.*, 1993).

Recently, plant extracts and antioxidants including vitamin E and vitamin C were suggested as materials for attenuation of alcohol-evoked negative effects (Cho *et al.*, 2001; McDonough, 2003). This study was conducted to evaluate the alcohol-metabolizing capacities of four medicinal plants.

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MATERIALS AND METHODS

Materials

Materials used for the study were leaves of *Lagerstroemia indica* L., fruit of *Terminalia chebula* R., bark of *Ulmus davidiana* Planchn var. *japonica* N., leaves of *Zelkova serrata* M., which were obtained from YoungGwang (Korea), KeongDong Market (originated from China and Korea) and NICS (Korea), respectively. Preparation of the plant extracts was conducted with aqueous or absolute ethanol.

All reagents used for the analysis of ADH, ALDH, MEOS activities and protein content of enzyme solution were purchased from Sigma Co. (USA). Assay kits(Asan Pharm. Co. Ltd, Korea) and guaranteed grade solvent were used for the analysis of GPT acitivity and extract preparation of plants, respectively.

Animals and treatment

Animals used for the experiment were four week-old Sprague Dawely male rats with 110~120 g body weight. Rats were randomly departed into 6 groups (n=8) and fed with experimental diets and intoxicated with 10% alcohol for 4 weeks. Basal diets were composed with 39.75% of corn starch, 20% of casein, 13.2% of dextrin, 10% of sucrose, 7% of soybean oil, 5% of fiber, AIN-mineral mixture, and AIN-vitamin mixture (α-tocopherol excluded) L-cystine and choline bitartarate. Diets for plant extracts (and α-tocopherol) supplemented groups were the same composition of basal diet except the addition of 1% of each extract (and tocopherol) with 4% of fiber. Rats were checked in their body weights at intervals of few days, and sacrificed at 28th days.

Preparations of liver homogenate, mitochondrial, microsomal and cytosol fraction

Rat liver was weighed, minced, and homogenated in 0.1 M phosphate buffer (pH 7.4). Homogenate was centrifuged at 3,000 rpm for 15 min. and the supernatant was again centrifuged at 10,000rpm for 20 min. The pellet was used for the analysis of aldehyde dehydrogenase (ALDH) activity and the supernatant was again centrifuged at 35,000 rpm for 60 min. The pellet (microsomal fraction) was used for analyzing microsomal ethanol-oxidizing system (MEOS) and the supernatant(cytosol fraction), for alcohol dehydrogenase (ADH) activity. Blood was stated at 4℃ for 30 min., centrifuged at 3,000 rpm for 10 min. for the preparation of serum for the analysis of glutamate-pyruvate transferase (GPT) activity.

Analysis of liver ADH, ALDH, and MEOS activity

Analysis of ADH activity of rat liver was conducted by the method of Lebsack *et al.* (1977) as following: liver cytosolic solution was incubated in reaction system containing 0.01 M NAD, 0.05 M semicarbazide and 0.01 M ethanol in 2 ㎖ of Tris buffer (pH 8.5). Formation of NADH in the reaction system was monitored at 340 ㎚ for 1 min. Enzyme activity expressed as nM NADH/min./protein, was calculated from adaption of extinction coefficient of NADH, ε340 of 6220 $M^{-1}cm^{-1}$ to the absorbance.

Activity of ALDH was analyzed by method of Grill *et al.* (1996) as stated below. The assay mixture contained 50 mM sodium pyrophosphate (pH 8.8), 1 mM NAD, 2 μM rotenone, 0.2 mM 4-methylpyrazole, and 1 mM magnesium chloride. The mixture was incubated with enzyme solution at 25℃ for 20 min. After the addition of 0.1 ㎖ of 5 mM acetaldehyde, the absorbance was measured at 340 ㎚ for 5 min. The activity of ALDH (nM NADH/min./㎎ protein) was calculated from the absorbance based on the extinction coefficient of NADH, ε340 of 6220 $M^{-1}cm^{-1}$.

Determination of MEOS activity was conducted by the method of Hasumura *et al.* (1975) as mentioned below. 15 mM semicarbazide hydrochloride in center well of 50 ㎖ flask was pre-incubated with 1.7 ㎖ of assay mixture composed of liver microsome, 0.3 mM NADP, 8 mN sodium isocitrate, 5 mM $MgCl_2$, and 0.2 ㎖ of isocitrate dehydrogenase (2 ㎎/㎖) in 0.1 M potassium phosphate buffer (pH 7.4) at 37℃ for 20 min. The assay mixture was incubated with 0.3 ㎖ of 500 mM ethanol for 0, 5 and 10 min. in shaking water bath and overnight at room temperature. A_{224} of acetaldehyde trapped by 0.6 ㎖ of 15 mM semicarbazide hydrochloride in center well was measured and used to calculate the activity of MEOS (nM acetaldehyde/min./㎎ protein) with calibration curve using acetaldehyde as standard.

Determination of serum GPT activity and protein content of enzyme solutions

Determination of activity of GPT(IU/ℓ), was conducted by the method of Reitman-Frankel (1957) using assay kits. The content of protein in homogenate, mitochondrial fraction, and cytosol fraction was determined by the method of Bradford (1976) using bovine serum albumin as a standard compound for calibration curve.

Statistical analysis of data

The results were exhibited as mean±standard deviation, and the statistical significance was verified by the one way ANOVA and Duncan's multiple range test at the level of $p<0.05$.

RESULTS AND DISCUSSION

Fig. 1 shows the activity of ADH, an enzyme which oxidizes ethanol to acetaldehyde. ADH activities in *Lagerstroemia*, *Ulmus*, and *Zelkova* extract-administrated rats were significantly higher than control group. ADH activities of *Terminalia* extract and tocopherol-administrated group did not showed significant effects at the level of $p<0.05$. Because ADH is a metabolizing-enzyme of ethanol, the activity is correlated with the capacity of ethanol-elimination from the body. The absence of ADH activity enhances the activity of MEOS by which many free radicals including superoxide anion radical can be made. Free radicals can act as the promotor of diseases such as cancer and inflammation (Shigeta *et al.*, 1983; Oneta *et al.*, 2002). Therefore, it is suggested that three plant, *Lagerstroemia, Ulmus*, and *Zelkova* extracts possessing higher ADH activities are more effective on ethanol-metabolism than *Terminalia* extract or tocopherol. The result was similar to the reports by Cho *et al.* (2001). It is supposed that two control groups, tocopherol-subtracted and supplemented groups which had the similar values with each other should be more studied their action modes on ethanol-metabolism.

The activity of ALDH measured in experimental groups was exhibited in Fig. 2. Rats supplemented with α-tocopherol showed the highest value of ALDH activity among the experimental groups and *Lagerstroemia*, and *Zelkova* extracts-supplemented rats also showed higher activities than control. Because ALDH, a key ethanol-metabolizing enzyme, can oxidize acetaldehyde to acetate, a milder

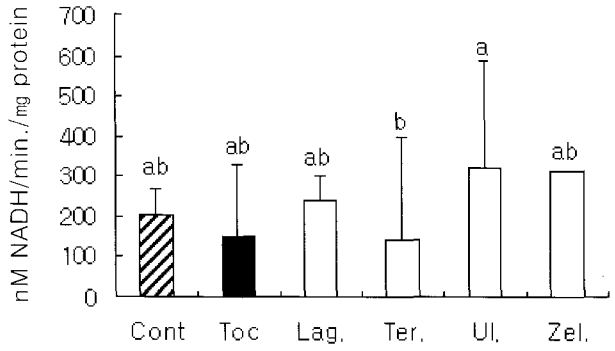

Fig. 1. Activity of alcohol dehydrogenase (ADH) determined in alcohol and medicinal plant extracts-treated rat liver (Cont, control; Toc, α-tocopherol; Lag., *Lagerstroemia indica*; Ter., *Terminalia chebula*; Ul., *Ulmus davidiana* var. *japonica*; Zel., *Zelkova serrata*; Values are mean±standard deviation (n=8); Different alphabets above the bar indicate significant difference at $p<0.05$ by Duncan's multiple range test).

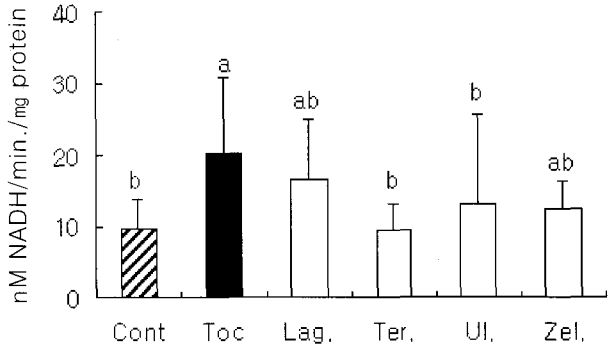

Fig. 2. Activity of aldehyde dehydrogenase (ALDH) determined in alcohol and medicinal plant extracts-treated rat liver (Cont, control; Toc, α-tocopherol; Lag., *Lagerstroemia indica*; Ter., *Terminalia chebula*; Ul., *Ulmus davidiana* var. *japonica*; Zel., *Zelkova serrata*; Values are mean±standard deviation (n=8); Different alphabets above the bar indicate significant difference at $p<0.05$ by Duncan's multiple range test).

metabolite than acetaldehyde, it is supposed that rats supplemented α-tocopherol, *Lagerstroemia*, and *Zelkova* extracts can be safe from the toxicity of acetaldehyde more than control. Therefore, it is suggested that two plant extracts and α-tocopherol with the high ALDH activity can efficiently eliminate ethanol from the body ethanol-metabolizing activities.

The effects of experimental groups on MEOS activity were shown in Fig. 3. The activity of MEOS in all of the plant extract-supplemented groups was higher than control, and that in *Terminalia* extract-supplemented group was the highest among the experimental groups. This result was correlated with the results that the activities of ADH and ALDH in *Terminalia* extract-supplemented group were lower than those in other three plant extract-supplemented groups. The increase in MEOS activity imply the increase of free radicals production, which can produce harmful effect on health. From these results, it is suggested that *Lagerstroemia*, *Ulmus*, and *Zelkova* extracts have some hepatoprotective activity on ethanol-toxicity.

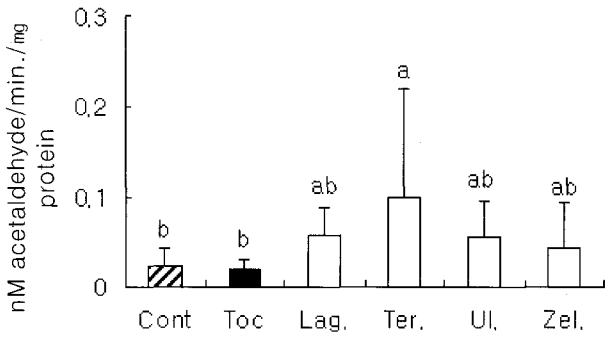

Fig. 3. Activities of microsomal ethanol-oxidizing system (MEOS) determined in alcohol and medicinal plant extracts-treated rat liver (Cont, control; Toc, α-tocopherol; Lag., *Lagerstroemia indica*; Ter., *Terminalia chebula*; Ul., *Ulmus davidiana* var. *japonica*; Zel., *Zelkova serrata*; Values are mean±standard deviation (n=8); Different alphabets above the bar indicate significant difference at $p<0.05$ by Duncan's multiple range test).

As shown in Fig. 4, all of the plant extracts showed significantly lower GPT activities than control groups. Since GPT is an enzyme increased in serum in the case of severe liver injury. It is supposed that the plant extracts experimented have protective activities on ethanol-induced liver injury.

In conclusion, it is suggested that all of the plants experimented have at least partly protective activities on liver injury, and the most effective one is leaves of *Lagerstroemia indica*.

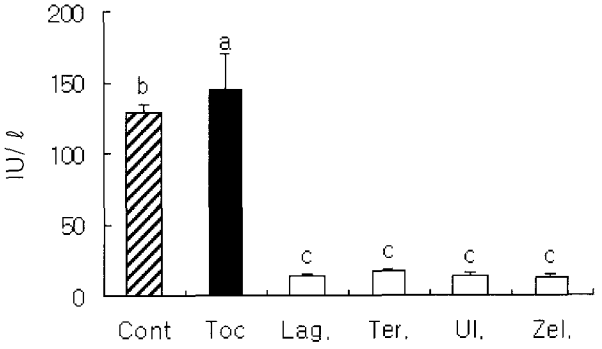

Fig. 4. Activities of glutamate-pyruvate transferase (GPT) determined in alcohol and medicinal plant extracts-treated rat liver (Cont, control; Toc, α-tocopherol; Lag., *Lagerstroemia indica*; Ter., *Terminalia chebula*; Ul., *Ulmus davidiana* var. *japonica*; Zel., *Zelkova serrata*; Values are mean±standard deviation (n=5); Different alphabets above the bar indicate significant difference at $p<0.05$ by Duncan's multiple range test).

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