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The Effect of Ground Cherry Extract on the Activity of Hepatic Aniline Hydroxylase in Mice

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Abstract

To evaluate the effect of ground cherry extract on the activity of aniline hydroxylase, we gave ground cherry extract in doses of 100, 200 or 400 mg/kg i.p to mice for 1, 2 or 4 days. The aniline hydroxylase activity in the group treated with ground cherry extract increased in a dose dependant manner in all experimental groups compared with the control group, and was significantly higher in the group treated with ground cherry extract at a dose of 200 mg/kg, which also exhibited a time dependant increase over 4 days. Enzyzme kinetic analysis was performed for hepatic aniline hydroxylase activity in the group treated with 200 mg/kg for 4 days. There was no change of the Km values for aniline hydroxylase between the experimental group and the control group, but the Vmax values for aniline hydroxylase was 21% lower in the experimental group compared with the control. The experimental group also showed lower lipid peroxide and reduced glutathione content, and there were no significant difference in serum alanine aminotransferase activity between the experimental group and the control. Aniline was injected into both the experimental group mice treated with ground cherry extract at a dose of 200 mg/kg for 4 days and the control group, and then the level of blood aniline was assayed at 1hr. The level of blood aniline was lower in the experimental than the control group. This study suggests that ground cherry extract induces hepatic aniline hydroxylase activity and might accelerate the scavenging system of reactive oxygen species. It is likely that ground cherry extract influences the metabolism of xenobiotics by activating AH activity substituted for CYP2E1.

Key words: ground cherry extract, aniline hydroxylase, kinetics

INTRODUCTION

We are faced with a variety of processed foods and a number of medications, which can cause health problems. Research has shown that various natural products act as effective agents for enhancing the immune system, reducing reactive oxygen species, accelerating alcohol metabolism, and functioning as an alternative or functional food (1-3).

Ground cherry, in the solanaceae family, is a widely distributed small annual herb indigenous to Korea. It is also known as *Physalis Fructus* or *Physalis alkekengi var franchetti (Masters) Hort* in traditional oriental medicine. Ground cherry had been utilized for alleviating fever, detoxication, and diuresis in ancient times, and it is a major component of remedies for the successful treatment for jaundice and eczema (4). Although it is known that the same genus of ground cherry is an anticancer agent (5,6), there has not been nearly enough study on the pharmacological effect of ground cherry. It has been well reported that there are two phases in the detoxification of xenobiotics. Phase I, which is critical in the oxidative metabolism of xenobiotics, is a microsomal P450 mixed function oxidase. Phase Ⅱ is involved in scavenging and conjugation (7,8). CYP2E1, one of the cytochrome P450 of Phase I enzymes, is more abundant in the liver than other organs and is involved in the physiological metabolism of xenobiotics as well as generating reactive oxygen species (ROS) during the metabolism of harmful substances (9). When CYP2E1 metabolizes xenobiotics such as ethanol it also causes liver damage due to toxic intermediates and ROS (10-12), which is an unavoidable consequence of Phase I oxidation of chemicals, detoxication of xenobiotics, or bioactivation of prodrugs (13,14).

The objective of this study was to assess the effects of ground cherry extract on the hepatic xenobiotic metabolism. Experimental animals were administered ground cherry extract i.p at doses of 100, 200 or 400 mg/kg for 1, 2 or 4 days. The CYP2E1-dependent aniline hydroxylase activity (15-17), the contents of reduced glutathione and lipid peroxide in liver, and serum alanine

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aminotransferase were determined.

MATERIALS AND METHODS

Extraction of ground cherry

Dry aerial parts of ground cherry were extracted with 70% methanol, and concentrated by a rotary evaporator. The extract was dissolved with distilled water and separated with ethylether to remove lipid soluble substance, and then the water layer was separated with n-butanol (18). The n-butanol fraction (ground cherry extract) was concentrated by rotary evaporation.

Animals

Male ICR mice with a mean body weight of 25 g were purchased from Oriental Co. Ltd. (Busan, Korea). Mice were provided food (Purina Chow, Korea) and water *ad libitum*. The experimental animals were administrated ground cherry extract, dissolved in physiological saline, i.p at doses of 100, 200 or 400 mg/kg for 1, 2 or 4 days. A control group was administrated the same volume of physiological saline i.p. Aniline was administered (110 mg/kg in physiological saline) to mice i.p 1 hr before euthanasia (19). The animals had fasted for 24 hrs before being killed by drawing blood from the inferior vena cava under ether anesthesia. The liver was used for the experiments. Mice were individually housed in stainless steel wire-bottom cages in a room maintained at $20\pm2^{o}C$ and $60\pm5\%$ relative humidity. The room was exposed to alternating 12-hour periods of light and dark. The experimental protocols were conducted in accordance with internationally accepted principles for laboratory animal use and care as found in the Korea Food and Drug Administration guidelines.

Preparations of sample

Livers were rinsed with cooled physiological saline and homogenized with 0.25 M sucrose by using a biohomogenizer. The 20% homogenate was centrifuged at $600\times g$ for 10 min and the supernatant was recentrifuged at $10{,}000\times g$ for 20 min and the supernatant was ultracentrifuged at $105{,}000\times g$ for 1 hr. The microsomal fractions were used for aniline hydroxylase (AH) activity.

Biochemical assay

Aniline hydroxylase (AH) activity was estimated by the method of Bidlack and Lowry (20) and expressed as p-amniophenol nmole per hr per mg protein. Kinetic parameters for the oxidation of aniline by rat liver aniline hydroxylase were determined spectrophotometrically from a Lineweaver-Burke double reciprocal plot. Lipid peroxide (LPO) content was estimated by the method of Ohkawa et al. (21) and reduced glutathione (GSH) content was determined by the method of Ellman (22). Serum alanine aminotransferase (ALT) activity was measured using a commercial Laboratories kit (Ansan Pharm. Co., Korea) and expressed as Karmen unit per mL of serum. Levels of blood aniline were measured by a modified method of Norwitz and Keliher (23) and expressed as μg aniline per mL of serum. Protein content was determined by the method of Lowery et al. (24).

Statistical Analysis

The results were expressed as mean±SD values for the six animals. Statistical comparison of differences between the different groups was carried out using one-way analysis of variance test followed by Duncan's multiple range test (SPSS statistical software package, version 12.0, SPSS, Chicago, IL).

RESULTS

Effect of ground cherry extract on hepatic AH activity

Dose dependency of AH activities as microsomal CYP2E1 in the groups treated with ground cherry extract is shown in Fig. 1; time dependence of AH activity at the dose of 200 mg/kg is shown in Fig. 2. Hepatic AH activities in all experimental groups compared with the control group are increased in a dose dependent manner. The most significant increase is in the group at a dose of 200 mg/kg (Fig. 1). At a 200 mg/kg dose, hepatic AH activity showed a time dependent in increase, and was the significantly higher in the group treated with ground cherry extract for 4 days (Fig. 2).

Effect of ground cherry extract on the kinetics of AH

The kinetics of hepatic AH, in the animals treated with ground cherry extract at the dose of 200 mg/kg for 4 days, was calculated from a double reciprocal plot with

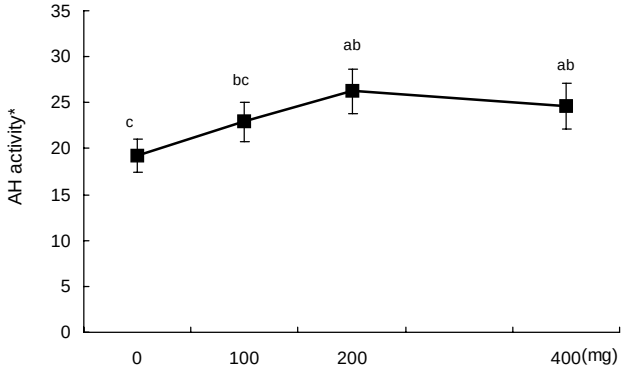

Fig. 1. Dose dependency of changes in hepatic microsomal AH activity in mice treated with ground cherry extract. Data are mean±SD values of six rats. Different superscripts within groups indicate a significant difference ($p<0.05$). *p-aminophenol formed nmole/hr/mg protein.

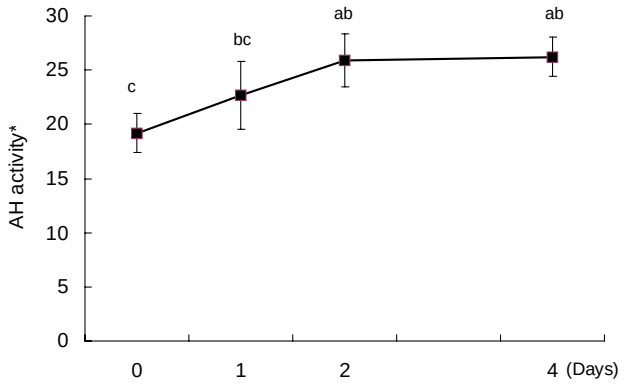

Fig. 2. Time dependency of changes in hepatic microsomal AH activity in mice treated with ground cherry extract at the dose of 200 mg/kg. Data are mean±SD values of six rats. Different superscripts within groups indicate a significant difference ($p<0.05$). *p-aminophenol formed nmole/hr/mg protein.

aniline (Fig. 3). Whereas there were no differences between the control (581.4 μM) and the experimental group (549.5 μM) on the Km values, the Vmax values were significantly increased by 42% in the experimental group (14.3 nmoles/hr/mg protein) compared to the control (10.1 nmoles/hr/mg protein).

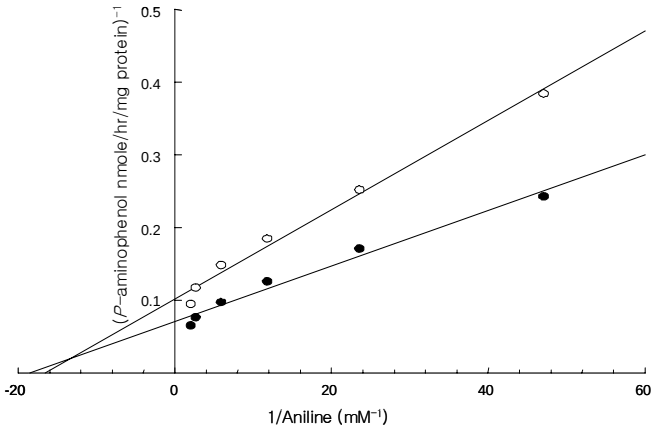

Fig. 3. Double reciprocal plots of microsomal AH in mice treated with ground cherry extract for aniline. Open circle: control, closed circle: group treated with ground cherry extract at the dose of 200 mg/kg for 4 days.

Hepatic contents of LPO and reduced GSH, and serum ALT activity

The hepatic contents of LPO and GSH, and serum ALT activity in the group treated with ground cherry extract at a dose of 200 mg/kg for 4 days are shown in Fig. 4. The hepatic LPO and GSH contents were significantly lower in the experimental group compared with the control group. There were no significant differences in the level of ALT between the experimental group and the control group.

Effect of ground cherry extract on the level of blood aniline

In the animals treated with aniline, the level of blood aniline at 1 hr was determined and shown in Fig. 5. Both the experimental and the control group were administered with aniline after the experimental group was

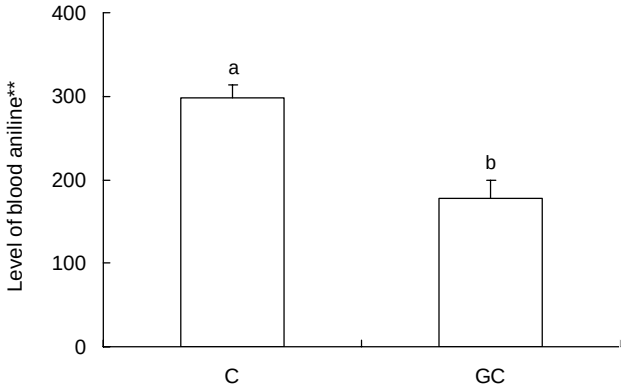

Fig. 5. Changes of blood aniline level in ground cherry extract pretreated mice. Data are mean±SD values of six rats. Different superscripts within groups (same color) indicate a significant difference ($p<0.05$). **Level of blood aniline: μg/mL of serum. The animals were sacrificed at 1 hr after administration of the aniline (110 mg/kg). C: control, GC: group treated with ground cherry extract at the dose of 200 mg/kg for 4 days.

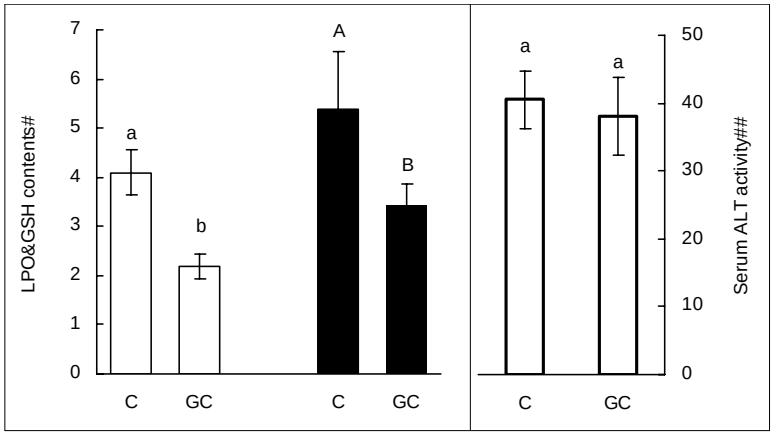

Fig. 4. Effect of ground cherry extract on the hepatic contents of LPO, reduced GSH, and serum ALT activity in mice. Data are mean±SD values of six rats. Different superscripts within groups (same colors) indicate a significant difference ($p<0.05$). White column: LPO, black column: reduced GSH, bold outline column: ALT activity. #LPO content: nmole/g of tissue, reduced GSH content: μmole/g of tissue. ##Serum ALT activity: Karmen unit/mL of serum. C: control, GC: group treated with ground cherry extract at the dose of 200 mg/kg for 4 days.

treated with ground cherry at a dose of 200 mg/kg for 4 days. The level of blood aniline was approximately 40% lower in the experimental group (178.2 μg/mL of serum) than in control (298.3 μg/mL of serum).

DISCUSSION

It is well documented that microsomal CYP2E1 is responsible for Phase Ⅰ reactions in the physiological metabolism of xenobiotics, such as acetaminophen and ethanol, but also generates ROS during the xenobiotics metabolism, and increased CYP2E1 influences the effective hepatic detoxification (25-28). In this study, we evaluated the effect of ground cherry extract on the hepatic drugs and xenobiotic metabolism in experimental animals administered ground cherry extract i.p. at 100, 200 or 400 mg/kg, and for 1, 2 or 4 days.

The CYP2E1-dependent aniline hydroxylase (15-17), which is one of cytochrome P450 isozymes, was determined in the group treated with ground cherry extract at doses of 100, 200 or 400 mg/kg to evaluate the dose dependency the effect of ground cherry extract. AH activity tended to increase in the experimental group, and increased in the group treated with ground cherry extract over a dose of 200 mg/kg. When ground cherry extract was given to mice at a dose of 200 mg/kg for 1, 2 or 4 days, AH activity increased in proportion to the injection period and the most significant increase occurred in the group treated for 4 days. Huh et al. (19) reported that natural herbs such as *Glycyrrhizae radix* have effects on the alleviation and the recovery of tissue damage and induce CYP2E1 in the animal model of tissue injury or aging. These results suggest that ground cherry extract plays an important role in accelerating the detoxification or the activation of xenobiotics through increased AH activity. To confirm the relation of the mechanism of changed AH activity to ground cherry extract, kinetic analysis was used and found that ground cherry extract at a dose of 200 mg/kg for 4 days which showed the highest increase in AH activity. Although the Km values were similar between the experimental group and control, the Vmax values were remarkably higher in the experimental group compared with the control group, which indicated an increase in the amount of AH enzymatic protein via stimulation of AH protein synthesis and/or inhibition of protein degradation. It is reported that during xenobiotics metabolism the produced ROS plays a role in the increased cell membrane damage leading to lipid peroxidation, and that the depletion of reduced GSH content is the direct parameter to protect the tissue (29,30). In this study, the experimental group compared to the control group exhibited significantly less lipid peroxidation and lower GSH content, which elicits the hepatic protection of ground cherry extract. There was no hepatic damage in the group treated with ground cherry extract compared with the control group as shown in serum ALT activity, in liver weight per body weight, hepatic protein content and the weights of other organs which were not itemized in the results. The level of blood aniline was significantly lower in the experimental group treated with ground cherry extract at a dose of 200 mg/kg for 4 days compared with the control group. These results suggest that ground cherry extract influences the metabolism of xenobiotics by activating AH activity. In conclusion, the results suggest that ground cherry extract affects the hepatic metabolism of xenobiotics including various drugs and organic solvents by activating CYP2E1. However, we could not confirm the main constituent of ground cherry extract involved with the hepatic microsomal CYP2E1 activition from the present results. Therefore, further study is needed to elucidate the main constituent of ground cherry extract and the mechanism of its effectiveness on AH activity via molecular techniques such as mRNA analysis.

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