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Effect of Hesperidin Supplementation on Lipid and Antioxidant Metabolism in Ethanol-fed Rats

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Abstract

This study examined the effect of hesperidin supplementation with an ethanol diet on lipid and antioxidant metabolism in rats. Male Sprague-Dawley rats were divided into two groups (n=10), and were assigned to one of two dietary categories: E_8, ethanol diet (50 g/L) for 8 wks; E_8H_4, ethanol diet for the first 4 wks and hesperidin (0.02%, w/w) supplemented ethanol diet for the last 4 wks. The plasma and hepatic lipids, hepatic cholesterol regulating enzyme activity, hepatic antioxidant enzyme activity and lipid peroxidation were determined. Supplementation with hesperidin for the last 4 wks during the 8 wks period of the ethanol diet, significantly increased the ADH activity. In conjunction with the chronic administration of ethanol, hesperidin supplementation resulted in a significant decrease in the hepatic cholesterol and triglyceride concentrations compared to the E_8 group. The hepatic HMG-CoA reductase and ACAT activities were significantly lower in the hesperidin-supplemented group. When comparing hepatic antioxidant enzyme activities, SOD, GSH-Px, and G6PD activities and GSH level were significantly higher in the E_8H_4 group than in the E_8 group. Plasma TBARS levels were significantly lower in rats fed ethanol with hesperidin compared to the rats fed only ethanol; however, the hepatic TBARS levels were not significantly different between the groups. Accordingly, the additional hesperidin supplement with an ethanol diet might be effective for improving the hepatic lipid metabolism and antioxidant defense system.

Key words: hesperidin, ethanol diet, lipid metabolism, antioxidant metabolism

INTRODUCTION

Alcohol abuse and alcoholism are serious health and socioeconomic problems throughout the world. Despite great progress, the development of suitable medications for the treatment of alcoholism remains a challenging goal for alcohol researchers. Ethanol is almost exclusively metabolized in the body by enzyme catalyzed oxidative processes. The accumulation of acetaldehyde in the liver after chronic alcohol ingestion is determined by its formation and removal rates as catalyzed by alcohol dehydrogenase (ADH) activity and aldehyde dehydrogenase (ALDH) activity, respectively (1). Chronic ethanol feeding causes the accumulation of lipids in the liver and lipid peroxide in other tissues. Alcohol also alters lipid metabolism, causing a profound inhibition of lipolysis (2). The acetaldehyde formed is further oxidized to acetate, which is then converted to carbon dioxide via the citric acid cycle. Ethanol or its metabolites can also cause autooxidation of the hepatic cells by acting as a prooxidant, resulting in marked hepatotoxicity (3). Hepatic oxidative stress resulting from lipid peroxidation has been identified as playing a pathogenic role in alcoholic liver disease (2).

Flavonoids are a group of naturally occurring compounds that are widely distributed as secondary metabolites in plants. They have been recognized for having interesting clinical properties, such as anti-inflammatory, antiallergic, antiviral, antibacterial, and antitumoral activities (4). The pharmacological effects of many traditional drugs are attributable to the presence of flavonoid compounds, which are due to their ability to inhibit certain enzymes and to their antioxidative activity (5,6). One of these flavonoids, hesperidin, a flavonone type of flavonoid abundant in citrus fruit, has biologically beneficial effects, such as cholesterol-lowering and anticarcinogenic activities (7-10).

In general, ethanol has been shown to both generate ROS and attenuate antioxidant enzymes activities (11).

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As a result, increased oxidative stress has been suggested as one of the mechanisms involved in the development of complications related to ethanol intoxication (12,13). Accordingly, the current study evaluated the effect of supplementary hesperidin against liver damage in ethanol administered rats based on the analysis of hepatic alcohol-metabolizing enzymes, antioxidant enzymes, lipid accumulation and cholesterol-regulating enzymes.

MATERIALS AND METHODS

Animals and diets

Twenty male Sprague-Dawley rats weighing between 140 and 150 g were purchased from Orient Inc. (Seoul, Korea). The animals were individually housed in stainless steel cages in an air-conditioned room with controlled temperature (20～23°C) and automatic lighting (alternating a 12-hr period of light and dark) and fed a pelletized chow diet for 1 week after arrival. Next, the animals were randomly divided into two dietary groups (n=10): E_8, ethanol diet for 8 wks; E_8H_4, ethanol diet for the first 4 wks and hesperidin supplemented ethanol diet for the last 4 wks (Fig. 1). The E_8 group consumed a nutritionally complete 1982 formulation of the LD ethanol liquid diet (14) shown in Table 1 containing 36% of energy as ethanol ad libitum. Animals received their LD diet in 150 mL graduated plastic bottles. Animals were allowed 24-hr access to diet, which was provided fresh daily between 1,600 and 1,700 hr. Ethanol was introduced into the diet gradually starting from 0% (w/v) and increasing to 5% (w/v) over a 1-week period. The E_8 group was fed an ethanol diet (50 g/L) for 8 weeks after the adaptation period. The rats in the E_8H_4 group received an ethanol liquid diet for 8 weeks along with hesperidin supplement (0.02%, w/w; Sigma Chemical Co.) for the last 4 weeks. The hesperidin dose was established by our preliminary study. Food consumption and weight gain were measured every day and once a week, respectively. At the end of the experimental period, the rats were anesthetized with ketamine-HCl following a 12-hr fast. Blood was drawn from the inferior vena cava into a heparin-coated tube, and the plasma was obtained by centrifuging the blood at 1,000×*g* for 15 min at 4°C. The livers were then removed, rinsed with physiological saline, and weighed for enzyme analysis and lipid measurement. The plasma and livers were stored at -70°C until analysis.

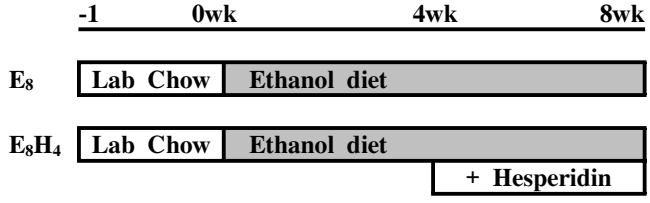

Fig. 1. Experimental schedule for feeding ethanol diet with flavonoid supplements.
E_8: ethanol diet for 8 wks, E_8H_4: ethanol diet for the 8 wks with hesperidin supplementation for 4 wks.

Table 1. Composition of Lieber-DeCarli liquid alcoholic diet[1)] and experimental diets (g/liter/1,000 calories)

	Ethanol diet (E_8)	Ethanol diet + Hesperidin (E_8H_4)
Casein	41.4	41.4
L-cystine	0.5	0.5
DL-Methionine	0.3	0.3
Corn oil	8.5	8.5
Olive oil	31.1	31.1
Dextrin maltose	25.6	25.6
Choline bitartrate	0.53	0.53
Fiber	10.0	10.0
Xanthan gum	3.0	3.0
Vitamin mix[2)]	2.55	2.55
Mineral mix[3)]	9.0	9.0
Ethanol	50	50
Hesperidin	-	0.06

[1)]The liquid diet is mixed nutritional ingredient in 1 L distilled water.
[2)]Vitamin mixture according to AIN-76.
[3)]Mineral mixture according to AIN-76.

Plasma and hepatic lipids

The plasma total and HDL-cholesterol concentrations were determined using a commercial kit (Sigma) based on a modification of the cholesterol oxidase method of Allain et al. (15). The HDL-fractions were separated using a Sigma kit based on the heparin-manganese precipitation procedure (16). The plasma triglyceride concentrations were measured enzymatically using a kit from Sigma Chemical Co., a modification of the lipase-glycerol phosphate oxidase method (17). The hepatic lipids were extracted using the procedure developed by Folch et al. (18). The dried lipid residues were dissolved in 1 mL of ethanol for cholesterol and triglyceride assays. Triton X-100 and a sodium cholate solution (in distilled H_2O) were added to 200 μL of the dissolved lipid solution to produce final concentrations of 5 g/L and 3 mmol/L, respectively. The hepatic cholesterol and triglycerides were analyzed with the same enzymatic kit used in the plasma analysis.

Plasma and hepatic lipid peroxidation (TBARS assay)

The TBARS (thiobarbituric acid-reactive substances) were monitored according to a previously described procedure (19). Briefly, 500 μL of plasma was well mixed with 3 mL of 5% trichloroacetic acid and 1 mL

of freshly prepared 60 mmol/L thiobarbituric acid (TBA). After incubation at 80°C for 90 min, the samples were cooled at room temperature, centrifuged at 1,000×*g* for 15 min at 4°C, and the supernatant absorbance was read at 535 nm.

Hepatic lipid peroxide levels were determined using the method of Ohkawa et al. (20) with a slight modification. Tissue homogenates were prepared based on a ratio of 1 g of wet tissue to 9 mL of a 1.15% KCl solution using a glass or Teflon Potter-Elvehjem homogenizer. A reaction mixture containing a 0.2 mL aliquot of the homogenates, 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), and 0.6 mL of distilled water was allowed to sit at room temperature for 5 min, then mixed with 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of a 0.8% aqueous solution of TBA, and finally heated at 95°C for 60 min. After cooling with tap water, 1 mL of distilled water and 5.0 mL of a mixture of n-butanol and pyridine (15:1, v/v) were added and the mixture was vigorously vortexed. Then, after centrifugation at 800×*g* for 10 min, the absorbance of the upper layer was measured at 535 nm. A malondialdehyde (MDA) solution freshly made by the hydrolysis of 1,1,3,3-tetramethoxypropane (TMP) was used as the standard. The results were expressed as the nmol MDA/mL plasma and nmol MDA/g liver.

Hepatic antioxidant enzyme activities

The enzyme sources were isolated using the following procedure. Two grams of liver tissue were homogenized with 10 mL of a 0.25 M sucrose buffer; then the homogenates were centrifuged at 600×*g* for 10 min to remove the nuclear fraction and the remaining separated supernatant was recentrifuged at 10,000×*g* for 20 min to collect the mitochondrial fraction (pellet) for a CAT assay. The supernatant was ultra-centrifuged at 100,000×*g* for 1 hr to isolate the cytosolic fraction for an SOD, GSH-Px, GR, and G6PD assay. The activities of the antioxidant enzymes were measured using the following methods.

The CAT activity was measured using Aebi's method (21), wherein the hydrogen peroxide decomposition to yield water and oxygen is measured. The absorbance of the samples was recorded for 5 min at a wavelength of 240 nm and the enzyme activities were expressed as the decrease in H_2O_2 nmol/min/mg protein. The SOD activity was estimated according to the method of Marklund and Marklund (22), which uses the color change due to the auto-oxidation of pyrogallol. The reaction was terminated by adding 0.1 mL of 1 N HCl, and one unit of enzyme activity was calculated as the protein content inhibiting 50% of the auto-oxidation of 15 mM pyrogallol without an enzyme source. The GSH-Px activity was assayed using Paglia and Valentine's method (23). The conversion of NADPH to NADP, as a measure of the GSH-Px activity, was followed by recording the changes in absorbance at 340 nm for 5 min after initiation of the enzyme reaction. The results were expressed as nmol NADPH/min/mg protein. The protein concentrations in the mitochondrial and cytosolic fractions were determined using the Bradford (24) method, and all the antioxidant enzyme assays were performed and facilitated at 25°C. Glutathione reductase (GR) activity was determined with the method of Pinto and Bartley (25) by monitoring the oxidation of NADPH at 340 nm. The reaction mixture contained 1 mM EDTA and 1 mM GSSG in 0.1 M potassium phosphate buffer (pH 7.4). The activity was expressed oxidized NADPH nmol/min/mg protein. Glucose-6-phosphate dehydrogenase (G6PD) activity was determined with the method of Pitkanen et al. (26). The reaction mixture contained 55 mM Tris-HCl (pH 7.8), 3.3 mM $MgCl_2$ buffer and 6 mM G-6-P. The activity was expressed reduced NADPH nmol/min/mg protein.

Hepatic alcohol metabolizing enzyme activities

The alcohol dehydrogenase (ADH) activity of cytosolic fraction was assayed using Bergmeyer's method (27). The conversion of NAD to NADH, as a measure of ADH activity, was followed by recording the changes in absorbance at 340 nm for 5 min after the initiation of the enzyme reaction. The aldehyde dehydrogenase (ALDH) activity of cytosolic fraction was assayed using Koivula and Koivusalo's method (28).

Measurement of glutathione (GSH) content

GSH content was measured using the method of Ellman (29). Five hundred microliters of the liver homogenate was mixed with 500 μL of 4% (w/v) sulfosalicylic acid and centrifuged at 600×*g* for 10 min. Three hundred microliters of the supermatant were added to 2.7 mL of a disulfide (5,5′-dithiobis-2-nitrobenzoic acid) reagent, and measured at 412 nm. Total GSH content was expressed as nmole/g of tissue.

HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase and ACAT (acyl-CoA: cholesterol acyltransferase) activities

The microsomes were prepared according to the method developed by Hulcher and Oleson (30) with a slight modification. One gram of liver tissue was homogenized in 3 mL of an ice-cold buffer (pH 7.0) containing 0.1 mol/L of triethanolamine, 0.02 mol/L of EDTA, and 2 mmol/L of dithiothreitol. The homoge-

nates were centrifuged for 15 min at 10,000×*g* and 12,000×*g* at 4°C. Next, the supernatants were ultracentrifuged twice at 100,000×*g* for 60 minutes at 4°C. The resulting microsomal pellets were then redissolved in 1 mL of a homogenation buffer for protein determination (31) and finally analyzed for their HMG-CoA reductase and ACAT activities.

The HMG-CoA reductase activities were determined as described by Shapiro et al. (32) with a slight modification using freshly prepared hepatic microsomes. The incubation mixture (60 μL) containing the microsomes (100～150 μg of protein) and 500 nmol of NADPH (dissolved in a reaction buffer containing 0.1 mol/L of triethanolamine and 10 mmol/L of EDTA) were preincubated at 37°C for 5 minutes. Next, 10 μL of 50 nmol of [^{14}C]HMG-CoA (specific activity, 2.1083 GBq/mmol; NEN™ Life Science Products, Boston, MA) were added, and the incubation continued for 15 minutes at 37°C. The reaction was terminated by the addition of 15 μL of 10 mol/L HCl, and the resultant reaction mixture was incubated at 37°C for an additional 15 minutes to convert the mevalonate into mevalonolactone. The incubation mixture was centrifuged at 10,000×*g* for 5 min, and the supernatant was spotted on a Silica Gel 60F_{254} TLC plate using mevalonolacetone as the standard. The plate was developed in benzene-acetone (1:1, v/v) and air-dried. Finally, the region R_f 0.3～0.6 was removed by scraping with a clean razor blade and its ^{14}C radioactivity was determined using a liquid scintillation counter (Packard Tricarb 1600TR; Packard Instrument, Meriden, CT). The results are expressed as picomol of mevalonate synthesized per min per mg protein.

The ACAT activities were determined using freshly prepared hepatic microsomes, as developed by Erickson et al. (33) and modified by Gillies et al. (34). To prepare the cholesterol substrate, 6 mg of cholesterol and 600 mg of Tyloxapol (Triton WR-1339, Sigma) were each dissolved in 6 mL of acetone, mixed well, and completely dried in N_2 gas. The dried substrate was then redissolved in 20 mL of distilled water to a final concentration of 300 μg of cholesterol/mL. Next, reaction mixtures containing 20 μL of the cholesterol solution (6 μg of cholesterol), 20 μL of a 1 mol/L of potassium phosphate buffer (pH 7.4), 10 μL of 0.6 mmol/L bovine serum albumin, 10 μg of the microsomal fraction, and distilled water (up to 180 μL) were preincubated at 37°C for 30 min. The reaction was then initiated by adding 20 μL of 5.62 nmol of [^{14}C]-oleoyl CoA (specific activity; 1.9795 GBq/mmol; NEM™ Life Science Products) to a final volume of 200 μL; the reaction time was 30 min at 37°C. The reaction was terminated by the addition 500 μL of isopropanol: haptane (4:1, v/v), 300 μL of haptane, and 200 μL of 0.1 mol/L potassium phosphate (pH 7.4), and the reaction mixture was allowed to stand at room temperature for 2 min. Finally, an aliquot (200 μL) of the supernatant was subjected to scintillation counting. The ACAT activities were expressed as picomol of cholesteryl oleate synthesized per min per mg protein.

Table 2. Effect of hesperidin supplementation on weight gain, food intake and organ weight in rats fed ethanol diet

	E_8	E_8H_4
Weight gain (g/day)	2.50±0.16	2.80±0.10
Food intake (mL/day)	63.68±1.77	67.04±1.00
Organ weight (g/100 g BW)		
Liver	3.70±0.10	3.44±0.12
Heart	0.32±0.01	0.33±0.01
Kidney	0.71±0.02	0.70±0.02

Mean±SE, n=10.
E_8: ethanol diet for 8 wks, E_8H_4: ethanol diet for the first 4 wks and hesperidin-supplemented ethanol diet for the last 4 wks.

Statistical analysis

All data are presented as the mean±SE. The statistical significance of the difference ($p<0.05$) between the groups was determined by a Student's *t*-test using the standard social science statistical packages (SPSS).

RESULTS

Effect of hesperidin supplementation on food intake, weight gain and organ weight

There were no significant differences in the amounts of food intake, weight gain, and organ weights between the groups (Table 2).

Effect of hesperidin supplementation on alcohol metabolizing enzyme activities

Hesperidin supplementation resulted in a significant increase in hepatic alcohol dehydrogenase (ADH) activity, whereas aldehyde dehydrogenase activity did not differ significantly between the groups (Fig. 2).

Effect of hesperidin supplementation on plasma and hepatic lipids

The levels of plasma total cholesterol, HDL-cholesterol, HDL-C/total-C ratio, triglyceride, and the atherogenic index were not different between the groups (Table 3). However, as shown in Table 3, the hepatic cholesterol and triglyceride levels were significantly lower in the E_8H_4 group than in the E_8 group. The hepatic cholesterol and triglyceride levels were 53% and 44% lower in E_8H_4 group compared to the E_8 group, respectively.

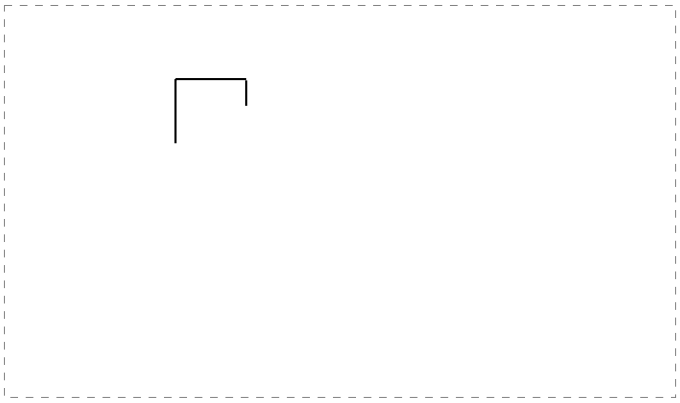

Fig. 2. Effect of hesperidin supplementation on hepatic alcohol metabolizing enzyme activities in rats fed ethanol diet.
Mean±SE, n=10.
E_8: ethanol diet for 8 wks, E_8H_4: ethanol diet for the first 4 wks and hesperidin-supplemented ethanol diet for the last 4 wks.
$^{*}p<0.05$ vs. E_8 group based on Student's t-test.

Table 3. Effect of hesperidin that was supplemented from the 5th week on the levels of plasma and hepatic lipids in the rat fed ethanol diet for 8 weeks

	E_8	E_8H_4
Plasma		
Total cholesterol (mmol/L)	2.62±0.09	2.37±0.08
HDL-cholesterol (mmol/L)	1.10±0.04	1.15±0.05
HDL-C/Total-C[1] (%)	42.06±0.91	48.60±2.22
Triglyceride (mmol/L)	0.51±0.03	0.49±0.05
Atherogenic index[2]	1.38±0.04	1.06±0.09
Liver		
Cholesterol (mmol/g)	0.270±0.009	0.128±0.009***
Triglyceride (mmol/g)	0.086±0.008	0.048±0.004***

Mean±SE, n=10.
E_8: ethanol diet for 8 wks, E_8H_4: ethanol diet for the first 4 wks and hesperidin-supplemented ethanol diet for the last 4 wks.
[1]HDL-cholesterol/total cholesterol.
[2](Total cholesterol minus HDL-cholesterol)/HDL-cholesterol.
$^{***}p<0.001$ vs. E_8 group based on Student's t-test.

Effect of hesperidin supplementation on hepatic HMG-CoA reductase and ACAT activities

The E_8H_4 diet resulted in lower HMG-CoA reductase and ACAT activities compared to the E_8 group (Fig. 3).

Effect of hesperidin supplementation on antioxidant enzyme activities, glutathione contents and lipid peroxidation

Hepatic SOD, GSH-Px and G6PD activities were significantly higher in the E_8H_4 group compared with the E_8 group (Table 4). However, both groups exhibited no significant differences in the CAT and GR activities. The hepatic total GSH contents were significantly higher in the E_8H_4 group than in the E_8 group.

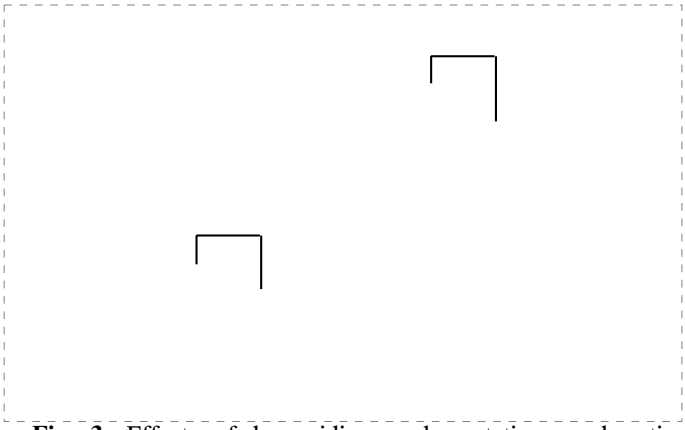

Fig. 3. Effects of hesperidin supplementation on hepatic HMG-CoA reductase and ACAT activities in rats fed ethanol diet.
Mean±SE, n=10.
E_8: ethanol diet for 8 wks, E_8H_4: ethanol diet for the first 4 wks and hesperidin-supplemented ethanol diet for the last 4 wks.
$^{*}p<0.05$ vs. E_8 group based on Student's t-test.

Table 4. Effect of hesperidin supplementation on hepatic antioxidant enzyme activities and hepatic and plasma TBARS levels in rats fed ethanol diet

	E_8	E_8H_4
SOD (units/mg)	0.60±0.06	1.17±0.09**
CAT (μmol/mg/min)	0.11±0.00	0.10±0.00
GSH-Px (nmol/mg/min)	7.89±0.35	10.01±0.26**
G6PD (nmol/mg/min)	11.79±0.76	16.26±1.18*
GR (nmol/mg/min)	52.97±1.26	54.26±1.51
GSH (μmol/g)	2.47±0.06	2.75±0.06*
Plasma TBARS (nmol/mL)	3.17±0.10	2.49±0.05*
Hepatic TBARS (nmol/g)	6.66±0.16	6.34±0.19

Mean±SE, n=10.
E_8: ethanol diet for 8 wks, E_8H_4: ethanol diet for the first 4 wks and hesperidin-supplemented ethanol diet for the last 4 wks.
SOD: superoxide dismutase, CAT: catalase, GSH-Px: glutathione peroxidase, G6PD: glucose-6-phosphate dehydrogenase, GR: glutathione reductase, GSH: glutathione, TBARS: thiobarbituric acid reactive substance.
$^{*}p<0.05$, $^{**}p<0.01$ vs. E_8 group based on Student's t-test.

The plasma and hepatic lipid peroxide levels were determined by measuring the TBARS concentrations. Plasma TBARS levels were significantly lower in rats fed the alcohol with hesperidin compared to the rats fed alcohol only, while the hepatic TBARS levels were not significantly different between the groups.

DISCUSSION

Alcohol is readily absorbed from the gastrointestinal track. Only 2~10% of that absorbed is eliminated through the kidneys and lungs; the rest is oxidized in the body, mainly in the liver. Except for the stomach,

extrahepatic metabolism of ethanol is very low (35).

ADH is the major metabolic enzyme for ethanol disposition in the liver (36). Even though ethanol can be efficiently converted into acetaldehyde by high ADH activity, as an ethanol-inducible enzyme, the subsequent conversion of acetaldehyde into acetate can be delayed due to low ALDH activity. In the current study, we demonstrated that hesperidin supplementation significantly enhanced ADH activity and tended to increase ALDH activity.

Flavonoids, compounds containing a 2-phenylbenzo (gamma) pyrane nucleus, are commonly distributed among vascular plants. There have been few reports on the effects of these compounds on cholesterol metabolism. As shown in tests with cholesterol-fed rats, hesperidin is a potent inhibitior of HMG-CoA reductase and ACAT, and is also beneficial for lowering plasma and hepatic cholesterol levels (37).

The two key enzymes involved in the regulation of cholesterol metabolism are HMG-CoA reductase, the rate-limiting enzyme in the cholesterol biosynthetic pathway, and ACAT, the cholesterol-esterifying enzyme in tissues, including the small intestine. The inhibition of HMG-CoA reductase decreases cholesterol synthesis and its inhibitors are very effective in lowering serum cholesterol in most animal species, including humans (38). The present study indicated that hesperidin supplementation significantly inhibited hepatic cholesterol biosynthesis and esterification. The blockade of cholesterol synthesis by an inhibitor of HMG-CoA reductase results in a lower intracellular supply of cholesterol, thereby triggering an over-expression of hepatic LDL receptors and enhancing the clearance of circulating LDL particles (39). ACAT is another key enzyme involved in the esterification and absorption of cholesterol, secretion of hepatic LDL-cholesterol, and cholesterol accumulation in the arterial wall (40,41). Accumulation of esterified cholesterol is one of the major metabolic changes in atherosclerosis (42). Wilcox et al. (43) and Borradaile et al. (44) have provided evidence that the citrus flavonoids naringenin and hesperetin not only decrease cholesterol synthesis but also inhibit ACAT activity in HepG2 cells. Thus, the inhibition of hepatic ACAT could be one of the mechanisms whereby hesperidin exerts its hypocholesterolemic and hypotriglyceridemic effects.

The development of a fatty liver, a characteristic early sign of alcoholic liver disease, is partially caused by the alcohol-induced suppression of lipid oxidation in the liver and by the increased influx of fat from the peripheral tissues. These early changes are also associated with the typical signs of alcoholic hyperlipidemia, which include elevated serum triacylglycerol levels caused by an increased hepatic secretion of very-low-density lipoproteins (VLDLs) and with the delayed removal of the VLDLs resulting from an impaired lipoprotein lipase (45). In the present study, hesperidin supplements were also found to significantly lower both the hepatic cholesterol and triglyceride levels compared to the ethanol control group.

Hesperidine lowered HMG-CoA reductase and may decrease hepatic triglyceride synthesis, thereby decreasing hepatic cholesterol and triglycerides levels. However, there was no difference in plasma cholesterol and triglyceride concentrations between the groups. These results may suggest that hesperidin supplement could not increase lipoprotein lipase and LDL receptor activity in ethanol fed rats although we did not measure these activities. Exact mechanism needs to be elucidated to verify this phenomenon in detail.

In the present study, the concentration of plasma TBARS were significantly higher after alcohol treatment of the rats, indicating the increase in oxygen free radicals could be due to either their increased production or decreased destruction (46). The level of plasma TBARS in hesperidin-supplemented rats significantly lower, indicating a decreased rate of the plasma lipid peroxidation. Lipid peroxidation has been implicated in a number of conditions including increased membrane rigidity, osmotic fragility, decreased cellular deformability, reduced erythrocyte survival, and lipid fluidity (47).

Abnormal levels of plasma of GOT (aspartate aminotransferase) and GPT (alanine aminotransferase) are of clinical and toxicological importance, being indicative of tissue damage by toxicants or disease conditions. The activities of liver function markers, such as plasma GOT and GPT, were not different between the groups in the current study (data not shown).

Superoxide ion and hydroxyl radical are known to cause marked injuries to the surrounding tissues and organs (48,49). Therefore, removing superoxide ions and hyroxyl radicals is probably one of the most effective defense mechanisms against a variety of diseases (48). Lowered activities of SOD and CAT will result in the accumulation of these highly reactive free radicals leading to deleterious effects such as loss of cell membrane integrity and membrane function (49). A study by Antonekov and Panchenko (50), also reported that hepatic SOD activity was evidently decreased in ethanol-fed rats. This decrease in the SOD activity may be associated with the elevation of the intracellular

concentration of H_2O_2 (50). The decrease in the SOD activity could be due to the oxidative inactivation of the enzyme as a result of excessive reactive oxygen species generation (51) or the generation of the α-hydroxy ethyl radical that inactivates the SOD enzyme (52). SOD activity, which helps scavenge superoxide ions, was significantly higher in hesperidin-supplemented rats (E_8H_4) as compared with control rats (E_8).

The current results showed that the GSH-Px activity was significantly higher in the E_8H_4 group than the E_8 group, indicating a compensatory mechanism in response to increased oxidative stress (53). H_2O_2 detoxification would seem to be critically dependent on GSH-Px in tissue lacking CAT activity (54).

GSH is a major nonprotein thiol in living organisms, which plays a central role in coordinating the antioxidant defense processes in the body. It is involved in the maintenance of normal cell structure and function, probably through its redox and detoxification reactions (55). In the current study, the alcohol-treatment group (E_8) had reduced levels of hepatic GSH as compared to the hesperidin-supplemented alcohol group (E_8H_4), which corresponds to the results reported by Shaw et al. (56). Ethanol administration induces a loss of GSH from the liver and a decrease in its hepatic content (57). These lowered levels may be due to the increased utilization of GSH by antioxidant enzymes such as GSH-Px which scavenge H_2O_2 (58). It is well established that the intracellular GSH level can be depleted under conditions of oxidative stress (59). Consequently, exposure to ethanol, which triggers oxidative stress, may inhibit GSH biosynthesis at a genetic level thereby resulting in a depressed hepatic GSH formation (60). Chronic ethanol feeding causes hepatic GSH depletion and lipid peroxide initiation (60), which agrees with the results of this study, since hepatic GSH content was significantly lower in the ethanol-treated rats. Hesperidin contributes to alleviating the adverse effect of ethanol ingestion by enhancing lipid metabolism as well as the hepatic antioxidant defense system.

For optimal GR activity, NADPH must be supplied in adequate concentrations. Thus, we looked for alterations in G6PD activity and found that it was decreased after chronic ethanol administration. This decrease could be partially explained by the findings of Szweda et al. (61), who described the inactivation of purified bacterial G6PD by trans-4-hydroty-2-nonenal, a toxic product of membrane lipid peroxidation. Considering the physiologic role of this enzyme in supplying NADPH, the coupling of GSH-Px and GR for glutathione recycling in the livers of rats chronically fed ethanol could be hampered by the lower G6PD activity, regardless of the change in GR activity (62).

The present study indicated that the addition of a hesperidin supplement to rats administered an ethanol diet had a significant effect on the hepatic antioxidant enzyme activity, SOD, GSH-Px, and G6PD, along with the plasma TBARS levels in rats. As a result, increased antioxidant enzyme activity induced by the hesperidin supplement can reduce the alcoholic damage by detoxifying reactive oxygen species. In addition, the level of plasma TBARS in the hesperidin group were significantly reduced, indicating decreased rate of lipid peroxidation.

In conclusion, the hesperidin supplement during chronic ethanol feeding led to a decrease in the levels of hepatic lipids and plasma TBARS as well as the hepatic HMG-CoA reductase and ACAT activities compared to the ethanol-fed control rats. This appeared, in part, to be related to changes in the hepatic SOD and GSH-Px activities that were higher in the hesperidin-supplemented ethanol-treated rats. Hesperidin supplementation seemed to be effective for improving the ethanol and lipid metabolism as well as the hepatic antioxidant defense system in rats fed an ethanol diet.

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