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Quercetin Supplement is Beneficial for Altering Lipid Metabolism and Antioxidant Enzyme Activities in the Middle of Ethanol Feeding in Rats

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The current study examined the effect of quercetin supplements on the lipid-lowering and antioxidant metabolism in ethanol-fed rats. The control group(E_8) received ethanol only diet for 8 wks, whereas the other group(E_8Q_4) received a diet including quercetin supplementation(0.05%, wt/wt) for 4 wks while on the ethanol diet for 8wks. The hepatic alcohol dehydrogenase activity was significantly higher in the E_8Q_4 group than in the E_8 group. Supplementation with quercetin significantly elevated the HDL- cholesterol concentration, the HDL-C/total-C ratio, and lowered the atherogenic index(AI) compared to the E_8 group. The hepatic triglyceride and cholesterol contents were significantly lowered by the quercetin supplement compared to those of the control group. The hepatic HMG-CoA reductase and ACAT activities of the E_8Q_4 group were significantly lower than those of the E_8 group. The overall potential for antioxidant defense was significantly enhanced by the quercetin supplement, as indicated by a decrease in plasma and hepatic TBARS levels. The hepatic GSH-Px and G6PD activities were significantly higher in the E_8Q_4 group compared to the E_8 group. The current results suggest that dietary quercetin leads to the inhibition of HMG-CoA reductase and ACAT, which in turn lowers cholesterol levels and normalizes antioxidant enzyme activities.

Key words: Quercetin, Ethanol-diet, HMG-CoA reductase, ACAT, Antioxidant enzyme activity

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INTRODUCTION

Chronic ethanol feeding causes the accumulation of lipids in the liver and lipid peroxides in other tissues. Alcohol also alters lipid metabolism, causing a profound inhibition of lipolysis.[1)] Various hepatic enzymes can catalyze the metabolism of alcohol. These pathways are located in different subcellular compartments of the hepatocytes. The alcohol dehydrogenase pathway (ADH) exists in the cytosol, the microsomal ethanol-oxidizing system (MEOS) is in the endoplasmic reticulum, and catalase is located in the peroxisomes.[2)] Each of these pathways produces specific metabolic and toxic disturbances, resulting in the production of acetaldehyde, a toxic metabolite. 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.[3)]

Flavonoids and phenolics exhibit antitumor and antibacterial activities,[4-5)] along with inhibitory effects on membrane lipid peroxidation.[6)] In the previous paper, we demonstrated that flavonoids, hesperidin, naringin, and its aglycones lowered plasma and hepatic cholesterol levels as well as HMG-CoA reductase activity.[7-8)]

Quercetin (3,5,7,3′,4′-pentahydroxy flavon) is the major flavonoid in the human diet and its daily intake with foods is estimated to be 50–500 mg.[9)] It prevents oxidant injury and cell death[10)] by several mechanisms including scavenging oxygen radicals,[11-12)] protecting against lipid peroxidation,[13-14)] and chelating metal ions.[15)] Researchers have demonstrated that quercetin, when co-administered with ethanol, reduces ethanol-induced hepatic steatosis and lipid peroxidation, suggesting that the gastroprotective effect of quercetin could be due to its antiperoxidative, antioxidant, and antihistaminic effects.[16)] Ethanol is almost exclusively metabolized in the body by enzyme catalyzed oxidative processes.

Accordingly, the present study investigated the

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Table 1. Composition of Lieber-DeCarli liquid alcoholic diet[1] and experimental diets

(g / liter / 1,000 calories)

	Ethanol diet(E)	Ethanol diet+Quercetin(EQ)
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[2]	25.6	25.6
Choline bitartrate	0.53	0.53
Fiber	10.0	10.0
Xanthan gum	3.0	3.0
Vitamin mix[3]	2.55	2.55
Mineral mix[4]	9.0	9.0
Ethanol	50	50
Quercetin	-	0.05

[1] The liquid diet is mixed nutritional ingredient in 1 L distilled water.
[2] Replaced by 25.6 g of dextrin maltose and 50 g of ethanol in the ethanol formula
[3] Vitamin mixture according to AIN-76
[4] Mineral mixture according to AIN-76

possible role of dietary quercetin on hepatic alcohol metabolizing enzyme activities, hepatic antioxidant enzyme activities, and the plasma and hepatic lipid profiles in rats fed an ethanol diet.

MATERIALS AND METHODS

1. Animals and Diets

Twenty male Sprague-Dawley rats weighing between 140 and 150 g were purchased from Orients. Inc. (Seoul, Korea). The animals were all individually housed in stainless steel cages in an air-conditioned room with controlled temperature (20~23 ℃) and automatic lighting (alternating a 12-h period of light and dark) and fed a pelletized chow diet for 1 week after arrival. Then, the animals were randomly divided into two groups (n=10), which were assigned to E_8: ethanol diet for 8 weeks and E_8Q_4: ethanol diet for the first 4 weeks and with quercetin supplemented ethanol diet for the last 4 weeks. The E_8 group consumed a liquid diet[17] shown in Table 1 providing 36% of energy as ethanol ad libitum. 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 given an ethanol diet (50 g/L) for the next 8 weeks after the adaptation period. The E_8Q_4 group was given an ethanol diet with quercetin supplement (0.05 g, w/v; Arian Quimica Co., Spain) for the last 4 weeks. 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-h 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 ℃. The livers were then removed, rinsed with physiological saline, and weighed for enzyme analysis, lipid measurement, and morphological analysis. The plasma and livers were stored at −70 ℃ until analysis.

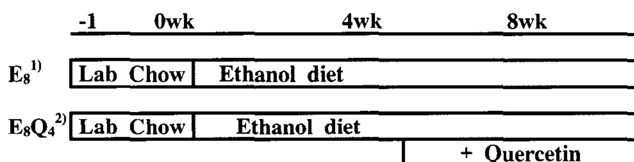

Fig. 1. Experimental schedule for feeding ethanol diet with quercetin supplements

[1] E_8: ethanol diet for 8wks
[2] E_8Q_4: ethanol diet for the 8wks with quercetin supplementation for 4wks

2. Hepatic Alcohol Dehydrogenase and Aldehyde Dehydrogenase Activities

The alcohol dehydrogenase (ADH) activity was assayed using Bergmeyer's method.[18] 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 was assayed using Koivula and Koivusalo's method.[19]

3. Plasma and Hepatic Lipids

The plasma total cholesterol and high-density lipoprotein (HDL)-cholesterol concentrations were determined using a commercial kit (Sigma) based on a modification of the cholesterol oxidase method of Allain et al.[20] The HDL-fractions were separated using a Sigma kit based on the heparin-manganese precipitation procedure.[21] The plasma triglyceride concentrations were measured enzymatically using a kit from Sigma Chemical Co., a modification of the lipase-glycerol phosphate oxidase method.[22] The hepatic lipids were extracted using the procedure developed by Folch et al..[23] 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 as used in the plasma analysis.

4. 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[24] 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 homogenates were centrifuged for 15 min at 10,000 × g and 12,000 × g at 4 ℃. Then, the supernatants were ultracentrifuged twice at 100,000 g for 60 minutes at 4 ℃. The resulting microsomal pellets were then redissolved in 1 mL of a homogenation buffer for protein determination[25] and finally analyzed for their HMG-CoA reductase and ACAT activities.

The HMG-CoA reductase activities were determined as described by Shapiro et al.[26] 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) was preincubated at 37 ℃ for 5 minutes. Then, 10 µL of 50 nmol [^{14}C]HMG-CoA (specific activity, 2.1083 GBq/mmol; NEN™ Life Science Products, Boston, MA) was added, and the incubation was continued for 15 minutes at 37 ℃. The reaction was terminated by the addition of 15 µL of 10 mol/L HCl, and the resultant reaction mixture was incubated at 37 ℃ 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 mevalonolactone 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 1600 TR; Packard Instrument, Meriden, CT). The results were 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.[27] and modified by Gillies et al.[28] 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. Then, reaction mixtures containing 20 µL of the cholesterol solution (6 µg of cholesterol), 20 µL of a 1 mol/L 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 ℃ for 30 min. The reaction was then initiated by adding 20 µL of 5.62 nmol [^{14}C]-oleoyl CoA (specific activity; 1.9795 GBq / mmol; NEM™ Life Science Products) to a final volume of 200 µL; and the reaction time was 30 min at 37 ℃. The reaction was stopped by adding 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.

5. 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; the homogenates was then 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 for a catalase (CAT) assay. The supernatant was ultra-centrifuged at 105,000 × g for 1 h to isolate the cytosolic fraction for superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) assay. The activities of the antioxidant enzymes were measured using the following methods.

The SOD activity was measured using Marklund and Marklund's[29] method with a slight modification. One hundred microliters of the cytosol supernatant were mixed with 1.5 mL of a Tris-EDTA-HCl buffer (pH 8.5); then 100 µl of 7.2 mmol/L pyrogallol was added and the reaction mixture was incubated at 25 ℃ for 10 min. The reaction was terminated by addition of 50 µl of 1 mol/L HCl and measured at 420 nm. One unit of enzyme activity was calculated as the protein content inhibiting 50% of the auto-oxidation of pyrogallol without an enzyme source. The activity was expressed as U/mg protein. CAT activity was measured using Aebi's[30] method with a slight modification, in which the disappearance of hydrogen peroxide was monitored spectrophotometrically at 240 nm for 5 min. A molar extinction coefficient of 0.041 $mM^{-1}cm^{-1}$ was used to determine the CAT activity. The activity was defined as the amount of enzyme that oxidized H_2O_2 µmol/min/mg protein. The GSH-Px activity was measured using Paglia and Valentine's[31] method with a slight modification. The

reaction mixture contained 2.6 mL of 0.1mol/L Tris-HCl (pH 7.2) buffer, 100 µl of 30 mmol/L glutathione, and 100 µl of 6 mmol/L NADPH. One hundred microliters of the cytosolic supernatant were added to 2.9 mL of the reaction mixture and incubated at 25 ℃ for 5 min. The reaction was initiated by addition of 100 µl of 7.5 mmol/L H_2O_2 and the absorbance was measured at 340 nm for 5 min. A molar extinction coefficient of 6.22 × 103 $(mmol/L)^{-1} \cdot cm^{-1}$ was used to determine the activity. One unit of GSH-Px was defined as the amount of enzyme which oxidized 1 µmol per min per mg protein. Glutathione reductase (GR) activity was determined with the method of Pinto and Bartley[32] 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 as oxidized NADPH nmol/min/mg protein. Glucose-6-phosphate dehydrogenase (G6PD) activity was determined with the method of Pitkanen et al.[33] 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 as reduced NADPH nmol/min/mg protein.

6. Plasma and Hepatic Lipid Peroxidation (TBARS assay)

The TBARS (thiobarbituric acid-reactive substances) were monitored according to the procedure previously described.[34] 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 ℃ for 90 min, the samples were cooled at room temperature, centrifuged at 1,000 × g for 15 min at 4 ℃, and the supernatant absorbance was read at 535 nm.

The levels of hepatic lipid peroxide were determined using the method of Ohkawa et al.[35] with a slight modification. Tissue homogenates were prepared in a ratio of 1 g of wet tissue to 4 mL of 1.15% KCl solution using a glass or Teflon Potter-Elvehjem homogenizer. The 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, and then mixed with 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of 0.8% aqueous solution of TBA, and finally heated at 95 ℃ 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 1000 × g rpm for 10 min, the absorbance of the upper layer was measured at 535 nm. A malondialdehyde (MDA) solution made freshly by the hydrolysis of 1,1,3,3-tetramethoxypropane (TMP, Sigma) was used as the standard. The results were expressed as the nmol MDA/mL plasma and nmol MDA/g liver.

7. Measurement of Glutathione (GSH) Content

GSH content was measured using the method of Ellman.[36] Five hundred microliters of the liver homogenate were mixed with 500 µL of 4% (w/v) sulfosalicylic acid and centrifuged at 600 × g for 10 min. Three hundred microliters of the supernatant 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.

8. Statistical Analysis

The parameter values were all expressed as the mean ± SE. Significant differences among the groups were determined by a Student's t-test using the SPSS package program. Statistical significance was considered at $p<0.05$.

RESULTS

1. Effect on Food Intake, Weight Gain and Liver Weights

There was no significant difference in the food intake, weight gain, or organ weight between the groups (Table 2). However, the heart weights of the E_8Q_4 group were significantly higher compared to those of the E_8 group.

2. Effect on Hepatic Alcohol Metabolizing Enzyme Activities

The hepatic alcohol dehydrogenase activity was significantly higher in the E_8Q_4 group than in the E_8 group, but aldehyde dehydrogenase activity was not significantly changed by quercetin supplementation as

Table 2. Effect of quercetin supplements on weight gains, food intake and organ weight in rats fed ethanol diet[1)]

	Groups	
	E_8[2)]	E_8Q_4
Weight Gain (g/day)	2.50 ± 0.16	2.08 ± 0.28
Food Intake (mL/day)	63.68 ± 1.77	63.60 ± 1.34
Organ Weight (g/100g B.W.)		
Liver	3.70 ± 0.10	3.84 ± 0.14
Heart	0.32 ± 0.007	0.36 ± 0.008*
Kidney	0.71 ± 0.02	0.75 ± 0.02

1) Mean ± SE, n=10
2) E_8: ethanol diet for 8wks, E_8Q_4: ethanol diet for the 8wks with quercetin supplementation for 4wks
* p<0.05 vs E_8

Table 3. Effect of quercetin that supplemented from the 5th week on hepatic alcohol metabolizing enzyme activities in rats fed ethanol diet for 8 weeks[1)] (nmol/mg/min)

	Groups	
	E_8[2)]	E_8Q_4
Alcohol dehydrogenase	3.57 ± 0.09	4.79 ± 0.34*
Aldehyde dehydrogenase	4.06 ± 0.47	4.79 ± 0.25

1) Mean ± SE, n=10
2) E_8: ethanol diet for 8wks, E_8Q_4: ethanol diet for the 8wks with quercetin supplementation for 4wks
* $p<0.05$ vs E_8

Table 4. Effect of quercetin that supplemented from the 5th week on plasma and hepatic lipids in the rat fed ethanol diet for 8 weeks[1)]

	Groups	
	E_8[2)]	E_8Q_4
Plasma		
Total cholesterol (mmol/L)	2.62 ± 0.09	2.57 ± 0.04
HDL-cholesterol(mmol/L)	1.102 ± 0.04	1.561 ± 0.06*
HDL-C / Total-C[3)] (%)	42.06 ± 0.91	60.73 ± 0.96*
Triglyceride (mmol/L)	0.51 ± 0.03	0.46 ± 0.04
Atherogenic index[4)]	1.38 ± 0.04	0.65 ± 0.06*
Liver		
Cholesterol (mmol/g)	0.27 ± 0.009	0.132 ± 0.018*
Triglyceride (mmol/g)	0.086 ± 0.008	0.060 ± 0.008*

1) Mean ± SE, n=10
2) E_8: ethanol diet for 8wks, E_8Q_4: ethanol diet for the 8wks with quercetin supplementation for 4wks
3) HDL-cholesterol/total cholesterol
4) (Total cholesterol minus HDL-cholesterol)/HDL-cholesterol
* $p<0.05$ vs E_8

Table 5. Effect of quercetin that supplemented from the 5th week on hepatic antioxidant enzymes and hepatic and plasma TBARS levels in rats fed ethanol diet for 8 weeks[1)]

	Groups	
	E_8[2)]	E_8Q_4
SOD (units/mg)	0.60 ± 0.06	0.87 ± 0.12
Catalase (μmol/mg/min)	0.11 ± 0.002	0.12 ± 0.003
GSH-Px (nmol/mg/min)	7.89 ± 0.35	11.14 ± 0.38*
G6PD (nmol/mg/min)	11.79 ± 0.76	16.76 ± 1.65*
GR (nmol/mg/min)	52.97 ± 1.26	55.26 ± 1.30
Plasma TBARS (nmol/mL)	3.17 ± 0.10	2.48 ± 0.04*
Hepatic TBARS (nmol/g)	6.66 ± 0.16	5.76 ± 0.21*

1) Mean ± SE, n=10
2) E_8: ethanol diet for 8wks, E_8Q_4: ethanol diet for the 8wks with quercetin supplementation for 4wks
* $p<0.05$ vs E_8

shown in Table 3.

3. Effect on Plasma and Hepatic Lipids

Supplementation of quercetin to the ethanol treatment significantly elevated the HDL-cholesterol concentration and the HDL-C/total-C ratios, so the atherogenic index was significantly lower than that of the E_8 group (Table 4). The concentrations of the plasma cholesterol and triglyceride tended to be lowered in the E_8Q_4 group compared to the E_8 group. However, the hepatic triglyceride and cholesterol contents were significantly lowered by quercetin supplement (Table 4).

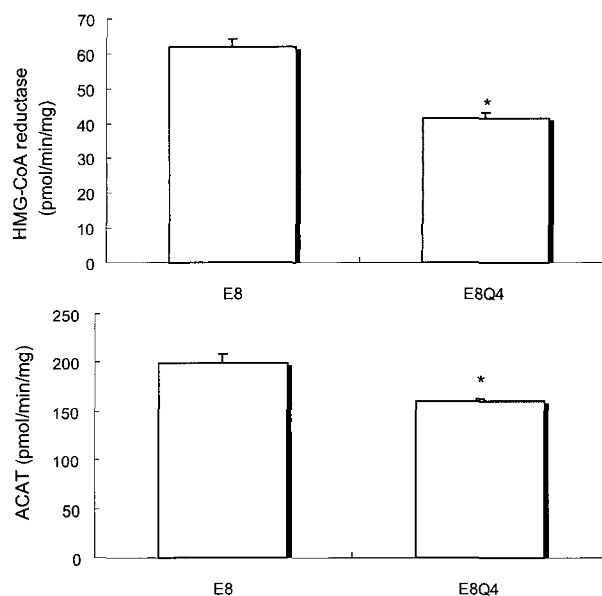

Fig. 2. Effects of quercetin that supplemented from the 5th week on hepatic HMG-CoA reductase (a) and ACAT (b) activities in rat fed ethanol diet for 8 weeks[1,2]
[1]Mean ± SE, n=10. [2]E_8: ethanol diet for 8wks, E_8Q_4: ethanol diet for the 8wks with quercetin supplementation for 4wks
* $p<0.05$ vs E_8

4. Effects on Hepatic HMG-CoA reductase and ACAT Activities

The hepatic HMG-CoA reductase and ACAT activities were significantly lowered in the E_8Q_4 group compared to the E_8 group (Figure 2). Accordingly, these hepatic cholesterol regulating enzyme activities were significantly suppressed by quercetin supplement.

5. Effects on Antioxidant Enzyme Activities, TBARS and Glutathione Contents

Regarding the hepatic antioxidant enzyme system, the hepatic GSH-Px and G6PD activities were significantly higher in the E_8Q_4 groups compared to the E_8 group. However, the hepatic SOD, GR and catalase activities were not different between the groups. In contrast, the plasma and the hepatic TBARS levels were significantly lower in the E_8Q_4 group than in the E_8 group (Table 5). Hepatic total glutathione content was significantly higher in the E_8Q_4 group than in the E_8 group (Figure 3).

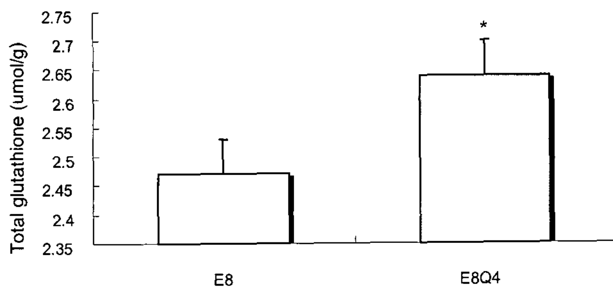

Fig. 3. Effect of quercetin that supplemented from the 5th week on hepatic total glutathione content in rats fed ethanol diet for 8 weeks

Mean ± SE, n=10, * $p<0.05$ vs E_8

E_8: ethanol diet for 8wks, E_8Q_4: ethanol diet for the 8wks with quercetin supplementation for 4wks

DISCUSSION

Alcohol abuse and alcoholism are serious health and socioeconomic problems. Despite great progress made in the treatment and prevention, the development of suitable medications for the treatment of alcoholism remains a challenging goal for alcohol research. The liver is considered to be the main organ responsible for oxidizing alcohol. Early changes are also associated with the typical signs of alcoholic hyperlipidemia, which include elevated serum triacylglycerol levels caused by increased hepatic secretion of very-low-density lipoproteins (VLDLs) and delayed removal of the VLDLs resulting from an impaired lipoprotein lipase.[37] The cholesterol-lowering activity of flavonoids was also identified in a preliminary study by the current authors.[7-8]

Supplementation with quercetin significantly raised the HDL-cholesterol level and HDL-C/total-C ratio, while it lowered the AI value. Dietary quercetin supplement was offset alcohol induction by increasing HDL-C level in addition to raising alcohol dehydrogenase activiry. Quercetin supplement in ethanol-treated rats significantly lowered both the hepatic cholesterol and triglyceride levels when supplemented in the middle of ethanol feeding.

The liver is the major site for the synthesis and net excretion of cholesterol using negative feedback control. 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. They play key roles in intracellular cholesterol storage, in lipoprotein assembly in the liver, and in the development of fatty streaks in arteries. Hence, the inhibition of ACAT may represent an attractive mechanism for inducing both hypolipidemic and antiatherosclerotic effects.[38-39] The present study demonstrated that the supplementation of quercetin significantly inhibited hepatic cholesterol biosynthesis and esterification. Quercetin is a potent agent for the inhibition of HMG-CoA reductase and ACAT activities, while also lowering plasma and hepatic lipid levels. As demonstrated by the current results 0.05% (wt/wt) quercetin supplementation is sufficient to exert its hypocholesterolemic effect in ethanol-treated rats.

Quercetin has a potent protective activity on injury triggered by xanthine/xanthine oxidase system,[40] cyclo-oxygenase and lipoxygenase enzymes,[41] and effectively scavenges ·OH and $O_2^{-\cdot}$, singlet oxygen, terminates the radical chain reaction, and chelates metal ions. The levels of plasma and hepatic TBARS in the quercetin-fed rats showed a significant reduction, indicating a decreased rate of lipid peroxidation. Therefore, quercetin, either by scavenging or by inhibiting the production of ROS, decreases the TBARS and increases the antioxidant levels. Dietary quercetin significantly affected hepatic GSH-Px activity, along with the plasma and hepatic TBARS levels. SOD converts superoxide radicals into hydrogen peroxide, which is then converted to water by both CAT and GSH-Px. Chronic ethanol feeding resulted in lowering GSH-Px activity with elevated GR activity. According to others results,[42] although the increase in the GR activity can promote the recycling of glutathione for the active detoxification of xenobiotics, the decrease in GSH-Px activity may attenuate the radical scavenging function. In the current study, the hepatic SOD, GR, and catalase activities were slightly upregulated by the quercetin supplementation through, in part, the detoxifying reactive oxygen species GSH is a major non-protein 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.[43] In the present study, rats on alcohol treatment exhibited low levels of tissue corresponding to the results reported by Shaw et al..[44] Regardless of exposure to ethanol, which triggers oxidative stress, quercetin supplement increased hepatic GSH content

In conclusion, quercetin supplement in the middle of an ethanol diet led to a decrease in the levels of plasma and hepatic lipids and plasma TBARS as well as the hepatic HMG-CoA reductase and/or ACAT activities

compared to rats fed only on ethanol. Quercetin supplement, as indicated by this study, apparently alleviates the adverse effects of ethanol ingestion by enhancing the lipid metabolism as well as the hepatic antioxidant defense system, even when added during later part of ethanol feeding in an experimental animal model.

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