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Comparative Effects of Dietary Quercetin and Rutin in Rats Fed with the Lieber-DeCarli Ethanol Diet

Su-Jeong Seo[1], Cheol-Ho Park[1], In-Young Ko[1], Yeon-Ho Jeong[1], and Yong-Soon Choi[1,2,*]

[1]*Division of Biomedical Convergence, College of Medical Biosciences, Kangwon National University, Chuncheon 24341, Korea*
[2]*Institute of Medical Bioscience, Kangwon National University, Chuncheon 24341, Korea*

Abstract – Flavonoids including quercetin and rutin are a group of naturally occurring compounds widely distributed in plants, especially in buckwheat. Thus, cereal and the leaf of the plant have increasingly used as a source of nutritional and functional foods such as noodle, cake or soup in Korea, Japan and other countries. This study investigated comparative effects of dietary rutin rich in buckwheat and its aglycone, quercetin, on serum biomarkers and antioxidant parameters in rats treated with chronic ethanol. Rats were fed with the liquid diets prepared by the method of Lieber Decarli. Serum alanine transaminase (ALT) and aspartate transaminase (AST) activities increased significantly by alcohol feeding. Dietary flavonoids including rutin, quercetin and their mixtures (1/1, v/v) decreased significantly the activities of serum ALT whereas the feeding of quercetin decreased only the activity of serum AST. The concentration of serum malondialdehydes elevated by chronic alcohol feeding decreased markedly in all the experimental groups that were fed with the flavonoids; however, the combined administration of quercetin or rutin, but not that of rutin or quercetin alone decreased significantly the concentration of liver malondialdehydes to the normal range in rats fed without ethanol. Our results suggested that dietary combined mixture of rutin and quercetin might be effective in ameliorating adverse responses seen in rats exposed to ethanol chronically.
Keywords – Ethanol, Liver, Quercetin, Rutin, Rats

Introduction

Flavonoids are a group of naturally occurring compounds widely distributed in plants. Especially, buckwheat is one of plants rich in rutin, the glycoside of quercetin, than others, whereas quercetin is abundant highly in fruits and vegetables such as apples and onions.[1-3]

Together with these, quercetin, aglycone structure of the rutin, is one of the most prominent dietary antioxidant, *in vitro* by chelating metal ions and/or scavenging free radicals, in vivo by scavenging highly reactive species such as peroxynitrite and the hydroxy radicals after absorption into the cells, suggesting the possible beneficial potential applications.[1-4]

It has reported that different sugar moiety of the flavonoids might influences primarily not only its retention time but also its absorption rates in the intestine: rutin was absorbed more slowly because of the digestion of microflora in the large intestine. Rutin is hydrolyzed to quercetin in the intestine, absorbed as quercetin and is present as the conjugate metabolites of quercetin, suggesting that rutin and quercetin might also affect differently the intestinal environments due to different absorption mechanism.[5-7]

There appears to be increasing support that ethanol toxicity may be associated with an increased production of reactive oxygen intermediates. Chronic drinking of ethanol impairs not only liver and intestine functions, but also their interactions, leading to organ damages.[8] The liver is the main metabolic organ of alcohol ingested, and therefore predisposed to liver cell damage. Thus, the biochemical markers in the liver and the serum has widely been used as effective methods of an early diagnosis of the liver diseases in determining functional components for prevention or treatment of hepatic lesions or chronic alcoholism.[1,2,9-11]

In this study, we evaluated the biological effects of dietary quercetin and rutin in rats exposed to ethanol chronically. Liquid diet used in this study has accepted as efficient regimens to elicit a reproducible animal model

*Author for correspondence
Yong-Soon Choi, Division of Biomedical Convergence, College of Medical Biosciences, Kangwon National University, Chuncheon 24341, Korea.
Tel: +82-33-250-6482; E-mail: yschoi@kangwon.ac.kr

for the study of several pathological events, especially observed in heavy drinkers.[12]

Experimental

Animals and diets – Male specific pathogen free Sprague-Dawley rats, 5 weeks old were purchased from Japan SLC (Shizuoka, Japan). They were acclimated to standard chow for 1 week, and then divided into four groups of six rats each, designated as alcohol-free group (Normal), ethanol group (Control), ethanol plus quercetin (Quercetin) , and ethanol plus rutin (Rutin), and ethanol plus the equivalent mixtures of rutin and quercetin (R+Q). The experimental diets were prepared according to the Lieber-Decarli rat liquid diet method,[12] and prepared fresh every day prior to use (Table 1). For experimental groups, final concentration of quercetin (0.5%, Sigma-Aldrich, St. Louis, USA), rutin (0.9%, Sigma-Aldrich, St. Louis, USA), and the mixtures of quercetin (0.25%) and rutin (0.45%) were adjusted to the liquid diet. The dose of rutin as aglycone was equivalent to that of quercetin among groups. Rats were housed individually in a temperature-controlled room (23 – 25 °C) with a 12/12 h light/dark cycle. Food intake and body weight were measured every 2 days. They had free access to food and water throughout the experimental period. The animal protocol used in this study was reviewed and approved by the Kangwon national University Institutional Animal Care and Use Committee (Approval Number: KIACUC-10-0001) regarding the care of the laboratory animals and ethical procedures. After 6 weeks of experimental diets, rats were fasted for 12 h and then decapitated.

Serum and hepatic biochemical assays – Livers were excised, washed and stored at −70 °C until analysis. Blood samples were collected in sterile test tubes and centrifuged at 1,500 × g for 15 min to obtain serum samples. Serum alanine transaminase (ALT) and aspartate transaminase (AST) activities were measured using commercially available kits (Asan Pharm., Co., LTD, Seoul, Korea). The concentration of serum total cholesterol, triacylglycerol (Asan Pharm., Seoul, Korea), high density lipoprotein (HDL)-cholesterol (Daiichi Pure Chemical Industries, Co. Ltd., Tokyo, Japan), and non-esterified fatty acid (NEFA, Wako Pure Chemical Industries, Co. Ltd., Osaka, Japan) were measured using commercially available kits. Liver lipid extracts were dissolved in acetone containing Triton X-100,[13] and then liver lipids were enzymatically determined described as above. Lipid peroxide levels of the serum and liver were determined following to the protocols, as described by Yagi.[14] The protein content was estimated by the method of Lowry *et al*.[15]

Statistical analysis – Values are presented by mean ± standard error of mean (SEM). Comparisons between groups were made using one-way analysis of variance. Differences between means were assessed by the least significant difference method when the F value was significant ($P < 0.05$).

Result

Table 2 shows the growth performance and food intake. In this study, rats were fed with the liquid diets prepared

Table 1. Diet composition (g/L)

	Normal	Experimental			
		Control	Quercetin	Rutin	Q+R
Casein	41.4	41.4	41.4	41.4	41.4
L-Cystine	0.5	0.5	0.5	0.5	0.5
DL-Methionine	0.3	0.3	0.3	0.3	0.3
Corn oil	8	8	8	8	8
Olive oil	15	15	15	15	15
Dextrin-maltose	153	64	64	64	64
Fiber(cellulose)	10	10	10	10	10
Xanthan gum	3	3	3	3	3
Choline bitartrate	0.53	0.53	0.53	0.53	0.53
Vitamin mix[1]	2.55	2.55	2.55	2.55	2.55
Mineral mix[2]	9	9	9	9	9
Ethanol[3]	0	48	48	48	48
Rutin	0	0	9	0	4.5
Quercetin	0	0	0	5	2.5

[1]AIN-76 vitamin mix, [2]AIN-76 Mineral mix, [3]Ethanol 95%.

Table 2. Body weight and growth parameters

Parameters	Normal	Experimental			
		Control	Quercetin	Rutin	Q+R
Initial body weight (g)	188 ± 5[a]	199 ± 4[a]	187 ± 3[a]	188 ± 3[a]	188 ± 3[a]
Weight gain (g)	244 ± 12[b]	125 ± 9[a]	133 ± 14[a]	136 ± 11[a]	132 ± 17[a]
Food intake (ml/days)	110 ± 3[b]	78.1 ± 1.7[a]	85.2 ± 3.5[a]	85.8 ± 2.7[a]	87.4 ± 3.2[a]
FER	0.054 ± 0.001[b]	0.039 ± 0.002[a]	0.038 ± 0.003[a]	0.038 ± 0.002[a]	0.036 ± 0.003[a]
Relative liver weight (g/100g BW)	2.73 ± 0.08[a]	2.92 ± 0.05[ab]	3.0 ± 0.1[bc]	3.24 ± 0.06[c]	3.16 ± 0.10[c]

Mean ± S.E. of 6 rats.
[a,b,c]Values in a same line without superscript letters denote significant difference ($p<0.05$).
FER: feed efficiency ratio.

Table 3. The concentration of serum lipids

	Normal	Experimental			
		Control	Quercetin	Rutin	Q+R
TC (mg/dL)	52.2 ± 2.6[a]	69.5 ± 4.8[b]	63.0 ± 3.8[b]	50.8 ± 1.9[a]	64.3 ± 3.5[b]
HDL-C (mg/dL)	33.2 ± 1.3[ab]	39.6 ± 3.3[b]	32.3 ± 2.1[ab]	27.8 ± 2.1[a]	28.5 ± 1.8[a]
HDL-C/TC (%)	63.9 ± 2.5[c]	55.5 ± 2.5[b]	54.7 ± 3.4[b]	51.3 ± 0.9[ab]	45.0 ± 3.6[a]
TG (mg/dL)	100 ± 20[a]	76.9 ± 10.1[a]	84.7 ± 8.1[a]	79.8 ± 6.9[a]	73.3 ± 6.0[a]
NEFA (mEq/L)	1188 ± 45[a]	1265 ± 127[a]	1072 ± 171[a]	1192 ± 124[a]	894 ± 146[a]

Mean ± S.E. of 6 rats.
[a,b,c]Values in a same line without superscript letters denote significant difference ($p < 0.05$).
TC: total cholesterol, HDL-C: high density lipoprotein-cholesterol, TG: triacylglycerol, NEFA: non-esterified fatty acid.

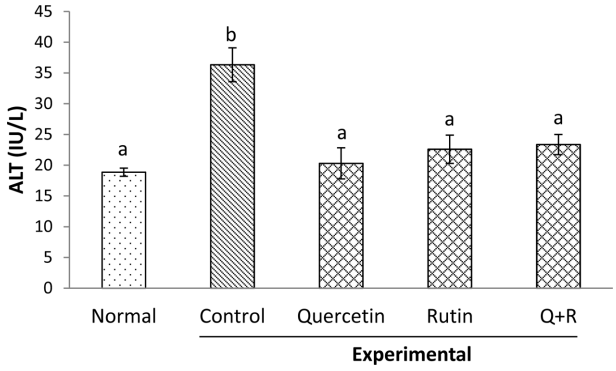

Fig. 1. The activities of serum ALT in rats treated with ethanol. [a,b]Values in a bar without superscript letters denote significant difference ($p < 0.05$).

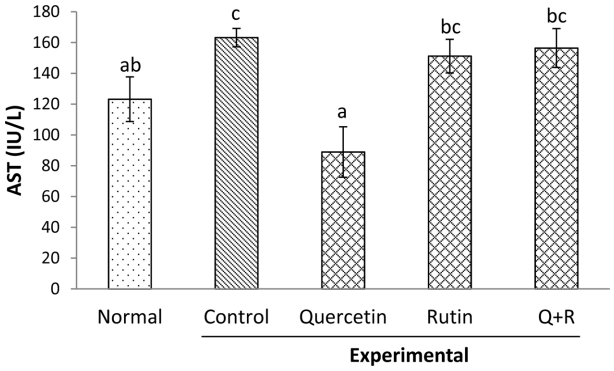

Fig. 2. The activities of serum AST in rats treated with ethanol. [a,b,c]Values in a bar without superscript letters denote significant difference ($p < 0.05$).

according to Lieber and DeCarli.[12] Ethanol feeding decreased significantly weight gain, food intake and FER, regardless of flavonoids ingestion, whereas it resulted in the higher relative liver weight in groups that were fed with rutin, and the mixture (Q+R).

Table 3 shows the concentration of serum lipids in rats treated with ethanol. Ethanol increased the concentration of total cholesterol in all the groups, but not in rutin-fed group, in which cholesterol level was similar to that of the normal group. On the other hand, ethanol decreased the ratio of serum HDL-/total-cholesterol and triacylglycerol, regardless of flavonoids feeding. Flavonoids seemed not to modulate largely ethanol effects in the serum lipids parameters. There were no significant differences in the concentration of serum free fatty acids.

Fig. 1 and 2 show the activities of serum ALT and AST in rats. Ethanol induced significantly the activities of serum AST and ALT in rats, indicating extensive cellular damages in organs of rats. Although the activity of serum ALT decreased significantly by all the flavonoid compounds tested, there were no differences in their responses. Interestingly, only the quercetin ingestion showed to

Table 4. The concentration of hepatic lipids

	Normal	Experimental			
		Control	Quercetin	Rutin	Q+R
Cholesterol (mg/g)	5.7 ± 0.5[a]	11.0 ± 1.9[b]	9.0 ± 1.3[ab]	9.1 ± 1.5[ab]	9.7 ± 2.2[ab]
Triacylglycerol (mg/g)	60.4 ± 7.2[a]	66.8 ± 8.1[a]	57.7 ± 10.8[a]	54.6 ± 10.3[a]	52.1 ± 10.9[a]

Mean ± S.E. of 6 rats.
[a,b]Values in a same line without superscript letters denote significant difference ($p < 0.05$).

Table 5. The concentration of liver and serum malondialdehydes (MDA).

	Normal	Experimental			
		Control	Quercetin	Rutin	Q+R
Serum (nmol/ml)	15.7 ± 7.8[ab]	33.0 ± 7.7[b]	12.5 ± 4.3[a]	13.7 ± 5.7[a]	16.7 ± 4.3[ab]
Liver (nmol/ mg protein)	8.3 ± 0.4[ab]	11.9 ± 1.3[abc]	13.7 ± 3.0[bc]	14.4 ± 2.9[c]	7.6 ± 0.9[a]

Mean ± S.E. of 6 rats.
[a,b,c]Values in a same line without superscript letters denote significant difference ($p < 0.05$).

decrease the activity of serum AST effectively to the range of normal rats not exposed to ethanol.

Table 4 shows the concentration of cholesterol and triacylglycerol in the liver. Ethanol increased significantly the concentration of liver cholesterol, and there were moderate lowering effects in all the flavonoids-fed groups. The concentration of liver triacylglycerol did not change largely by ethanol or flavonoids ingestion.

Table 5 shows the concentration of serum malondialdehydes (MDA) in the liver and serum. Ethanol increased significantly the level of the serum MDA, whereas the flavonoids lowered efficiently the concentration of serum MDA induced by ethanol ingestion. On the other hand, ethanol tended to increase the liver MDA content. The level of the liver MDA of rats ingested with the mixtures of rutin and quercetin were similar to that of the liver MDA of the normal group.

Discussion

This study conducted to investigate the biological effects of quercetin, rutin and/or their mixture (Q+R) on chronic ethanol-induced liver injury in rats. Our results showed that supplementation of the mixture (Q+R), rather than that of quercetin or rutin alone, might exert more beneficial effects, judged from the response of antioxidative actions, on chronic ethanol-induced liver injury in rats. As shown in our results, all ethanol fed groups had the lower food intake and FER than normal group, and flavonoids ingested did not influence meaningfully on growth parameters. It has reported that the reduction in weight gain in rats fed with ethanol was due to the loss of the adipose tissue content.[16,17]

In this study, an ethanol-dependent increase in the concentration of serum cholesterol and free fatty acids, and hypocholesterolemic action of rutin were previously reported in animals including rats.[1,17,18] On the other hand, the concentration of serum triacylglycerol showed a contradictory response to that of serum cholesterol. An impaired hepatic production of lipoproteins by ethanol might lead ultimately to the reduction of the secretion of products containing triacylglycerol into the circulation.[18,19] In this study, favorable effects of rutin and/or quercetin were obscure in lipids parameters determined, in contrast to the data reported previously.[9,10] It may be possible that the differences in dosage or drinking pattern of ethanol affected biological potentials of dietary flavonoids.

An alteration in activities of serum ALT, which is only present in the hepatocyte cytoplasm or AST, which distribute in both the hepatocyte cytoplasm and mitochondria, has regarded as the clinical indicator of the leakage of cellular enzymes occurred from the enhanced permeability, and injury and necrosis of tissues. Especially, serum ALT has been regarded as a sensitive marker for the diagnosis of alcohol-induced hepatic diseases.[11,16] Our data showed that all the feeding of quercetin, rutin or the mixture reduced effectively the activities of serum ALT to the normal extent, suggesting an effectiveness of quercetin and rutin as potential applications.[1,3,4] Furthermore, dietary quercetin seemed to protect effectively hepatic subcellular damage induced by ethanol, in agreement with the data reported previously by Tang *et al*.,[9] in which they demonstrated hepato-protective effects of quercetin through hepatic mitochondrial damage in rats fed with ethanol.

Liver injuries due to ethanol feeding seems to be initiated by a hepatic necro-inflammatory cascade, which

is triggered by circulating gut-derived endotoxin; ethanol promotes intestinal oxidative stress, giving rise to the impairment of intestinal barrier.[20-22] Available reports[6-8] have indicated that rutin, unlike quercetin, is slowly absorbed in the large intestine after additional hydrolysis procedure. On the other hand, dietary quercetin is absorbed from rat stomach.[5] It suggests that quercetin may be fast acting than rutin, when they are administered as single dose. Instead, long-term feeding of rutin that stays in intestinal lumen longer than quercetin may create an environment that does not favor oxidative stress in gut cells. Alternately, dietary rutin may lessen portal influx of oxidative products occurred from ethanol-induced oxidative injury in the intestine into the liver.[23] It may ameliorate hepatic metabolic loads to handle possible oxidative products in status of chronic ethanol feeding.

In this study, the level of serum MDA, but not liver MDA, increased significantly by ethanol feeding. Anti-oxidant effects of flavonoids ingested were obvious in concentration of serum MDA, but the combined mixture of rutin and quercetin lowered only the concentration of liver MDA to normal ranges, indicating synergistic effects of the combined mixtures of rutin and quercetin against ethanol-induced oxidative stress. It is possible that rutin would enhance the hepato-protective effects of quercetin.

Lipid peroxidation, measured by evaluating the concentration of serum MDA may reflect the MDA products originated from oxidative injury of the liver to some extent. [9,10,20] However, our results suggested that the concentration of liver MDA did not necessarily correlate to the concentration of serum MDA or the activities of serum ALT and AST. Interestingly, the apparent response of serum ALT was similar to that of serum MDA among groups. It is possible that markers such as serum MDA and serum ALT indicated generally the size of the hepatic damages, partly including possible damages of other tissues of the body evoked by ethanol.[8,16] Vuppalanchi et al.[24] showed that oxidative stress analyzed from blood samples was poorly reflective of liver tissue oxidative stress.

In conclusion, our results showed that dietary rutin and quercetin showed to ameliorate and elevation of the activities of serum enzyme markers and the content of serum MDA; the combined administration of quercetin and rutin strengthened especially their antioxidative action in the liver than that of quercetin or rutin alone. Our results suggested that dietary combined mixture of rutin and quercetin might be effective in protecting the adverse responses seen in organs of rats exposed to ethanol chronically.

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