Effects of Korean *Citrus junos* and Medicinal Herbs on Liver Protection and Lipid Metabolism of Alcohol Fed Rats

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Abstract - **In order to investigate whether or not the alcohol-treated rat liver cells can be protected by Korean *Citrus junos* and medicinal herbs, We compared the serum biochemistry of rats administered both alcohol and the complex of Korean *Citrus junos* and medicinal herbs to control rats treated with alcohol alone. The activities of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in the citron 3 (Citron 3, less mellowed citron which was ripened for three months)+*Phellinus linteus, Alnus japonica, Dendropanax morbifera* and citron 4 (Citron 4, completely mellowed citron which was ripened for four months)+*Phaseolus radiatus, Cordyceps militaris* group were significantly low when compared with the negative control group ($p<0.05$). The levels of triglyceride (TG) in all experimental groups were significantly lower than the negative control group ($p<0.05$). The concentrations of total cholesterol in the citron 3+*Phellinus linteus, Alnus japonica, Dendropanax morbifera* and citron 4+*Phaseolus radiatus, Cordyceps militaris, Phellinus linteus* were lower than the negative control group ($p<0.05$). The activities of alcohol dehydrogenase (ADH) in all experimental groups were significantly high when compared with the normal control group ($p<0.05$). These results suggest that the complex of Korean *Citrus junos* and medecinal herbs could be an excellent candidate for protecting rat liver cell damage induced by alcohol.**

Key words : Korean *Citrus junos*, medicinal herbs, rat liver cell, alcohol protection effect

INTRODUCTION

As the number of drinkers has been increased, the more interested we become in the alcohol influence to human health recently. Temperate drinking helps to relive the fatigue and stress, and to stimulate the secretion of digestive fluid to promote the appetite for food. Recently, it's suggested that moderate taking alcohol results in high concentration of HDL-cholesterol and can protect the cardiovascular disease like arteriosclerosis (Schapiro *et al.* 1965). However, the extreme alcohol drinking could injure such internal organs as liver, heart and pancreas and at last it can lead a drinker alcoholism. The excessive alcohol intake, in a short time, might cause the accumulation of aldehyde, hypertension, arrhythmia and shock. It also hinders the medicinal metabolism within the vesicle of the lipid oxidation within the liver. As a result, clonic alcoholism hurts the liver cells (Rosser and Gores 1995). In other words, the degree of liver damage depends on the amount of alcohol and the term of taking it (Rao and Larkin 1997).

Alcohol and fat metabolism have a close relationship. The increasement of NADH in the liver by alcohol disintegration ferment effects on the fat metabolism and

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results in the triglyceride accumulation within the liver and blood. Figueroa's research (1962) showed that the activity of alcohol disintegration ferment of rats, injured 3～5 ml of 20% of alcohol for 12 weeks, dropped much more than those injected the same amount for three month. This showed that long term alcohol intake influences considerably the triglyceride accumulation within the liver and blood. According to Schapiro's research (1965) on alcohol sufferers, the amount of triglyceride of the alcohol sufferers reached two times more than that of normal person within the blood.

Hyperlipidemia can be said to be the state of abnormal fat increasement such as cholesterol and triglyceride in the blood plasma. It's reported that the clonic alcohol intake brings about the abnormal fat metabolism, hyperlipidemia and the morbid state of internal organs which appears in the various undernourishment (Chait *et al.* 1972; Fargubar and Reaven 1974).

This hyperlipidemia causes the abnormal lipoprotein metabolism, and specially causes the abnormal LDL metabolism. Considering those of causes, various medicines for the prevention or the treatment of hyperlipidemia are now being developed. Hyperlipidemia is a long-term induced disease, so a long-term treatment is needed. Chemical medicines have defects causing on adverse reaction. Almost all kinds of medicines are taken through the mouth and are absorbed through the small intestine and the colon. Some of them are carried to the liver through the front hepatic portal vein, most of them are regarded as extraneous substances, and are removed within the liver, and some of them are excreted through the kidney. So most of taken medicines effects the liver. Therefore natural medicines are embossed as the treatment medicines.

Nowadays, researches for the development of liver function promotion medicines for cooling crapulence and of hyperlipidemia prevention medicines are lively put into practice. Mungbean have a medical function for detoxication and antiphlogistic. Choi's research (1997) showed that AST and ALT activities are considerably dropped by having Mungbean juice within rats which have hurt liver by cadmium. Cho's research (1993) showed that the density of cholesterol and the content of triglyceride become lower by having Mustard leaf (*Brassica Juncea*) juice within rats. Kim (1996) studied the Mugwort influence to the rats that have hurt liver by ethanol injection. He reported that when *Artemissia selengensis* extract were treated to long-term alcohol fed rats, AST and ALT activities of blood serum of rats were considerably decreased. It showed that the internal peroxide injury caused by free radical that was formulated by ethanol was improved by *Pueraria radix* extract and *Pueraria labata* extract (Lee 1999; Lee 2000). Ahn (1997) showed that *Phellinus linteus* extract made increased rat's alcohol disintegration ability to 13 percent. He also showed the same influence to the man and woman.

In this research, we tested the synergy influence of the complex of Korean *Citrus junos* and medicinal herbs whether it helps the liver function of alcohol fed rats or not and whether it helps the lipid metabolism of alcohol fed rats or not. And major finding of this paper is that the complex of Korean *Citrus junos* and medicinal herbs protect damage of alcohol-fed liver cells, resulting in curing liver diseases and in improving lipid metabolism.

MATERIALS AND METHODS

1. Preparation and treatment of *Citrus junos* and medicinal herb extract

Each medicinal herb extract was prepared from dried Korean medicinal herbs (*Phaseolus radiatus, Cordyceps militaris, Phellinus linteus, Alnus japonica, Dendropanax morbifera*). Each volume (100g) of every herb were added to 1200ml of sterilized water, and boiled for 150 min by using herbal medicine decocter (Daewoong Co. Ltd., Seoul). The aqueous extracts from each sample were centrifuged at 6000 $\times$g for 15min and filtered through 3MM filter papers (Whatman, England). Final volume was adjusted volumetrically to around 600 ml to prepare and appropriate volume for administration (10 ml kg^{-1} body weight). The extracts were administered orally at dose of clinical use for a consecutive 5 weeks.

Korean *Citrus junos* was purchased from Seungil farm (Wando, Korea) at October (ripened for three month) of 2002 and November (ripened for four month) of 2002. Less mellowed citron (ripened for three months, so not mellowed completely, afterward it will be marked citron 3) and mellowed citron (ripened for four months, so mel-

lowed completely, it'll be marked by citron 4) were grinded properly by using mixer (Hanil Co. Ltd., Korea). The grinded samples were centrifuged at 6,000 × g for 15 minutes and supernatant were filtered by using gauze. The filtrates were stored at 4°C and used for experiment. The filtrated *Citrus junos* extracts were administered orally at dose of 5 ml kg^{-1} rat body weight once a day for 5 weeks.

2. Animal models

Young adult male Sprague Dawley rats, initial weight 130 ~ 150 g, were purchased from the Daehan Biolink Co. Ltd. (Seoul). Animals were housed in individual cages under conditions of constant temperature (22 ± 2 °C) and humidity (55 ± 5%). They were kept on a 12 h light/dark cycle and acclimatized to the housing situation for four weeks before experiments. Rats were divided into thirteen groups (n = 6). In normal control group, rats were fed with water. For negative control group rats were pair-fed with alcohol/water. Rats in positive group were pair-fed with alcohol and hangover cure solution (Condition : Cheil je dang Co. Ltd., Seoul). In each group of experiments, rats were pair-fed with ethanol and experimental materials for 5 weeks (Table 1). Ethanol-fed rats were induced to consume 40% ethanol in water. An intake of 5 g ethanol kg^{-1} day^{-1} was achieved. The body weight and general condition of the animals were monitored every days. Rats were sacrificed for determination of biochemical test by anesthetizing with diethyl ether. After blood was obtained from abdominal vein, the liver was rapidly removed and rinsed in cold physiological saline and stored at −70°C.

3. Biochemical determinations

Blood was set aside in an EDTA-free tube for half an hour. The serum was separated from the blood with a centrifuge at 3,000 × g for 15 minutes. AST or ALT activity in the serum was determined using the AST kit (Boehringer Mannheim, Germany) or ALT kit (Boehringer Mannheim, Germany). Triglyceride was measured using the TG kit (Boehringer Mannheim, Germany), and the enzymatic colorimetric test for cholesterol content was also conduced using the Total Cholesterol kit (Boehringer Mannheim, Germany).

Table 1. Composition of experimental groups

Group	No. of exam	Treatment
Normal Control	6	None-alcohol
Negative Control	6	Alcohol + Distilled Water
Positive Control	6	Alcohol + HCS*
Experimental group 1	6	Alcohol + citron 3 + *Phaseolus radiatus*
Experimental group 2	6	Alcohol + citron 3 + *Cordyceps militaris*
Experimental group 3	6	Alcohol + citron 3 + *Phellinus linteus*
Experimental group 4	6	Alcohol + citron 3 + *Alnus japonica*
Experimental group 5	6	Alcohol + citron 3 + *Dendropanax morbifera*
Experimental group 6	6	Alcohol + citron 4 + *Phaseolus radiatus*
Experimental group 7	6	Alcohol + citron 4 + *Cordyceps militaris*
Experimental group 8	6	Alcohol + citron 4 + *Phellinus linteus*
Experimental group 9	6	Alcohol + citron 4 + *Alnus japonica*
Experimental group 10	6	Alcohol + citron 4 + *Dendropanax morbifera*

HCS* : Hangover cure solution (Condition: Cheil-je-dang Co., Ltd., Seoul) commercially available in Korea.

4. Determination of ADH in the liver

The rats were killed by decapitation and bled, and then liver cells were transferred to 0.25 M sucrose buffer and homogenized for determination of the ADH. Homogenized solution centrifuged at 14,000 × g for 15 min. Achieved supernatant was filtered by a 0.45 μm membrane filter (Millipore, France) and stored at 4°C.

5. Statistical analysis

All results were shown as mean standard deviation. Statistical evaluation of data was performed by Duncan's multi-range test to make comparisons between groups.

RESULTS AND DISCUSSION

1. Weight gain and ratio of liver weight to body weight

Table 2 showed that the body weight change and liver damage were observed after chronic alcohol administra-

Table 2. Total body weight gains and the weight ratio of liver

Group	Body weight change (g)	Liver index (liver/body weight %)
Normal control[3)]	59.8±5.49[a,1),2)]	3.98±0.40[a]
Negative control[4)]	58.5±12.49[a]	4.06±0.24[b]
Positive control[5)]	56.2±11.50[a]	4.34±0.13[b]
Experimental group 1	43.2±10.65[b]	4.20±0.16[b]
Experimental group 2	52.0±5.76[b]	3.80±0.28[a]
Experimental group 3	65.0±22.43[c]	3.72±0.11[a]
Experimental group 4	39.2±8.16[b]	4.39±0.30[b]
Experimental group 5	38.9±7.54[b]	3.63±0.71[a]
Experimental group 6	46.8±14.41[a]	3.65±0.13[a]
Experimental group 7	46.5±12.66[a]	3.53±0.19[a]
Experimental group 8	58.0±10.13[a]	4.39±0.26[b,c]
Experimental group 9	35.2±9.89[b]	3.57±0.17[a]
Experimental group 10	40.2±8.82[b]	3.60±0.16[a]

1)Value are Mean±S.D. (standard deviation) of six rats.
2)Means with different superscript letters within a column and significantly different from each other at P<0.05 as determined by Duncan's multiple-range test.
3)Heathy normal control group.
4)Pair-fed with alcohol/water.
5)Pair-fed with alcohol and hangover cure solution (Condition: Cheil-je-dang Co. Ltd., Seoul).

Table 3. Enzyme activity of AST and ALT in serum

Group	AST (U L^{-1})	ALT (U L^{-1})
Normal control[3)]	60.33±4.98[a,1),2)]	19.17±2.14[a]
Negative control[4)]	117.00±20.02[b]	45.83±7.14[b]
Positive control A[5)]	95.33±4.46[c]	43.50±8.62[b]
Positive control B	104.33±19.34[b,c]	40.33±5.79[b]
Experimental group 1	92.67±7.94[c]	35.17±5.42[b]
Experimental group 2	101.00±21.45[c,d]	29.50±5.61[c]
Experimental group 3	68.50±11.04[a]	20.83±5.08[a]
Experimental group 4	54.83±5.88[a]	20.33±3.50[a]
Experimental group 5	63.17±14.59[a]	18.83±3.19[a]
Experimental group 6	80.33±24.20[a]	18.50±1.05[a]
Experimental group 7	71.00±15.22[a]	18.67±3.56[a]
Experimental group 8	86.50±18.97[c]	28.83±7.84[c]
Experimental group 9	137.33±18.21[b]	36.50±5.72[b]
Experimental group 10	116.67±26.96[b,e]	29.50±6.09[c]

1)Value are Mean±S.D. (standard deviation) of six rats.
2)Means with different superscript letters within a column and significantly different from each other at P<0.05 as determined by Duncan's multiple-range test.
3)Heathy normal control group.
4)Pair-fed with alcohol/water.
5)Pair-fed with alcohol and hangover cure solution (Condition: Cheil-je-dang Co. Ltd., Seoul).

tion. The normal control group was determined 59.8±5.49 g, the negative control group was determined 58.5±12.49 g, the positive control group was determined 56.2±11.50 g. The experimental group 3 (citron 3+*Phellinus linteus*) was showed a greater gain than negative control groups in body weight (65.06±22.43 g).

According to previous researches (Pikarr *et al.* 1987; Mezey 1980), It was reported that body weight gain decreased in alcohol-treated rats (Pikarr *et al.* 1987) and body weight decreased by 50% alcohol ingestion instead of sugar in total energy source of man, suggesting that oxygen consumption and metabolic rate were increased, ATP production was decreased in microsome by excessive alcohol ingestion (Mezey 1980). In this study, the body weight of negative control group was a little decreased compared to that of normal control group. And in the experimental group 3, significant recovery of body weight was appeared (65.0±22.43 g).

The ratio (%) of liver weight to body weight was shown in table 2. In the ratio (%) of liver weight to body weight, the normal control group was 3.98±0.40%, the negative control group was 4.06±0.24%, and the positive control group was 4.34±0.13%. The experimental group 1 (4.20±0.16%), 4 (4.39±0.30%), 8 (4.39±0.26%) were not significant compared to the negative group. But the other groups were significantly lower than the negative control group ($p<0.05$). Generally in alcohol-fed animal, the ratio (%) of liver weight to body weight was increased because lipid and fiber were accumulated in liver (Leo *et al.* 1983)

Liver weight to body weight of the experimental group 2, 3, 5, 6, 7, 9 and 10 were lower than that of the negative control group. It seemed that these experimental materials prevent accumulating fiber and lipid in the alcohol fed rat liver cells. So these groups may have helped liver regeneration, when it were damaged with alcohol.

Especially in the citorn 3+*Phellinus linteus* group (experimental group 3), total body weight gains was significantly high and the ratio of liver weight to body weight was significantly low, so this group have assumed a great effect on reduction of alcohol toxicity.

2. Activity of AST and ALT

As to the AST level in the blood serum, the normal group was determined at 60.33±4.98 unit, the negative group determined at doubled 117.00±20.02 unit, the positive group was determined 95.33±4.46 unit (Table

3). The AST level of experimental group 3 (68.50±11.04 unit), 4 (54.83±5.88 unit), 5 (63.17±14.59 unit) and 7 (71.00±15.22 unit) were significantly lower than the that of negative control group ($p<0.05$).

As shown in Table 3, the ALT amount was 19.17±2.14 unit for the normal group, whereas the negative and positive control group were significantly ($p<0.05$) high at 45.83±7.14 unit and 43.50±8.62 unit, respectively. The experimental group 3 (20.83±5.08 unit), 4 (20.33±3.50 unit), 5 (18.83±3.19 unit), 6 (18.50±1.05 unit) and 7 (18.67±3.56 unit) demonstrated significantly low ALT level in compared to the negative control group at near-normal level.

The AST and ALT are essential enzymes determining the status of liver diseases. In general, decreasing function of the liver is signaled by the increased AST and ALT amount in blood serum (Kien and Ganther 1983; Thompson and Scott 1970). It is reported that the activities of AST and ALT was increase in the result of problem with liver metabolism and loss of liver cell by alcohol intake (Zimmerman 1981).

In negative control group, these two enzymes were higher value than normal control group. This result was caused with alcohol-intake. However, as to the AST enzyme in table 3, the intake of experimental group 3 (citron 3+*Phellinus linteus*), 4 (citron 3+*Alnus japonica*), 5 (citron 3+*Dendropanax morbifera*) and 7 (citron 4+*Cordyceps militaris*) with alcohol can effectively decrease the activities of AST enzymes. The experimental materials of these four groups seem to have an effect of protecting the liver.

And experimental group 3 (citron 3+*Phellinus linteus*), 4 (citron 3+*Alnus japonica*), 5 (citron 3+*Dendropanax morbifera*), 6 (citron 4+*Phaseolus radiatus*) and 7 (citron 4+*Cordyceps militaris*) were significantly low in compared to the negative control group for ALT enzyme activity ($p<0.05$). This result showed that some elements in the citron and medicinal herbs complexes are presumed to protect the liver and reduce the rate of damaged liver by alcohol, efficiently.

3. Serum triglycerides and total cholesterol content

A number of reports have demonstrated that alcohol intake increases the triglyceride and total cholesterol level significantly in the liver and the blood (Belfrage *et al.* 1977). Given the results of the studies in this report (Table 4), alcohol intake leads to increase a significant amount of triglyceride and cholesterol in the blood and fatty liver. The triglyceride contents in the blood serum were determined for each group in order to examined the citron and medicinal herbs effects on the lipid metabolism of an animal with liver lesions. The triglyceride amount in the normal, negative and positive control group were 14.17±6.80 mg dl^{-1}, 71.33±44.27 mg dl^{-1}, 76.33±23.57 mg dl^{-1}, respectively, whereas in all experimental groups, triglyceride was significantly reduced in compare to the negative control group ($p<0.05$).

Table 4. Concentration of serum lipid in rat.

Group	TG (mg dl^{-1})	T-CHOL (mg dl^{-1})
Normal control[3)]	14.17±6.80[a,1),2)]	42.83±13.99[a]
Negative control[4)]	71.33±44.27[b]	70.50±6.19[b]
Positive control A[5)]	76.33±23.57[b]	81.17±7.00[b]
Positive control B	58.17±20.15[b]	76.67±8.24[b]
Experimental group 1	56.33±14.90[b]	70.67±3.08[b,c]
Experimental group 2	54.17±15.01[b]	66.83±8.54[b,c]
Experimental group 3	27.83±7.47[a,c]	39.33±5.24[a]
Experimental group 4	28.67±5.82[a,c]	43.50±2.74[a]
Experimental group 5	20.67±8.12[a,c]	45.33±10.15[a]
Experimental group 6	18.67±4.89[a,d]	49.33±7.97[a]
Experimental group 7	18.00±5.66[a,d]	47.00±12.49[a]
Experimental group 8	18.00±4.47[a,d]	52.00±7.40[a]
Experimental group 9	19.67±5.16[a,d]	68.67±6.09[b,d]
Experimental group 10	15.50±3.21[a,d]	69.33±11.76[b,d]

[1)]Value are Mean±S.D. (standard deviation) of six rats.
[2)]Means with different superscript letters within a column and significantly different from each other at $P<0.05$ as determined by Duncan's multiple-range test.
[3)]Heathy normal control group.
[4)]Pair-fed with alcohol/water.
[5)]Pair-fed with alcohol and hangover cure solution (Condition: Cheil-je-dang Co. Ltd., Seoul).

The concentration of serum cholesterol for normal control group was 42.83±13.99 mg dl^{-1}, for the negative control group was 70.50±6.19 mg dl^{-1}, and for the positive control group was 81.17±7.00 mg dl^{-1}. In positive control group, triglyceride and cholesterol levels were not much different from negative control group significantly ($p>0.05$). But total cholesterol concentration of experimental group 3 (citron 3+*Phellinus linteus*, 39.33±5.24 mg dl^{-1}), 4 (citron 3+*Alnus japonica*, 43.50±2.74 mg dl^{-1}), 5 (citron 3+*Dendropanax morbifera*, 45.33±10.15 mg dl^{-1}), 6 (citron 4+*Phaseolus radiatus*, 49.33±

Table 5. Activity of alcohol dehydrogenase in rat liver

Group	ADH (U L^{-1})
Normal control[3)]	$0.54 \pm 0.62^{a,1),2)}$
Negative control[4)]	1.05 ± 0.44^{b}
Positive control A[5)]	1.31 ± 0.60^{b}
Positive control B	1.41 ± 0.29^{b}
Experimental group 1	1.78 ± 0.62^{b}
Experimental group 2	1.63 ± 0.57^{b}
Experimental group 3	1.82 ± 1.04^{b}
Experimental group 4	1.74 ± 0.94^{b}
Experimental group 5	1.44 ± 0.40^{b}
Experimental group 6	1.99 ± 0.29^{b}
Experimental group 7	1.51 ± 0.67^{b}
Experimental group 8	2.69 ± 0.93^{c}
Experimental group 9	0.91 ± 0.48^{a}
Experimental group 10	1.97 ± 0.84^{b}

[1)]Value are Mean±S.D. (standard deviation) of six rats.
[2)]Means with different superscript letters within a column and significantly different from each other at $P < 0.05$ as determined by Duncan's multiple-range test.
[3)]Heathy normal control group.
[4)]Pair-fed with alcohol/water.
[5)]Pair-fed with alcohol and hangover cure solution (Con-dition: Cheil-je-dang Co. Ltd., Seoul).

7.97 mg dl^{-1}), 7 (citron 4+*Cordyceps militaris*, 47.00± 12.49 mg dl^{-1}) and 8 (citron 4+*Phellinus linteus*, 52.00 ±7.40 mg dl^{-1}) were significantly lower than that of negative control group ($p < 0.05$). These above results suggest that experimental group 3, 4, 5, 6, 7 and 8 could be an excellent candidate for protecting rat liver cell damage induced by alcohol and treating the high lipid contents of the blood.

4. Activity of ADH

In Table 5, the ADH (alcohol dehydrogenase) level of the negative control group was determined at 1.05± 0.44 U L^{-1} and of the normal control group was determined with 0.54±0.62 U L^{-1}, while the ADH level was about three or four fold as high as that of the normal control group at the experimental groups.

ADH is an enzyme that oxidizes ethanol, and the enzyme's activity changes by the length of ethanol administration (Koivula and Lindors 1975). Kim (1985) and Lee (2000) reported that the ADH activity increases resulting from a chronic intake of alcohol in animal tests. It is widely known that poisons caused by alcohol is closely related to alcohol metabolism (Nanji and Zakim 1996). The damages to organic tissue are caused by the acetaldehyde created during the metabolic process. The results (Table 5) showed that a long-term intake of alcohol may lead to an accumulation of acetaldehyde, and that the intake of citron and medicinal herbs may promotes alcohol metabolism. It is suggested that the citron and medicinal herbs intake can enhance alcohol metabolism, so they discourage the accumulation of acetaldehyde and prevent alcohol poisoning.

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