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Effects of the *Zizyphus jujuba* Seed Extract on the Lipid Components in Hyperlipidemic Rats

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Abstract

The purpose of this study was designed to observe the effects of *Zizyphus jujuba* seed extract on the concentrations of the lipids and blood glucose in the S.D. rats fed the experimental diets for 4 weeks. Concentrations of total cholesterol, atherosclerotic index, LDL, LDL-cholesterol, free-cholesterol, cholesteryl ester, triglyceride (TG), phospholipid (PL) and blood glucose in serum were significantly higher in the cholesterol administration groups (group 2 (cholesterol+water), group 3 (cholesterol+*Zizyphus jujuba* seed extract)) than those in the control group (group 1, basal diet+water). But the concentrations of total cholesterol, atherosclerotic index, LDL, LDL-cholesterol, free-cholesterol, cholesteryl ester, TG, PL and blood glucose in serum were remarkably lower in the group 3 than those in the group 2. In the ratio of HDL-cholesterol concentration to total cholesterol and HDL-cholesterol concentration, *Zizyphus jujuba* seed extract administration group was higher percentage than in the group 2. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) in serum were rather lower in the *Zizyphus jujuba* seed extract administration group (group 3) than in the cholesterol diet group (group 2). From the above research, *Zizyphus jujuba* seeds were effective on the improvement of the blood glucose, lipid compositions in serum of dietary hyperlipidemic rats. And particularly, *Zizyphus jujuba* seeds were more effective as a therapeutic regimen for the control of metabolic derangements in adult disease.

Key words: hyperlipidemic rat, *Zizyphus jujuba* seed, lipid components, aminotransferase, lactate dehydrogenase, alkaline phosphatase

INTRODUCTION

Hyperlipidemia is a disease in which the concentration of lipids in serum increases, and is clinically important. Hypercholesterolemia and hypertriglycemia occur frequently, and are associated with liver disease, kidney disease, diabetes, internal secretion disease, diet, lack of exercise, aging, and environmental influences (1-4). Cholesterol is composed of cell membrane. It is significant because of the precursor of steroid hormones and vitamin D. Steroid hormones and vitamin D are factors related to the cause of heart circulation disease: hyperlipidemia, arteriosclerosis, heart disease, cholelithiasis. Cholesterol is synthesized in the liver or brought into the body through diet. About 80% of the bodies total cholesterol is excreted from liver, and brought into cholesterol metabolism, as bile acid (2,5-8).

The concentration of cholesterol is a major factor of hyperlipidemia. And, there are indications that concentration of triglyceride, lipoprotein and formation of blood plasma thromboxane A_2 (TX A_2) are factors in hyperlipidemia (9-17).

Polyunsaturated fatty acids are the form that serum cholesterol takes as it travels in the body. These fatty acids lower levels of LDL-cholesterol and VLDL-cholesterol. The LDL-cholesterol and VLDL-cholesterol are accumulated in the arteries of the body, and promote hyperlipidemia (18-21). HDL-cholesterol, in cholesterol ester form, remove cholesterol that have accumulated in the peripheral tissues and arteries. The newly removed cholesterol is then transferred to the liver, and excreted as bile acid. This consequently lowers the concentration of cholesterol in the serum (22-24).

Zizyphus jujuba is from the Rhamnaceae family. It grows in the wild from Europe to Asia. The plants origin is China, and about 40 different species, 300~400 different breeds of *Zizyphus jujuba* are in the tropics, subtropics, and temperate zone. The fruit of the plants were used for medicinal purposes and processed food as fresh or dry fruits. Old documents (Hyangyakdaesachon, Tonguibokam, Pangyakhappon, Bonchohak, Hyangyakchipsongbang, Uibangyuchwi) and folk lore say the effect of *Zizyphus jujuba* are calming and hypnotic. It may help with the following: heart asthenia, gastrospasm, circu-

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lation disease, liver inflammation, liver cirrhosis, urination, tonic, relaxation, anemia, lack of appetite, nervous asthenia and hysteria, muscle urgent, biliousness, bronchitis, tuberculosis, bleeding disorder, expectorant, blood pressure descent, and antiinflammation (25-27). However, there are few scientific studies about the effect of *Zizyphus jujuba*. As reported by Kim et al. (28), the physiological activity material of the *Zizyphus jujuba* seeds were mostly carbohydrates, but the seeds also contain Mg, Mn, K, Ca, P, Fe, Zn, proline, aspartic acid, glutamic acid, glycine, lysine, leucine, serine, alanine, valine, arginine, linoleic acid, oleic acid, and eicosapentaenoic acid.

In this study, we tested the effect of the *Zizyphus jujuba* seeds that residue of *Zizyphus jujuba* as a medicine for hyperlipidemia and the possibility of its use as a health beverage.

MATERIALS AND METHODS

Preparation of *Zizyphus jujuba* seed extract

The *Zizyphus jujuba* was purchased (Gyeongnam, Miryang, Korea), and the sarcocarp removed. The *Zizyphus jujuba* seed were dried and stored at 4°C until use. The *Zizyphus jujuba* seeds, weighing 35 g, were heated with 700 mL of distillated water in a flask until 450 mL, then the extraction was poured into another flask. The extraction was heated with 500 mL distillated water until reduced to 350 mL. Remained seeds were heated with 400 mL distillated water until reduced to 200 mL. The volume of compounded extraction was completed to 1000 mL, concentrated to 3.5 g% and stored 4°C until use.

Experimental animals

Sprague-Dawley male rats weighing about 60±5 g were supplied from Hallym Experimental Animal Inc. (Suwon, Gyeonggido, Korea). The animals ate a basal diet containing 5% corn oil (Dongbang Euryang) for 10 days, then were housed individually in metabolic cages (JD-C-71, Jeongdo Industry Inc, Korea) with a randomized complete block design into 3 groups each group had 6 rats, the temperature was 20±1°C, a humidity level of 50±10%, with a light controlled room with a 12-hr light-dark cycle (07 : 00~19 : 00). Water and *Zizyphus jujuba* seed extract were provided *ad libitum*.

Diets and experimental group

The compositions of the experimental diets are shown in Table 1. Group 1 was provided basal diet plus water, group 2 was provided basal diet plus water plus lard and cholesterol for hyperlipidemia, and group 3 was provided cholesterol diet plus *Zizyphus jujuba* seed extract.

Treatment of experimental animals

At the end of study, food was withheld from their cages for 7 hours. Each rat was anesthetized by the inhalation of ether. Blood samples were collected from the heart with non-heparinized syringes and incubation on ice water for 1 hr. The serum was separated from the collected blood by centrifugation at 3,000 rpm for 15 min at 4°C.

Table 1. Experimental groups and compositions of basal and experimental diet (g/kg diet)

Ingredient	Basal diet	Cholesterol diet
Casein	200	200
DL-methionine	3	3
Corn starch	150	150
Sucrose	500	490
Cellulose powder	50	50
Mineral mixture[1)]	35	35
Vitamin mixture[1)]	10	10
Choline bitartrate	2	2
Corn oil	50	-
Lard	-	50
Cholesterol	-	7.5
Sodium cholate	-	2.5

Group 1: Basal diet + Water
2: Basal diet + Cholesterol + Water
3: Basal diet + Cholesterol + *Zizyphus jujuba* seed extract
[1)]According to AIN-76™ diet compositon.

Total choleterol, free cholesterol and cholesterol ester in serum

The levels of total cholesterol and free cholesterol in serum determined respectively using total cholesterol assay kit (Cholestezyme-V, Eiken, Tokyo, Japan) and free cholesterol assay kit (Free-cholestezyme-V555, Eiken, Tokyo, Japan), the levels of cholesterol ester in the serum expressed as the levels of total cholesterol in the serum minus the levels of free cholesterol in serum.

Analysis of LDL, LDL-cholesterol and HDL cholesterol

The levels of LDL in the serum was determined using LDL assay kit (β-lipoprotein C-Test, Wako, Osaka, Japan), the levels of LDL-cholesterol expressed as multiply the levels of LDL in the serum by 0.35, and the levels of HDL-cholesterol in the serum determined using HDL-cholesterol assay kit (HDL-C555, Eiken, Tokyo, Japan).

Analysis of triglyceride and phospholipid

Triglyceride and phospholipid in the serum were determined respectively using triglyceride assay kit (Triglyzyme-V, Eiken, Tokyo, Japan) and phospholipid assay kit (PLzyme, Eiken, Tokyo, Japan).

Analysis of blood glucose

The levels of blood glucose were determined using commercial kit (GLzyme, Eiken, Tokyo, Japan).

Analysis of aminotransferase activity

Aspartate aminotransferase activity (AST, EC 2.6.1.1)

and alanine aminotransferase (ALT, EC 2.6.1.2) in the serum were measured by method of Reitman and Frankel (29) using commercial kits (serum transaminase assay kit, Eiken, Tokyo, Japan), the values were expressed as Karmen unit per 1mL (30).

Analysis of lactate dehydrogenase activity

Lactate dehydrogenase (LDH, EC 1.1.1.27) activity in the serum was determined using serum LDH assay kits (LDH, Neo D, Eiken, Tokyo, Japan), the values were expressed as Wroblewski unit.

Analysis of alkaline phosphatase activity

Alkaline phosphatase (ALP, EC 3.1.3.1) activity in the serum was determined using serum ALP assay kits (NEW-K-PHOS, Eiken, Tokyo, Japan), the values were expressed as King-Armstrong unit (31).

Statistical analysis

Data from individual experiments calculated mean and standard deviation, significant difference between individual experiments were determined by using Duncan's multiple range test that $p<0.05$ was judged to be statistically significant.

RESULTS AND DISCUSSION

Total cholesterol and HDL-cholesterol concentration

The concentration of total cholesterol and HDL-cholesterol, the ratio of HDL-cholesterol/total cholesterol, and atherogenic index in the serum are shown in Table 2. The concentration of total cholesterol in the serum was higher in the cholesterol experimental groups than in the control group. However, the level of total cholesterol in the serum of group 3 (128.4 mg/dL) was significantly lower than that of group 2 (149.4 mg/dL).

The concentration of the HDL-cholesterol in the serum in group 3 (22.9 mg/dL) showed an increase, where as group 2 (21.0 mg/dL) remained the same. This is because of the administering of the *Zizyphus jujuba* seed extract. The ratio of the HDL-cholesterol/total cholesterol in group 3 was higher than that in group 2. Atherogenic index in group 3 was lower than that in group 2, but did not attain the level of the control group.

Hyperlipidemia is a disease in which the levels of cholesterol, triglycerides, and lipoproteins are increased by any impairment in lipid metabolism. The causes of impairment are due to diabetes, smoking, lack of exercise, excess intake of calorie, alcoholism, and liver disease (32,33). It is known that serum cholesterol concentration is closely associated with coronary heart disease and is affected by the kind and amount of dietary fatty acids (34,35).

From the results, *Zizyphus jujuba* seed extract was effective in improving the serum lipid profiles. Decreases in total cholesterol concentration, atherogenic index, and an increase in HDL-cholesterol concentration were seen in the hyperlipidemic rats.

Low density lipoprotein (LDL) and LDL-cholesterol concentration

Serum LDL concentration in group 3 (250.6 mg/dL) did not attain the level of the control group (183.4 mg/dL), but was significantly lower than that in group 2 (312.3 mg/dL). It was shown that serum LDL-cholesterol concentration among groups was similar to serum LDL concentration.

Serum LDL are bound to the specific receptors exposed to the outside of the cell and removed in the liver and other tissues (36). It is said that the lesion of LDL-receptor

Table 2. Effects of *Zizyphus jujuba* seed extract on lipid components and concentrations of blood glucose in serum of rats fed the experimental diets for 4 weeks (mg/dL)

Group[1)]	1	2	3
Total cholesterol (A)	92.5±3.4[a2)]	149.4±5.7[c]	128.4±4.8[b]
HDL-cholesterol (B)	24.1±1.8[a]	21.0±1.4[a]	22.9±1.7[a]
(B)/(A) × 100 (%)	26.1	14.1	17.8
A.I.[3)]	2.8	6.1	4.6
Low density lipoprotein	183.4±12.8[a]	312.3±14.9[c]	250.6±14.0[b]
LDL-cholesterol	64.2±4.4[a]	109.3±5.2[c]	87.7±4.9[b]
Free cholesterol	17.4±1.8[a]	26.8±2.2[b]	22.8±1.9[ab]
Cholesteryl ester	75.1±3.0[a]	122.6±3.9[c]	105.6±3.1[b]
Cholesteryl ester ratio (%)[4)]	81.2	82.1	82.2
Triglyceride	81.4±3.4[a]	131.2±4.7[c]	109.8±2.9[b]
Phospholipid	113.4±3.9[a]	145.8±5.0[c]	130.2±4.4[b]
Blood glucose	166.8±7.2[a]	197.2±8.1[b]	185.5±7.8[ab]

[1)]See the legend of Table 1.
[2)]Mean±SE (n=6). Means in the same column not sharing common superscript letters are significantly different ($p<0.05$).
[3)]Atherosclerotic index; (Total chol. - HDL-chol.) / HDL-chol.
[4)]Cholesteryl ester / Total cholesterol × 100.

and reduced activity of LDL-receptor complex causes LDL to not bind to their receptor sites and are released into the blood, and therefore serum LDL concentration increases (37). In addition, Smith (21) reported that LDL-cholesterols were the main cholesterol carriers, and enhanced the delivery and deposition of cholesterol in arterial walls and peripheral tissues. Therefore, LDL-cholesterols are the main factor in the likelihood of developing atherosclerosis.

Free-cholesterol and cholesteryl ester concentration

Serum free-cholesterol and cholesteryl ester concentration were lower in group 3 than in the group 2. It was shown that the cholesteryl ester ratio (%) is analogous in the cholesterol administration groups except the control group. Cholesterol including phospholipid is a major constituent of the cell membrane. Cholesterol consists of about 70% cholesteryl ester, the binding type of fatty acid and ester, and about 30% free-cholesterol in the blood (32). The reduced cholesteryl ester ratio (%) is a diagnostic index of liver diseases, and the cholesteryl ester ratio shows an increase in a case of hypercholesterolemia (38).

Triglycerides and phospholipids concentration

The concentrations of triglyceride and phospholipid in the serum were higher in the cholesterol administration groups than those of the control group. However, serum triglyceride and phospholipid concentration in group 3 were significantly lower than those in group 2. Chylomicron and VLDL are hydrolyzed by the lipoprotein lipase, which are located on the surface of the capillary endothelium. Thus, the level of triglyceride in the serum is decreased by the enzyme lipoprotein lipase (38). PUFA are effective in lowering serum phospholipid concentration because they stimulate the synthesis of bile acids from the phospholipids (39).

The concentration of blood glucose

The concentration of blood glucose of rats maintained on diets containing *Zizyphus jujuba* seed extract (group 3,185.5 mg/dL) was lower than that in the hyperlipidemic rats (group 2, 197.2 mg/dL) and higher than that of the control group (166.8 mg/dL), but it did not have any statistical significance.

However, the change in blood glucose concentration between group 2 and group 3 has shown that *Zizyphus jujuba* seed extract was effective in lowering blood glucose concentration.

The activities of aminotransferase (AST, ALT)

The effects of *Zizyphus jujuba* seed extract in the activities of AST and ALT in serum are shown in Fig. 1. The activities of AST in serum were higher in group

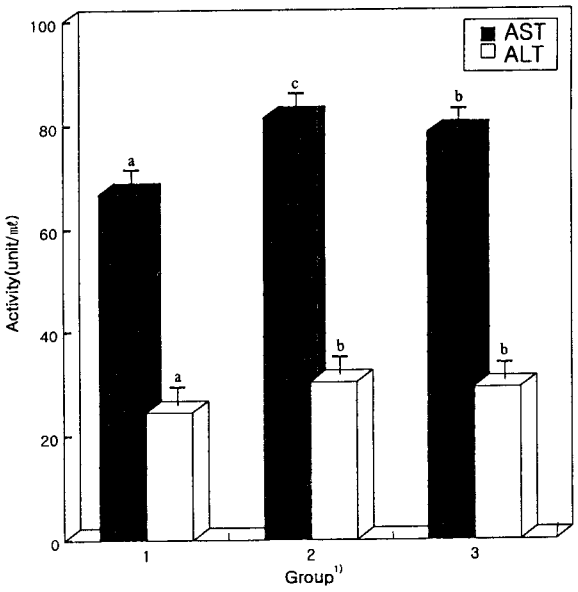

Fig. 1. Effect of *Zizyphus jujuba* seed extract on aspartate and alanine aminotransferase activities in serum of rats fed the experimental diets for 4 weeks.
[1)]See the legend of Table 1.

2 (81.3 Karmen unit/mL) and in group 3 (78.5 Karmen unit/mL) than in the control group (66.8 Karmen unit/mL). But it was shown that *Zizyphus jujuba* seed extract decreases the activities of AST.

The activities of serum ALT in the control group (24.4 Karmen unit/mL) were lower than those in group 2 (30.2 Karmen unit/mL) and those in group 3 (29.2 Karment unit/mL), but it did not have any statistical significance.

The increased activities of AST (ALT), an indicator of liver disease, is due to lower of lipid metabolism, and is correlated to the lesion of hepatocytes. The causes of AST(ALT) being released into the blood are known to be liver diseases, myocardiac infarction, fatty liver, obstructive jaundice, and hemolysis.

The activities of lactate dehydrogenase (LDH)

The activities of lactate dehydrogenase are shown in Fig. 2. The activities of LDH in group 3 (1214.5 Wroblewski unit/mL) were significantly lower than those in group 2 (1369.2 Wroblewski unit/mL), and higher than those in the control group (889.7 Wroblewski unit/mL).

It was that the activities of LDH were due to the biliousness that occurred as a result of hyperlipidemia and the deposition of lipid in the liver. It was shown that the administering of *Zizyphus jujuba* seed extract reduced the activities of LDH.

The activities of alkaline phosphatase (ALP)

The changes of serum ALP activities are shown in Fig. 3. The activities of ALP in group 3 (28.0 King-Armstrong unit/mL) were lower than those in group 2

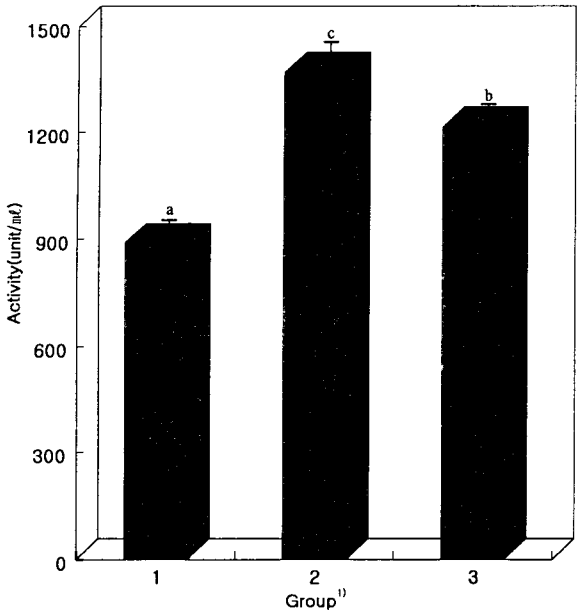

Fig. 2. Effect of *Zizyphus jujuba* seed extract on lactate dehydrogenase activity in serum of rats fed the experimental diets for 4 weeks.
[1)]See the legend of Table 1.

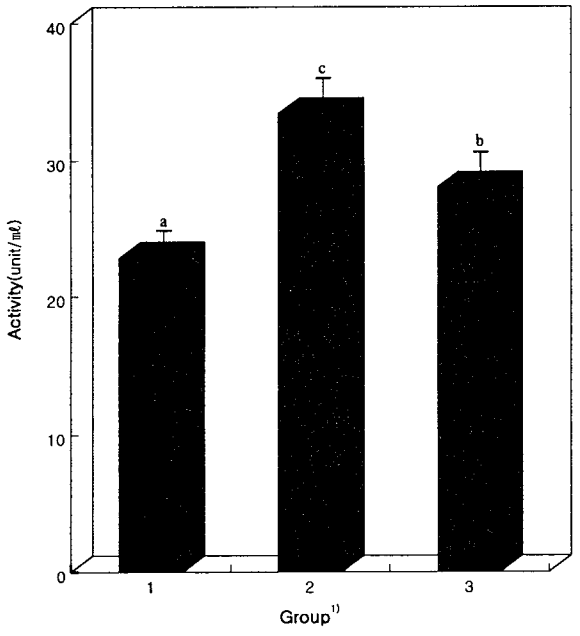

Fig. 3. Effect of *Zizyphus jujuba* seed extract on alkaline phosphatase activity in serum of rats fed the experimental diets for 4 weeks.
[1)]See the legend of Table 1.

(33.4 King-Armstrong unit/mL), and higher than those in the control group (22.8 King-Armstrong unit/mL).

Serum ALP activities increase in the case of bile duct obstruction, hyperlipidemia, and liver diseases. Therefore, the levels of cholesterol in serum increase by an abnormal excretion of bile salt in the liver (38).

SUMMARY

For testing the effect of the *Zizyphus jujuba* seeds that residue of *Zizyphus jujuba* as a medicine and processed food on hyperlipidemia and its possible use as a health beverage, Sprague Dawley male rats were fed with either basal diet (group 1), basal diet plus lard and cholesterol (group 2), cholesterol diet plus *Zizyphus jujuba* seeds extract 3.5 g% for 4 weeks. Concentrations of total cholesterol, atherogenic index, LDL, LDL-cholesterol, free-cholesterol, cholesteryl ester, triglyceride, phospholipid and blood glucose in the serum were significantly lower that in group 3 (cholesterol + *Zizyphus jujuba* seed extract 3.5 g%) than those in group 2 (cholesterol + water). The ratio of HDL-cholesterol concentration to total cholesterol and HDL-cholesterol concentration, was higher in the *Zizyphus jujuba* seed extract administration group than in group 2. The activities of AST, ALT, LDH and ALP in the serum were significantly lower in the *Zizyphus jujuba* seed extract administration group (group 3) than in the cholesterol diet group (group 2). From the stated research, the *Zizyphus jujuba* seed extract was effective in improving the levels of the blood glucose, lipid compositions in the serum of dietary hyperlipidemia, and particularly, it was an effective therapy regimen for the control of metabolic derangements in adult disease.

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