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원 저

赤小豆가 高脂肪食餌로 유발된 비만 흰쥐의 생화학적 및 조직화학적 변화에 미치는 효과

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Effect of *Phaseoli Angularis Semen* on Biochemical and Histological Changes of Rats Fed High Fat Diet

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목적 : 적소두가 비만에 관하여 어떠한 효능을 발휘하는 가를 살펴보고자 하였다.
방법 : 적소두의 추출물을 고지방식이 흰쥐에 투여한 후 체중, 혈청 중의 변화 및 조직화학적 변화에 미치는 영향을 살펴보았다.
결과 : 체중에 미치는 영향을 살펴보면, 대조군에 비하여 체중이 감소되었으나, 유의성은 없었다. 혈청 중 triglyceride, free fatty acid 함량이 대조군에 비하여 유의성있게 감소하였다. 부고환 지방세포의 평균 면적에서는 대조군에 비하여 감소되었으나 유의성은 없었으며, 간소엽내 지방면적 비율의 감소는 대조군에 비하여 유의성이 있었다.
결론 : 적소두는 임상에서 비만증의 치료에 응용할 수 있을 것으로 생각되며, 특히, 利水消腫의 치료법이 필요할 경우에 더 좋은 효능을 발휘할 수 있을 것으로 사료된다. *(J Korean Oriental Med 2002;23(4):1-8)*

Key Words: Body weight-regulation, Obesity, Phaseoli Angularis Semen

Introduction

Obesity is defined as an abnormal state that lipid tissues in body is excessively increased. In one word, obesity does not merely mean overweighted state, it means excessive accumulation of lipid in body by metabolic disorder[1].

The cause of obesity is that because intake of calorie exceeds the energy needed for the body function and the growth[2], triglyceride as a form of lipid is stored in lipid tissue too much, in other words, the imbalance between

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the amount of calorie and energy[3].

For the present time being, the obese are showing a tendency to increase gradually due to the enhancement of standards of living and its effect, the change of diet habit[4].

The obesity rate of Korean children is lower than that of the Western, however, their surplus plasma lipid density has become something that we cannot disregard any more.

Not only abnormal lipid metabolism but also hypertension and diabetes are foreseeing the adults diseases in children is on the increase. That is to say, the obese children is becoming the serious social problem[5].

In addition, the obesity like this is withering individuals socially and mentally at the same time. It sometimes induces uneasiness and dejection. The obesity can be a disease in itself, and can be a major factor that increases the risk of adults disease such as high cholesterol, fatty liver, abnormal liver function, stenocardia, cardiac infarction, arteriosclerosis, coronary heart disease, stroke, hypertension, diabetes, hyperinsulinemia, decreased growth hormone secretion, Pickwick syndrome, and gout[6,7,8].

Besides, the mechanical and physical stress of the obesity is often the origin of various diseases and it induces these diseases; Osteoarthritis, lumbago, thrombosis, abdomen hernia, cholelithiasis[7].

In addition, the mortal rate of the obese is much higher than that of the normal. When it is supposed that the mortal rate of the normal is 100, that of the 10% obese is 120. In the case of 20% is 125, 30% is 145, and 40% is 170 or so. In other words, we can have the conclusion that the mortal rate gets higher in accordance with the higher obesity[9].

Actually, the statistics in U.S said that the death rate of the under-standard weight is lower by 20% compared to the average and that of the obese is higher by 20~40% compared to the average[10].

These days, the ingredients of staple foods are becoming proteins or lipids of high quality caused by the economic development. As a consequence of it, the obesity like this has appeared as a disease to be studied significantly in both oriental and western medical fields.

Hereby, the *Phaseoli Angularis Semen*(PAS) has effects of inducing diuresis to alleviate edema. And we had experiment with *Phaseoli Angularis Semen*(PAS) in order to find cure effects for an obesity. We investigated the effect of Phaseoli Angularis Semen on biochemical and histological changes of rats fed high fat diet.

Materials and method

1. Materials

1) Experimental Extracts Preparation

The extract of *Phaseoli Angularis Semen*(PAS), made of 350g herb medicines were added to 3000㎖ water and decocted for 4 hours. The extract of decoction liquid was condensed by evaporator. The condensed liquid was frozen by deep freezer for 12 hours. The frozen extracts was dried by lyophilization apparatus(Model 104, ALPHA, W, German) for 36 hours. And finally the extracts was 34g.

2) animals and diets

Male Sprague-Dawley rats were purchased from Korean Experimental Animals(DJ 1617, Korean)and acclimated in a 53±3% humidity-controlled room maintained at 22±2℃ with a 12-h light dark cycle. Before the experiment, rats were allowed free access to commercial chow diet(Samyang, Korean) for two weeks. Then rats were selected 240±5g body weights. The rats of normal group were allowed free access to commercial chow diet and water for 8 weeks.

The rats of control group were fed high fat diet(Harlan, TD94095, USA) for 8 weeks. The rats of experimental group were fed high fat diet(Harlan,

TD94095, USA) with each Phaseoli Angularis Semen(PAS: 7.26㎎/100g b.w) for 8 weeks. The composition of high fat diet is shown as follows:

Composition	Dosage
Casein, high protein	260.0g
DL-Methionine	3.9g
Sucrose	161.7g
Corn Starch	160.0g
Beef Tallow	300.0g
Cellulose	50.0g
Mineral Mix, AIN-76	45.5g
Calcium Carbonate	3.9g
Vitamin Mix, Teklad	13.0g
Choline dihydrpgen citrate	2.0g
total	1000.0g

2. Analytical Method

1) Blood collection and serum separation

At the end of the experiment, the rats were sacrificed to determine their chemical composition. Blood was collected immediately after decapitation and serum was centrifuged 3500 rpm for 10 minutes.

2) Measurement of total cholesterol contents

Serum total cholesterol contents were measured by enzymatic colorimetry CHOD-PAP method[11] using Hitachi 747(Japan).

3) Measurement of triglyceride contents

Serum triglyceride contents were measured by enzymatic colorimetry CHOD-PAP method[12] using Hitachi 747(Japan).

4) Measurement of free fatty acid contents

Serum free fatty acid contents were measured by enzymatic colorimetry ACS-ACOD method[3] using Hitachi 747(Japan).

5) Measurement of total lipid contents

Serum total lipid contents were measured by enzymatic colorimetry Sulfo-phospho vanillin method[13] using Hitachi 747(Japan).

6) Measurement of phospholipid contents

Serum phospholipid contents were measured by enzymatic colorimetry method[3] using Hitachi 747(Japan).

7) Measurement of HDL-cholesterol contents

Serum HDL-cholesterol contents were measured by enzymatic colorimetry CHOD-PAP method[14] using Hitachi 747(Japan).

8) Measurement of LDL-cholesterol contents

Serum LDL-cholesterol contents were measured by enzymatic colorimetry CHOD-PAP method[14] using Hitachi 747(Japan).

3. Histological Observation

1) Staining of Epididymal fat cells

Epididymal fat cells were cut in each experimetal animals. The epididymal fat cells were fixed in Bouil's solution for 8 hours, rinsed with ethanol completely, and the paraffined sections were cut 6~8㎛ thickness and stained in 2% anilin blue[15].

2) Determination of adipocyte

The size of epididymal fat cells was determined by electron microscope using computer image analyser(Leica 500MC, German).

3) Staining of Fat in Liver Tissues

In the 8th week after the beginning of experimental period, the rats were anesthetized with ketamin, the livers of rats were cut and then refrigerated with liquid nitrogen. The refrigerated tissues were cut at 10㎛ thickness by ultra-microtomes(Jung frigocut 2800N, German), and stained by oil-red-O[16].

4) Determination of the Area Ratio of Fat Drops in Hepatic Lobule

The area of fat drop in stained hepatic lobule was determined by electron microscope using computer image analyser(Leica 500MC, German). And determined extents was fixed square(202500㎛^{2}) centering around hepatic lobule. We utilized the means of all samples.

5) Stastical analysis

Significant differences were determined by using student t-test with graphpad prism(USA).

Table 1. Change on the Body Weight of Rats Fed High Fat Diet

Group	Body weight(g)								
	0 week	1 week	2 week	3 week	4 week	5 week	6 week	7 week	8 week
Normal	234.1±1.9[A]	262.9±4.9	270.0±8.1	294.5±7.5	321.8±7.0	332.3±5.7	353.6±7.7	385.9±8.3	407.5±6.7
Control	243.5±2.2###	288.4±8.1#	331.7±11.6###	359.5±12.2###	386.6±9.2###	409.1±14.5###	453.0±13.0###	481.5±15.1###	517.7±132###
PAS	243.7±2.0	283.5±4.3	310.5±6.5	333.2±7.5	361.0±10.4	390.5±11.5	436.0±11.8	454.4±11.4	484.4±10.1

[A] : Mean ± Standard Error
Normal : Group fed normal diet
Control : Group fed high fat diet and administered normal saline during 8 weeks
PAS : Group fed high fat diet and administered 7.26㎎/100g extract of Phaseoli Angularis Semen during 8 weeks
: Statistically significant difference with normal group (# : P<0.05, ## : P<0.01, ### : P<0.001)

Table 2. Effect on the Serum Total Cholestrol Level of Rats Fed High Fat Diet

Group	Total cholesterol(㎎/*dl*)	Decrease(%)
Normal	65.8±2.3[A]	
Control	83.0±2.7###	
PAS	77.2±3.3	7.0

Other legends are the same as Table 1
: Statistically significant difference with normal group (### : P<0.001)

Results

1. Body Weight

Weekly body weights of PAS group are respectively 243.7±2.0g, 283.5±4.3g, 310.5±6.5g, 333.2±7.5g, 361.0±10.4g, 390.5±11.5g, 436.0±11.8g, 454.4±11.4g, 484.4±10.1g. Body weight of PAS groups are decreased compared with those of control group, but the rates are not significant(Table 1).

2. Serum total cholesterol

Serum total cholesterol of control group(83.0±2.7㎎/*dl*) is much increased compared with that of normal group(65.8±2.3㎎/*dl*). Serum total cholesterol of PAS group(77.2±3.3㎎/*dl*) is decreased by 7.0% compared with that of control group(Table 2).

Serum total cholesterol level of experimental group are decreased compared with that of control group, but the rates are not significant. This results suggest that if the obese index is high, serum total cholesterol is increased.[17,18] Therefore Phaseoli Angularis Semen may effect on the reduction of obesity.

3. Serum Triglyceride

Triglyceride is a combination of 3 fatty acids connected by esterification[3]. It is the chief element of every fatty tissue, and controls the energy preservation in body. Measurement of triglyceride level can also explain the cause of abnormal lipid metabolite[3].

The variation of serum triglyceride level may be the index of obesity because it is well known that if the obese index is high, serum triglyceride is increased[17,19,20].

Serum triglyceride of control group(130±7.0㎎/*dl*) is much increased compared with that of normal group(71.0±5.7㎎/*dl*). Serum triglyceride of PAS group(103.6±9.0㎎/*dl*) is decreased by 21.1% compared with that of control group(Table 3).

4. Serum free fatty acid

Serum free fatty acid is only 4~5% of total fatty acid in lipid. It usually forms a combination with albumin, and is energy source of peripheral tissue[3]. It is measured excessively in a patient with obesity, diabetes or liver disease[3]. The higher the obese rate is, the higher the serum triglyceride density[21].

Serum free fatty acid of control group(783.5±47.9μEq/l) is much increased compared with that of normal group(599.5±10.0μEq/l). Serum free fatty acid of PAS group(630.1±45.9μEq/l) is decreased by 19.6% compared with that of control group. And decreasing rates of PAS groups is significant(Table 4).

Table 3. Effect on the Serum Triglyceride Level of Rats Fed High Fat Diet

Group	Triglyceride(㎎/*dl*)	Decrease(%)
Normal	71.0±5.7[A)]	
Control	130.5±7.0[###]	
PAS	103.0±9.0[*]	21.1

Other legends are the same as Table 1
[#] : Statistically significant difference with normal group ([###] : P<0.001)
[*] : Statistically significant difference with control group ([*] : P<0.05)
Decrease(%) : (Control - Sample)/Control x 100

Table 4. Effect on the Serum Free Fatty Acid(FFA) Level of Rats Fed High Fat Diet

Group	FFA(*μ*Eq/ *l*)	Decrease(%)
Normal	599.5±10.0[A)]	
Control	783.5±47.9[##]	
PAS	630.1±45.9[*]	19.6

Other legends are the same as Table 1
[#] : Statistically significant difference with normal group ([##] : P<0.01)
[*] : Statistically significant difference with control group ([*] : P<0.05)
Decrease(%) : (Control - Sample)/Control x 100

Table 5. Effect on the Serum Total Lipid Level of Rats Fed High Fat Diet

Group	Total lipid(㎎/*dl*)	Decrease(%)
Normal	301.8±11.1[A)]	
Control	432.8±45.2[#]	
PAS	393.6±23.7	9.1

Other legends are the same as Table 1
[#] : Statistically significant difference with normal group ([#] : P<0.05)
Decrease(%) : (Control - Sample)/Control x 100

Table 6. Effect on the Serum Phospholipid Level of Rats Fed High Fat Diet

Group	Phospholipid(㎎/*dl*)	Decrease(%)
Normal	124.2±4.2[A)]	
Control	145.5±6.6[#]	
PAS	132.4±3.9	9.0

Other legends are the same as Table 1
[#] : Statistically significant difference with normal group ([#] : P<0.05)
Decrease(%) : (Control - Sample)/Control x 100

Table 7. Effect on the Serum HDL- Cholestrol Level of Rats Fed High Fat Diet

Group	HDL- cholesterol(㎎/*dl*)	Decrease(%)
Normal	51.7±2.6[A)]	
Control	61.4±1.9[##]	
PAS	64.4±3.0	4.9

Other legends are the same as Table 1
[#] : Statistically significant difference with normal group ([#] : P<0.05)
Decrease(%) : (Control - Sample)/Control x 100

5. Lipid

When an obese person loses the body weight, total lipid amount is decreased[22].

Total lipid amount of control group(432.4±45.2㎎/*dl*) is much increased compared with that of normal group(301.8±11.1㎎/*dl*). Total lipid amount of PAS group(393.6±23.7㎎/*dl*) is decreased by 9.1% compared with that of control group. But decreasing rates of PAS groups is not significant(Table 5).

6. Serum Phospholipid

Phospholipid is related to many body functions such as the formation of cellular wall, emulsification or absorption of lipid, blood coagulation, choline metabolism, etc. It varies its level, when the lipidic metabolic disorder occurs[3]

Serum Phospholipid of control group(145.5±6.6㎎/*dl*) is much increased compared with that of normal group(124.2±4.2㎎/*dl*). Serum Phospholipid of PAS group(132.4± 3.9㎎/*dl*) is decreased by 9.0% compared with that of control group. But decreasing rates of PAS group is not significant(Table 6).

7. Serum HDL-cholesterol

HDL-cholesterol contributes to the elimination of cholesterol stored in body cells[3]. It is recently reported that HDL-cholesterol prevents arteriosclerosis[3]. And if the obese index is high, serum HDL-cholesterol is decreased[3,17,19].

Serum HDL-cholesterol of control group(61.4±1.9 ㎎/*dl*) is significantly increased compared with that of normal group (51.7± 2.6㎎/*dl*). Serum HDL-cholesterol of PAS group(64.4±3.0㎎/*dl*) is increased by 4.9%, compared with that of control group. But decreasing rates of PAS groups is not significant(Table 7).

8. Serum LDL-cholesterol

If the obese index of a person is high, LDL-

Table 8. Effect on the Serum LDL-Cholestrol Level of Rats Fed High Fat Diet

Group	LDL-cholesterol(㎎/*dl*)	Decrease(%)
Normal	8.1±0.4[A)]	
Control	10.5±0.7[##]	
PAS	9.6±0.5	8.6

Other legends are the same as Table 1
' : Statistically significant difference with normal group ([##] : P<0.01)
* : Statistically significant difference with control group (* : P<0.05)
Decrease(%) : (Control - Sample)/Control x 100

Table 9. Effect on the Average Size of Epididymal Fat Cells of Rats Fed High Fat Diet

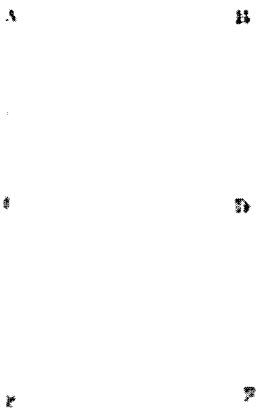
Group	Average size of fat cells(μm^2)	Decrease(%)
Normal	2136±226.0[A)]	
Control	3128±201.4[##]	
PAS	2671±136.4	14.6

Other legends are the same as Table 1
' : Statistically significant difference with normal group ([##] : P<0.01)
Decrease(%) : (Control - Sample)/Control x 100

Table 10. Effect on the Area % of Fat Drops in Hepatic Lobule of Rats Fed High Fat Diet

Group	Area % of fat drops	Decrease(%)
Normal	0.0±0.0[A)]	
Control	6.4±0.4[###]	
PAS	4.3±0.7[*]	32.8

Other legends are the same as Table 1
' : Statistically significant difference with normal group ([###] : P<0.001)
* : Statistically significant difference with control group (* : P<0.05)
Decrease(%) : (Control - Sample)/Control x 100



Legends of the figures
A. The epididymal fat cell of normal rat(x100, 2% aniline blue)
B. The epididymal fat cell of control rat(x100, 2% aniline blue)
C. The epididymal fat cell of PAS group rat(x100, 2% aniline blue)
D. The fat dorps in hepatic lobule of normal rat(x200, Oil-red-O)
E. The fat dorps in hepatic lobule of control rat(x200, Oil-red-O)
F. The fat dorps in hepatic lobule of PAS group rat(x200, Oil-red-O)

cholesterol concentration of him is also high[17,21)], and high lipid diet inceases especially LDL-cholesterol concentration[23)].

Serum LDL-cholesterol of control group(10.5±0.7㎎/*dl*) is much increased compared with that of normal group(8.1±0.4㎎/*dl*). Serum LDL-cholesterol of PAS group(9.6±0.5㎎/*dl*) is decreased by 8.6% compared with that of control group. But decreasing rate of experimental group are not significant(Table 8).

9. Effects on the Average Size of Epididymal Fat Cells of Rats

Recently in a research of obesity fat cells is very important[24)], because these'role is very important in obesity[25,26)]. Rats fed high fat diet have been getting fatter, and increasing fat cell numbers, fat cell size[27)]. And seemingly with this good reason, if clearly judge fat cells, it's very easy to cure the obesity[24)]. Based on this reason, in this studies, we had experiment with Phaseoli Angularis Semen(PAS) in order to find reducing for an obesity. We investigated the effects of Phaseoli Angularis Semen on epididymal and liver fat cells of rats.

The average size of epididymal fat cells of control group(3128±201.4μm^2) was significantly increased that of normal group(2136±226.0μm^2). The average size of epididymal fat cells of PAS group(2671±136.4μm^2) is decreased by 14.6% compared with that of control group. But Decreasing rates of PAS groups is not significant(Table 9).

10. Effects on the Area % of Fat Drops in

Hepatic Lobule of Rats

There is showing that fat is stored in liver if rats fed high fat diet[28], because this raises free fatty acid above so called increasing of fat's moving from adipose tissue[28]. In addition to, it is reporting that rats fed high carbohydrate diet were stored lipid in liver tissues. As the results it is increasing triglyceride contents and total cholesterol contents in blood[29,30].

The area % of fat drops in hepatic lobule of control group(6.40±0.40%) was significantly increased that of normal group(0.00±0.00%). The area % of fat drops in hepatic lobule of PAS group was decreased by 32.8% compared with that of control group. And decreasing rates of PAS groups is significant(Table 10).

Discussion

We carried out to investigate the effect of Phaseoli Angularis Semen(PAS) on biochemical and histological changes of rats fed high fat diet. We got the following result investigating the effects of PAS.

The serum triglyceride, free fatty acid level in Phaseoli Angularis Semen group showed a significant decrease in comparison with control group. The area % of the fat drops in hepatic lobule of rats in Phaseoli Angularis Semen group showed a significant decrease in comparison with control group.

Summarizing these results, it seems likely that Phaseoli Angularis Semen may be used to prevent or cure the obesity induced by high fat diet.

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