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Beneficial Effect of Anti-obese Herbal Medicine Mixture with Chitosan in High Fat Diet-induced Obese Rats

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Abstract

This study was carried out to investigate the dietary effects of chitosan mixture (CM), an herbal medicine mixture with *Sukjihwang* (HS), and CM containing HS (CHS) on obesity in an induced obese model of rats fed high-fat only (HF), in which supplemented diets of 5% CM (HCM), 5% HS (HHS), or 2.5% CM-2.5% HS (HCH) was tested for 6 weeks. Body weight gains, obesity indexes, and body fat contents in the experimental groups (HCM, HHS, HCH) were decreased compared with HF group. The levels of serum triglyceride, total lipid, total cholesterol and LDL-cholesterol in the experimental groups were markedly decreased, however HDL-cholesterol levels in the experimental groups were slightly increased compared with HF group. In addition, although serum ALT and AST activity, and relative organ weights were lower than those of HF group, serum albumin contents were not significantly different in all experimental groups including the normal control group (NC). In conclusion, there are improved effects on obesity in the obese model of animals with all experimental diets supplementations, and the improvement degrees on obesity depend on the content and compositions of the herbal medicine mixture. Further study is needed on the anti-obesity mechanism of these diets.

Key words: chitosan, herbal mixture, anti-obesity, high-fat diet

INTRODUCTION

Obesity is a global epidemic in both highly developed and underdeveloped countries. It is well known that obesity is an excess of fat accumulation in the body caused by energy imbalance through a high-calorie diet intake or deficient energy consumption. Many studies suggest that obesity is thought to be an definite risk factor for cardiovascular diseases (1,2), diabetes (3,4), and certain cancers (5). Furthermore, direct and indirect health budget costs caused by obesity have increased in highly developed countries. It is widely accepted that reduced calorie intake, increased physical activity and medication affect lipid utilization or absorption as prevention and therapy for obesity. Body weight is rapidly regained by many people, however, following discontinuation of healthy diets, medication or physical activity through a counter-regulatory mechanism in the body (6). Some healthy foods or drugs have adverse effects or side effects, but this is not the case with anti-obesity activity (7,8).

Thus, new healthy food or drugs with minimized adverse effects or side effects are required for prevention and therapy for obesity. Recently, it was reported that some traditional herbs have an improved effect on obesity without any noticeable side effects (9-12).

In this study, experimental diets are prepared with traditional Korean anti-obese herbal medicine mixtures containing *Sukjihwang* composed of *Rehmanniae Radix* (13), *Coicis Semen* (14), *Eucommia Cortex* (15), *Euryale ferox Salisbury* (16), *Ginseng Radix* (17,18) and *Atractylodes Rhizoma* (19) and having functionality of hypolipidemic, hypoglycemic, anti-obesity, anti-oxidative and/or anticancer activity. Also a mixture of chitosan, a substance which has been known to have antidiabetic (20,21), hypolipidemic (21), hypochloesterolemic (22), antiulcerogenic (23), hepatoprotective (24) and lipid absorption inhibiting effects (7).

We observed weight gain, obesity index, serum lipid profile, aminotransferase activity, and albumin level in the obese model of rats being fed a high-fat diet with and without the addition of herbal medicine mixtures containing *Sukjihwang* and/or chitosan mixtures for 6 weeks.

MATERIALS AND METHODS

Materials

Water souble α-chotosan (MW: 1 kDa) and acid soluble α-chotosan (MW: 746 kDa) were purchased from

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Kitto Life (Seoul, Korea). The aquatic plant calcium came from Celticsea Minerals (Ireland), sea tangle extract from Saerom-Bio (Korea), green tea extract from MSC (Korea), and the dried yeast and hibiscus extract was purchased from Saewon Trade Co. (Korea). Herbs such as *Rehmanniae Radix*, *Coicis Semen*, *Eucommia Cortex*, *Euryale ferox Salisbury*, *Ginseng Radix*, *Atractylodes Rhizoma Alba*, etc. were purchased from the traditional medicines market in Daegu, Korea. All other materials and chemicals were of the highest commercially available purity.

Preparation and compositions of CM, HS, and CH

CM (chitosan mixture) was composed of 2 kinds of α-chitosan (1 and 746 kDa of molecular weight), aquatic plant calcium, sea tangle, ascorbic acid, L-carnitine, L-tyrosine, dried yeast and hibiscus extracts. All materials for preparation of HS (herbal medicine mixture with *Sukjihwang*), and CH (CM plus HS) were filtered through a 100-mesh stainless steel sieve after drying at 50°C over night in the hot wind dry oven (Samhwa Co., Daegu, Korea). The compositions and content of the materials are shown in Table 1.

Animal experiment and diets

Male Sprague-Dawley rats (110±10 g) were supplied by Oriental Co., Ltd. (Busan, Korea). The rats were fed a standard rodent pellet chow and acclimatized to their environment for 1 week before commencement of the experiments. Next, the rats were randomly divided into 2 groups; normal control (n=6), and a high-fat diet group (n=24). The high-fat diet group was fed a standard pellet chow with an added 15% lard supplemented diet for 4 weeks for induction of obesity. After obesity was confirmed by checking obesity indexes, the animals were divided into 5 groups (n=6); first, the normal control (NC), and then taken from the obese model of rats, a high-fat diet obesity control group (HF), 5% chitosan mixture supplemented high-fat diet group (HCM), 5% herbal medicine mixture with *Sukjihwang* supplemented high-fat diet group (HHS), and 2.5% chitosan mixture plus 2.5% herbal medicine mixture with *Sukjihwang* supplemented high-fat diet group (HCH) for 6weeks as seen in Table 2. The rats were individually housed in stainless steel wire-bottom cages in a room maintained at 20±2°C and 60±5% relative humidity. The room was exposed to alternating 12-hr periods of light and dark. The experimental protocols were conducted in accordance with internationally accepted principles for laboratory animal use and care as found in the Korea food and drug administration guidelines.

Table 1. Ingredients of chitosan mixture, herb medicine mixture with *Sukjihwang*, and chitosan mixture plus herb medicine mixture with *Sukjihwang* for supplement diet (g/100 g)

Ingredients	CM[1)]	HS[2)]	CH[3)]
α-Chitosan (1 kDa)	33.3	−	16.6
α-Chitosan (746 kDa)	30.3	−	15.1
Calcium from sea weeds	9.0	−	4.5
Dry yeast	7.5	−	3.7
Hibiscus extract	4.8	−	2.4
Sea tangle extract	7.5	−	3.7
Ascorbic acid	3.3	−	1.6
L-carnitine	1.2	−	0.6
L-tyrosine	0.6	−	0.3
Green tea extract	1.5	−	0.8
Magnesium stearate	1.0	−	0.5
Rehmannia glutinosa	−	16.3	8.2
Coicis semen	−	16.3	8.2
Eucommia ulmoides Oliver	−	10.9	5.4
Euuryale ferox Salisbury	−	16.3	8.2
Atractylodes japonica	−	16.3	8.2
Zingiber nigrum Gaertner	−	3.8	1.9
Panax ginseng C.A. Meyer	−	10.9	5.5
Schizandra chinensis Baillon	−	3.8	1.9
Macrocarpium officinale Sieb. et Zucc.	−	3.8	1.9
Amomum xanthioides Wallich	−	1.6	0.8
Total	100	100	100

[1-3)]CM: chitosan mixture, HS: herb medicine mixture with *Sukjihwang*, CH: chitosan mixture plus herb medicine mixture with *Sukjihwang*.

Feed intakes, weight gain and feed efficiency ratio (FER)

Body weight and feed intake were measured every day at the same hour during all experimental periods. The FER was calculated as daily weight gain (g)/ daily dietary intake (g).

Obesity index and content of body fats

In order to calculate the obesity index, the length from the tip of the nose to the anus and body weight of rats were measured every week according to Kim and Chung (25) during experimental periods. The Rőhrer index (25), Lee index (26) and TM index (25) were calculated for the obesity index. And the content of body fat was estimated using TM index (25).

$$\text{Rőhrer index} = \frac{\text{Body weight (g)}}{\text{Naso-anal length (cm)}^3} \times 10^3$$

$$\text{Lee index} = \frac{\text{Body weight (g)}^{1/3}}{\text{Naso-anal length (cm)}} \times 10^3$$

$$\text{TM index} = \frac{\text{Body weight (g)}}{\text{Naso-anal length (cm)}^{2.823}} \times 10^3$$

$$\text{Body fat content} = 0.581 \times \text{TM index} - 22.03$$

Preparation of analytical samples

After 6 weeks on the experimental diets, rats were

Table 2. Experimental groups (g/kg)

Ingredients	Experimental groups[1)]				
	NC	HF	HCM	HHS	HCH
Casein	200.00	200.00	186.98	195.60	191.29
Corn starch	150.00	150.00	150.00	150.00	150.00
Sucrose	500.00	500.00	473.42	463.94	468.68
Cellulose	50.00	50.00	50.00	50.00	50.00
Corn oil	50.00	35.00	34.46	31.91	33.19
AIN mineral mixture[2)]	35.00	35.00	35.00	35.00	35.00
AIN vitamin mixture[2)]	10.00	10.00	10.00	10.00	10.00
DL-methionine	3.00	3.00	3.00	3.00	3.00
Choline bitartrate	2.00	2.00	2.00	2.00	2.00
Lard	−	15.00	15.0	15.0	15.0
CM[3)]	−	−	50.0	−	−
HS[4)]	−	−	−	50.0	−
1/2CM+1/2HS	−	−	−	−	50.0
Total calorie (kcal)	4050.0	4050.0	4050.0	4050.0	4050.0

[1)]NC: normal control, HF: high fat diet, HCM: high fat diet with 5% chitosan mixture, HHS: high fat diet with 5% herb medicine mixture with *Sukjihwang*, HCH: high fat diet with 2.5% chitosan mixture plus 2.5% herb medicine mixture with *Sukjihwang*.
[2)]Rat and Mouse 18% (PMI Nutrition International, LLC, Brentwood, Newhampshire, USA).
[3,4)]CM: chitosan mixture, HS: herb medicine mixture with *Sukjihwang*.

fasted for 24 hr, and blood was collected from the abdominal aorta while the rats were under anesthetic with ether. The collected blood was centrifuged at 2,500 rpm for 10 min at room temperature and the separated serum was kept frozen at -70°C.

Biochemical analysis

Content of triglyceride, total cholesterol and HDL-cholesterol in serum were measured by using kit reagents (AM 157S-K, AM 202-K, AM 203-K, Asanpharm Co., Korea). And the content of LDL-cholesterol was calculated by using the method of Friedewald et al. (27). The atherogenic index (AI) was calculated from (total cholesterol−HDL-cholesterol)/ total-cholesterol]. Activities of serum aminotransferase were estimated by using kit reagents (Elitech, Division of Seppim S.A., France) and the content of serum albumin and total protein were analyzed by commercial kit reagents (Elitech, Division of SEPPIM S.A France & Quant-iT^{TM}, Invitrogen, USA).

Statistical analysis

The results were expressed as mean±standard deviation (SD) of the 6 animals. Statistical comparisons of differences between the different groups was carried out by doing a two-way ANOVA test followed by Duncan's multiple range test using SPSS statistical software package (Version 12.0, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Induction of obesity

In order to confirm the obese animal model, we observed the content of body fat, and the Rőhrer, Lee and TM indexes for the obesity index in high-fat diet pretreated rats for 4 weeks. The Rőhrer index (30.3～30.5), Lee index (311.4～314.8) and TM index (52.3～54.2) in the high-fat diet feeding animals were significantly higher than those of normal control animals which showed a Rőhrer index of 27.8, a Lee index of 298.8, and a TM index of 46.5. The content of body fat in the high-fat diet animals also increased 67.1～89.2% compared with normal control animals (NC). Interestingly, four obese indexes in the HCH group were lower than those of the other groups at 6 weeks.

Obesity is confirmed in the case of a Rőhrer index of over 30, a Lee index of over 300, and a TM index of over 55 (25). Therefore, we can confirm that the experimental animals had become obese by high-fat diet feeding over 4 weeks. Furthermore, the anti-obese effects in the HCH group were shown to have a synergistic effect by mixture of anti-obese herbal medicines and chitosan. Under these experimental conditions, we observed the effects of experimental diets on the obese animal model.

Weight gains, feed intakes and feed efficiency ratio

The dietary effects of experimental diets on weight gain, feed, calorie intake, and the feed efficiency ratio (FER) in high-fat diet induced obese rats is shown in Table 3. Final body weight and weight gain in all experimental diet groups (HCM, HHS & HCH) were decreased 7.2～10.5% and 10.2～27.4% respectively, when compared with the HF group after 6 weeks.

Table 3. Weight gain, feed intakes and feed efficiency ratio (FER) in obese rats fed high fat diet containing 5% chitosan mixture, 5% herb medicine mixture with *Sukjihwang*, and 2.5% chitosan mixture plus 2.5% herb medicine mixture with *Sukjihwang* for 6 weeks

Parameters	Experimental groups[1)]				
	NC	HF	HCM	HHS	HCH
Initial weight (g)	311.17±16.80[b2)]	378.08±25.67[a]	356.58±19.28[a]	369.75±42.12[a]	349.00±11.00[a]
Final weight (g)	459.08±33.48[c]	562.92±42.13[a]	522.58±28.00[ab]	504.08±33.52[b]	514.50±34.70[b]
Weight gain (g/day)	3.36±0.63[ab]	4.20±0.60[a]	3.77±0.49[ab]	3.05±0.80[b]	3.76±0.79[ab]
Feed intake (g/day)	24.63±1.41[a]	21.56±1.58[ab]	20.50±1.67[b]	20.48±2.25[b]	19.49±1.42[b]
Calorie intake (g/day)	99.75±5.71[a]	87.32±6.40[b]	83.03±6.76[b]	82.94±9.11[b]	78.93±5.7[b]
FER	0.13±0.02[b]	0.19±0.02[a]	0.18±0.01[a]	0.16±0.03[a]	0.19±0.03[a]

[1)]See Table 2.
[2)]Each value is mean±SD of experimental group, n=6. Different alphabets in each values show statistically difference at $\alpha=0.05$ by Duncan's multiple range test.

However, feed, calorie intake, and FER in experimental groups were not significantly different from the HF group (Table 3). Of note, feed intake in HCH was higher than those of the other experimental groups.

Obesity index and content of body fats

Fig. 1 shows the effects of experimental diets on the obesity index and content of body fat in high-fat diet induced obese rats. Although the Rőhrer index, among obesity indexes in the HF group, was substantially increased from 30.4 to 31.6, in experimental groups it was significantly decreased from 30.3～30.5 to 27.8～28.2 after 6 weeks with supplementation of the experimental diets. Likewise, the Lee index in the HF group was increased from 311.6 to 316.0, but in the experimental diet groups it was decreased from 311.4～314.8 to 296.7～303.8. In addition, the TM index showed similar pattern as the Lee index. Furthermore, content of body fat in the HF group was markedly higher than that of NC group, but in experimental diet groups it was decreased compared with the HF group.

Presently, many medical clinicians have warned that the mortality rate has markedly increased due to metabolic syndrome caused by obesity (28). Furthermore, it is reported that when body weight decreases by 10 kg,

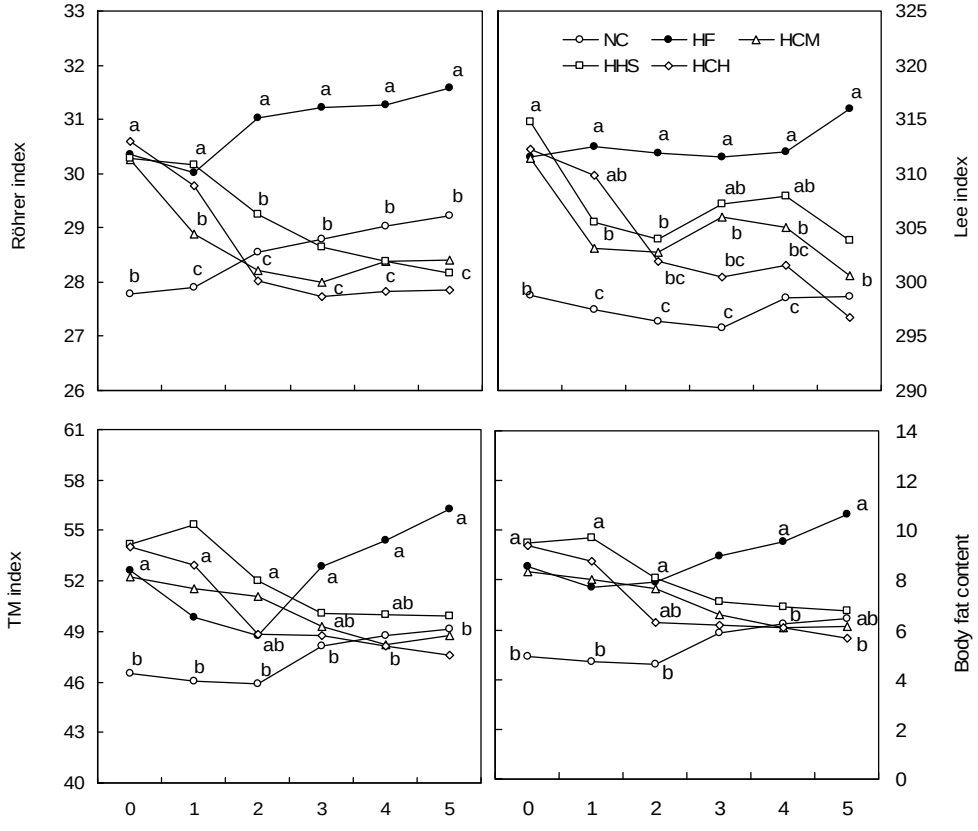

Fig. 1. Changes of obesity indexes in rats fed high fat diet with 5% chitosan mixture (CM), 5% herb medicine mixture with *Sukjihwang* (HS), and 2.5% HS plus 2.5% CM for 6 weeks. Abbreviations: See Table 2. Each value is mean±SD of experimental group, n=6. Different alphabets in each values show statistically difference at $\alpha=0.05$ by Duncan's multiple range test.

diabetes related-mortality reduces by 30～40% (29) and as well, diabetes, hypertension and cardiovascular diseases could significantly improve (29-31).

It is well known that decisive Rőhrer, Lee, and TM indexes among obesity indexes or body fat content in obese rats are over 30 (32), 300 (26) and 55 or 10 (25), respectively. The results of this study indicate that obesity was improved by the experimental diets such as chitosan mixture (CM), herbs mixture with *Sukjihwang* (HS), and CM plus HS (CH), especially it was considered that the mixed diet of herbs-chitosans (CH) could have synergistic effects on obesity.

Levels of serum lipid

Serum lipid profiles in high-fat diet, induced obesity rats are shown in Table 4. Levels of serum triglyceride, total lipid, total cholesterol, LDL-cholesterol and the atherogenic index in experimental diet groups were significantly decreased with levels of 46.0～51.1%, 13.0～19.8%, 11.7～26.1%, 52.0～65.0% and 31.7～51.7% compared with HF group, respectively. In addition, HDL-cholesterol levels in experimental groups were increased by 28.1～49.4% when compared with the HF group.

Overweight and obesity result from imbalance between food intake and energy expenditure (33). Many factors have been attributed to an epidemic of obesity, including a high-fat diet, consumption of large amount of fast food, and sedentary lifestyle (34). It is indicated that obesity is a risk factor for diseases like hyperlipidemia, hypertension, atherosclerosis, diabetes, certain cancers, osteoarthritis, gallstones, nonalcoholic fatty liver disease, sleep apnea, and asthma (35-38). Generally, while serum lipid profiles such as total lipid, TG, total-cholesterol, LDL-cholesterol and AI index in obesity were increased, HDL-cholesterol were decreased (39, 40).

It is generally accepted that increased serum VLDL and LDL levels caused by excessive intakes of fat could induce atherosclerosis in both macro- and micro-vessels. In addition, it is well documented that accumulation of triglycerides in tissues is a major factor of obesity (41). On the other hand, selected herbs are known to have hypolipidemic, hypoglycemic, anti-obesity and/or anti-oxidative effects (42,12). Chitooligosaccharides could prevent obesity via blockage of lipid absorption in the gastrointestinal tract (43). Chitooligosaccharides also have the effects of being antidiabetic (20,44,45), hypolipidemic, hypochloesterolemic (22) and hepatoprotective (24). And extract of hibiscus could reduce lipid accumulation through inhibition of body fat synthesis (46). Therefore, our results suggest that diets containing these herbal extracts could have improved effects on obesity and obesity-related diseases through direct or indirect regulation of lipid absorption and/or metabolism due to the hypolipidemic, hypoglycemic or anti-oxidative effects of herbs and/or chitosan. Generally, it is known that obese indexes and serum lipid profiles were decreased by hypolipidemic materials (47). In this study, obese indexes and serum lipid profiles such as TG, total cholesterol and LDL-cholesterol were decreased by all experimental diets. These results suggest that all experimental diets in this study showed anti-obese and hypolipidemic effects. However, serum lipid lowering effects in the HCH group was not higher than those of HCM and HHS group. At this time, we can not describe beneficial or synergistic effects of mixtures of anti-obese herbal medicines and chitosan on serum lipid lowering action. Further investigations into these areas are planned.

Organ weights, serum aminotransferase activities, albumin and protein contents

Table 5 and Fig. 2 show the effect of the experimental diets on the relative organ weights, serum aminotransferase activities, albumin and the total protein con-

Table 4. Serum lipids content in obese rats fed high fat diet containing 5% chitosan mixture, 5% herb medicine mixture with *Sukjihwang*, and 2.5% chitosan mixture plus 2.5 herb medicine mixture with *Sukjihwang* for 6 weeks

Parameters	Experimental groups[1)]				
	NC	HF	HCM	HHS	HCH
TG (mg/dL)	$39.00 \pm 1.41^{bc3)}$	78.33 ± 3.51^{a}	39.75 ± 3.30^{bc}	38.33 ± 1.53^{bc}	42.33 ± 3.51^{b}
Total lipid (mg/dL)	215.25 ± 27.32^{cd}	311.20 ± 22.13^{a}	249.60 ± 30.54^{bc}	251.40 ± 29.83^{bc}	270.75 ± 30.77^{b}
Total chol (mg/dL)	56.25 ± 0.50^{bc}	70.75 ± 4.11^{a}	55.00 ± 3.00^{c}	62.50 ± 5.45^{b}	52.25 ± 4.92^{c}
HDL-chol (mg/dL)	31.75 ± 3.30^{b}	29.67 ± 2.08^{b}	38.50 ± 9.26^{ab}	44.33 ± 4.16^{a}	38.00 ± 7.81^{ab}
LDL-chol (mg/dL)	12.50 ± 1.29^{cd}	33.30 ± 1.98^{a}	11.67 ± 1.15^{d}	16.00 ± 1.00^{b}	12.00 ± 1.00^{d}
AI[2)]	0.43 ± 0.05^{b}	0.60 ± 0.02^{a}	0.35 ± 0.03^{d}	0.29 ± 0.04^{e}	0.41 ± 0.01^{bc}

[1)]See Table 2.
[2)]AI (Atherogenic index) = (total chololesterol − HDL-cholesterol)/ total-cholesterol.
[3)]Each value is mean ± SD of experimental group, n = 6. Different alphabets in each values show statistically difference at $\alpha = 0.05$ by Duncan's multiple range test.

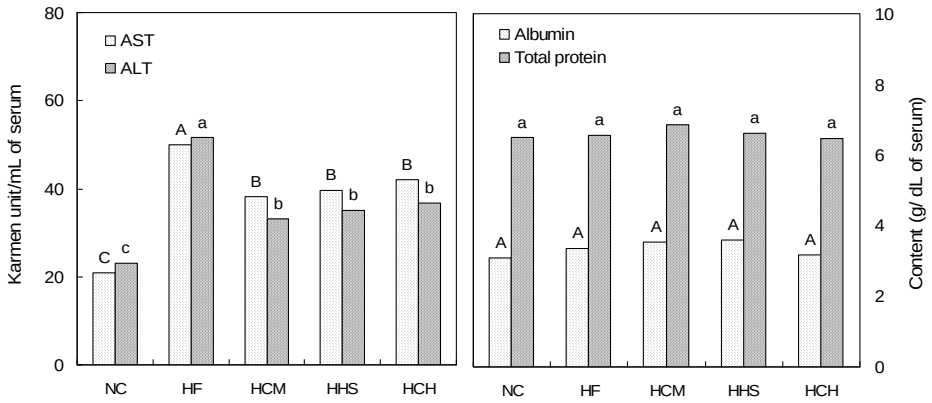

Fig. 2. Changes of serum aminotransferases (ALT & AST) activities, albumin and total protein contents in rats fed high fat diet with 5% chitosan mixture (CM), 5% herb medicine mixture with *Sukjihwang* (HS), and 2.5% HS plus 2.5% CM (HCM) for 6 weeks. Abbreviations: See Table 2. Each value is mean±SD of experimental group, n=6. Different alphabets in each values show statistically difference at $\alpha=0.05$ by Duncan's multiple range test.

Table 5. Organs weight in obese rats fed high fat diet with 5% chitosan mixture, 5% herb medicine mixture with *Sukjihwang*, and 2.5% chitosan mixture plus 2.5% herb medicine mixture with *Sukjihwang* for 6 weeks

(g/100 g body weight)

Experimental groups[1)]	Liver	Kidney	Heart
NC	2.52±0.19[NS2)]	0.59±0.04[ab3)]	0.30±0.02[ab]
HF	2.60±0.39	0.63±0.03[a]	0.33±0.02[a]
HCM	2.41±0.11	0.56±0.06[b]	0.30±0.04[ab]
HHS	2.55±0.16	0.54±0.03[b]	0.28±0.02[b]
HCH	2.56±0.14	0.55±0.04[b]	0.28±0.02[b]

[1)]See Table 2.
[2)]NS: not significant.
[3)]Each value is mean±SD of experimental group, n=6. Different alphabets in each values show statistically difference at $\alpha=0.05$ by Duncan's multiple range test.

tents in obese rats. Although liver, heart and kidney weight per body weight in the HF group had tendencies to increase compared to the NC group, all experimental diet groups were significantly decreased compared to the HF group, but not with significant differences among experimental diet groups themselves.

Whereas the activities of serum aminotransferase (ALT and AST) and content of serum albumin and protein in HF group were slightly higher than those of the NC group, activities of ALT and AST, and contents of serum albumin and total protein in the experimental diet groups were at the same levels found in the NC group, while the content of serum albumin and total protein were not changed in all experimental groups compared to the normal control group.

It is well known that hypoalbuminemia is very common in many illnesses such as liver diseases and renal diseases (48). Furthermore, activities of serum aminotransferases are increased, and activity of ALT is higher than AST in liver disease (49). Following cardiac damage, an increased level of AST, except ALT, appears in serum (50,51). It is also reported that relative liver weight was increased in rats fed the high-fat diet (52,53). Considering the reports, all experimental diets could modulate accumulation of lipid as well as metabolic functions in active metabolic organs such as the liver, heart and kidney in cases where high-fat diets are consumed and without severe organ toxicities.

It is reported that *Rehmannia glutinosa* extract prevents high fat diet (HFD)-induced weight gain and adiposity in rats, but also inhibits preadipocyte differentiation and adipogenesis in cultured cells and in rodent models of obesity (54,55). Also ginsenoside Rh2, one of the active components of ginseng, effectively inhibits adipocyte differentiation via PPAR-gamma inhibition by Hwang et al. (56). Furthermore, it is well known that chitosan inhibits obesity induced by feeding a high-fat diet in mice (57). Moreover, *hibiscus* water extract has an anti-obese effect (58).

These reports suggest that anti-obese effects in this experiment may be related to components of *Rehmanina glutinosa*, ginseng, chitosan, and/or *hibiscus*.

In conclusion, although the exact mechanisms of the anti-obesity effects of the experimental diets are unknown, the results provide a basis for developing efficacious healthy food or medicines for anti-obesity using chitosan and traditional herbs such as *Rehmanniae Radix*, *Coicis Semen*, *Eucommia Cortex*, *Atractylodes Rhizoma Alba*, and *Gingseng Radix*. Further study is needed to elucidate the anti-obesity mechanism of these diets.

REFERENCES

1. Alpert MA, Hashimi MW. 1993. Obesity and heart. *Am J Med Sci* 306: 117-123.
2. Sjostrom CD, Lissner L, Sjostrom L. 1997. Relationships between changes in body composition and changes in cardiovascular risk factors: the SOS intervention study. *Obese Res* 5: 519-530.
3. Felber JP, Golay A. 2002. Pathways from obesity to diabetes. *Int J Obes Rela Metab Disord* 26: S39-S45.

4. Virtanen KA, Iozzo P, Hallsten K, Huupponen R, Parkkola R, Janatuinen T, Lonnqvist F, Viljanen T, Ronnemaa T, Lonnroth P, Knuuti J, Ferrannini E, Nuutila P. 2005. Increased fat mass compensates for insulin resistance in abdominal obesity and type 2 diabetes: a positron-emitting tomography study. *Diabetes* 54: 2720-2726.
5. Schapira DV, Clark RA, Wolff PA, Jarrett AR, Kumar NB, Aziz NM. 1994. Visceral obesity and breast cancer risk. *Cancer* 74: 632-639.
6. Van der Ploeg LH. 2000. Obesity: an epidemic in need of therapeutics. *Curr Opin Chem Biol* 4: 452-460.
7. Pittler MH, Emst E. 2004. Dietary supplements for body-weight reduction: a systematic review. *Am J Clin Nutr* 79: 529-536.
8. Pittler MH, Schmidt K, Ernst E. 2005. Adverse events of herbal food supplements for body weight reduction: systematic review. *Obse Rev* 6: 93-111.
9. Kang MK, Oh JW, Lee HK, Chung HS, Lee SM, Kim CS, Lee HJ, Yoon DW, Choi H, Kim HY, Shin MK, Hong MC, Bae HS. 2004. Anti-obesity effect of PM-F2-OB, an anti-obesity herbal formulation, on rats fed a high-fat diet. *Biol Pharm Bull* 27: 1251-1256.
10. Heber D. 2003. Herbal preparations for obesity: Are they useful? Prim. *Care* 30: 441-463.
11. Choi HJ, Eo HK, Park KC, Jin MR, Park EJ, Kim SH, Park JE, Kim SY. 2007. A water-soluble extract from *Cucurbita moschata* shows anti-obesity effects by controlling lipid metabolism in a high fat diet-induced obesity mouse model. *Biochem Biophys Res Comm* 359: 419-425.
12. Chae MH, No JG, Jhon DY. 2006. Effect of high fat diet containing Hangbisan on the body weight and blood lipid composition in the rats. *J Kor Soc Nutr* 35: 416-421.
13. Chao JCJ, Chiang SW, Wang CC, Tsai YH, Wu MS. 2006. Hot-water extracted *Lycium barbarum* and *Rehmannia glutinosa* inhibit proliferation and induce apoptosis of hepatocellular carcinoma cells. *World J Gastroenterol* 12: 4478-4484.
14. Kwak CS, Lim SJ, Kim SA, Park SC, Lee MS. 2004. Antioxidative and antimutagenic effects of Korean buckwheat, sorghum, millet and job's tears. *J Korean Soc Food Sci Nutr* 33: 921-929.
15. Park SA, Choi MS, Kim MJ, Jung UJ, Kim HJ, Park KK, Noh HJ, Park HM, Park YB, Lee JS, Lee MK. 2006. Hypoglycemic and hypolipidemic action of Du-zhong (*Eucommia ulmoides* Oliver) leaves water extract in C57BL/KsJ db/db mice. *J Ethnopharmacol* 107: 412- 417.
16. Lee SE, Ju EM, Kim JH. 2002. Antioxidant activity of extracts from *Eurya leferox* seed. *Exp Mol Med* 34: 100-106.
17. Wang W, Zhao Y, Rayburn ER, Hill DL, Wang H, Zhang R. 2007. *In vitro* anti-cancer activity and structure-activity relationships of natural products isolated from fruits of *Panax ginseng*. *Cancer Chemother Pharmacol* 59: 589-601.
18. Krau N, Reifen R, Kerem Z. 2007. Weight gain reduction in mice fed *Panax ginseng* saponin, a pancreatic lipase inhibitor. *J Agric Food Chem* 55: 2824-2828.
19. Konno C, Suzuki Y, Munakata E, Hikino H. 1985. Isolation and hypoglycemic activity of atractans A, B and C, glycans of *Atractylodes japonica rhizomes*. *Planta Med* 51: 102-103.
20. Liu B, Liu WS, Han BQ, Sun YY. 2007. Antidiabetic effects of chitooligosaccharides on pancreatic islet cells in strepozotocin-induced diabetic rats. *World J Gastroenterol* 13: 725-731.
21. Miura T, Usami M, Tsuura Y, Ishida H, Seino Y. 1995. Hypoglycemic and hypolipidemic effect of chitosan in normal and neonatal streptozotocin induced diabetic mice. *Biol Pharm Bull* 18: 1623-1625.
22. Ausar SF, Morcillo M, Leon AE, Ribotta PD, Masih R, Vilaro Mainero M, Amigone JL, Rubin G, Lescano C, Castagna LF, Beltramo DM, Diaz G, Bianco ID. 2003. Improvement of HDL- and LDL-cholesterol levels in diabetic subjects by feeding bread containing chitosan. *J Med Food* 6: 397-399.
23. Santhosh S, Sini TK, Anandan R, Mathew PT. 2006. Effect of chitosan supplementation on antitubercular drugs-induced heaptotoxicity in rats. *Toxicol* 219: 53-59.
24. Anandan R, Nair PG, Mathew S. 2004. Anti-ulcerogenic effect of chitin and chitosan on mucosal antioxidant defence system in HCl-ethanol-induced ulcer in rats. *J Pharm Pharmacol* 56: 265-269.
25. Kim HS, Chung JS. 2001. Effects of dietary zinc and iron levels on serum trace minerals and obesity index in high fat diet-induced obese rats. *Korean J Food Sci Nutr* 30: 325-330.
26. Duduc PU. 1981. Non-essential role of dietary factors in the development of diabetes in ob/ob mice. *J Nutr* 111: 1742-1748.
27. Friedewald WT, Levy RI, Fredrickson DS. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18: 499-502.
28. Lim MJ. 2000. Effects of 8 week-exercise on fat distribution, cardio-vascular fitness, lipid profiles and plasma homocysteine concentration in eldery obese women. *J Korean Soc Study Obes* 9: 237-245.
29. Koo BJ. 2006. Pharmacotherapy for obesity. The 19th Annual Spring Meeting of Korean Clinical Diabetes. p 255-258.
30. Halford JCG. 2006. Pharmacotherapy for obesity. *Appetite* 46: 6-10.
31. Goldstein DJ. 1992. Benefical health effects of modest weight loss. *Int J Obesity* 16: 397-415.
32. Park SJ, Kang MH. 2003. The effect of dietary nuddle with glucomannan on the weight loss in high fat diet induced obese rats. *J Korean Soc Food Sci Nutr* 32: 893-898.
33. Woods SC, Seeley RJ, Porte D Jr, Schwarts MW. 1998. Signals that regulate food intake and energy homeostasis. *Science* 280: 1378-1383.
34. Grundy S. 1998. Multifactorial causation of obesity: implication for intervention. *Am J Clin Nutr Suppl* 67: 563S-572S.
35. Saltiel AR. 2001. You are what you secrete. *Nat Med* **7**: 887-888.
36. Leonhardt M, Hrupka B, Langhans W. 1999. New approaches in the pharmaco logical treatment of obesity. *Eur J Nutr* 38: 1-13.
37. Bray GA. 2004. Medical consequences of obesity. *J Clin Endocrinol Metab* 89: 2583-2589.
38. Stein CJ, Colditz GA. 2004. The epidemic of obesity. *J Clin Endocrinol Metab* 9: 2522-2525.
39. Kwon JY, Ann IS, Park KY, Cheigh HS, Song YO. 2005. The beneficial effects of pectin on obesity *in vitro* and *in vivo*. *J Korean Soc Food Sci Nutr* 34:13-20.
40. Park SJ, Kang MH. 2005. The dietary effect of patty made with added glucomannan in high fat diet-induced obese

rats. *J East Asian Soc Dietary Life* 15: 40-48.
41. Tom B. 1999. *Obesity*. Nutrional Biochemistry. 2nd ed, Academic Press, San Diego, USA. p 379-419.
42. Kim DH, Soh KS. 2007. Experimental study of *Pinus densiflora* Siebold et Zuccarini on hyperlipidemia and lipid in rats. *J Korean Institute of Herbal Acupuncture* 10: 109-119.
43. Lee JM, Son BK. 1998. Effects of chitosan of different molecular weights on lipid metabolism in rats. *Korean J Nutr* 31: 143-152.
44. Lee HW, Park YS, Choi JW, Yi SY, Shin WS. 2003. Antidiabetic effects of chitosanoligosaccharides in neonatal streptozotocin-induced noninsulin-dependent diabetes mellitus in rats. *Biol Pharm Bull* 26: 1100-1103.
45. Hayashi K, Ito M. 2002. Antidiabetic action of low molecular weight chitosan in genetically obese diabetic KK-Ay mice. *Biol Pharm Bul* 25: 188-192.
46. Ishihara K, Oyaizu S, Onuki K, Lim K, Fushiki T. 2000. Chronic (-)-hydroxycitrate administration spares carbohydrate utilization and promotes lipid oxidation during exercise in mice. *J Nutr* 130: 2990-2995.
47. Jeon JR, Kim JY. 2006. Effect of pine needle extract on differentiation of 3T3-L1 preadipocytes and obesity in high-fat diet fed rats. *Biol Pharm Bull* 29: 2111-2115.
48. Bauer JD. 1982. *Albumin in Clinical Laboratory Methods*. 9th ed. Mosby CV Co., St. Louis, USA. p 493-497.
49. De Ritis R, Giusti G, Piccinino F, Cacciatore L. 1965. Biochemical laboratory tests in viral hepatitis and other hepatic diseases. Evaluation and follow-up. *Bull World Health Organ* 32: 69-72.
50. Tiez NW. 1986. The aminotransferases (transaminases). In *Textbook of Clinical Chemistry*. Saunders WB Co., Philadelphia, USA. p 669-678.
51. Kibe O, Nilsson NJ. 1967. Observations on the diagnostic and prognostic value of some enzyme tests in myocardial infarction. *Acta Med Scand* 182: 597-610.
52. Lee HS, Kim HD, Ryu BH. 2000. Effects of soybean germ on the lipid composition of serum in cholesterol fed rats. *Korean J Food Nutr* 13: 312-318.
53. Sung IS, Kim MJ, Cho SY. 1997. Effect of *Quercus acutissima carruthers* extracts on the lipid metabolism. *J Korean Soc Food Sci Nutr* 26: 327-333.
54. Guo XN, Zhang RX, Jia ZP, Li MX, Wang J. 2006. Effects of *Rehmannia glutinosa* oligosaccharides on proliferation of 3T3-L1 adipocytes and insulin resistance. *Zhon Gguo Zhobg Tao Za Zhi* 31: 403-407.
55. Jiang L, Zhang NX, Mo W, Wan R, Ma CG, Li X, Gu YL, Yang XY, Tang QQ, Song HY. 2008. *Rehmannia* inhibits adipocyte differentiation and adipogenesis. *Biochem Biophys Res Commum* 371: 185-190.
56. Hwang JT, Kim SH, Lee MS, Kim SH, Yang HJ, Kim MJ, Kim HS, Ha J, Kim MS, Kwon DY. 2007. Anti-obesity effects ginsenoside Rh2 are associated with the activation of AMPK signaling pathway in 3T3-L1 adipocyte. *Biochem Biophys Res Commun* 364: 1002-1008.
57. Sumiyoshi M, Kimura Y. 2006. Low molecular weight chitosan inhibits obesity induced by feeding a high-fat diet long-term in mice. *J Pharm Pharmacol* 58: 201-207.
58. Alarcon-Aguilar FJ, Alejandro Zamilpa A, Perez-Garcia MD, Almanza-Perez JC, Romero-Nu˜nez E, Campos-Sepulveda EA, Vazquez-Carrillo LI, Roman-Ramos R. 2007. Effect of *Hibiscus sabdariffa* on obesity in MSG mice. *J Ethnopharmacol* 114: 66-71.

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