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RESEARCH NOTE

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Effects of Hangbisan, an Oriental Medicine, on Body Weight Gain in Diet-Induced Obese (DIO) rats

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Abstract This study was designed to determine possible weight loss effects of Hangbisan, an oriental medicine, on Sprague-Dawley (SD) rats. SD rats that were fed a high-fat diet for 6 weeks to induce obesity and subsequently fed with a basic diet containing 10%(w/w) Hangbisan or 10%(w/w) cellulose for 8 weeks. The Hangbisan fed rats demonstrated a significantly (p<0.05) reduced weight gain compared to the cellulose fed rats as well as a reduced level of plasma cholesterol and triglycerides and an increased level of (HDL)-cholesterol. These results suggest that dietary Hangbisan has an anti-obesity effect in the high-fat diet-induced obesity (DIO) rat and therefore a potential use as an anti-obesity agent in the application of oriental medicine compounds.

Key words: hangbisan, oriental medicine, obesity

Introduction

In the field of oriental medical practice, medicines for reinforcing Yang-qi, dynamic and progressive phenomenon of human body, have a warming effect of five viscera (liver, heart, spleen, lungs, and kidneys) and six bowels (small intestine, large intestine, gall bladder, stomach, bladder, and pancreas) and they are therefore used to treat various symptoms due to the low body temperature (1, 2). Of the various oriental medical science materials, sulfur is generally regarded as a heat-generating material in the body and is often used for the treatment of cold sensations in all four limbs (3). In the application of western medical science, sulfur has been used to treat dermatitis, constipation and hemorrhoids, but elemental sulfur is harmful (3). Processed sulfur, however, is generally known to be safe and could be used in treating osteoporosis (4) and possible complementing the Yang-qi in oriental medical sciences (3).

There have been several reports on the use of oriental medicines in anti-obesity and weight-loss studies (5-10) and the effects of processed sulfur on bone disease (4). Since processed sulfur apparently increases energy consumption in humans by acting as a heat-generating material (1), we have prepared a Hangbisan for study that contains 18 oriental herbs and 30%(w/w) processed sulfur to investigate a potential role for Hangbisan in the treatment of obesity. Our experimental animal model was the high fat diet induced obese laboratory rat. After the induction of obesity, we provided a basic diet that contained an additional 10%(w/w) of Hangbisan or cellulose and compared changes in body weight, liver histology, and blood composition over an eight week study period.

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Materials and Methods

Animals Four-week-old male Sprague-Dawley rats (n=16) weighing 100-110 g were used in this study and were purchased from the Daihan Biolink Co. (Daejeon, Korea). The rats were housed in individual cages for 1 week and fed a basic diet and then fed a high fat diet for 6 weeks to promote obesity as previously described by Jang and Choi (11). The diet-induced obese (DIO) rats were separated into two groups of eight each to receive either the Hangbisan diet (HD) or the cellulose diet (CD). The rats were maintained for an 8 week period under a temperature controlled environment at 22±2°C with a 12:12hr light-dark cycle. Food and water were available ad libitum throughout the whole experimental period.

Experimental diets The basic and high fat diets were purchased from the Harlan Co. (Indianapolis, IN, USA); the composition of the 2 diets are detailed in Table 1. The diets were stored at 4°C until used. Hangbisan was prepared by the Baekdu Oriental Medical Center (Gwangju, Korea). The composition of the Hangbisan is *Carex macrandrolepis* 5%, *Bupleureum Chinese* 5%, *Citrus aurantium* 5%, *Aristolochia debilis* 5%, *Cnidii rhizoma* 4%, *Carthamus tinctorius* 4%, *Plantago asiatica* 4%, *Polyporus umbellatus* 4%, *Alisma plantago-aguatica* 4%, *Poria cocos* 5%, *Pinellia ternate* 3%, *Arisaema consanguineum* 3%, *Atracylis orata* 4%, *Magnolia officinalis* 4%, *Perilla frutescens* var. *acuta* 4%, *Glycyrrhizae radix* 4%, *Sulphur* 30%, and 3% of *Burnt alumen.*

Measurement of body weights and food intake Rat body weights and food intakes were measured twice weekly during the entire experimental period. Food consumption was calculated as the difference between food supplied and food remaining and littered at each time point. A food efficiency ratio (FER) was calculated according to the equation: FER (%) = increase of animal

Table 1. Composition of the basic and the high-fat diets (g/ 100 g)

Ingredients	Basic diet	High-fat diet
Casein	20	29
Corn starch	60	10
Sugar	0	10
Lard	0	35
Corn oil	9	5
Cellulose	5.0	5.0
Mineral mixture[1)]	3.5	3.5
Vitamin mixture[1)]	1.0	1.0
Cholesterol	1.0	1.0
DL-methionine	0.3	0.3
Choline	0.2	0.2

[1)]AIN mixture, Nutritional Biochemicals, ICN Life Science Group (Cleveland, OH, USA).

body weight (g)/food consumed (g).

Biochemical analyses The rats were anesthetized using diethyl ether and the blood collected by heart puncture at the time of sacrifice. Commercial reagent kits for blood analysis were purchased from the Asan Pharmaceutical Company (Seoul, Korea). Bloods were centrifuged using a microcentrifuge to obtain the sera, which were used for the measurement of triglyceride (ASAN TG-Lq Reagents), total cholesterol (ASAN T–CHO-Lq Reagents) and HDL-cholesterol (HDL-cholesterol Kit). LDL-cholesterol was calculated according to Friedewald's equation (12):

LDL- cholesterol = total cholestero – HDL cholesterol-(triglyceride/5)

Blood glucose measurements were performed with the V-glucose kit; GOT and GPT levels were determined using a GOT·GPT assay kit.

Liver histology Livers were weighed after being flushed to remove blood by using phosphate buffered saline (PBS) buffer (pH 7.4). Liver tissue excised from the central lobe was used for histochemical analysis. Liver tissues were fixed in 4% paraformaldehyde, embedded in paraffin, the sections cut at 4-5 μm, stained by the standard hematoxylin and eosin (H & E) process and examined at 100x magnification.

Statistical analysis Data were expressed as the means ± standard deviation (SD). The analyses were performed using the Student's *t*-test (SPSS 10.0, SPSS Inc., Chicago, IL, USA). A value of $p<0.05$ was considered significant.

Results and Discussion

Effects of Hangbisan on body weights and food intake We were careful to observe any changes in appearance, behavior, and outbreak of disease during the study period for the two (CD and HD) animal groups. No apparent side effects such as disease or toxicities were noted nor were there differences in appearance (fur) and locomotion.

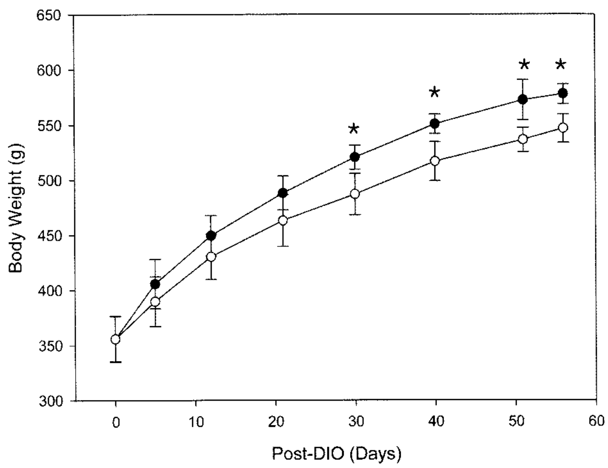

Fig. 1 Effects of Hangbisan, an oriental medicine on the body weights of diet-induced obese (DIO) rats. The results are expressed as Mean ± SD of 8 rats. CD (–●–) group fed a basic diet with added 10% cellulose after diet-induced obesity. HD (–○–) group fed a basic diet with added 10% Hangbisan after diet-induced obesity.

Figure 1 indicates body weights of the two groups of rats over the 8 week experimental diet feeding period. Each data point is the mean ± SD of 8 rats. Rats fed the high fat diet for 6 weeks attained an average body weight of 356 ± 17.3 g. The rats, now termed DIO, were randomly separated into two groups (HD and CD of eight each). As shown by the weight gain curves in Fig. 1, the HD group trended lower than the CD group from the first week onward of the 8 week experimental period, reaching significance at 4 weeks. Table 2 summarizes the changes in body weights, food intake, and the food efficiency ratio of the animals at the end of the study. The body weight gain in the HD group was lower than in the CD group, however, there was no significant difference in their daily food intake. The FER in the HD group (0.49%), therefore, was lower than the CD group (0.52%). It is well known that cellulose is one of the dietary fibers for weight loss (13, 14). *Kang et al.* (14) reported that the administration of 10%(w/w) cellulose in diet produced a 16% decrease in body weight compared to the control group. Based upon our results, Hangbisan was a more effective weight loss material than cellulose.

Sulfur-containing compounds reportedly enhance thermogenesis by uncoupling protein content and adrenaline secretion (15-17). Therefore, we have speculated that processed sulfur, a major component of our Hangbisan, can induce body weight loss through thermogenesis.

Effects of Hangbisan on serum lipids The serum lipid profiles of rats in the two study groups at the end of the 8 week experiment are presented in Table 2. Both the serum total cholesterol (176.75±5.06 mg/dL) and serum triglyceride (112.38±10.32 mg/dL) levels in the HD group were significantly reduced ($p<0.05$) compared to those in the CD group (184.71±5.25, 124.57±8.04 mg/dL). Castro *et al.* (18) recently reported that the addition of 5%(w/w) cellulose into a rat control basic diet decreased the serum

Table 2. Effects of the CD and HD diets on body weight, food intake and several blood variables in the high fat diet-induced-obese rats

	CD[1)]	HD
Weight (g)	578.00±9.01[2)]	546.75±7.63*
Weight gain (g/day)	3.86±0.53	3.41±0.42
Food intake (g/day)	7.38±0.17	7.19±0.12
FER[3)] (%)	0.52±0.07	0.49±0.08
Weight of liver (g/100g body weight)	3.50±0.47	4.15±0.54
HDL-cholesterol (mg/dL)	71.00±6.16	71.63±3.74
LDL-cholesterol (mg/dL)	88.80±3.65	82.65±4.12
Cholesterol (mg/dL)	184.71±4.25	176.75±3.06*
Triglyceride (mg/dL)	124.57±8.04	112.38±10.32*
Glucose (mg/dL)	290.25±48.60	257.88±48.27
GOT[4)] (IU/L)	13.75±2.96	19.71±6.55
GPT[5)] (IU/L)	12.75±4.92	14.25±3.96

1)CD, 10% cellulose diet; HD, 10% Hangbisan diet.
2)Values are means ± standard deviation of 8 rats.
3)FER (Food Efficiency Ratio) = body weight gain (g/day)/food intake (g/day).
4)GOT; glutamate oxaloacetic transaminase.
5)GPT; glutamate pyruvate transaminase.
*Significantly different between the values in the same row ($p<0.05$).

cholesterol (17%) and triglycerides (62%) when compared to rats fed the basic diet. Our results suggest that our Hangbisan was more effective in lowering the triglyceride and cholesterol levels than was the cellulose.

There were no significant differences in either HDL- or LDL-cholesterol levels between the two study diet groups. Martinez-Flores *et al.* (19) have reported that the addition of 8%(w/w) cellulose to the control basic diet in a hamster study increased HDL-cholesterol by 43% compared to the basic diet group. Similarly, Li *et al.* (20) reported that the administration of cellulose to the diet (0.2 g/kg-body weight) of women resulted in a 21% decrease in LDL-cholesterol when compared to the controls. Our Hangbisan, therefore, may be as effective as cellulose in improving serum lipid profiles.

Total cholesterol levels are generally increased and HDL-cholesterol levels decreased in obese humans (21, 22). Thus, the serum lipid composition is regarded as an important biomarker of obesity. Several studies have indicated that garlic, which contains several sulfur compounds is effective as a hypolipidemic agent (23-25). It is speculated, therefore, that the sulfur added to the Hangbisan diet might change the lipid profiles by acting as a hypolipidemic agent.

The GOT levels in the CD and HD groups were 13.75 ± 2.96 IU/L and 19.71 ± 6.55 IU/L, while GPT levels were 12.75 ± 4.92 IU/L and 14.25 ± 3.96 IU/L, respectively. These levels were not significantly different between the two diet groups. The administration of either Hangbisan or cellulose to DIO rats was not observed to cause any liver toxicity as observed by cellular damage via light microscopy (data not shown). This being the case, both study diets may similarly affect serum lipid composition without inducing liver toxicity.

Effects of Hangbisan on the liver It has been reported (26) that the liver weights of rats fed a 10%(w/w) cellulose diet were not significantly different from a basic diet group. In the present study the liver weights of the HD group were not significantly different from the CD group, thereby suggesting that the livers of the HD group are normal.

The livers of the CD group when examined histologically, however, showed mild fatty changes compared to the HD group (data not shown). It is well known that a high fat diet induces fatty changes in the liver (27, 28) and as a result changes observed in this experiment would reflect the DIO diet while suggesting that Hangbisan might lessen the fatty accumulations. Examination of the cellular structure of the liver in both CD and HD groups found essentially no differences. Although elemental sulfur is known to be harmful to man (3), processed sulfur has been shown to be safe (29). Kim and Seo (4) have reported the use of processed sulfur in treating osteoporosis. We have shown by our experiment that the addition of a total of 3% processed sulfur into the Hangbisan complemented basic diet was without toxicity in the experimental rats. This result is similar to that previously reported which found that processed sulfur was not toxic to humans (4). Therefore, the results presented here for the rodent suggest that Hangbisan might be beneficial in the treatment of obesity in man.

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