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Feeding Studies of Rats with Some Drinks Containing Ginseng Extract

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인삼 추출물 함유 액체식품에 의한 백쥐 사육시험

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Abstract

To study the effects of diets containing Ginseng extracts as foods, Sprague-Dawley strain rats were fed several diets containing the Ginseng extracts(Ginseng nectar, Ginseng orange juice, Ginseng drink) for 12 weeks. The Ginseng diets contained 555 mg or 1,110 mg of Ginseng extracts per kg of the diet. During the feeding, growth rate, feed efficiency ratio, organ weight, hematocrit value, SGOT and SGPT activity were compared with those of the control rats. As results of growth rate, feed efficiency ratio, organ weight, hematocrit value and SGPT activity showed no statistical significance between Ginseng fed animals and the controls. The experimental animals showed slightly lower SGOT activity and higher serum cholesterol than the controls did. The difference was, however, not statistically significant. No abnormalities of liver, spleen and kidney tissues were observed in the rats fed with the diets containing the Ginseng extract. As result of swimming test, a statistically significant increase of the swimming time was observed in the experimental animal groups, especially in Group V.

Introduction

Research works on the application of Ginseng products as food are very scarce. Recently several Ginseng products as food such as tea, caramel, nectar and drinks containing Ginseng are commercialized in the market.

Authors had studied to find the feeding effects in the rats fed with Ginseng confectioneries (1). In this continued work we studied the feeding effects in the rats fed with orange juices and nectars containing the Ginseng extract. Data of growth rate, feed efficiency ratio, organ weight change, hematocrit value, serum cholesterol level, SGOT (Serum glutamic-oxaloacetic transaminasec, SGPT (Serum glutamic-pyruvic transaminase) and swimming tests are presented in this report.

Materials and Methods

1. Animal and treatment

Animals were treated as the method described previously(1). Fourty healthy weanling male youngsters of Sprague-Dawley strain, weighing 76~85g each were divided in 5 groups. Each group consisted

of 8 male youngesters. Commercial stock diets were fed for 4 days before the experimental diet was fed. The animals were fed ad libitum for 12 weeks with the special diets containing Ginseng extract.

2. Sample preparation

Preparation of Ginseng nectars:

To prepare Korean Ginseng nectar (Bosung Nongsusan Co. LTD.), fresh Ginseng was grinded and added sugar, honey and Vitamin C. After canning, it was sterilized. Reducing sugar content of this nectar was 9.8% and total saponin content was 1 mg/ml of Ginseng nectar.

Preparation of Ginseng orange juice:

Ginseng extract 0.5% was added to natural orange juice and filled in cans and then sterilized. It contained 9.5% of reducing sugar.

Preparation of Ginseng drink:

In this experiment, WON KI SAM-D (Cheil General IND., Co. products) was used. It's components were 14% sucrose solution, Ginseng extract 0.4%, Sodium benzoate 0.05%, Sodium citrate 0.05%, Vitamin B_1 0.0042%, Vitamin B_2 0.005%, Drink flavor 0.05%, Niacin 0.0083%, Citric acid 0.25% and Caramel 0.1%.

Preparation of Ginseng Extract:

The Ginseng extracts used in this work were prepared as follows; 150 g of Ginseng powders was added to 500 ml of 70% ethylalcohol in the 1,000 ml of the round flask of a refluxed extractor. The extraction was carried out for 3~4 hrs. at 75~80°C. This

Table 1. Composition of experimental diet (%)

Constituent	Group				
	I	II	III	IV	V
Carbohydrate[1]	64.9	64.9	64.9	64.9	64.9
Sucrose	20	18.6	17.5	18.4	17.1
Glucose	10	10	10	10	10
Ginseng nectar A[2]		(10 ml)			
Ginseng nectar B			(20 ml)		
Ginseng orange juice[3]				(12 ml)	
Ginseng drink[4]					(15 ml)
Protein					
Casein	18	18	18	18	18
DL-methionine	0.1	0.1	0.1	0.1	0.1
Fat					
Soybean oil	5	5	5	5	5
Tallow	5	5	5	5	5
Salt mixture[5]	4	4	4	4	4
Vitanin mixture[6]	1	1	1	1	1
Celluflor	2	2	2	2	2

1. Starch: Sucrose: Glucose = 70:20:10
2. Ginseng nectar containing crushed fresh Ginseng root
3. Ginseng extract added 0.5%
4. Ginseng extract added 0.4%
5. Hubble Mendel Wakeman mixtures (g in 100g)
   Calcium carbonate 50.30 Magnesium carbonate 2.50
   Magnesium sulfate 1.60 Sodium chloride 6.30
   Potassium chloride 11.20 KH_2PO_4 21.20
   Ferric phosphate 2.05 Potassium iodide 0.008
   Manganese sulfate 0.035 Sodium fluoride 0.01
   Aluminium potassium sulfate 0.017
   Cupric sulfate 0.009
6. Vita-M: Manufactured by Yu-Yu Industrial Co. Ltd., Seoul, Korea.
   P-amino benzoic acid 50 mg, biotin 0.2 mg, inositol 100 mg per kg of diet were supplemented.
   Ginseng extract content in diets was as follows
   Group II : 555 mg of Ginseng extract per kg of diet
   Group III : 1.110 mg of Ginseng extract per kg of diet
   Group IV : 600 mg of Ginseng extract per kg of diet
   Group V : 600 mg of Ginseng extract per kg of diet

extraction was repeated 6 times using the fresh solvent. The extracts were combined and concentrated at 60° C with a rotary evaporator. The resulted concentrate was a brownish syrup. The yield was 35%.

3. Diets

Diets containing various amounts of Ginseng extract were prepared according to the previous report(1). The percentage of dietary components were carbohydrate 64.9, protein 18, fat 10, DL-methionine 0.1, salt mixture 4, Vitamin mixture 1 and cellulose flour 2. The amount of Ginseng exract in diets were 555 ~1,110mg per kg diet. The composition of diet was shown in Table 1.

4. Measurements of parameters

Determination of growth rate, feed efficiency ratio, organ weight, hematocrit value, serum cholesterol and histological analysis were carried out according to the procedures described in the previous report[1]. Statistical analysis was performed by a computer to obtain t-Test, mean value and standard deviation. Activities of SGOT and SGPT were detrmined by the method of Reitman *et al*[3]. Swimming capability was measured by the method of Rueckert[4].

Results and Discussion

1. Growth rate

Table 2 shows the effects of feeding with various diets containing Ginseng extract on increase of body weight after 12 week-feeding. As results, control showed the highest body weight increase, 352.1 g, compared to 341.4 g for diets containing Ginseng nectar (group II), 316.9 g for diets containing Ginseng drink (group V) and 313.6 g for diets containing Ginseng orange juice (group IV). It was concluded, therefore, that feeding with diets containing Ginseng extract showed no statistically signficant effects on body weight increase in the rat.

Kim *et al*[1] also reported that statistical analysis showed no significant body weight increase between groups fed with diets of Ginseng extract to compare with control when they fed rats with various diets of Ginseng extract for 6weeks. In their work they found 198-231 g of body weight increase when the animals were fed with diets of Ginseng extract (800-1,600 mg Ginseng extract/kg diet) for 6 weeks. In the present work it was found that body weight increase was 316.9-352.1 g when animals were fed with diets of Ginseng extract (555-1,110 mg Ginseng extract/kg diet) for 12 weeks. In both experiments there were no statistical significances in body weight increase.

Park *et al*[5] observed that the addition of Ginseng powder (3%) in protein-low diet (protein content 6.8%) did not increase body weight to compare the control. But Ginseng powder supplement (3%) in protein-high diet (protein content 12.8% or 17.9%) showed increase in body weight.

Feeding effects of diets containing Ginseng extract, however, have been controversial[6,7,8,9].

2. Food intake and Feed efficiency ratio(FER)

Table 3 shows food intakes and FER during 12 week-feeding experiment with various Ginseng extract containing diets. As shown in Table 3 group II (diets containing Ginseng nectar) showed the highest food intake (2,120 g) while control showed the lowest (2,003.1 g). However data indicated that there were

Table 2. Body weight gain (g)[1]

Group	Initial body weight(g)	Final body weight(g)	Body weight gain(g)	t-Test*
I	76.5±13.6	428.8±76.5	352.1±69.2	
II	77.1±10.9	420.7±36.6	341.4±36.9	NS
III	85.7±13.4	432.8±28.2	340.4±32.1	NS
IV	77.3±16.9	396.4±80.0	313.6±78.0	NS
V	78.2±17.1	397.8±46.3	316.9±34.5	NS

1. Mean±standard deviation
* Compared to group I, $P < 0.05$
NS Non significant

Table 3. Total food intake and feed efficiency ratio (FER)

Group	Total food intake (g)	t-Test*	FER[1]	t-Test
I	2003.1		0.182±0.094	
II	2120.2	NS	0.181±0.080	NS
III	2048.7	NS	0.174±0.067	NS
IV	2060.0	NS	0.158±0.067	NS
V	2031.8	NS	0.163±0.074	NS

1 Mean±standard deviation
* Compared to group I, $P < 0.05$
NS Non significant

Table 4. Organ weight (g)[1]

Group	Liver	t-Test*	Kidney	t-Test	Heart	t-Test	Spleen	t-Test
I	13.050±2.525		2.775±0.330		1.500±0.141		0.800±0.082	
II	14.525±1.664	NS	2.750±0.191	NS	1.500±0.082	NS	0.725±0.096	NS
III	14.075±0.699	NS	2.650±0.173	NS	1.375±0.150	NS	0.725±0.096	NS
IV	14.225±2.746	NS	2.650±0.311	NS	1.400±0.216	NS	0.775±0.171	NS
V	13.875±0.995	NS	2.575±0.150	NS	1.375±0.050	NS	0.700±0.082	NS

1 Mean±standard deviation * Compared to group I, $p < 0.05$ NS Non significant

no significant statistical differences in food intakes between Ginseng fed animals and control.

FER results were as follows; 0.182 for control, 0.181 for Ginseng nectar diet (group II), 0.158 for Ginseng orange juice diet (group IV), 0.174 for Ginseng nectar diet(group III) and 0.163 for Ginseng drink diet (group V). Previous report[1] found FER was 0.225-0.255 for 6 week-feeding experiment while FER in this experiment was 0.158-0.182 for 12-week feeding This slight decrease may be caused by extending the feeding period.

3. Organ weight

In Table 4 the results of organ weight change were presented after 12-week feeding experiment was com pleted. Results were as follows; liver weight ranged between 13.05-14.525 g in 12-week feeding while they showed 10.45-12.428 g in 6-week feeding[1], kidney weights ranged 2.575-2.775 g, heart weights ranged 1.375~1.50 g and spleen weights ranged 0.7~0.8 g. As results no great differences were observed between control and Ginseng fed groups, and no certain statistical significance was observed in organ weights between experimental groups. This result was very similar to those of Kim *et al*(1), and other investigators(5, 6, 10).

4. Hematocrit value and serum cholesterol content

Table 5 shows the hematocrit values and serum cholesterol content of rats fed with Ginseng containing diets for 12 weeks. Hematocrit values ranged 44.75~49.50. No considerable differences were found between in rats fed with Ginseng products and control. Although control and group II(Ginseng nectar diet) indicated slightly lower values and Ginseng orange juice and drink diet groups showed higher values, there was no statistical significant differences

Table 5. Hematocrit value(%) and serum cholesterol level (mg%)

Group	Hematocrit value	t-Test*	Serum cholesterol	t-Test
I	44.75±1.258(4)[2]		116.25±16.070	
II	44.75±1.258(4)	NS	123.25±17.153	NS
III	47.00±2.160(4)	NS	138.00±32.357	NS
IV	49.50±7.325(4)	NS	138.66±17.161	NS
V	45.75±0.957(4)	NS	162.00±18.000	S

1 Mean±standard deviation
2 Number of animal used
* Compared to group I, $P<0.05$
NS Non significant
S Significant

between rats fed with Ginseng and control. Hematoc rit value did not change upon the feeding period. The value of 12-week feeding animals was 44.75~49.50 while that of 6-week feeding animals was 46.00~47.50[1] which were good agreement with the results of other investigators.[10,11]

As shown in Table 5 the animals fed with Ginseng diet showed slightly higher serum cholesterol than control animals did. The amounts of cholestrol were as follows; 116.25 mg% for the control, 123.25 mg% for the Ginseng nectar diet group (II), 138.00 mg% for the Ginseng nectar diet group (III), 138.66 mg% for the Ginseng orange juice diet group (IV) and 162.00 mg% for the Ginseng drink diet group (V). Specially group V showed the highest cholesterol and the difference was statistically significant. Extension of feeding of Ginseng diet increased cholesterol content in serum slightly. The content of serum cholesterol of rats fed with various Ginseng products for 6 weeks was 94.00~99.50 mg%[1].

As previously discussed Park *et al*[5] fed rats with protein diets (protein content 6.4, 12.8 and 17.9%) containing Ginseng powder (3%). They found that

the cholestrol content of Ginseng diet group was higher than that of control diet group, without being affected by protein content in the diet.

To study the effect of Ginseng diet on fatty acid metabolism in rats, Ju *et al*[12] injected acetate-1, 2-^{14}C into rats fed with control diet and Ginseng containing diet respectively. They found that half life of cholesterol and fatty acid was 30 min and 70 min respectively in the control whereas 19 min and 44 min were observed in the rats fed with Ginseng saponin. It was, therefore, suggested that Ginseng saponin may increase the metabolic activity of fatty acid.

Kang *et al*[13], however, reported the decrease of blood cholesterol level when they fed rats with Ginseng powder for 4 weeks which was contrast of other results. More intensive research should be carried out to elucidate the exact effect on the change of cholesterol by Ginseng containing diets.

5. Serum glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase activities

Two transaminases are intracellular enzymes and the serum level of GOT and GPT are normally very low. Any significant tissue breakdown gives rise to high serum transaminase levels. For example, there is an increase in the serum level of GOT. There are alterations in SGOT and SGPT levels in some of the liver diseases. One also finds high serum levels of these enzymes in conditions where there is damage to skeletal muscle. Although for a thousands of years Ginseng has been as a kind of drug, there was no definite answer for the therapeutic properties. To see an any indication of protective effect of Ginseng on liver damage, determinations of SGOT and SGPT levels had been tried by many investigators,

Table 6 shows the results of SGOT and SGPT levels after 12-week feeding experiment was completed with various Ginseng extract containing diets. As results the control exhibited the highest SGOT activity(156) and that of Ginseng nectar (group II) 141, Ginseng drink diet (group V) 126 and Ginseng orange juice diet (group IV) 111. Although there was no statistically significant difference in SGOT level between control and Ginseng extract containing diets, lower SGOT levels were observed when animals were fed with Ginseng extract, except activity of SGOT in Ginseng orange juice diet was significantly lower than control.

Table 6. Serum GOT and GPT activity[1]

Group	GOT Activity	t-Test*	GPT Activity	t-Test*
I	156±48.24		38.5±6.45	
II	141±53.77	NS	42.5±5.44	NS
III	124.5±27.00	NS	41.2±5.62	NS
IV	111±31.17	S	38.5±3.00	NS
V	126±28.14	NS	39.5±5.66	NS

1 Mean±standard deviation
* Compared to group I, $P<0.05$
NS Non significant
S Significant

Table 7. Swimming time

Group	Body weight (g)	Swimming time (min.)[1]	t-Test*
I	426.5	21.5±9.19	
II	417.5	23.5±6.36	NS
III	390.5	31.5±13.43	NS
IV	429.0	35.0±22.62	NS
V	444.0	36.0±1.41	S

1 Mean±standard deviation
* Compared to group I, $p<0.05$
NS Non significant
S Significant

SGPT levels were between 38.5-42.5 which showed no statistically sigoificant difference in all experimental animals.

According to the results of Kang *et al*[14] SGOT level decreased greatly, in which they used fed higher level of Ginseng extract than in the present work. They also observed that SGPT did not change significantly.

6. Pathology

After 12-week feeding with experimental diets, tissues of organ selected were checked under the microscope. Selected organs after autopsy were included liver, kidney and spleen from all experimental groups. As results no abnormalities were observed. Thes results were agreed well with the previous work[1] and the results of Shibata *et al*[6].

7. Swimming test

After feeding experiments were completed for 12 weeks, 2 rats from each group were taken and swi-

mming test was carried out by the method of Rueckert[4]. The results are presented in Table 7.

Swimming times obtained were as follows; control 22.5 min, Ginseng nectar diet group 23.5 min, Ginseng orange iuice diet group 35 min and Ginseng drink diet group 36 min. These results were good agreement with the other results[4,7] which feeding rats with Ginseng containing diets increased swimming times than control.

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요 약

인삼 nectar, 인삼추출물이 함유된 orange juice, 인삼 drink 제등을 정상식이에 첨가 (555 mg Ginseng extract/kg of diet, 1,110 mg Ginseng extract/kg diet) 하여 이유 직후의 Sprgue-Dawley strain 백쥐 40마리를 8마리씩 5군으로 나누어 12주간 사육하였다. 12주 후에 체중증가율, 사료효율, 장기의 무게, hematocrit value, serum cholesterol content, serum GOT and GPT 활성등을 측정하여 정상식이군과 비교한 결과 체중증가율, 사료효율, 장기의 무게, hematocrit value, SGOT 활성등은 모두 정상식이군에 비해서 실험군이 통계적으로 유의차를 보이지 않았으나, s-GOT 활성은 대조군에 비해 실험군이 다소 낮은 경향을 관찰할 수 있었고, s-cholesterol 농도는 대조군에 비해 다소 높은 경향이었으나 통계적인 유의차는 없었다.

백쥐 각군의 간, 신장, 비장등의 조직세포를 검사한 바 하등의 이상 조직을 발견하지 못하였다.

12주 실험이 끝난 후 체중을 측정하고 swimming test를 실시한 결과 대조군에 비해 실험군의 수영시간이 연장되었으며 V군에서는 유의성있게 연장되었다.

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