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Feeding Studies in Rats with Confectionery Products Containing Ginseng Extract

by

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인삼 함유 제과류에 의한 백쥐 사육시험

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Abstract

Ginseng jellies and candies were prepared by adding Ginseng extracts as final concentration of 0.5% and 1% for jellies and 0.7% for candies. Feeding experiments of the diets containing Ginseng extract were carried out for evaluation in respects of growth rate, feed efficiency ratio, organ weight change, hematocrit value, and blood cholesterol content by feeding rats for 6-week.

The results of Ginseng supplemented diets were not significantly different from those for the control. Liver, spleen and kidney cells of Ginseng products feeding rats appeared no abnormalities to compare to the control rats.

Introduction

Ginseng (*Panax ginseng*) has been used in Korea, China, Japan and other Asian countries for thousands of year under various presentations, such as teas, tinctures, wines, pills and unguents, for prevention of aging, tireness, headaches, amnesia, tuberculosis, diabetes and other diseases[1]. Thus the scientific systematic studies on the therapeutic properties and biochemical constituents of Ginseng have been carried out and many findings are reported[1,2].

Recently their products such as teas, Ginseng-candy, Ginseng-jelly and ginseng-nectars have been developed by food industry and that local industrial companies search the foreign market. Rapid distributions continued to United States and western European countries in these days. The products of Ginseng, however, are not classified yet as food in foreign countries such as United States and certain European countries, while they are classified as food in Korea.

Nevertheless few researches on food properties of Ginseng products including safety problems were carried out and thus this study was aimed to find the feeding effects in the rats fed with confectioneries containing Ginseng extracts. Data of growth rate, changes of hematocrit value and serum cholesterol, and histological changes of certain organs of animal are presented in this report.

Materials and Methods

1. Animal and treatment

Healthy weanling female and male youngsters of Sprague-Dawley strain, weighing 65 to 69 g each were used for the experiments. Commercial stock diet and water were fed at least 4 days after arrival.

Youngsters were randomly divided into 6 groups of

48 animals (♂ 4 and ♀ 4 per group). The *ad libitum* fed animals were started to feed for 6 weeks with the specified diet.

2. Sample preparation

Preparation of jellies containing Ginseng extract The ratio of mixture for ginseng-jelly was sucrose 33.0%, maltose syrup 64.5%, agar 2% and Ginseng extract 0.5%. when ginseng extract was used 1.0%, sucrose used was 33.5%. To prepare the jelly, agar was melted by boiling at water bath and sucrose and maltose syrup were added, following concentration (Brix 76 %). When cooled to 80°C Ginseng extract was mixed and mixture was cooled for gelation. After sanding with sugar it was dried for 12 hrs at 58-63°C.

Ginseng extract used was prepared by extraction with 95% ethyl alcohol at 75-80°C for 3-4 hrs. Extraction was repeated 6 times. Extract was combined and concentrated at 60°C under vacuum(yield 60%).

General composition of jellies prepared was shown in Table 1.

Table 1. General composition of jellies

	Ginseng extract added (%)	Moisture (%)	Ash (%)	Total sugar (%)
Jelly A(control)	0	15.6	0.13	79.7
Jelly B	0.5	16.6	0.13	80.7
Jelly C	1.0	17.0	0.10	79.2

Preparation of candies containing Ginseng extract: The ratio of mixture for Ginseng-candy was sucrose 59.1%, maltose syrup 40% caramel pigment 0.2% and Ginseng extract 0.7%. To prepare candy sucrose and maltose syrup were heated at 145°C in autoclave. Mixture was cooled and Ginseng extract which was prepared as described previously was added. Finishing candy was followed by the conventional method. General composition of candy prepared was shown in Table 2.

Table 2. General composition of candy

	Ginseng extract added (%)	Moisture (%)	Ash (%)	Total sugar (%)
Candy A(control)	0	6.1	0.05	92.8
Candy B	0.7	5.9	0.05	91.0

3. Diets

The percentages of dietary components were as follows: carbohydrate 64.9, protein 18, fat 10, DL-methionine 0.1, salt mixture 4, Vitamin mixture 1 and cellulose flour 2. For the specified diets Ginseng-jelly or Ginseng-candy was substituted for sucrose (20% of total carbohydrate). The composition of the diets is shown in Table 3. As shown in Table 3 the amounts of Ginseng extract in group III, IV and VI were 810, 1,620 and 987 mg per kg of diet respectively.

4. Measurements

Growth rate; body weight was determined weekly on the same day in order to minimize any possible day to day fluctuations.

Feed efficiency ratio: it was determined as follows:

$$\text{FER}=\frac{\text{body weight increase (g)}}{\text{food intake (g)}}$$

Hematocrit value: blood was centrifuged by micro-hematocrit centrifuge and packed cell was separated from serum. Volume of packed cell was determined by micro-capilary reader.

Serum cholesterol: cholesterol was determined by the method of Frankel *et. al.* (3). To 0.2 ml containing serum 0.1 ml and dist. H_2O 0.1 ml, and 6ml of color reagent (acetic anhydride: glacial acetic acid =30:20 V/V, containing 5 parts of conc. H_2SO_4) and measure absorbancy at 640 nm after 1min. For standard cholesterol 0.1 ml of cholesterol solution (160 mg cholesterol per 100ml of glacial acetic acid) was used. The amount of cholesterol in serum was determined as follows:

$$\text{mg of cholesterol per 100ml}=\frac{OD_{640}\text{ of serum sample}}{OD_{640}\text{ of standard}}\times 160$$

Liver, heart, spleen and kidney weight: After 6 weeks when other determination was completed animals were decapitated and fresh organs isolated were weighted.

Histological analysis: organs isolated were quickly fixed in 10% buffered formalin and dehydrated with anhydrous ethyl alcohol. Tissue was sliced into pieces of 6 μ thickness and stained with hematoxylin-eosin solution. Stained tissue was subjected to microscopic observation.

Statistic analysis: data obtained were analysed by

Table 3. Composition of experimental diet(%)

Constituent	Group					
	I	II	III	IV	V	VI
Carbohydrate[1]	64.9	64.9	64.9	64.9	64.9	64.9
Sucrose	(20)	—	—	—	—	—
Jelly A[2]	—	(20)	—	—	—	—
Jelly B[3]	—	—	(20)	—	—	—
Jelly C[4]	—	—	—	(20)	—	—
Candy A[5]	—	—	—	—	(20)	—
Candy B[6]	—	—	—	—	—	(20)
Casein	18	18	18	18	18	18
DL-methionine	0.1	0.1	0.1	0.1	0.1	0.1
Soybean oil	5	5	5	5	5	5
Tallow	5	5	5	5	5	5
Salt mixture[7]	4	4	4	4	4	4
Vitamine mixture[8]	1	1	1	1	1	1
Cellulose flour	2	2	2	2	2	2

1 : Starch : Sucrose : Glucose=70 : 20 : 10
2 : Control for Ginseng-jelly group(no Ginseng extract contained)
3 : Ginseng extract added 0.5%
4 : Ginseng extract added 1.0%
5 : Control for Ginseng-candy group (no Ginseng extract contained)
6 : Ginseng extract added 0.7%
7 : Hubble Mendel Wakeman mixtures (g in 100g)

Calcium carbonate	54.30	Magnesium carbonate	2.50
Magnesium sulfate	1.60	Sodium chloride	6.30
Potasium chloride	11.20	KH_2PO_4	21.20
Ferric phosphate	2.05	Potasium iodide	0.008
Manganese sulfate	0.035	Sodium fluoride	0.01
Aluminium potasium sulfate			0.017
Cupric sulfate			0.009

8 : 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:
Jelly B : 810 mg of Ginseng extract per Kg of diet
Jelly C : 1620 mg of Ginseng extract per Kg of diet
Candy B : 987 mg of Ginseng extract per Kg of diet

computer to obtain t-Test, mean value and standard error.

Results and Discussion

1. Growth rate

Table 4 shows the effect of feeding with diets containing Ginseng extract on increase of body weight in female and male rats after 6 week-feeding. Throughout this experiment body weight increase was significantly higher in the male than female rats in all experimental groups. Statistical analysis, however, showed no significance between groups fed with diet of Ginseng extract to compare with control.

For male rats body weight increase of control was 215.3g (group I) while Ginseng-jelly diets showed 198.0g and 196.8g respectively when Ginseng extract in the diets were 0.5% and 1.0%. But control for Ginseng-jelly diet (group II) showed 231.0g of increase. Control for Ginseng-candy diet (group V) showed 195.6g of increase in 6-week feeding and Ginseng-candy diet which contained 0.7% Ginseng extract showed 222.5g of increase. For female rats there was also insignificance of body weight increase statistically between the groups fed Ginseng confectionery products and control.

The amounts of Ginseng extract intake are average 52mg-182mg per final Kg of body weight per day. Feeding effects of diets containing Ginseng have been controversial. Shibata, *et. al.*[4] observed noconsiderable effect of Ginseng products on body weight increase and Kim[5] reported that effect was noticeable when feeding was continued for long period of time. Oh, *et. al.*[6], however, reported high increase of body weight in chickens fed with composite feeds containing Ginseng. Hong *et. al.*[7] also observed significant increase of rat's body weight statistically when experimental rats were fed with Ginseng products for 2 weeks and that animals lived longer to compare with control animal. But Han *et. al.*[8] reported decrease of body weight in rats fed with Ginseng products. Park[9] carried out experiments in which rats were injected subcutaneously with ethanol extract of Ginseng. He observed increase of body weight and prevention of loss of body weight when animals were subjected to stress such as coldness, high temperature, injuries or ACTH treatment.

Although feeding results are depending on period of experiment, kinds of animal used or amount of specified nutrient added generally, Ginseng was considered to have clinical effects to human beings or animals and that this was not considered as nutrients.

2. Food intake and feed effieiency ratio(FER)

Table 4. Body weight gain[1] (g)

Sex	Group	Initial body weight	Final body weight	Body weight gain	*t-Test
M.	I	68.325	283.625	215.300±17,700(4)[2]	
	II	66.825	299.125	231.325± 4.054(4)	NS
	III	67.950	265.975	198.025±13.764(4)	NS
	IV	67.825	264.675	196.850± 4.315(4)	NS
	V	67.650	262.825	195.675±21.017(4)	NS
	VI	68.275	290.800	222.525± 7.812(4)	NS
F.	I	67.533	196.833	129.300± 5.187(3)	NS
	II	66.825	203.225	136.400± 5.888(4)	NS
	III	67.100	202.450	135.350± 1.672(4)	NS
	IV	65.167	206.500	141.333±38.225(3)	NS
	V	69.700	197.533	127.833± 7.362(3)	NS
	VI	67.467	192.333	124.867± 2.580(3)	NS

1 Mean±standard error
2 Number of animal used
* Compared to group 1 $P<0.05$
NS non significant

Table 5 shows food intake and FER during 6-week feeding experiment. As shown in Table 5 data indicated that there were no statistical significance of food intake and FER between Ginseng fed animals and control. Results showed male rats took higher food intakes than females and in addition same results were shown in FER.

FER results were as follows: 0.235 for control(group I), 0.255 for Ginseng-jelly control, 0.227-0.231 for Ginseng-jelly diets (group II and IV), 0.225 for Ginseng-candy control, 0.245 for Ginseng-candy diet(group VI) in male rats: 0.15-0.17 for female rats. Ha *et. al.*[10] reported similar results ranging 2.00-2.33 for male rats and 1.60-1.82 for female rats. Both experiments of food intake and FER are in good agreement with that male rats showed higher increase of body weight than females in the result of growth rate as described previously.

3. Organ weight

Animals were autopsied after 6-week feeding experiment was completed and the weights of selected organs were recorded. Results are presented in Table 6. Generally weights of male rats were higher than females in all organs selected. But no certain statistical significance was observed between Ginseng product fed groups and control. Results are follows: liver weights ranged between 10.45~12.425g for males and between 7.6~8.95g for females; heart weights ranged between 1.0~1.175g for males and between 0.867~0.975 for females; spleen weights ranged between 0.575~0.7g for males and between

Table 5. Total food intake and feed efficiency ratio[1]

Sex	Group	Total food intake (g)	t-Test*	FER	t-Test*
M.	I	912.250±20.569(4)[2]		0.235±0.019(4)	
	II	905.000±11.438(4)	N S	0.255±0.006(4)	N S
	III	885.000±82.156(4)	N S	0.227±0.020(4)	N S
	IV	857.250±50.348(4)	N S	0.231±0.012(4)	N S
	V	866.250±37.699(4)	N S	0.225±0.018(4)	N S
	VI	908.750±19.839(4)	N S	0.245±0.012(4)	N S
F.	I	851.667±19.954(3)		0.151±0.005(3)	
	II	857.250±20.369(4)	N S	0.159±0.005(4)	N S
	III	815.500±26.082(4)	N S	0.166±0.005(4)	N S
	IV	807.333±65.540(3)	N S	0.170±0.032(3)	N S
	V	831.000±30.022(3)	N S	0.154±0.011(3)	N S
	VI	834.333±20.004(3)	N S	0.150±0.004(3)	N S

1 Mean±standard error
2 Number of animal used
* Compared to group 1 $P<0.05$
N S Non significant

Table 6. Organ weight[1]

Sex	Group	Liver		Heart		Spleen		Kidney	
		Weight(g)	t-Test*	Weight(g)	t-Test*	Weight(g)	t-Test*	Weight(g)	t-Test*
M.	I	11.575±0.847(4)[2]		1.100±0.071(4)		0.575±0.048(4)		2.225±0.111(4)	
	II	12.425±0.533(4)	NS	1.150±0.029(4)	NS	0.675±0.048(4)	NS	2.250±0.075(4)	NS
	III	11.775±0.762(4)	NS	1.000±0.122(4)	NS	0.700±0.041(4)	NS	2.250±0.179(4)	NS
	IV	10.450±0.574(4)	NS	1.150±0.150(4)	NS	0.650±0.150(4)	NS	2.100±0.071(4)	NS
	V	10.775±0.530(4)	NS	1.125±0.144(4)	NS	0.650±0.065(4)	NS	2.300±0.079(4)	NS
	VI	11.500±0.436(4)	NS	1.175±0.048(4)	NS	0.625±0.025(4)	NS	2.150±0.083(4)	NS
F.	I	8.667±0.721(3)		0.900±0.085(3)		0.500±0.058(3)		1.667±0.145(3)	
	II	8.275±0.744(4)	NS	0.975±0.111(4)	NS	0.625±0.058(4)	NS	1.650±0.065(4)	NS
	III	8.950±0.303(4)	NS	0.900±0.041(4)	NS	0.525±0.095(4)	NS	1.675±0.130(4)	NS
	IV	8.233±0.233(3)	NS	0.867±0.088(3)	NS	0.433±0.088(3)	NS	1.700±0.082(3)	NS
	V	7.600±0.340(3)	NS	0.867±0.033(3)	NS	0.533±0.033(3)	NS	1.667±0.098(3)	NS
	VI	8.033±0.438(3)	NS	0.900±0.058(3)	NS	0.500±0.058(3)	NS	1.633±0.072(3)	NS

1 Mean±standard error
2 Number of animal used
* Compared to group 1 $P<0.05$
NS Non significant

Table 7. Hematocrit value(%) and serum cholesterol level (mg%)[1]

Sex	Group	Hematocrit value	t-Test*	Serum cholesterol	t-Test*
M.	I	46.250±0.479(4)[2]		94.000± 2.858(4)	
	II	46.000±0.707(4)	NS	94.500± 4.173(4)	NS
	III	47.500±0.866(4)	NS	99.500± 3.014(4)	NS
	VI	47.250±1.109(4)	NS	98.750± 5.543(4)	NS
	V	46.500±0.289(4)	NS	95.000± 2.799(4)	NS
	VI	47.250±0.762(4)	NS	97.750± 5.750(4)	NS
F.	I	46.000±0.577(3)		99.333± 1.333(3)	
	II	47.750±0.854(4)	NS	103.250± 7.330(4)	NS
	III	46.250±1.109(4)	NS	102.500± 4.173(4)	NS
	IV	46.333±0.334(3)	NS	101.000± 5.508(3)	NS
	V	47.000±1.000(3)	NS	103.333±15.191(3)	NS
	VI	48.000±0.577(3)	NS	106.000± 2.082(3)	NS

1 Mean±standard error
2 Number of animal used
* Compared to group 1 $P<0.05$
NS Non significant

0.433~0.625g for females; kidney weights ranged between 2.1~2.3g for males and 1.633~1.7g for females. This result was very simillar to those of Shibata *et. al.*.[4] and Han[11].

4. Hematocrit value and serum cholesterol

Hematocrit values and serum cholesterol content of male and female youngsters fed with Ginseng confectionery products were presented in Table 7.

Hematocrit value ranged between 46.250 to 48.0. No considerable differences were observed both in

males and females and that there were no statistical significant differences between rats fed with Ginseng products and control. Oh *et. al.*[12] and Han *et. al.*[11] also reported that hematocrit values did not change during the experimental period when they fed rats with various products of Ginseng.

As shown in Table 7 female rats showed a slight higher serum cholesterol than males. For male rats group III, which was fed with Ginseng-jelly of 0.5%, contained 99.5 mg% of serum cholesterol, group IV, which was with Ginseng 1.0%, 98.75mg% of cholesterol, 0.7% Ginseng-candy group (group VI) 97.75mg % of cholesterol and control 94.0mg% of cholesterol. For females control showed 99.33mg% of serum cholesterol, the lowest and 0.7% Ginseng-candy group (group IV) contained 106.0mg%, the highest. However there was no considerable statistic significance in difference.

Cho *et. al.*[13] studied on effect of Ginseng saponin to serum cholesterol of rabbits. They found insignificant differences between control and Ginseng saponin fed animals. Lee *et. al.*[14] also reported a similar result in chickens. They fed Ginseng orally and animals were irradiated by γ-rays. Throughout the experiment they observed no change of serum cholesterol in Ginseng-fed chickens.

5. Pathology

After 6-week feeding with experimental diets, tissues of organ selected were stained hematoxylin-eosin solution after they fixed in 10% buffered-formalin solution. Results showed no gross histological changes to compare with control. Selected organs after autopsy were included: liver, spleen and kidney from all experimental groups. No abnormalities were observed. Shibata *et al.*[4] reported that no abnormalities were found in organs of rat fed with Ginseng extracts. It seemed that feeding of Ginseng extract affect no gross histological changes.

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요 약

인삼 추출물을 첨가하여 제조한 인삼 젤리 (첨가량 0.5% 및 1.0%)와 인삼 캔디(첨가량 0.7%)로 정상식이 탄수화물의 20%를 대체한 사료로서 이유 직후의백쥐암수 48마리를 6주간 사육한 바 체중증가율, 사료효율, 장기의 무게, Hematocrit값, 혈청 콜레스테롤 농도등에 관하여 정상식이군과 비교하였다.

각항이 모두 정상식이군에 대해서 실험군이 통계적으로 유의차를 보이지 않았으며, 또한 실험군 백쥐의 liver, spleen, kidney의 조직세포를 검사한 결과 이상조직을 발견하지 못하였다.

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