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Plasma and Liver Cholesterol Lowering Substances in Gyrophora Esculenta (Sogi)

I. Effects of Gyrophora esculenta (Sogi) on the plasma and liver cholesterol in rats

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石茸버섯(Gyrophora eseulanta) 中에 含有되여있는 肝臟 및 血漿콜레스테롤의 低下 生理活性物質에 關한 硏究

— 第1報 —

식이버섯이 흰쥐의 혈장과 간 콜레스테롤에 미치는 효과

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국 문 초 록

Sprague-Dawley系 숫놈 흰쥐에게 석이버섯 분말을 사료에 첨가 (0.5 %, 1.0 %, 3.0 %, 5.0 %, 群) 하여 6주간 사육한후 혈장 및 간장중에 콜레스테롤의 變化를 測定한 결과 다음과 같다.

1) 석이버섯 분말 첨가군에 있어 기본식이군이나 대조군에 비하여 체중증가나 사료섭취량에 특별한 차이나 영향을 나타내지 않았다.

2) 그러나 혈장 및 간장지질량, 혈장 및 간장콜레스테롤치에 있어서는 현저하게 저하했음을 보였다.

3) 이러한 低下는 첨가량 증가에 따라 저하율이 뚜렸했으나 간지질량 측정에서는 3.0 % 첨가군이 가장 효과를 나타냈다.

INTRODUCTION

In recent years, the relationship between cardiovascular diseases and cholesterol level in blood has been widely discussed and many researches in food which may help lower the plasma chol esterol level have also been reported. There are number of papers dealing with the effects of

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fatty acid in food. As is generally known, lipid containing linoleic acid mainly lowers the cholesterol level while lipid containing palmitic acid raises it. In addition, dietary factors affecting the liver or serum cholesterol have been reported in numerous articles.

They may generally be divided into the following : substance accelerating the turnover of cholesterol, substance resisting the absorption of cholesterol intake, and substance inhibiting the biosynthesis of cholesterol from isoprenoidic substance in the body. Though the mechanism of those substances which may lower cholesterol varies, it is reported that the cholesterol level is lowered, as a whole, because these substances are in the diet. They contain a considerable amount of pectin and lignin which may be classified as edible fibers, especially the changes in cholesterol levels observed by giving pectin to man have been reported.

Suzuki & Tsuji,[1] Kiriyama & Yoshida[2] and Okimasu[3] et al. have reported on the effect of konnyaku (a paste made from the starch of the devel's tongue) mannan on the lowering of serum or liver cholesterol levels. Whereas the lowering of cholesterol was also confirmed by ingestion of dietary fiber and pectin (Keys, A.,[4] Palmer, G.H.,[5] and Durrington, P.N.,[6] et al.) and guar gum (Fahrenbach, M.J.,[7] and Jeckins, D.J.A.,[8] et al.) And also Moore, J.H.[9] & Cookson, F.B., et al.[10] and Key. R.M.,[11] et al. have reported on the effects of alfalfa or dietary roughage on serum cholesterol levels and aortic atherosis in rabbits or man. Kaneda and Tokuda[12] have also reported a similar effect of Cortinellus Puogo. The structure of substance contained in Cortinellus Pyogo which has a lowering effect of the cholesterol levels was identified and named as Eritadenine. In Korea, Sogi (Gyrophora esculenta Miyoshi) has been widely used as health food from ancient times, especially for the sick and pregnant women. According to Bcnchogangmok[13] (literally a general outline of herbal medicine), Sogi is sweet in taste, invigorates when eaten habitually for a long period of time, relieves invariably from a hungry feeling and lessens excretion even at senescence. It also improves sight and increases vitality. Judging from this classics of herbal medicine, it seems that Sogi was also used as health food for the aged in old China. In Japan, Otomo et al. reported the history of Sogi used in the past and present. According to them, Sogi is a typical edible lichen belonging to Sogi family, produced indigenously in small quantity in eastern Asia. The history of Sogi is as old as it was mentioned in an account of food published in 1664. It may be seen from this that Sogi was rather prevailed as a common dish among the genreal public in the early Yedo period (1603 – 1867). However, it was simply used as a delicacy in Japan without taking into account the characteristic efficacy of its own like in Korea and China. There is no report which indicates definitely the reason why Sogi was favored as health food for old people in China and Korea. however. There has also not been reported yet that cholesterol lowering substance is contained in lichens. Therefore it was determined to perferm experiments with animals in order to see whether cholesterol lowering substance is contained in Sogi.

Rats selected for experiments were fed a diet containing cholesterol to find out whether Sogi contains such substance restraining the absorption of dietary cholesterol or inhibiting the biosynthesis of cholesterol. The changes in plasma and liver cholesterol levels were measured.

EXPERIMENTAL PROCEDURES

1) Assay, composition of diet, and feeding method

Male Sprague-Dawley rats, weighing 110 - 120g on the average, obtained from Matsumoto Experimental Animal Center in Chiba Prefecture of Japan were used and each group consisted of 12 rats. An individual rat was placed in a metal cage in animal feeding room airconditioned at

20 ℃. The composition of diet is as shown in Table 1. Dried Sogi from Kangwondo province of Korea, bought from a local market and pulverized Sogi was used for the present experiments. The pulverized Sogi was further ground with mixture of diet component and properly steamed, then made into dumbling shape. Feed and water were given ad libitum. The animals were fed for 6 weeks. Feed containing no cholesterol and no Sogi was determined as the basal group and that to which feed containing 1 % cholesterol was determined as the control group. The test groups consisting of Ⅰ, Ⅱ, Ⅲ, and Ⅳ were determined by adding 0.5 %, 1.0 %, 3.0 %, and 5.0 % of pulverized Sogi, respectively, to the feed for the control group (Table 1). The weight of feed was properly adjusted by reducing the content of starch. The feed intake and change in weight of the rate were measured and recorded at feeding time every day.

2) Separation of samples for assay and measurement

After feeding for 6 weeks, the rate were anesthesized with nembutal and blood was drawn directly from their abdominal aorta with heparin treated syringes. The plasma was centrifugally separated. The livers were taken out immediately after drawing blood and wrapped with aluminium foil after weighing. These samples were frozen at -20 ℃ and kept in cold storage prior to assay. Liver and plasma lipid was extracted three times repeatedly by using chloroform-methanol mixture (2 : 1) in accordance with the Folch et al. method[15], washed with 0.5 % NaCl solution combining the extracted fluid and only the chloroform layer was separated. This chloroform extract was dried with sodium acetic anhydride and evaporated for concentration by using a rotary evaporator. The remaining fluid was dissolved with a small amount of n-Hexane and removed in a fixed amount into pre-weighed test tubes. n-Hexane was blown off under a nitrogenous flow, dried under vacuum condition in a decicator and its weight obtained was determined as the amount of lipid. The measurement of total cholesterol and isolated cholesterol was made by the Nieft-Deuel variation of the Sperry-Schoenheimer's coloring method[16] using concentrated sulfuric acid. Sample fluid made by removing protein by addition of 14ml ethanol-acetone mixture (1 : 1) per 1ml plasma was used for test. Fatty substance extracted by chloroform-methanol mixture (2 : 1) was further washed with 0.5 % NaCl solution and chlorform layer thus separated was used. This layer was dried in a rotary evaporator and the residue was dissolved in n-Hexane and messed up in a fixed amount for test.

Table 1. Composition of diets (%)

	Basal	Control	Ⅰ (0.5% Sogi)	Ⅱ (1.0% Sogi)	Ⅲ (3.0% Sogi)	Ⅳ (5.0% Sogi)
Starch	66.8	65.79	65.29	64.79	62.79	60.79
Casein	20	20	20	20	20	20
Lard	5	5	5	5	5	5
Salt mix	4	4	4	4	4	4
Vitamin mix	2	2	2	2	2	2
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2
Cellulose powder	2	2	2	2	2	2
Na – cholate	–	0.01	0.01	0.01	0.01	0.01
Cholesterol	–	1	1	1	1	1
Sogi powder	–	–	0.5	1.0	3.0	5.0

DISCUSSION

Figure 1 shows the changes in weight of the experimental animals of each group during the period of experiments. Table 2. also shows the average feed consumption, increase in weight and weight of the liver per day of each group. The weight increase curves of each animal group almost the same, indicating a favorable growth. The increase in weight per day was between 5.2g and 6.0g. There was no significant difference between each group in weight gain. The daily intake of feed was between 36.8g and 38.5g, especially the addition of dried Sogi did not bring about any change in eating habit(pattern) and each group consumed about the same amount. The liver weight of the basal group was 13.0g and that of the control group was 14.7g, showing a slight gain. In the test groups, it was ranging from 13.6g to 15.6g nearly closed to that of the control group. The ratio of liver weight to the body weight was 3.4% in the basal group, 4.3% in the control group and 4.0% to 4.5% in the test groups, respectively. Comparing the basal group with the other groups, it was considered that the ratio of the liver to its weight and body weight became slightly higher because the test groups were given cholesterol controlling additives. However, no significant difference was observed in the control and the test groups. The

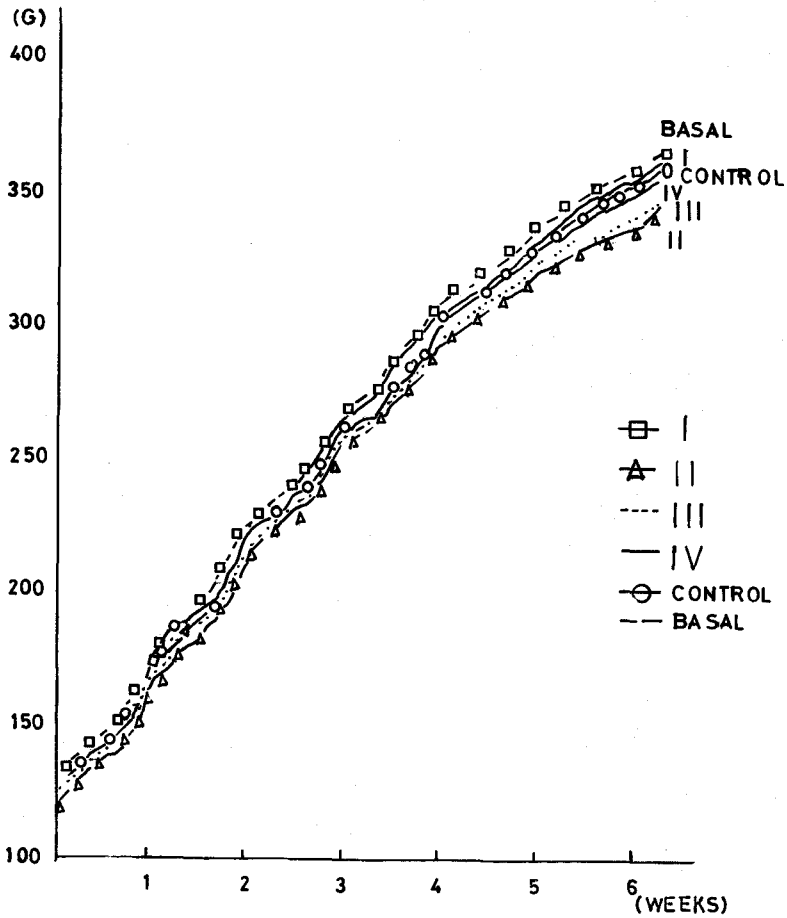

Fig. 1. Growth curve.

lipid content resulted in the liver and plasma is presented in Table 3.

It may be seen that the levels of cholesterol in the liver and plasma lipid become invariably high in all of the test groups fed cholesterol compared with that of the basal group. This indicates a marked effect of cholesterol. There was no significant difference in lipid content between the control group and Sogi fed groups, I and II. However, the lipid content was significantly lower in groups III and IV than that of the control group. It was also observed that the lipid content of group III fed 3.0% Sogi was lower than that of group IV fed 5.0%. Therefore, difference was recognized at 1% risk between group III and the control group. A significant difference was also recognized at 5% risk between group III and IV.

Similar results of Sogi feeding could also be seen in respect of the lipid content. Comparing the control group and the test groups, the lipid content of Sogi fed groups II, III and IV was lower at 1% significance level than that of the control group.

The data on the plasma and level cholesterol are summarized in Table 4. The total cholesterol content in plasma was approximately 90mg% in the basal group. It went up to 193mg%, 2.16 times, in the control group as cholesterol was added to the diet. However, the increase was prevented by adding dried Sogi and 5% Sogi group became 106.5mg%. When compared with the total cholesterol of the basal group, it was 119%. It has become clear that about 84% of increase in plasma cholesterol level of the control group could be attributed by adding 5.0% dried Sogi. Therefore it may be presumed that the plasma cholesterol lowering substance is in Sogi. The liver cholesterol level also showed the same tendency, especially it went up 10 times by adding cholesterol to the diet. The liver cholesterol level was also lowered as in the plasma cholesterol by adding dried Sogi. It has also become apparent that about 72% of increase in the liver cholesterol level could be inhibited by adding 5.0% dried Sogi. The relationship beween these

Table 2. Diet intake, weight gain, and liver weight of rats feeding different levels of sogi

Item / Group	Diet Intake/day (g)	Weight Gain/day (g)	Liver Weight (g)
Basal	38.5± 1.7	6.0± 0.7	13.0±1.4
Control	37.4± 1.4	5.6± 0.4	14.7±2.5
I (0.5% Sogi)	37.1± 2.1	5.7± 0.7	15.6±2.5
II (1.0% Sogi)	36.8±12.6	5.2± 0.7	15.6±1.9
III (3.0% Sogi)	36.9± 9.5	5.2± 0.4	13.6±1.7
IV (5.0% Sogi)	37.5± 2.5	5.5± 0.6	13.9±1.4

* Mean ± S.D.

Table 3. Lipid contents in liver and plasma of rats feeding different levels of sogi

Group	Sogi (%)	Liver lipid (%)	Plasma lipid (%)
Basal	–	3.61±0.25	0.27±0.04
Control	–	6.26±1.64	0.44±0.07
I	0.5	6.13±0.94	0.40±0.06
II	1.0	5.53±1.78	0.27±0.06
III	3.0	4.43±1.11	0.23±0.07
IV	5.0	4.86±1.15	0.19±0.04

* Mean ± S.D.

Table 4. Cholesterol contents in liver and plasma of rats feeding different levels of sogi

Group	Sogi (%)	Liver cholesterol (mg%)	Plasma cholesterol	
			Total (mg%)	Free (mg%)
Basal	–	39.4± 12.1*	89.6±15.9	16.8±4.0
Control	–	388.5±154.9	193.3±28.9	18.6±2.8
I	0.5	329.6± 82.2	174.8±41.3	26.9±7.8
II	1.0	265.2±136.3	149.4±28.2	22.6±7.7
III	3.0	182.6±104.8	118.9±29.1	21.7±7.6
IV	5.0	137.2± 73.7	106.5±20.4	15.5±2.9

* Mean ± S.D.

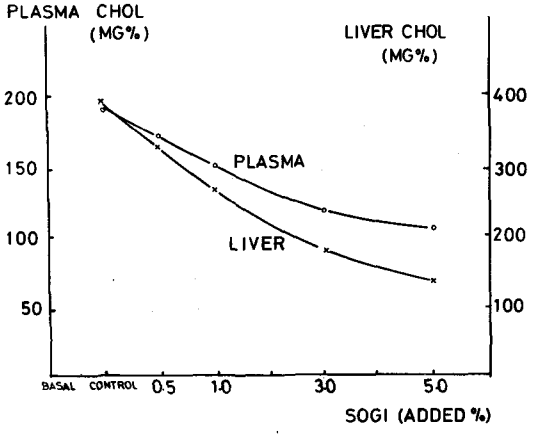

Fig. 2. Effect of dried sogi powder on plasma and liver cholesterol levels

factors is illustrated in Fig. 2. These results make it possible to consider that dried Sogi contains certain substance which may prevents the absorption of cholesterol in food or inhibits the biosynthesis of cholesterol. Also, from view of the fact that the Sogi additive lowers signifcantly the lipid levels in both the plasma and liver, it is not difficult to be considerd to contain some substances which act on cholesterol removing-elements to eliminate lipid from the body system. Further studies of these facts are necessary to clarify their functional mechanism.

CONCLUSION

The results obtained are summarized as follows:

1) The addition of dried Sogi to the diet showed no effect on the increase of weight and feed intake.

2) The lipid content of plasma and liver and their cholesterol levels were markedly lowered by Sogi.

3) The lowering effect became great as the amount of Sogi increased. The lipid content of the liver reached the lowest level when 3.0% Sogi was added.

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