J. Food Sci. Nutr.
Vol. 4, No. 3, p. 188~192 (1999)

Effects of Methanol Extract of Prosomillet on Cholesterol and Fatty Acid Metabolism in Rat

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Abstract

To study effects of methanol extract of prosomillet on lipid metbolism, five groups of male Sprague-Dawley rats weighing 116±9 g were fed test diets for four weeks. The five diets consisted of one low fat (5% w/w) diet containing starch as carbohydrate source (normal) and four high fat diets (15% w/w) containing 40.5% (w/w) sucrose (control) and additional 80% methanol extract of prosomillet at the levels of 0.3% and 1% (w/w) or prosomillet powder at the level of 20% (w/w). Serum level of total cholesterol was a little higher but that of triglyceride was 41% lower in 20% (w/w) prosomillet powder group than in the control group. The cholesterol levels of two groups fed methanol extract were not different from the control but their triglyceride levels tended to be reduced. Liver cholesterol levels were lower and phospholipid levels higher in all three prosomillet groups than the control but triglyceride level was significantly low only in the prosomillet powder group. Fecal excretion of bile acid was most increased in the prosomillet powder group among all five test groups. Activity of liver microsomal 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase was significantly lower in 0.3% methanol extract fed group than the control and also appeared to be reduced in 1% extract fed one, whereas those of 20 cholesterol 7α-hydroxylase were not different among the five groups. Activities of liver cytosolic glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme were decreased in 0.3% prosomillet methanol extract and 20% powder groups. The results indicate that in addition to fiber, certain active components in prosomillet have potential to exert hypolipidemic effects via regulating hepatic cholesterogenesis and lipogenesis.

Key words: prosomillet, hypolipidemic effect, HMG CoA reductase, G6PDH, malic enzyme

INTRODUCTION

Epidemiological data indicate that individuals consuming diets rich in crude cereals are at low risk for cardiovascular disease (1). The effects of these cereals are attributed mainly to dietary fiber which plays a role in reducing serum cholesterol. In regards to types of dietary fiber, those with high content of water-soluble β-glucans are believed to decrease absorption of dietary lipids and increase fecal excretion of bile acids and neutral sterols through their viscosity in aqueous solution. But there are other data suggesting that the hypocholesterolemic effect of cereal is not solely due to viscosity resulting from soluble fibers. Zhang et al. (2) have shown that feeding brewer's spent grain which was low in water-soluble β-glucan decreased plasma cholesterol and increased fecal steroid loss in human subjects with ileostomies. Reports from Topping and his coworkers (3-5) have supported the result of Zhang et al. (2) and also conceded the findings of Qureshi et al. (6) that lipid components from barley were involved in the hypocholesterolemic effects of cereals via inhibitory action on hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34). In their subsequent works, Qureshi et al. (7,8) have identified α-tocotrienol as one of the active constituents and later shown that γ-tocotrienol is as potent as α-isomer in human studies, although Pearce et al. (9) have argued that γ-isomer was much greater inhibitory activity toward cholesterol biosynthesis in the HepG2 cell *in vitro*. It is well known that cereal grains contain various types of polyphenolic compounds other than tocotrienols (10). Among them tannic acid and morin (11) have been reported to reduce serum levels of total cholesterol and triglyceride

Cereals consumed in Korea are of various kinds and some of them have been reported to have hypocholesterolemic effects (12,13). But active materials other than dietary fibers for the effects have been rarely investigated. In our recent work, we have examined the inhibitory action on microsomal HMG-CoA reductase using *in vitro* assay system of 80% methanol extracts from twelve kinds of cereal and six kinds of legume grown in Korea and found prosomillet (*panicum milaceum*) most potent (14). In the present study we report that *in vivo* effects of the methanol extract of prosomillet on the activities for cholesterol biosynthesis and degradation and fatty acid synthesis in rat liver as well as serum and liver lipid status in comparison with prosomillet powder in experimental diets.

MATERIALS AND METHODS

Animals and diets

Male Sprague-Dawley rats were obtained from Korea Re-

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search Institute of Bioscience and Biotechnology, Taejon, Korea weighing about 100 g were acclimated to the facility for 1 week. The rats were divided into five groups, each group comprising of 8~9 rats in stainless steel cages with wire mesh bottoms in an environment of constant temperature (22±1°C) and lighting (light on, 0800~2000 h). They were allowed free access to test diets and water for 4 weeks. Food intake was measured daily and body weight every three days; feces were collected during the last 1 week and kept frozen at -50°C. The experiment was done appropriately under the guideline of animal experiments provided by Catholic University of Taegu-Hyosung.

Diets

The five test diets consisted of one low fat-starch diet (normal) and four kinds of high fat-sucrose diets which were based on the AIN-76 diet (15) as shown in Table 1. Casein, vitamin and mineral mixtures were purchased from Teklad Test Diets (Madison, WI, USA) and cellulose, DL-methionine and choline bitartarate from Sigma-Aldrich Chemical (St. Louis, MO, USA). Corn starch, sucrose, corn oil and lard were obtained from the supermarket. The normal diet had 5% (w/w) fat and corn starch as carbohydrate source while the four high fat diets had 15% (w/w) fat and 40.5% (w/w) sucrose in total 55.5% (w/w) carbohydrate. The high fat-sucrose diet without additional prosomillet component was used as the control. The clean prosomillet were ground into 40 mesh powder and added to the diet either as such or extracted with 80% methanol of ten times volume for 16 hours with continuous shaking. The resultant extract was filtered, concentrated under vacuum at 60°C and finally freeze-dried to remove solvent before added to the diets. To three high fat-sucrose diets, methanol extracts of prosomillet were added at levels of 0.3 and 1% (w/w) and prosomillet powder at the level of 20% (w/w), respectively. Contents of starch, cellulose, and soybean oil in the prosomillet powder diet were adjusted as in Table 1, considering those present in the prosomillet (16). Since 2.9 g of the methanol extract was obtained from 100 g prosomillet powder, 20% (w/w) prosomillet powder in diet was assumed to have a methanol extractable component at the level of 0.58% (w/w) in diet which was close to the average of two levels of the methanol extract used in diets described above.

Table 1. Composition of experimental diets (g/1000 g diet)

	Normal	Control	1% MeOH extact	0.3% MeOH extract	20% Prosomillet powder
Casein	200	200	200	200	177
DL-Methionine	3	3	3	3	3
Starch	650	150	150	150	0
Sucrose	0	405	405	405	405
Cellulose	50	50	50	50	46
Lard	0	95	95	95	95
Soybean oil	50	50	50	50	46
Mineral mix	35	35	35	35	35
Vitamin mix	10	10	10	10	10
Choline	2	2	2	2	2
Prosomillet methanol extract	0	0	10	3	0
Prosomillet powder	0	0	0	0	200
kcal/1000 g	3850	4330	4240	4240	4080

Preparation of tissues for analysis

After 4 weeks of the experimental diets the rats were anesthetized with ether at 0700~0800 h. Blood was drawn from abdominal *vena cava* and serum was prepared by centrifugation and stored at -50°C before lipid determination. The liver was excised after removing blood by passing saline through the portal vein, blotted dry and one part of it quickly frozen in liquid nitrogen for subsequent measurements of lipids. Another part of the liver was homogenized and underwent differential centrifugation to prepare microsomal and cytosolic fractions which were stored at -70°C before measuring enzyme activities.

Measurements of lipids and bile acid

Serum total cholesterol, HDL-cholesterol and triglyceride were measured by using an enzymatic kit (Shinyang Chemical Co. Seoul, Korea). Hepatic lipids were extracted by the method of Folch et al. (17). Cholesterol (18) and phospholipid (19) were measured colorimetrically, and triglyceride was measured by using an enzymatic kit (Shinyang Chemical Co.) by the aid of a detergent, triton X-100 (20). Bile acid from dry feces was extracted by the method of Crowell and Macdonald (21) and determined by using enzymatic kit (Sigma).

Assay of hepatic enzymes for chloesterol and fatty acid metabolism

Activity of HMG-CoA reductase was measured as described by Qureshi et al. (22) with a slight modification. Ten μl of microsome suspensions (about 200 μg protein) were incubated at 37°C in a vol of 35 μl of assay mixture containing 5.5 μmol potassium phosphate/buffer pH 7.4, 20 nmol dithiothreitol, 12 nmol DL-3-hydroxymethyl-[3-^{14}C] glutaryl CoA (0.06 μCi, NEN, Boston, Mass, USA) and 200 nmol NADPH. After 30 min, 5 μl of 6 N HCl was added to end the reaction. DL-Mevalonolactone produced was isolated by thin layer chromatography and radioactivity measured. Activity of cholesterol 7α-hydroxylase was measured using incorporation of liposome solublized cholesterol isotope ([4-^{14}C] cholesterol, Amersham, Buckinghamshire, England) into microsomal preparations (23) but azolectin for liposome was prepared from phosphatidylcholine (Sigma # P3644). Five grams of phosphatidylcholine was placed in an amber bottle and stirred overnight in 100 ml anhydrous acetone plus 0.5 mg buthylated hydroxytoluene. After filtration, resultant phosphatidylcholine crystal was dissolved with anhydrous ethyl ether that was later evaporated off. Final azolectin crystal was ground to powder and stored under vacuum at room temperature until use. Activities of glucose-6-phosphate dehydrogenase and malic enzyme in cytosol were measured by recording NADP reduction (24,25). Protein concentrations of microsome and cytosol preparations were estimated spectrophotometrically using bovine serum allbumin as standard (26).

Table 2. Effects of dietary prosomillet on growth of rats

	Initial body weight (g)	Body weight gain (g)	Feed efficiency (g/100 g diet)	Relative liver weight (g/100 g bw)
Normal	114± 9[1)]	195±10	39.1±1.5	4.51±0.40
Control	122±10	196±11	41.0±3.4	4.81±0.59
0.3% MeOH extract of prosomillet	106±11	184±18	39.3±3.2	4.59±0.62
1% MeOH extract of prosomillet	123±13	193±16	40.2±2.8	4.51±0.51
20% prosomillet powder	114± 9	177±17	37.3±4.2	4.49±0.91

[1)]Mean±SD of 8~9 rats per group.

RESULTS AND DISCUSSION

Growth and liver weight

As shown in Table 2, there was no significant difference in feed efficiencies and relative liver weights among the five experimental groups, although feed efficiency seemed slightly lower in rats fed 20% prosomillet powder.

Effects of dietary prosomillet on serum and liver lipids and fecal excretion of bile acid

Table 3 shows serum lipid levels of five experimental groups. Levels of total cholesterol were not significantly different among normal, control groups and groups supplemented with two levels of methanol extract of promillet but a little higher in the group fed 20% prosomillet powder. But HDL-cholesterol levels in the prosomillet powder group tended to be higher than the other three high fat groups including the control so that HDL-cholesterol/total cholesterol ratio (0.59±0.02) was in the same ranges of the three groups (0.61 ±0.03 for control, 0.58±0.04 and 0.64±0.03 for 0.3 and 1% methanol extract fed groups). However, triglyceride levels were the lowest in the prosomillet powder group and had tendency to be low in the methanol extract fed groups compared with the control. Qureshi et al. (6) have reported hypocholesterolmic effects of various lipid soluble fractions from barley but have not measured serum triglyceride level concomitantly. Among plant constituents, capsaicin of red pepper has been recognized earlier to have a hypotriglyceridemic effect (27) which was followed by tannic acid and morin (11), but underlying mechanisms for the hypotriglyceridemic effects were suggested to be different.

Table 3. Serum total cholesterol, triglyceride, and HDL cholesterol

	Total cholesterol	Triglyceride (mmol/L)	HDL cholesterol
Normal	2.46±0.10[1)a2)]	1.79±0.22[b]	1.66±0.10
Control	2.31±0.09[a]	2.64±0.25[a]	1.43±0.10
0.3% MeOH extract of prosomillet	2.45±0.13[a]	1.79±0.18[b]	1.43±0.09
1% MeOH extract of prosomillet	2.37±0.08[a]	1.97±0.24[ab]	1.53±0.07
20% prosomillet powder	2.67±0.08[b]	1.55±0.41[b]	1.58±0.07

[1)]Mean±SEM of 7~9 rats per group.
[2)]Values in the same column not sharing common superscript letters are significantly different at $p<0.05$ by Tukey's test.

Hepatic lipid concentrations and fecal bile acid excretions were shown in Table 4. In contrast to serum levels, liver cholesterol was reduced in all prosomillet groups compared with control group. Liver triglyceride levels were almost the same among control and two prosomillet metanol extract groups while that of 20% prosomillet powder group was as low as normal group. As described in Materials and Methods, 20% prosomillet powder in diet was equivalent to 0.58% methanol extract which was about midpoint of 0.3 and 1% of the extract used in diets. A very small change (0.4% or less in weight of toal diet) in type of dietary fiber that originated from 20% prosomillet powder diet was distinguished in type from sole cellulose used in the two diets containing methanol extract. It is of question that the small change caused the difference in the liver levels of triglyceride between the prosomillet powder and two methanol extract groups. Liver phospholipid levels were higher in all prosomillet groups than the control group. The higher contents of phospholipid may have been related to the lower content of triglyceride in the prosomillet groups but this relationship remains to be evaluated.

The fecal bile acid excretion of rats fed the 20% prosomillet powder was higher than those of rest groups except a group fed 1% prosomillet methanol extract. Intakes of dietary fibers from grains have been reported to increase fecal bile acid exceretion (3). Dietary fiber originated from prosomillet may have influenced the increase although minute in quantity as described above. The other constituents from

Table 4. Hepatic contents of cholesterol, triglyceride, and phospholipid and fecal bile acid excretion

	Cholesterol	*Triglyceride* (μmol/liver)	*Phospholipid*	*Fecal bile acid* (mg/day)
Normal	21.2±1.3[1)ab2)]	102.8±12.0[b]	172.7±12.6[NS]	7.17±0.45[b]
Control	25.8±2.8[a]	159.1±21.5[a]	153.7± 5.2	6.80±0.54[b]
0.3% MeOH extract of prosomillet	19.4±1.5[ab]	142.8± 7.9[ab]	164.0±12.9	6.60±0.80[b]
1% MeOH extract of prosomillet	20.4±1.4[ab]	153.7± 7.0[a]	183.1±14.4	7.78±0.94[ab]
20% prosomillet powder	18.5±1.5[b]	93.1± 7.3[b]	161.9±12.9	8.34±0.54[a]

[1)]Mean±SEM of 7~9 rats per group.
[2)]Values in the same column not sharing common superscript letters are significantly different at $p<0.05$ by Tukey's test.

grains including tocotrienols have been rarely studied for their effects on bile acid excretion even though both lipid and water soluble fractions of barley have been shown to decrease activity of cholesterol 7α-hydroxylase (6,7).

Hepatic enzyme activities for cholesterol and bile acid synthesis

As shown in Fig. 1, activities of liver microsomal HMG-CoA reductase were lower in groups fed methanol extract of prosomillet both at the level of 0.3% of control and tended to be lower in those fed 1.0% extract, but that of group fed diet containing 20% prosomillet powder did not differ from control. This result is agreed well with those obtained from *in vitro* addition of the methanol extract to rat liver microsome suspension (14). The methanol extract used in the present study was prepared with 80% methanol and was analysed to contain water soluble fraction at the level of 29% (w/w). However, suppressive effects on *in vitro* HMG-CoA reductase activity were observed only in lipid soluble fractions such as hexane, chloroform and ethyl acetate extracts (unpublished observation). However, a few water-soluble compounds from garlic (28) and artichoke (29) have recently been shown to inhibit HMG-CoA reductase activity in isolated rat hepatocytes. The reason for no change by 20% powder diet on enzyme activity was not clear but may have been due to slow release of active material from grain texture during digestion and absorption. On the other hand, there was no effect by dietary prosomillet either as methanol extract or powder at the given levels on activities of liver microsomal cholesterol 7α-hydroxylase. Increases in bile acid excretion by dietary fibers (30) have often been accompanied by elevated activity of cholesterol 7α-hydroxylase (31). In the present study, however, bile acid excretion of 20% prosomillet powder group was higher than the other groups but activity of cholesterol 7α-hydroxylase was not increased. This discrepancy may be due to a smaller increase in bile acid excretion by dietary prosomillet powder in the present study in comparison with larger changes in bile acid pool shown in studies for dietary fibers (30). Activity of hepatic cholesterol 7α-hydroxylase has been shown to be stimulated by dietary cholesterol (31), under which condition the enzyme activity could react to prosomillet components differently.

Hepatic activities of lipogenic enzymes

Activities of liver cytosolic glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme are shown in Fig. 2. Both were reduced either by dietary methanol extract of prosomillet or powder at the levels used in the present study. The present results can be compared with those of Qureshi et al. (6) showing different effects on the activity of fatty acid synthetase of two active compounds isolated from petroleum extracts of barley powder although both of them had the same inhibitory effect on HMG-CoA reductase. One of the active compound from their study, α-tocotrienol increased the activity of fatty acid synthetase and the other decreased activity. G6PDH and malic enzyme are lipogenic enzymes generally known to be inducible at the same pattern as fatty acid synthetase (32), although it was not the case in a study with panthethine supplementation (33). Therefore, it is more likely that the active component(s) in prosomillet is (are) not tocotrienols.

Low activities of G6PDH and malic enzyme could play a role in reducing serum and liver levels of triglyceride in groups fed prosomillet either as methanol extract or as powder. It is not confirmed whether active materials for reducing lipogenic enzyme activities are polar or nonpolar. But the action of polar compounds can not be excluded because inhibitory effects on fatty acid synthetase have been shown by polar fractions from garlic (34).

In conclusions, prosomillet contains potential hypolipidemic component(s), apart from dietary fiber and probably different from those found in barley (6,7). These components are regarded to play roles via regulating hepatic HMG CoA reductase and lipogenic enzymes possibly within optimal concentrations, since 0.3% methanol extract showed better results than 1% extract in most parameters measured in the present study.

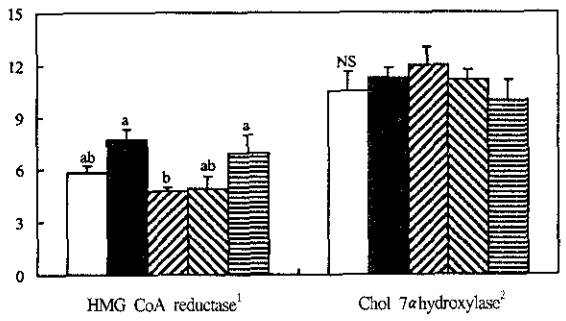

Fig. 1. Activities of liver microsoml HMG-CoA reductase and cholesterol 7α-hydroxylase. □, Normal; ■, Control; ▨, 0.3% prosmillet MeOH-extract; ▧, 1% prosmillet MeOH-extract; ▤, 20% prosmillet powder. [1]pmol of mevalonic acid/min/mg microsomal protein. [2][14C]cholesterol into [14C]7α-hydroxycholesterol/min/mg microsomal microsomal protein. Mean±SEM of 6~8 rats per group. Different alphabet letters in HMG-CoA reductase activities denotes significant difference among groups. NS: not significant.

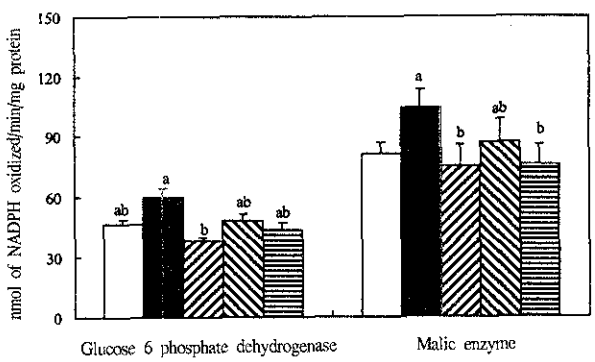

Fig. 2. Activities of liver cytosolic glucose 6-phosphate dehydrogenase and malic enzyme. □, Normal; ■, Control; ▨, 0.3% prosmillet MeOH-extract; ▧, 1% prosmillet MeOH-extract; ▤, 20% prosmillet powder. Mean±SEM of 7~9 rats per group. Different alphabet letters in the same enzyme activities denotes significant difference among groups.

ACKOWLEDGEMENTS

We thank the Agricultural R & D Promotion Center, Korea and RRC program of MOST and KOSEF for its support in the accomplishment of this study and Department of Genetic Engineering, Kyungbuk National University for its accomodation of experimental facilities for using radioactive materials.

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(Received August 9, 1999)