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Hypolipidemic Effect of Soluble Fiber Isolated from Seeds of *Cassia tora* Linn. in Rats Fed a High-Cholesterol Diet

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Soluble fibers isolated from the seeds of *Cassia tora* Linn. (SFC) have attracted considerable attention in recent years due to their phenomenal rheological behavior. In this study were investigated the effects of SFC on lipid metabolism. Male Sprague−Dawley rats were fed one of three experimental diets, a normal diet, a high-cholesterol diet, or a high-cholesterol diet with 5% SFC, for 5 weeks. The serum concentration of total cholesterol in rats fed SFC was 27% lower ($p < 0.05$) compared to that of the control group, but the serum high-density lipoprotein cholesterol level was increased in the SFC group. Liver total cholesterol and triglyceride levels were reduced significantly ($p < 0.05$) in rats fed the SFC diet. In addition, fecal bile acid and lipid excretion was significantly increased by SFC consumption. These results indicate that SFC enhances fecal lipid excretion and may cause a reduction in serum and hepatic lipid concentrations in rats.

KEYWORDS: Soluble fiber; seeds of *C. tora*; hypolipidemic effect

INTRODUCTION

Water-soluble fibers, including gums, have proven to be effective in lowering serum and liver cholesterol concentrations (*1*). Evidence for a cholesterol-lowering effect of water-soluble fiber in humans and other animals has been accumulating in recent years, especially with viscous fibers such as guar gum, psyllium, and oat bran (*2*−*4*). The effect varies depending on the amount and properties of the gums and the cholesterol levels of the diet. In many studies, using soluble fibers to enhance the hypocholesterolemic effects of fat-restricted diets can decrease the level of serum cholesterol by 20−30% (*4*). This degree of reduction in humans would enable most individuals with elevated serum cholesterol levels to manage their hypercholesterolemia satisfactorily without drugs. Plant gums derived from seeds, such as guar and panwar, have attracted considerable attention in recent years due to their phenomenal rheological behavior. During the past two decades, guar gum acquired great importance as a source of industrial polysaccharides (*5*). *Cassia tora* Linn. is a small shrub that grows as a common weed in Asian countries. The *C. tora* seed is composed of hull (27%), endosperm (32%), and germ (41%) (*6*). It has been demonstrated that some of the properties of *C. tora* gum are as effective as those of guar gum (*6*); for example, they both exhibit good gelling properties and fairly stable viscosity in solution at pH 5−9 (*7*). Methylation studies have shown that the backbone of the polysaccharide consists of 1→4-linked D-mannopyranose and D-glucopyranose units (*5*). The seed extracts are reported to have hypotensive (*8*), antimicrobial, and antihepatotoxic activities (*9*). Several polyherbal formulations including *C. tora* seeds are available at Chinese markets for preventing the formation of atherosclerosis plaques (*10*). Although hypolipidemic properties of the *C. tora* seed ethanol extract were reported by Patil et al. (*11*), not much is known about the hypolipidemic effects of soluble fiber of *C. tora* seeds. Therefore, to clarify the hypolipidemic effect of soluble fiber isolated from seeds of *C. tora* Linn. (SFC), we examined the changes in serum and liver lipid levels and in fecal lipid excretion in rats fed a high-cholesterol diet and a 5% SFC high-cholesterol diet.

MATERIALS AND METHODS

Materials. The seeds of *C. tora* Linn. originating in Korea were purchased from Kyung-dong Oriental medicine market (Seoul, Republic of Korea). Casein, cellulose, and vitamin and mineral mixtures were purchased from Harlan Teklad (Madison, WI). DL-Methionine and choline chloride were obtained from Sigma-Aldrich Chemical (St. Louis, MO). All other chemicals were of analytical grade or purer.

Preparation of Soluble Fiber from *C. tora* Linn. Seeds. The *C. tora* seeds were ground into 40-mesh powder, and soluble dietary fiber was isolated according to the method described by Prosky et al. (*12*).

Animals and Diets. Six-week-old male Sprague−Dawley rats, purchased from Daehan Experimental Animal (Eumsung, Korea), were initially fed the chow diet for 7 days. After acclimation, the rats (weighing 200−220 g) were randomly divided into three groups and fed a normal diet (N) and two high-cholesterol diets (HC, 5-SFC) for 5 weeks, respectively. The high-cholesterol diet group consisted of two

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Table 1. Composition of Experimental Diets

ingredient	N[a] (g/kg of diet)	HC[b] (g/kg of diet)	5-SFC[c] (g/kg of diet)
casein	200	200	200
corn oil	50	50	50
mineral mix[d]	35	35	35
vitamin mix[c]	10	10	10
choline chloride	2	2	2
methionine	3	3	3
cellulose	50	50	50
sucrose	200	200	200
corn starch	450	438.75	388.75
cholesterol		10	10
taurocholic acid		1.25	1.25
SFC[f]			50

[a] Normal diet group. [b] High-cholesterol diet group. [c] High-cholesterol diet with 5% SFC group. [d] AIN-76 mineral mixture. [e] AIN-76 vitamin mixture. [f] Soluble dietary fiber isolated from the seeds of *C. tora*.

groups, without (HC) or with 5% (w/w) SFC (5-SFC). The compositions of experimental diet (**Table 1**) were based on the AIN-76 diet (American Institute of Nutrition, 1980). The high-cholesterol diet was prepared by supplementing 1% cholesterol into the normal diet.

Rats were maintained at a temperature and humidity of 23 ± 2 °C and 55 ± 5%, respectively, with a 12-h/12-h light/dark cycle (lights on from 6:30 a.m. to 6:30 p.m.). All animal procedures were conducted in accordance with the Guidelines for Animal Experimentation of the Korea Food Research Institute.

Sample Preparation. After 5 weeks of experimental diet feeding, the rats were fasted for 12 h and sacrificed under diethyl ether anesthesia. Feces were collected for 3 consecutive days before sacrifice. Fecal samples were weighed, dried, milled, and stored at −20 °C until analysis. Blood from the abdominal aorta was collected into a tube and centrifuged at 1500*g* for 15 min to separate the serum. Serum was stored at −70 °C until analysis. The livers were excised, weighed, and stored at −70 °C until use.

Lipid Analyses. Serum total cholesterol, high-density lipoprotein (HDL)-cholesterol, free cholesterol, free fatty acid, and triglyceride levels were measured using commercial enzymatic kits (Eiken, Japan). Hepatic lipids were extracted according to the method of Folch et al. (*13*). Hepatic total cholesterol and triglyceride levels were measured using commercial enzymatic kits (Eiken, Japan).

Activities of Alanine Transaminase (ALT) and Aspartate Transaminase (AST). The activities of AST and ALT in serum were measured with the aid of an enzymatic kit (Shinyang Chemical, Republic of Korea).

Measurement of Lipid Peroxide Contents. Serum lipid peroxide content was assayed according to the method of Yagi (*14*). For the analysis of hepatic lipid peroxide, 1 g of liver tissue was homogenized in 5 volumes of 1.15% KCl solution with a Teflon−Elvehjem homogenizer and centrifuged at 600*g* at 4 °C for 10 min to obtain postnuclear supernatant. The supernatant was used to determine hepatic lipid peroxide content. Lipid peroxidation in the liver was determined by the production of thiobarbituric acid-reactive substances according to the method of Ohkawa et al. (*15*). Malondialdehyde, which has been identified as the product of lipid peroxidation, is reacted with thiobarbituric acid, and the resultant absorbance is determined at 532 nm.

Activities of Hepatic Enzymes for Fatty Acid Metabolism. One gram of liver tissue was homogenized in 10 volumes of 0.25 M sucrose/0.5 M EDTA buffer (pH 7.4) with a Teflon−Elvehjem homogenizer and centrifuged at 10000*g* at 4 °C for 30 min to obtain postmitochondrial supernatant. The supernatant was used to measure the activities of glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme. The activities of G6PDH and malic enzyme were assessed by measuring the rate of nicotinamide adenine dinucleotide phosphate (NADP) reduction (*16*, *17*). Protein concentration was determined according to the method of Lowry et al. (*18*).

Determination of Fecal Lipid and Bile Acid. Fecal lipids were extracted according to the method of Folch et al. (*13*), and total cholesterol and triglyceride were measured using commercial enzyme kits (Eiken, Japan). Fecal bile acid was extracted according to the method of DeWeal et al. (*19*) and determined with the aid of bile acid assay kits (Kyokudo, Tokyo, Japan).

Statistics. All statistical analyses were carried out using ANOVA and Duncan's multiple-range test; a p value of <0.05 was selected as the limit of statistical significance. The statistical program used was SAS (Cary, NC).

Table 2. Change in Body Weight, Food Intake, and Liver and Adipose Tissue Weights of Rats Fed Soluble Fiber Isolated from *C. tora* Seeds

	N[a]	HC[b]	5-SFC[c]
initial body weight (g)	205.2 ± 6.7 ns[d]	205.1 ± 4.8	205.6 ± 2.9
weight gain (g/5 weeks)	131.8 ± 6.7 ns	138.1 ± 7.0	134.1 ± 4.4
food intake (g/5 weeks)	422.5 ± 8.8 ns	411.9 ± 7.2	409.6 ± 6.2
feed efficiency ratio	0.31 ± 0.02 ns	0.34 ± 0.02	0.33 ± 0.01
tissue weight			
epididymal fat pad (g)	2.94 ± 0.26 b	3.64 ± 0.14 a	2.93 ± 0.13 b
liver (g)	9.36 ± 0.23 b	14.34 ± 0.52 a	10.90 ± 0.57 b

[a] Normal diet group. [b] High-cholesterol diet group. [c] High-cholesterol diet with 5% SFC group. [d] Means ± SE of nine animals per diet; values in the same row not sharing common letters are significantly different at $p < 0.05$, as assessed using Duncan's multiple-range test. ns, not significant.

Table 3. Effect of Soluble Fiber Isolated from *C. tora* Seeds on the Serum Levels of Lipids and Activities of Transamination in Experimental Rats

	N[a]	HC[b]	5-SFC[c]
serum lipid level			
total cholesterol (mg/dL)	93.0 ± 1.41 b[d]	142.7 ± 6.84 a	92.2 ± 3.45 b
free cholesterol (mg/dL)	10.2 ± 0.97 ns	12.2 ± 0.49	10.0 ± 0.75
triglyceride (mg/dL)	40.9 ± 2.71 b	80.4 ± 9.33 a	79.4 ± 2.48 a
HDL-cholesterol[e] (mg/dL)	66.7 ± 0.53 a	30.1 ± 2.38 c	41.8 ± 1.16 b
free fatty acid (μequiv/L)	598.4 ± 25.1 c	968.1 ± 56.1 a	799.3 ± 35.0 b
enzyme activity			
AST[f] (karmen units)	61.8 ± 4.32 b	121.0 ± 17.31 a	63.8 ± 4.33 b
ALT[g] (karmen units)	30.8 ± 2.17 b	38.8 ± 1.91 a	31.6 ± 1.02 b

[a] Normal diet group. [b] High-cholesterol diet group. [c] High-cholesterol diet with 5% SFC group. [d] Means ± SE of nine animals per diet; values in the same row not sharing common letters are significantly different at $p < 0.05$, as assessed using Duncan's multiple-range test. ns, not significant. [e] HDL, high-density lipoprotein. [f] AST, aspartate transaminase. [g] ALT, alanine transaminase.

RESULTS

Diet Consumption, Growth, and Tissue Weight. There were no significant differences in the diet intakes, body weight gains, and feed efficiency ratios among the experimental groups (**Table 2**). The rats fed high-cholesterol diets had higher epididymal fat pad weight than those of the normal diet (**Table 2**). Epididymal fat pad weight, however, was significantly lower ($p < 0.05$) in the group fed the 5% SFC diet compared to the HC group. The liver weight of the HC group was 53% higher than that of the normal group; however, the rats fed the 5% SFC diet had significantly lower liver weight than those of HC group.

Serum Lipids. Table 3 shows the serum lipid levels of experimental rats. The total cholesterol level was increased by 52% in the HC group compared to the normal group, but total cholesterol concentration was significantly reduced in the 5-SFC group, compared to the HC group. There was no significant difference in serum free cholesterol level among the three experimental groups. The HC group achieved serum triglyceride levels that were 100% higher than in the normal group, but there was no significant difference between the HC and 5-SFC group. The 5-SFC group exhibited a 17% reduction in serum free fatty

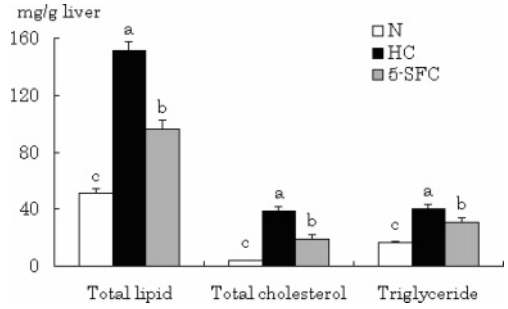

Figure 1. Effect of soluble fiber from *C. tora* seeds (SFC) on hepatic lipid levels in experimental rats fed a hyperlipidemic diet for 5 weeks. Bars with different letters differ significantly ($p < 0.05$, $n = 9$). N, normal diet group; HC, high-cholesterol diet group; 5-SFC, high-cholesterol diet with 5% SFC group.

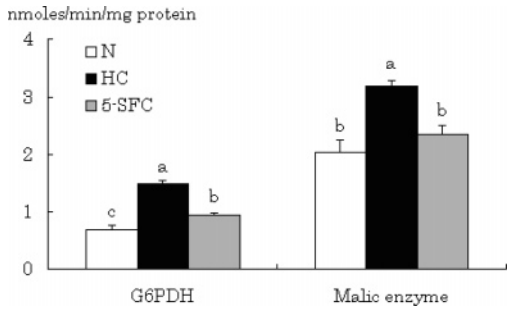

Figure 2. Effect of soluble fiber from *C. tora* seed (SFC) on the activities of lipogenic enzyme in experimental rats fed a hyperlipidemic diet for 5 weeks. Bars with different letters differ significantly ($p < 0.05$, $n = 9$). G6PDH, glucose-6-phsphate dehydrogenase; N, normal diet group; HC, high-cholesterol diet group; 5-SFC, high-cholesterol diet with 5% SFC group.

acid concentration compared to the HC group. The HDL-cholesterol level was increased by 37% (significant at $p < 0.05$) after feeding of the 5% SFC diet, compared to the HC group.

Activities of AST and ALT. The activities of AST and ALT (representing the biochemical parameters of liver damage) are given in **Table 3**. The HC group had significantly higher AST and ALT activities compared with the normal group; however, the 5% SFC diet lowered significantly the activities of both AST and ALT compared with HC group. The lowering effect of the SFC diet on AST and ALT activities suggests that treatment with the 5% SFC diet prevents the hepatic damage caused by hyperlipidemia.

Liver Lipids and Activities of Lipogenic Enzymes in the Liver. Figure 1 shows the effect of SFC on liver total cholesterol, triglyceride, and total lipid in the rats. Total cholesterol content in the liver exhibited tendencies simliar to that obtained for serum total cholesterol concentration. Liver total cholesterol and triglyceride contents were elevated by about 13 and 2.5 times, respectively, in rats that consumed the high-cholesterol diet, compared to the normal diet. Liver total cholesterol levels were significantly reduced (50%, $p < 0.05$) in the group fed the 5% SFC diet compared to those of HC group; liver triglyceride levels were also significantly lower in this group (30.9 mg/g of liver) than in the HC group (40.0 mg/g of liver). The activities of liver cytosolic G6PDH and malic enzyme are shown in **Figure 2**. G6PDH and malic enzyme activities were elevated in the HC group and significantly reduced by consumption of the 5% SFC diet compared to rats fed the high-cholesterol diet (39 and 28%, respectively; $p < 0.05$). Thus, the addition of 5% SFC in the high-cholesterol diet suppresses somewhat the high-cholesterol-induced increase in the activities of both enzymes.

Table 4. Effect of Soluble Dietary Fiber Isolated from *C. tora* Seeds on Serum and Liver Levels of Thiobarbituric Acid Reactive Substances (TBARS) in Rats

	N[a]	HC[b]	5-SFC[c]
serum TBARS (nmol/dL)	61.9 ± 3.1 b[d]	84.06 ± 7.0 a	62.3 ± 1.94 b
liver TBARS (nmol/g of liver)	93.8 ± 5.0 b	119.3 ± 9.1 a	113.9 ± 10.1 ab

[a] Normal diet group. [b] High-cholesterol diet group. [c] High-cholesterol diet with 5% SFC group. [d] Means ± SE of nine animals per diet; values in the same row not sharing common letters are significantly different at $p < 0.05$, as assessed using Duncan's multiple-range test.

Table 5. Effect of Soluble Fiber Isolated from *C. tora* Seeds on the Fecal Excretions of Lipid and Bile Acid in Experimental Rats

	N[a]	HC[b]	5-SFC[c]
total lipid (mg/day)	78.8 ± 3.75 c[d]	315.5 ± 19.15 b	626.7 ± 49.04 a
total cholesterol (mg/day)	14.4 ± 1.10 c	100.0 ± 5.94 b	151.1 ± 10.85 a
triglyceride (mg/day)	13.4 ± 0.27 b	13.3 ± 0.30 b	17.0 ± 0.49 a
bile acid (μmol/day)	31.1 ± 0.87 c	75.9 ± 0.55 b	88.8 ± 2.61 a

[a] Normal diet group. [b] High-cholesterol diet group. [c] High-cholesterol diet with 5% SFC group. [d] Means ± SE of nine animals per diet; values in the same row not sharing common letters are significantly different at $p < 0.05$, as assessed using Duncan's multiple-range test.

Serum and Liver Lipid Peroxide. Table 4 gives the values for lipid peroxide in the serum and liver of rats fed the experimental diets for 5 weeks. Significantly lower serum lipid peroxide values were found in the SFC group when compared with those fed the high-cholesterol diet. No significant difference in liver lipid peroxide values was apparent, however, between the HC group and the 5-SFC group.

Fecal Excretions of Lipids and Bile Acid. As shown in **Table 5**, significantly higher fecal total lipid, cholesterol, and triglyceride contents were found in the 5-SFC group compared to the HC group. Fecal cholesterol excretion was 51% greater in rats fed the 5% SFC diet compared to those fed the high-cholesterol diet (**Table 5**). Fecal excretion of total lipid and triglyceride was markedly increased (by 99 and 28%, respectively) in the 5-SFC group compared with the HC group, and more bile acid excretion (17% increase) was found in the 5-SFC group than in the HC group (**Table 5**).

DISCUSSION

This study was conducted to examine the hypolipidemic effects of soluble fiber extracted from *C. tora* Linn. seeds. SFC decreased the serum total cholesterol level in the hypercholesterolemic model by 36%, but did not affect triglyceride and free cholesterol concentrations in these rats. The serum cholesterol-lowering effects of the diet containing SFC are in accord with the results of studies carried out by other researchers (*2*−*4*). Patil et al. (*11*) have reported the hypolipidemic effects of ethanol extracts from *C. tora* seeds on Triton-induced hyperlipidemia in rats. In their hyperlipidemic model, total cholesterol levels in the group with water-soluble fraction of ethanol extraction were decreased by up to 71% compared to the initial, baseline level. HDL-cholesterol levels, however, were increased compared to initial levels, which is in accord with our results. In the present study, serum HDL-cholesterol levels were significantly higher in the group fed the 5% SFC diet (41.82 mg/dL) compared to the HC group. A higher content of HDL-

cholesterol is very important in humans because it is correlated with a reduced risk of coronary heart disease (*20*). The increased HDL facilitates the transport of cholesterol from the serum to the liver, where it is catabolized and excreted from the body. The possible mechanism for the hypolipidemic action of soluble fibers may be enhancement of the activity of lecithin:cholesterol acyltransferase and inhibition of the action of hepatic triglyceride-lipase on HDL, which may contribute to the rapid catabolism of blood lipids through extrahepatic tissues (*20*).

Dietary manipulations in rats can elicit changes in liver cholesterol concentration, which is a strong indicator of whole-body cholesterol metabolism. In the study, we observed that 5% SFC diet reduced serum and liver cholesterol concentrations. These results are similar to those of Fernandez et al. using guar gum (*3*). As shown in **Figure 2**, the 1% cholesterol-induced increase in the activity of G6PDH was partially suppressed and that of malic enzyme was totally suppressed by 5% SFC supplementation. These enzymes are lipogenic enzymes that are generally known to be inducible in the same pattern as fatty acid synthetase (*21*). Low activities of G6PDH and malic enzyme in the SFC group could play a role in reducing liver levels of triglyceride. Specific mechanisms by which water-soluble fibers reduce serum and liver lipid levels remain uncertain, but SFC is thought to play a role via the regulation of liver lipogenic enzymes, possibly within optimal concentrations.

In this study, fecal bile acid excretion in rats was significantly greater in animals fed the SFC diet. Furthermore, higher fecal cholesterol levels were observed after SFC feeding in the present study. It is hypothesized that the lower liver cholesterol found in rats after SFC feeding might be related to lower cholesterol absorption. This suggests that SFC may influence hepatic cholesterol metabolism by affecting cholesterol absorption, which might lead to a lower liver weight. In addition, Favier et al. (*22*) reported greater fecal neutral steroid excretion in animal models fed viscous fibers such as guar gum. The possible mechanism by which increased viscosity promotes neutral steroid excretion is by inhibiting cholesterol absorption (*23*). Nishimura et al. (*24*) presumed that an enhanced fecal steroid excretion would be at the least in part responsibility for the hypocholesterolemic effects of certain dietary fibers. Another possible mechanism is its ability to form a gel that interferes with the absorption of lipids; the cholesterol (*25*) created in the feces is thus removed from the enterohepatic cycle, and smaller amounts of bile salts are available for lipid absorption. Moreover, serum and liver cholesterol are used for bile acid synthesis to compensate for depleted pools (*4*), and cholesterol absorption itself could also be depressed by soluble fibers, resulting in a greater excretion of neutral sterols.

Some investigators (*26*−*28*) have reported that short-chain fatty acid (SCFA), the fermentation product of dietary fiber in the cecum, may be involved in the serum cholesterol-lowering effect. Roy et al. (*29*) demonstrated that induction of hepatic 7α-hydroxylase activity, which is the initial and rate-limiting enzyme in the bile acid synthesis pathway, by psyllium, guar gum, and pectin intake is one of the major secondary mechanisms that may account, in part, for the hypocholesterolemic effect of soluble fibers. Although we did not measure SCFA and 7α-hydroxylase activity, one possibility may be that the changes in SCFA and 7α-hydroxylase activity by soluble fibers may be influenced by serum cholesterol level. Further studies are needed to provide details of this mechanism.

Another interesting finding in this study is that TBARS, an index of lipid peroxidation, are significantly increased in serum by high-cholesterol diet administration, and this increase is significantly inhibited by the supplementation of SFC. This result is similar to that reported by Naito et al. (*30*), who found that the increase in TBARS in the mice colonic mucosa was inhibited by guar gum. Chiang et al. (*31*) have demonstrated that a change in cholesterol absorption may alter liver lipid content and lipid peroxidation in cholesterol-fed rats. Therefore, it is suggested that a decrease of serum TBARS in rats fed SFC may be related to lower serum lipid concentration.

In conclusion, the administration of 5% SFC in the diet resulted in significantly lower levels of total cholesterol in the serum and liver of rats. Furthermore, serum HDL-cholesterol concentrations were increased in the SFC group, but fecal lipids and bile acid excretion were also significantly increased. From these results, it is suggested that intake of 5% SFC plays an important role in lowering the serum and hepatic levels of cholesterol by increasing fecal bile acid excretion and down-regulating the production of lipogenic enzymes. Further studies must be carried out to identify the more precise mechanisms underlying the hypolipidemic effect of SFC.

ABBREVIATIONS USED

SFC, soluble fiber isolated from seeds of *Cassia tora* Linn.; HC, high cholesterol; HDL, high-density lipid cholesterol; AST, aspartate transaminase; ALT, alanine transaminase; G6PDH, glucose-6-phosphate dehydrogenase; NADP, nicotinamide adenine dinucleotide phosphaste; SCFA, short-chain fatty acid; TBARS, thiobarbituric acid reactive substances.

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