Research Article

Effect of water-soluble gummy fiber, water-insoluble neutral detergent fiber isolated from *Syzygium cumini* seeds on biliary and fecal bile acids and sterols in rats fed a high cholesterol diet

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Abstract

Background: Highly viscous water-soluble fibers present in plant materials have been shown to reduce serum levels of blood cholesterol and lipid levels in animals and human beings. The water-insoluble neutral detergent fibers had shown no significant hypocholesterolemic and hypolipidemic effects.

Objective: Effect of water-soluble gummy fiber, water-insoluble neutral detergent fiber isolated from Syzygium cumini seeds on biliary and fecal bile acids and sterols in rats fed a high cholesterol diet

Materials and Methods: Effects of cholesterol-supplemented and cholesterol-unsupplemented diets containing 6%, 12%, and 18% water-soluble gummy fibers and 2.25%, 5%, and 10% water-insoluble detergent fibers were studied in both normal and diabetic rats for 21 days

Results: Significant increase in biliary content and fecal excretion of bile acids was observed in rats fed for 21 days on cholesterol-supplemented and cholesterol-unsupplemented diets containing 6%, 12%, and 18% water-soluble gummy fibers, but 2.25%, 5%, and 10% water-insoluble neutral detergent fibers isolated from *Syzygium cumini* seeds had no significant effect on the above parameters.

Conclusion: The increased biliary content and fecal excretion of bile acids and sterols in our study suggest that the dietary water-soluble gunny fiber of *S. cumini* seeds may enhance the breakdown of cholesterol, leading to an increased conversion of cholesterol to bile acids and salts that are excreted in alimentary canal.

KEY WORDS: *Syzygium cumini* seed fiber, neutral detergent fiber, biliary bile acids, fecal bile acids, fecal sterol

Introduction

Hypocholesterolemic and hypolipidemic effects of *Syzygium cumini* seeds have been attributed to the water-soluble gummy fibers present in them. The viscosity measurement studies indicated that water-soluble gummy fiber isolated from *S. cumini* seeds is a highly viscous material.[1] Highly viscous water-soluble fibers present in plant materials have been shown to reduce serum cholesterol levels and lipid levels in animals and human beings[2–4]. The water-insoluble neutral detergent fibers (NDFs) had no significant hypocholesterolemic and hypolipidemic effects. This is based on the observations of our research study that 6%, 12%, and 18% water-soluble gummy fibers lowered blood cholesterol and lipid levels whereas 2.25%, 5%, and 10% water-insoluble NDFs did not exhibit any such activity.[5] Because cholesterol is the precursor for the synthesis of bile acids, bile salts, and sterols, this study was conducted to investigate the effect of 6%, 12%, and 18% water-soluble gummy fibers on biliary and fecal bile acids and sterols in normal and diabetic rats fed on high-cholesterol diet.

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Materials and Methods

Materials

Cholesterol, chloroform, chromotropic acid, sodium arsenite, and calcium chloride were purchased from Merck, Germany. Rest of the chemicals used were of analytical grade.

Methods

The collected samples were dried in an incubator at 37°C for 48 h, powdered and weighed, homogenized, and extracted with absolute alcohol. This extract was used for the estimation of fecal sterols and bile acids (cholic acid and deoxycholic acid) by the methods of Zlatkis *et al*.[6] and Snell and Snell,[7] respectively. Cholic acid and deoxycholic acid were determined spectrophotometrically at 320 and 385 nm, respectively, after heating in 2:1 sulfuric acid/conjugate. Biliary sterols and bile acids were also estimated using another method.[8]

Animals and Diets

The male albino rats of Wistar strain, between 150 and 170 g, were divided into two sets. One set meant for investigating the effect of 6%, 12%, and 18% water-soluble gummy fibers and 2.25%, 5%, and 10% water-insoluble NDFs with cholesterol-supplemented diet, and another set for investigating the effect of 6%, 12%, and 18% water-soluble gummy fibers and 2.25%, 5%, and 10% water-insoluble NDFs with cholesterol-unsupplemented *S. cumini* seed diet. Each set was further divided into seven groups each of normal rats as Group I N, Group II N, Group III N, Group IV N, Group V N, Group VI N, and Group VII N and diabetic rats as Group I D, Group II D, Group III D, Group IV D, Group V D, Group VI D, and Group VII D. Animals were made diabetic by the method of Pandey and Khan.[1] Groups I N and I D rats from both the sets served as control and were fed the control diet. Groups II–VII of both normal and diabetic rats of one set were fed the experimental cholesterol-supplemented diets, and Groups II–IV of both normal and diabetic rats of another set were fed experimental cholesterol-unsupplemented diets containing 6%, 12%, 18% water-soluble gummy fibers and 2.25%, 5%, and 10% water-insoluble NDFs. Rats were fed 20 g of all the diets daily for 21 days. The composition of the control and experimental diets are given in Table 1. At the end of 21 days, rats of both the sets were fasted for 18 h. After fasting, the rats were stunned by blow at the back of neck and killed by decapitation.

A 48-h sample of feces was collected from all the animals before kill. Bile was collected for 60 min by cannulation of the bile duct after anesthetizing the animals with Nembutal.

Result

Feeding of cholesterol-supplemented and cholesterol-unsupplemented diets each containing 6%, 12%, and 18% water-soluble gummy fibers isolated from *S. cumini* seeds to the normal and diabetic rats for 21 days significantly increased the biliary content and fecal excretion of bile acids and sterols when compared with the respective control rats [Table 2].

The increased biliary content and fecal excretion of bile acids and sterols suggest that the water-soluble gummy fibers may enhance the breakdown of cholesterol leading to an increased conversion to bile acids and bile salts that are excreted in the alimentary canal.

The increase in the amount of water-soluble gummy fibers in the cholesterol-unsupplemented and cholesterol-supplemented diets from 6 g% to 12 g% and 18 g% had pronounced enhancing effect. The normal and diabetic rats fed on the cholesterol-supplemented diets showed highly significant ($p < 0.01$) increase in biliary content and fecal excretion when

Table 1: Composition of control and experimental diets

Ingredients	Control diet	Experimental cholesterol-unsupplemented diets						Control diet	Experimental cholesterol-supplemented diets					
		6%	12%	18%	2.25%	5%	10%		6%	12%	18%	2.25%	5%	10%
Casein	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Water-soluble gummy fibers isolated from *Syzygium cumini* seeds		6.0	12.0	18.0					6.0	12.0	18.0			
Water-insoluble neutral detergent fibers isolated from *Syzygium cumini* seeds					2.25	5.0	10.0					2.25	5.0	10.0
Hydrogenated oil	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Starch	65.0	59	53	62	62.75	60	55	63.3	57.73	51.3	45.3	61.05	58.3	53.3
Cholesterol								1.0	1.0	1.0	1.0	1.0	1.0	1.0
Cholic acid								0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt mixture	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mixture	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Cholic acid								0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt mixture[a]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mixture[b]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Calcium chloride								0.2	0.2	0.2	0.2	0.2	0.2	0.2

Experimental cholesterol-unsupplemented and cholesterol-supplemented diets contain water-soluble gummy fibers and water-insoluble neutral detergent fibers isolated from *Syzygium cumini* seeds.
Each diet contains (%w/w) [a]salt mixture (4 g) and [b]vitamin mixture (1 g).

Table 2: Effect of feeding cholesterol-supplemented and cholesterol-unsupplemented diets containing water-soluble gummy fibers and water-insoluble neutral detergent fiber isolated from *Syzygium cumini* seed diets on the concentration of fecal sterols and bile acids in normal and diabetic rats

(A)

Groups	Cholesterol unsupplemented					Cholesterol supplemented				
	Fecal weight	Fecal sterol	Cholic acid	Deoxycholic acid	Total bile acid	Fecal weight	Fecal sterol	Cholic acid	Deoxycholic acid	Total bile acid
Group I D (6), control diet fed	2.23 ± 0.07	33.16 ± 1.67	21.55 ± 1.68	24.37 ± 1.98	45.92 ± 3.66	4.84 ± 0.32	67.23 ± 4.53	23.05 ± 1.90	29.11 ± 3.02	52.16 ± 4.92
Group II D (6), diets containing 6% water-soluble gummy fiber	4.19** ± 0.13	43.33** ± 2.28	29.79 ± 2.85	19.61 ± 1.74	49.40** ± 4.54	6.37** ± 0.50	74.69** ± 5.11	29.41 ± 2.60	25.03 ± 3.02	54.44** ± 5.77
Group III D (6), diets containing12% water-soluble gummy fiber	4.04** ± 0.52	53.54** ± 2.91	32.33 ± 2.75	25.00 ± 1.96	57.33** ± 4.71	7.35** ± 0.80	93.00** ± 5.79	31.06 ± 4.01	27.99 ± 2.50	59.05** ± 6.51
Group IV D (6), diets containing 18% water-soluble gummy fiber	5.14** ± 0.75	69.00*** ± 3.96	37.10 ± 3.24	34.53 ± 2.54	71.63*** ± 5.78	7.98** ± 0.68	110.00*** ± 4.47	39.25 ± 5.98	37.70 ± 6.29	69.95*** ± 12.27
Group V D (6), diets containing 2.25% water-insoluble neutral detergent fiber	2.25^{NS} ± 0.15	30.40^{NS} ± 2.02	17.39 ± 1.59	20.43 ± 2.00	37.82^{NS} ± 3.47	4.28^{NS} ± 0.58	64.11^{NS} ± 4.24	20.08 ± 2.00	22.88 ± 2.77	42.96^{NS} ± 5.02
Group VI D (6), diets containing 5% water-insoluble neutral detergent fiber	2.42^{NS} ± 0.15	32.47^{NS} ± 2.27	21.71 ± 1.64	20.39 ± 1.98	42.10^{NS} ± 3.63	4.69^{NS} ± 0.40	67.11^{NS} ± 4.37	23.91 ± 2.05	28.97 ± 2.69	52.88^{NS} ± 5.99
Group VII N (6), diets containing 10% water-insoluble neutral detergent fiber	2.20^{NS} ± 0.15	31.63^{NS} ± 2.10	17.54 ± 1.69	22.29 ± 2.00	39.83^{NS} ± 3.66	5.20^{NS} ± 0.41	66.41^{NS} ± 4.49	22.39 ± 2.24	25.06 ± 2.44	47.45^{NS} ± 6.00

(B)

Groups	Cholesterol unsupplemented					Cholesterol supplemented				
	Fecal weight	Fecal sterol	Cholic acid	Deoxycholic acid	Total bile acid	Fecal weight	Fecal sterol	Cholic acid	Deoxycholic acid	Total bile acid
Group I D (6), Control dietfed	2.23 ± 0.07	33.16 ± 1.75	21.55 ± 2.06	24.37 ± 2.16	45.92 ± 4.22	4.84 ± 0.38	67.23 ± 4.60	23.05 ± 2.04	29.11 ± 3.52	52.16 ± 5.52
Group II D (6), diets containing 6% water-soluble gummy fiber	4.19** ± 0.13	43.33** ± 2.28	29.79 ± 2.85	19.61 ± 1.74	49.40** ± 4.54	6.37** ± 0.50	74.69** ± 5.11	29.41 ± 2.60	25.03 ± 3.02	54.44** ± 5.77
Group III D (6), diets containing 12% water-soluble gummy fiber	4.33** ± 0.14	55.67** ± 2.94	43.85 ± 4.20	27.15 ± 2.41	64 46** ± 5.92	7.91** ± 0.62	85.39** ± 5.84	33.09 ± 2.92	28.97 ± 3.50	62.06** ± 6.57
Group IV D (6), diets containing 18% water-soluble gummy fiber	5.44** ± 0.17	71.00*** ± 3.74	42.12 ± 4.03	33.57 ± 2.98	75.69*** ± 6.95	8.27** ± 0.65	93.29*** ± 6.38	41.71 ± 3.69	32.73 ± 3.95	74.44*** ± 7.88
Group V D (6), diets containing 2.25% water-insoluble neutral detergent fiber	2.31^{NS} ± 0.07	31.79^{NS} ± 1.68	20.47 ± 1.96	20.28 ± 1.80	40.75^{NS} ± 3.74	4.23^{NS} ± 0.33	66.71^{NS} ± 4.56	23.65 ± 2.09	28.62 ± 3.46	52.24^{NS} ± 5.53
Group VI D (6), diets containing 5% water-insoluble neutral detergent fiber	2.42^{NS} ± 0.08	32.47^{NS} ± 1.71	21.71 ± 2.08	20.39 ± 1.81	42.10^{NS} ± 3.87	4.69^{NS} ± 0.37	67.11^{NS} ± 4.59	23.91 ± 2.11	28.97 ± 3.50	52.88^{NS} ± 5.60
Group VII N (6), diets containing 10% water-insoluble neutral detergent fiber	2.36^{NS} ± 0.08	32.83^{NS} ± 1.73	22.67 ± 2.17	21.36 ± 1.90	44.03^{NS} ± 4.04	4.89^{NS} ± 0.38	67.99^{NS} ± 4.65	24.26 ± 2.14	29.37 ± 3.55	53.63^{NS} ± 5.67

The results presented in the table are mean ± SD of six rats each. Number of animals used is given in parenthesis.
The data were analyzed statistically using Student *t*-test.
Fecal weight, fecal sterol, and bile acid content of cholesterol-unsupplemented and cholesterol-supplemented normal and diabetic rats compared with the fecal weight, fecal sterol, and bile acid content of cholesterol-unsupplemented and cholesterol-supplemented normal and diabetic control rats for statistical analysis.
$p < 0.01$; ***$p < 0.001$.

compared with the normal and diabetic control rats fed on the cholesterol-unsupplemented diets. Our study is supported by the reports available on the characteristic property of plant fiber, that is viscosity. It would be pertinent to mention that viscosity of water-soluble gummy fiber isolated from *S. cumini* seeds was found to be 14 centipoise (cP),[1] which is nearer to that of highly viscous gummy fiber, that is guar gum (16–20 cP).

The nonsignificant effect of water-insoluble NDFs in our study is due to the very low amount of lignin present in water-insoluble NDFs isolated from *S. cumini* seeds, which are unable to bring about the effect [Table 2]. The amount of lignin in NDFs was determined by Goering and Vansoest, and it was estimated to be 6.66%.[9] The earlier reports also indicated that insoluble plant fiber generally had no effects on cholesterol and lipid levels in humans and experimental animals.[10,11]

Discussion

The increased biliary content and fecal excretion of bile acids and sterols in our study suggest that the dietary water-soluble gunny fibers of *S. cumini* seeds may enhance the breakdown of cholesterol, leading to an increased conversion of cholesterol to bile acids and salts that are excreted in the alimentary canal. These bile acids and salts and also the cholesterol bind to the dietary water-soluble gummy fibers resulting in the increased fecal excretion of bile acids and sterols,[12] which will result into their decreased absorption. This factor may also be responsible for reduced absorption of cholesterol because bile salts and bile acids are required for the absorption of cholesterol.

Conclusion

The increased biliary content and fecal excretion of bile acids and sterols in our study suggest that the dietary water-soluble gunny fibers isolated from *S. cumini* seeds may enhance the breakdown of cholesterol, leading to an increased conversion of cholesterol to bile acids and salts that are excreted in the alimentary canal.

References

1. Pandey M, Khan A. Hypoglycaemic effect of defatted seeds and water soluble gummy fibre from the seeds of *Syzygium cumini* (Linn.) skeels in alloxan diabetic rats. Indian J Exp Biol 2002;40:1178–82.
2. Anderson JW, Chen WL. Plant fiber: Carbohydrate and lipid metabolism. Am J Clin Nutr 1979;32:346–63.
3. Mathur KS, Khan NA, Sharma KO. Hypocholesterolaemic effect of Bengal gram: A long-term study in man. Brit Med J 1968; 1:30–1.
4. Fahrenbach MJ, Riccardi BA, Grant WC. Hypocholesterolaemic activity of mucilagenous polysaccharides in White Leghorn cockerels. Proc Soc Exp Biol Med 1966;123:321–6.
5. Agnihotri MA, Khan A. In Press; 2009.
6. Zlatkis A, Zak B, Boyle J. A new method for direct determination of serum cholesterol. J Lab Clin Med 1953;41:486–92.
7. Snell FD, Snell GT. Estimation of cholic acid deoxycholic acid. In: *Colorimetric Methods of Analysis*, Vol 34. New York: Van Nostrand, 1961.pp. 351–62.
8. Trowell H, Painter N, Burkitt D. Am J Digest Dis 1978;18:864.
9. Goering HK, Van Soest PJ. Forage fiber analysis. In: *Agriculture Handbook No. 379,* Washington, DC: Agricultural Research Service, United States Department of Agriculture, 1970.
10. Eastwood MA, Kirkpatrick JR, Mitchell WD, Bone A, Hamilton T. Effects of dietary supplements of wheat bran and cellulose on faeces and bowel function. Br Med J 1973;4:392–4.
11. Munoz JM, Sandstead HH, Jacob LK, Johnson L, Mako ME. Effect of dietary fibre on glucose tolerance in normal man. Diabetes 1979;28:496–502.
12. Menon PV, Kurup PA. Dietary fibre and cholesterol metabolism: Effect of fibre rich polysaccharide from blackgram (*Phaseolus mungo*) on cholesterol metabolism in rats fed normal and atherogenic diet. Biomedicine 1976;24:248.

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