Research Article

Effects of cholesterol-supplemented and unsupplemented diets containing unextracted and extracted Syzygium cumini seeds on lipid profiles of alloxan-induced diabetic albino rats

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Received January 3, 2014. Accepted April 4, 2014

Abstract

Background: Hypercholesterolemia and hyperlipidemia are the major problems associated with diabetes. Various agents are being tried to solve these so that problems related to micro- and macroangiopathy can be averted. Plant fibers particularly water-soluble gummy fibers have been reported earlier to reduce blood lipids and cholesterol levels in normal and diabetic animals and humans.

Objective: Effects of cholesterol-supplemented and unsupplemented diets containing unextracted and extracted Syzygium cumini seeds on lipid profiles of alloxan-induced diabetic albino rats

Material and Methods: Powder of *Syzygium cumini* seeds was successively extracted using organic solvents, and hypolipidemic and hypocholesterolemic activities of 15% unextracted, 15% extracted, and 15% degummed *S. cumini* seeds with cholesterol-supplemented and unsupplemented diets were assayed in normal and alloxan-induced diabetic rats.

Result: In this study, significant decrease in the levels of total serum cholesterol, serum triglycerides, serum phospholipids, and total cholesterol and triglycerides in tissues of liver, heart, and aorta was observed. Also, significant increase was observed in the phospholipid levels in tissues of liver, heart, aorta, and biliary, and fecal bile acid content and sterols in normal and alloxan-induced diabetic rats.

Conclusion: The hypocholesterolemic and hypolipidemic effects of *S. cumini* seeds may be attributed to their water-soluble gel-forming gummy fiber acting by decreasing cholesterol absorption and breakdown leading to an increased fecal excretion of bile acids and sterols.

KEY WORDS: *Syzygium cumini* seed (intact), extracted (defatted) seed powder, hypolipidemic and hypocholesterolemic activity, male albino rats, neutral detergent fiber

Introduction

Hypercholesterolemia and hyperlipidemia are the major problems associated with diabetes.[1,2] Hyperlipidemia is a risk factor for the development of coronary heart disease, cerebrovascular disease, hypertension, and peripheral vascular disease. Therefore, preventive measures against diabetes should aim at lowering not only blood glucose levels but also serum cholesterol and triglyceride levels. Conventional insulin and oral glycemic drug although reduced glucose levels but failed to prevent microvascular and neurological complications in diabetes.[3]

Plant fibers particularly water-soluble gummy fibers have been reported earlier to reduce blood lipids and cholesterol levels in normal and diabetic animals and humans.[4–10] No attempts appear to have been made to isolate and investigate the hypocholesterolemic and hypolipidemic effects of water-soluble gummy fibers from the seeds of *Syzygium cumini* Skeels (Myrtaceae), a plant traditionally used to treat diabetes, although a fair amount of fiber has been reported in the seeds.[11] The reports available on the confirmed hypocholesterolemic effects of certain types of plant fibers and the presence of fair amount of fibers in *S. cumini* seeds tempted us to speculate that fibers present in *S. cumini* seeds are responsible for the hypocholesterolemic and hypolipidemic effects.

Access this article online

Website: http://www.ijmsph.com

DOI: 10.5455/ijmsph.2015.030120149

Quick Response Code:

In one of our research studies, the quantitative estimation of the amount of fibers showed the presence of 40 g% water-soluble gummy fibers and 15 g% water-insoluble neutral detergent fibers (NDFs) in *S. cumini* seeds.[10] Keeping this in view, this study was designed to investigate the hypocholesterolemic and hypolipidemic effects of *S. cumini* seeds and to determine whether the hypocholesterolemic and hypolipidemic effects, if any, were due to the presence of water-soluble gummy fibers or water-insoluble NDFs or constituents other than these two types of fiber.

Materials and Methods

1. *Solvent fractionation*: In one of our research studies, *S. cumini* seeds were subjected to a solvent fractionation[11] to remove lipids and saponin (defatting) and water-soluble gummy fibers (degumming). Then, these defatted, degummed, and unextracted (intact) seeds were investigated for their hypocholesterolemic and hypolipidemic effects in normal and diabetic rats.
2. *Acute toxicity test*: Feeding *S. cumini* seed diets to the rats at the dose levels of 5, 10, and 15% was found to be quiet safe and nontoxic as evident by the hematological and biochemical investigations and growth rate of rats. No gross behavioral changes were found in these rats. The hypoglycemic effects were more pronounced in the rats fed 15% *S. cumini* seed diet. Hence, 15% *S. cumini* seed diets were selected in our study. Feeding 20% and 25% *S. cumini* seed diets caused growth arrest in the rats.[12]

Materials

Cholesterol, chloroform, glycerol, potassium hydroxide, sodium periodate, sodium arsenite, and chromotropic acid were purchased from SD Fine-Chem (Bombay, India). Sodium phosphotungstate and sodium sulfate were purchased from E. Merck (Germany).

Methods

The levels of serum and tissue cholesterol were estimated by the method described by Zlatkis *et al*.[13] The levels of serum and tissue triglycerides were estimated by the method given by Van Handel and Zilversmit.[14] The levels of serum and tissue phospholipids were estimated by the method described by Zilversmit and Davis.[15] 48-h collected fecal samples were dried in the incubator at 37°C, powdered, weighed, homogenized, and extracted with hot absolute alcohol. The extract was then used for the estimation of fecal sterol and bile acids (cholic and deoxycholic acids) contents by the method described by Zlatkis *et al.*[13] and Snell and Snell,[16] respectively. Cholic and deoxycholic acids were determined spectrophotometrically at 320 and 365 nm, respectively, after heating in 2:1 sulfuric acid/conjugate. Biliary sterol and bile acids were also estimated by the methods described earlier.[17]

Animals and Diet

To determine the effects of cholesterol-supplemented and unsupplemented *S. cumini* seed diets, we divided the male albino rats into two sets. Each set was further divided into four groups each containing normal rats as Group I N, II N, III N, and IV N and diabetic rats as Group I D, II D, III D, and IV D. The rats were made diabetic using the method of Lazaro and Pallay.[11] The rats of Group I N and Group I D from both the sets were fed the control diet. The number of rats in each group is mentioned in the tables within parenthesis. Groups II, III, and IV of both normal and diabetic rats of one set were fed experimental cholesterol-supplemented diets and Groups II, III, and IV of both normal and diabetic rats of another set were fed experimental cholesterol-unsupplemented diets containing 15% unextracted,

Table 1: Composition of control and experimental diets

Ingredients	Control diet	Experimental cholesterol-unsupplemented *Syzygium cumini* seed diets			Control diet	Experimental cholesterol-supplemented *Syzygium cumini* seed diets		
		15% Unextracted	15% Extracted	15% Degummed		15% Unextracted	15% Extracted	15% Degummed
Casein	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Unextracted	15.0					15.0		
Extracted			15.0				15.0	
Degummed				15.0				15.0
Hydrogenated oil	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Starch	65	50	50	50	63.30	48.30	48.30	48.3
Cholesterol					1.0	1.0	1.0	1.0
Cholic acid					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
Vitamin mixture	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Choline chloride					0.2	0.2	0.2	0.2

Each diet contains (%w/w) salt mixture (4 g) and vitamin mixture (1 g).[11]

15% extracted, and 15% degummed seeds separately [Table 1]. All the rats had free access to deionized distilled water. At the end of 21 days, the rats of both the sets were fasted for 18 h. After fasting, they were stunned by blow at the back of neck and killed by decapitation. The heart, liver, kidney, and aorta were removed and kept in ice-chilled containers for the analysis of cholesterol, triglyceride, and phospholipid levels. Fasting samples of blood were collected immediately after killing the rats by decapitation and the serum was separated.

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

Result

Feeding both cholesterol-supplemented and unsupplemented control diets to the diabetic control rats showed a significant increase in the levels of total serum cholesterol, serum triglycerides, serum phospholipids, and total cholesterol and triglycerides in the tissues of the liver, heart, and aorta when compared with their respective normal controls [Tables 1–4]. Both normal and diabetic rats fed on cholesterol-supplemented diets containing 15% unextracted and 15% extracted *S. cumini* seeds showed significant increase in the total serum cholesterol, serum triglyceride, serum phospholipid, and total cholesterol and triglyceride levels in the tissues of the liver, heart, and aorta when compared with their respective normal and diabetic rats fed on cholesterol-unsupplemented diets containing 15% unextracted and 15% extracted *S. cumini* seeds.

The levels of cholesterol and triglyceride in kidney remain unaffected by feeding cholesterol-supplemented and unsupplemented diets containing 15% unextracted, 15% extracted, and 15% degummed *S. cumini* seeds to both normal and diabetic rats [Tables 3 and 4].

Feeding cholesterol-supplemented and unsupplemented diets containing 15% unextracted and 15% extracted *S. cumini* seeds to both normal and diabetic rats significantly reduced the total serum cholesterol, serum triglyceride, and serum phospholipids [Tables 2 and 3], and total cholesterol and triglyceride [Tables 3 and 4] levels in the tissues of the liver, heart, and aorta when compared with their respective controls. In addition, a significant increase was observed in the biliary content [Table 5] and fecal excretion of bile acids and sterols [Table 6] and also in the phospholipid levels [Table 7] of the liver, heart, aorta, and kidney of both normal and diabetic rats when compared with their respective controls.

Table 2: Effect of cholesterol-unsupplemented and supplemented *Syzygium cumini* seed diets on serum cholesterol, triglyceride, and phospholipid levels of normal and diabetic rats

Groups	Cholesterol unsupplemented			Cholesterol supplemented		
	Serum cholesterol (mg/100 ml)	Serum triglyceride (mg/100 ml)	Serum phospholipids (mg/100 ml)	Serum cholesterol (mg/100 ml)	Serum triglyceride (mg/100 ml)	Serum phospholipids (mg/100 ml)
Normal						
Group I N (6), control	196.30 ± 12.40	117.50 ± 9.06	143.70 ± 10.91	347.00 ± 9.67	138.00 ± 10.88	210.00 ± 15.70
Group II N (6), 15% extracted	130.92** ± 10.06	94.34** ± 7.66	120.39* ± 9.25	125.00*** ± 9.99	98.56*** ± 8.71	162.00** ± 11.60
Group III N(6), 15% extracted	126.00** ± 8.59	92.00** ± 7.85	122.57* ± 9.98	114.51*** ± 9.66	100.05*** ± 9.09	160.00** ± 12.95
Group IV N (6), 15% degummed	197.25^{NS} ± 11.69	118.06^{NS} ± 9.72	141.96^{NS} ± 2.62	347.23^{NS} ± 19.25	140.00^{NS} ± 10.98	212.03^{NS} ± 14.63
Diabetic						
Group I N (6), control	299.00 ± 17.00	193.00 ± 11.15	216.08 ± 15.44	441.6 ± 22.02	215. 19 ± 16.18	227.15 ± 17.00
Group II N (6), 15% extracted	191.00** ± 10.79	154.00** ± 10.90	185.00* ± 14.61	198.06*** ± 12.72	112.00*** ± 10.14	177.00** ± 13.65
Group III N(6), 15% extracted	190.78** ± 10.66	150.08** ± 10.84	186.40* ± 14.70	197.55*** ± 12.70	112.45*** ± 10.80	176.00** ± 14.18
Group IV N (6), 15% degummed	305.50^{NS} ± 17.29	194.00^{NS} ± 11.20	214.36^{NS} ± 15.74	438.81^{NS} ± 21.76	218.00^{NS} ± 16.77	228.12^{NS} ± 18.04

The serum cholesterol, triglyceride, and phospholipid levels of normal and diabetic rats fed on cholesterol-unsupplemented and supplemented diets compared with those of normal and diabetic control rats fed on cholesterol-unsupplemented and supplemented diets for statistical analysis.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
The serum cholesterol, triglyceride, and phospholipid levels of diabetic control rats fed on cholesterol-unsupplemented and supplemented diets compared with those of normal control rats fed on cholesterol-unsupplemented and supplemented diets for statistical analysis.
•$p < 0.05$; ••$p < 0.01$.

Table 3: Effect of cholesterol-supplemented and unsupplemented *Syzygium cumini* seed diets on tissue cholesterol levels in the liver, aorta, heart, and kidney of normal and diabetic rats

Groups	Cholesterol unsupplemented				Cholesterol supplemented			
	Total cholesterol (mg/100 g wet tissue ± SD)							
	Liver	Aorta	Heart	Kidney	Liver	Aorta	Heart	Kidney
Normal								
Group I N (6), control diet	1515.50 ± 16.71	336.05 ± 1.96	232.08 ± 1.16	257.21 ± 1.81	2920.50 ± 34.30	379.25 ± 2.19	271.06 ± 1.36	303.33 ± 2.13
Group II N (6), 15% unextracted	1185.32** ± 26.74	270.17** ± 1.48	193.3** ± 1.2	257.16^{NS} ± 0.61	2504.18** ± 36.42	280.78** ± 1.43	231.08** ± 1.43	305.33^{NS} ± 0.55
Group III N (6), 15% extracted	1174.00** ± 20.55	269.11** ± 2.79	145.25** ± 3.56	256.49^{NS} ± 2.53	2360.51** ± 41.27	256.35** ± 2.65	139.24 ** ± 3.41	308.54^{NS} ± 3.09
Group IV N (6), 15% degummed	1489.00^{NS} ± 28.97	340.37^{NS} ± 0.99	241.42^{NS} ± 0.74	259.28^{NS} ± 1.88	2893.50^{NS} ± 56.23	380.25^{NS} ± 1.11	269.11^{NS} ± 0.83	304.16^{NS} ± 2.30
Diabetic								
Group I N (6), control diet	2560.41 ± 27.70	400.29 ± 1.65	278.38 ± 0.69	271.47 ± 0.98	3797.50 ± 40.97	457.02 ± 1.89	329.33 ± 0.82	327.16 ± 1.18
Group II N (6), 15% unextracted	1990.43** ± 24.11	366.67** ± 4.03	225.95** ± 4.09	273.45^{NS} ± 1.50	3197.16** ± 38.73	331.17** ± 4.47	273.66** ± 5.32	325.16^{NS} ± 1.16
Group III N (6), 15% extracted	2000.17** ± 26.02	367.50** ± 4.26	222.73** ± 1.06	273.94^{NS} ± 2.50	3198.66** ± 41.61	329.83** ± 3.78	270.16** ± 9.00	325.59^{NS} ± 2.16
Group IV N (6), 15% degummed	2497.28^{NS} ± 37.12	398.65^{NS} ± 3.31	280.19^{NS} ± 3.53	275.56^{NS} ± 2.03	3808.31^{NS} ± 56.92	453.33^{NS} ± 3.76	327.35^{NS} ± 4.13	326.66^{NS} ± 1.17

The cholesterol content in the liver, heart, and aorta of normal and diabetic rats fed on cholesterol-unsupplemented and supplemented diets compared with that of normal and diabetic control rats fed on cholesterol-unsupplemented and supplemented diets for statistical analysis.
*$p < 0.05$; **$p < 0.01$.
The cholesterol content in the liver, heart, and aorta of diabetic control rats fed on cholesterol-unsupplemented and supplemented diets compared with that of normal control rats fed on cholesterol-unsupplemented and supplemented diets for statistical analysis.
$p < 0.05$.

Table 4: Effect of cholesterol-unsupplemented and supplemented *Syzygium cumini* seed diets on tissue triglyceride levels of normal and diabetic rats

Groups	Cholesterol unsupplemented				Cholesterol supplemented			
	Triglyceride (mg/100 g wet tissue ± SD)							
	Liver	Aorta	Heart	Kidney	Liver	Aorta	Heart	Kidney
Normal								
Group I N (6), control diet	209.83 ± 2.71	380.17 ± 1.60	96.83 ± 1.47	62.17 ± 1.98	477.75 ± 6.17	421.25 ± 1.77	138.01 ± 2.09	68.29 ± 2.17
Group II N (6), 15% unextracted	160.66** ± 6.41	223.17** ± 2.48	61.17** ± 1.16	55.17^{NS} ± 0.56	427.09** ± 5.51	387.30** ± 1.67	90.07** ± 1.36	69.16^{NS} ± 2.13
Group III N (6), 15% extracted	157.25** ± 5.48	224.44** ± 2.26	60.83** ± 4.17	54.50^{NS} ± 1.32	425.34** ± 14.82	395.46** ± 3.98	90.78** ± 6.22	68.46^{NS} ± 2.18
Group IV N (6), 15% degummed	203.16^{NS} ± 3.31	378.17^{NS} ± 1.73	95.67^{NS} ± 0.82	60.31^{NS} ± 1.03	475.94 ^{NS} ± 7.75	428.00^{NS} ± 1.95	137.12^{NS} ± 1.18	67.07^{NS} ± 2.13
Diabetic								
Group I N (6), control diet	331.83 ± 0.40	722.67 ± 2.06	161.37 ± 1.33	136.50 ± 0.85	509.15 ± 0.61	924.75 ± 2.64	235.66 ± 1.94	211.58 ± 1.32
Group II N (6), 15% unextracted	261.51** ± 1.38	514.62** ± 0.52	118.50** ± 0.84	131.27^{NS} ± 0.63	326.25** ± 1.72	599.11** ± 2.08	175.25** ± 1.24	197.75^{NS} ± 0.95
Group III N (6), 15% extracted	258.44** ± 2.37	510.84** ± 1.33	116.83** ± 0.98	128.97^{NS} ± 23.30	324.75** ± 2.98	595.25** ± 2.06	172.31** ± 1.45	196.39^{NS} ± 3.55
Group IV N (6), 15% degummed	329.328^{NS} ± 1.21	719.17^{NS} ± 2.79	157.52^{NS} ± 2.43	135.52^{NS} ± 1.17	585.75^{NS} ± 2.16	922.94^{NS} ± 3.81	230.04^{NS} ± 3.55	215.36^{NS} ± 1.86

The triglyceride content in the liver, heart, and aorta of normal and diabetic rats fed on cholesterol-unsupplemented and supplemented diets compared with that of normal and diabetic control rats fed on the cholesterol-unsupplemented and supplemented diets for statistical analysis.
**$p < 0.01$.

Table 5: Effect of cholesterol-unsupplemented and supplemented *Syzygium cumini* seed diets for 21 days on concentration of biliary sterol and bile acid in normal and diabetic rats

Groups	Cholesterol unsupplemented		Cholesterol supplemented	
	Biliary sterol (mg/rat/100 ml bile)	Total bile acid (mg/rat/100 ml bile)	Biliary sterol (mg/rat/100 ml bile)	Total bile acid (mg/rat/100 ml bile)
Normal				
Group I N (6), control diet	18.92 ± 0.40	235.00 ± 3.87	23.00 ± 0.59	240.00 ± 3.85
Group II N (6), 15% unextracted	34.84** ± 0.82	269.00* * ± 4.10	48.35** ± 1.24	285.00** ± 4.58
Group III N (6), 15% extracted	36.07** ± 0.86	269.80** ± 4.15	48.11** ± 1.23	283.45** ± 4.55
Group IV N (6), 15% degummed	36.07** ± 0.42	269.80** ± 3.93	48.11** ± 0.63	283.45** ± 3.89
Diabetic				
Group I N (6), control diet	21.34 ± 0.55	236.07 ± 3.80	24.15 ± 0.61	42.12 ± 3.89
Group II N (6), 15% unextracted	39.80*** ± 0.94	273.15*** ± 4.31	51.09*** ± 1.31	288.00*** ± 4.63
Group III N (6), 15% extracted	41.06*** ± 10.13	274.01*** ± 4.47	50.75*** ± 1.30	285.21*** ± 4.58
Group IV N (6), 15% degummed	22.57^{NS} ± 0.57	240.16^{NS} ± 3.96	25.66^{NS} ± 0.66	241.63^{NS} ± 3.88

The results presented in table are mean ± SD of six rats each. The biliary sterol and bile acid contents of normal and diabetic rats fed on cholesterol-unsupplemented and supplemented diets compared with those of normal and diabetic control rats fed on cholesterol-unsupplemented and supplemented diets for statistical analysis.
**$p < 0.01$; ***$p < 0.001$.

Table 6: Effect of feeding of cholesterol-supplemented and unsupplemented *Syzygium cumini* seed diets on concentration of fecal and bile acids in normal and diabetic rats

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
Normal										
Group I N (6), control diet	1.14 ± 0.051	20.00 ± 1.62	10.65 ± 0.90	15.71 ± 1.39	26.36 ± 2.29	2.26 ± 0.10	39.90 ± 2.99	14.30 ± 1.38	19.25 ± 1.09	33.55 ± 1.19
Group II N (6), 15% unextracted	2.97** ± 0.18	49.71** ± 4.75	16.99 ± 1.46	12.74 ± 0.99	29.73** ± 2.45	5.70** ± 0.25	70.00 ± 2.43	19.65 ± 1.24	15.71** ± 1.03	35.36** ± 2.30
Group III N (6), 15% extracted	3.15** ± 0.26	48.50** ± 5.60	17.61 ± 1.69	19.11 ± 1.53	37.42** ± 3.21	5.90** ± 0.39	70.75** ± 3.00	21.00 ± 2.60	20.17 ± 1.96	41.17** ± 4.56
Group IV N (6), 15% degummed	1.20^{NS} ± 0.06	20.45^{NS} ± 1.84	25.43 ± 2.04	17.00 ± 1.86	42.43^{NS} ± 3.90	2.30^{NS} ± 0.15	42.65^{NS} ± 3.21	32.19 ± 2.45	22.31 ± 2.00	54.63^{NS} ± 4.45
Diabetic										
Group I N (6), control diet	1.40 ± 0.06	22.00 ± 1.782	13.15 ± 1.11	16.91 ± 1.49	30.06 ± 2.61	3.37 ± 0.15	41.11 ± 3.08	16.42 ± 1.58	22.20 ± 1.26	38.62 ± 1.37
Group II N (6), 15% unextracted	3.03** ± 0.183	52.71** ± 5.036	20.81 ± 1.788	13.55 ± 1.050	34.36** ± 2.83	8.06** ± 0.35	131.90** ± 2.48	22.61 ± 1.44	16.98 ± 1.11	39.59** ± 2.58
Group III N (6), 15% extracted	3.70** ± 0.305	51.06** ± 5.894	24.33 ± 1.902	17.76 ± 1.69	42.09** ± 3.61	7.84** ± 0.52	129.63** ± 3.04	30.46 ± 2.83	1.80 ± 2.12	52.26** ± 4.95
Group IV N (6), 15% degummed	1.82^{NS} ± 0.091	30.89^{NS} ± 2.779	29.74 ± 2.385	18.02 ± 1.97	47.76^{NS} ± 4.39	3.68^{NS} ± 0.24	44.71^{NS} ± 3.37	33.94 ± 2.58	23.55^{NS} ± 2.11	57.49 ± 4.68

Fecal weight, fecal sterol, and bile acid contents of normal and diabetic rats fed on cholesterol-unsupplemented and supplemented diets compared with those of normal and diabetic control rats fed on cholesterol-unsupplemented and supplemented diets for statistical analysis.
**$p < 0.01$.

Table 7: Effect of feeding of cholesterol unsupplemented and supplemented *Syzygium cumini* seed diets on tissue phospholipid levels of normal and diabetic rats

Groups	Cholesterol unsupplemented				Cholesterol supplemented			
	Phospholipids (mg/100 g wet tissue ± SD)							
	Liver	Aorta	Heart	Kidney	Liver	Aorta	Heart	Kidney
Normal								
Group I N (6), control diet	1576.09 ± 38.32	975.12 ± 2.73	1386.15 ± 1.56	996.00 ± 1.39	2690.11 ± 50.40	1438.88 ± 4.03	2323.33 ± 2.65	1848.67 ± 9.34
Group II N (6), 15% unextracted	2422.118** ± 44.12	1210.45** ± 3.22	1617.39** ± 5.85	1323.28*** ± 6.02	3262.83** ± 59.44	1894.40** ± 5.02	2924.16** ± 10.57	2415.00*** ± 12.20
Group III N (6), 15% extracted	2405.75** ± 43.77	1198.00** ± 2.91	1585.67** ± 1.65	1280.168*** ± 6.52	3284.33** ± 59.83	891.76** ± 4.59	010.50** ± 3.14	2432.33*** ± 12.38
Group IV N (6), 15% degummed	1606.25^{NS} ± 36.41	973.75^{NS} ± 3.11	1317.07^{NS} ± 1.67	1000.82^{NS} ± 1.99	2599.61^{NS} ± 48.93	1556.49^{NS} ± 4.97	2440.66^{NS} ± 3.10	1852.67^{NS} ± 9.12
Diabetic								
Group I N (6), control diet	2274.37 ± 41.70	1560.29 ± 2.89	1857.26 ± 1.77	1462.36 ± 1.42	3498.65 ± 63.74	2397.16 ± 4.45	3242.00 ± 3.09	2812.50 ± 14.10
Group II N (6), 15% unextracted	2731.55** ± 50.06	1771.63** ± 2.26	2125.51** ± 2.74	1699.92*** ± 3.34	3999.00** ± 72.85	3000.50** ± 3.83	3975.00** ± 5.12	3403.67*** ± 16.68
Group III N (6), 15% extracted	2707.60** ± 49.64	1750.41** ± 1.33	2203.74** ± 4.44	1680.19*** ± 3.53	3900.00** ± 72.05	2979.50** ± 2.27	3798.67** ± 7.49	3350.26*** ± 17.03
Group IV N (6), 15% degummed	2709.44^{NS} ± 49.96	1512.17^{NS} ± 3.09	1802.44^{NS} ± 3.53	1608.72^{NS} ± 0.64	3492.83^{NS} ± 63.46	2403.41^{NS} ± 4.92	3173.27^{NS} ± 6.22	3000.62^{NS} ± 1.37

The phospholipid content in the liver, heart, and aorta of normal and diabetic rats fed on cholesterol-unsupplemented and supplemented diets compared with that of normal and diabetic control rats fed on the cholesterol-supplemented and unsupplemented diets for statistical analysis.
$p < 0.01$; ***$p < 0.001$.

Discussion

Feeding cholesterol-supplemented and unsupplemented diets each containing 15% unextracted and 15% extracted *S. cumini* seeds with water-soluble gummy fibers of both normal and diabetic rats significantly decreased the total serum cholesterol, serum triglyceride, serum phospholipid, and total cholesterol and triglyceride levels in the liver, heart, and aorta. In addition, this diet significantly increased the biliary content and fecal excretion of bile acids and sterol and the phospholipids levels in the liver, heart, aorta, and kidney. In contrast, feeding cholesterol-supplemented and unsupplemented diets containing 15% degummed seeds (devoid of water-soluble gummy fibers but containing water-insoluble NDFs and all other constituents) had no significant effect on total serum cholesterol, serum triglyceride, serum phospholipid, and total cholesterol, triglyceride, and phospholipid levels in tissues of the liver, heart, aorta, and kidney. But biliary content and fecal excretion of bile acids and sterols clearly indicated that the significant hypocholesterolemic and hypolipidemic effects of *S. cumini* seeds were due to water-soluble gummy fiber and not due to water-insoluble NDF fibers and other constituents of seeds.

Saponins have been reported to show hypocholesterolemic effect.[17,18] In our study, a hypocholesterolemic effect due to saponin was ruled out because even when saponin was removed, the hypocholesterolemic effect was still observed. If the hypocholesterolemic effect of unextracted seed is due to saponin, this effect should be absent in the extracted seeds devoid of saponin. But the seeds devoid of saponin exhibited hypocholesterolemic effects very much similar to that by unextracted *S. cumini* seeds.

The result of this study indicates that cholesterol-supplemented and unsupplemented diets containing 15% *S. cumini* seeds significantly decrease the tissue and serum cholesterol, tissue and serum triglyceride, and serum phospholipids both in normal and diabetic rats. In addition, these significantly increase the biliary content and fecal excretion of bile acids and sterols and the tissue phospholipids levels both in normal and diabetic rats. The hypocholesterolemic and hypolipidemic effects of *S. cumini* seeds may be attributed to their water-soluble gel-forming gummy fiber acting by decreasing cholesterol absorption and breakdown, leading to an increased fecal excretion of bile acids and sterols.

Conclusion

The hypocholesterolemic and hypolipidemic effects of *S. cumini* seeds may be attributed to their water-soluble gel-forming gummy fiber acting by decreasing cholesterol absorption and breakdown, leading to an increased fecal excretion of bile acids and sterols.

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How to cite this article: Agnihotri MA, Khan A. Effects of cholesterol-supplemented and unsupplemented diets containing unextracted and extracted Syzygium cumini seeds on lipid profiles of alloxan-induced diabetic albino rats Int J Med Sci Public Health 2015;4:27-34

Source of Support: Nil, **Conflict of Interest:** None declared.