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Flavonoids from *Garcinia cambogia* lower lipid levels in hypercholesterolemic rats

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Abstract

Administration of flavonoids from *Garcinia cambogia*, at a dose of 1 mg 100 g^{-1} body weight day^{-1}, significantly lowered lipid levels in rats fed normal and cholesterol-containing diets. β-Hydroxy β-methyl glutaryl coenzyme A reductase showed significant reduction in normocholesterolemic rats. Activities of glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase were reduced significantly. Highly stimulated activities of the enzymes lipoprotein lipase and plasma lecithin cholesterol acyl transferase were noted in flavonoid-administered animals. Hepatic and fecal bile acids and fecal neutral sterols were elevated substantially, indicating a higher rate of degradation of cholesterol. Thus hypolipidemic activity of these flavonoids may be due to a lower rate of lipogenesis and higher rate of degradation.

Keywords: Flavonoids; *Garcinia cambogia*; HMG CoA reductase; LPL; Plasma LCAT; Hypolipidemic

1. Introduction

Flavonoids are a class of nutrients that are within a broader class of aromatic compounds called "polyphenols". A myriad of nutritional benefits has been attributed to these phytochemicals. Several reports are available on the beneficial effects of flavonoids (Brasseur, 1989; Di carlo, Mascolo, Izzo & Capasso, 1999; Sudheesh, Sandhya, Koshy & Vijayalakshmi, 1999). Flavonoids can inhibit the various stages thought to be involved in the initiation of atherosclerosis, endothelial damage, leucocyte activation, platelet adhesion, aggregation and secretion (Beretz & Cazenave, 1988). The plasma total cholesterol and atherogenic index were reduced by supplementation of 1–2% tea catechins to rats fed cholesterol containing diet (Muramatsu, Fukuyo & Hara, 1986). Tannic acid and morin can cause favourable changes in plasma lipid profiles of the type that have been correlated with coronary heart disease (Yugarani, Tan, Teh & Das, 1992). Hypolipidemic activity of flavonoids from various sources has been reported by several workers (Chan, Fong, Cheung, Huang, Ho & Chen, 1999; Choi, Yokozawa & Oura, 1991; Kono, Shinchi, Ikeda, Yanai & Imanishi, 1992; Sudheesh, Presanna Kumar, Vijaya Kumar & Vijayalakshmi, 1997). In this study an attempt is made to evaluate the hypolipidemic activity of flavonoids from *Garcinia cambogia* fruit, which is listed in indigenous medicine as having high therapeutic value and is even now used in remedies for various diseases. *G. cambogia* (malabar tamarind) is seen abundantly in the evergreen forests of Konkan in South India. Many traditional recipes in Kerala use it for its distinct flavour. *Garcinia* species are employed in traditional medicine for treatment of hepatitis, laryngitis and mouth infections (Iwu & Igboko, 1982). The biflavonones are the most dominant components in most *Garcinia* species (Waterman & Hussain, 1983). Aqueous extracts of the stem bark of *Garcinia huillensis* are used in Zairean traditional medicine against venereal diseases, sores, bronchitis, pneumonia, angina, measles and dermatitis (Bakana, 1984)

2. Materials and methods

All the biochemicals used in this experiment were purchased from Sigma Chemical Company Inc., St. Louis, MO, USA, and the chemicals from BDH, India.

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Flavonoids were extracted by the method described by Ansari, Rahman, Barraclough, Maynard and Scheinman (1976). Dried peel of mature fruits of *G. cambogia* were completely extracted with light petroleum (bp 40–60°C). The treated material was dried and exhausted with boiling acetone, thrice. The combined acetone extracts were concentrated and then extracted successively with light petroleum (bp 40–60°C), benzene and hot water to remove non-flavonoid and resinous matter. The residual mass was then refluxed with ethyl acetate for 10 h and the mixture was filtered. The filtrate was evaporated. Flavonoid content was then estimated according to the procedure of Eskin, Hoehn and Frenkel (1978) using quercetin as standard.

Male Sprague-Dawley rats weighing 80–100 g, bred in our animal house, were used for the studies. Animals were housed in polypropylene cages and maintained in a controlled environment (28–32°C) with 12 h of light and 12 h of dark each day. The animals were handled according to the guidelines for care and use of laboratory animals (CPCSEA) rules by the Government of India.

2.1. Experiment I

The rats were divided into two groups with 12 rats in each group. A standard pellet diet (Gold-Mohur rodent chow) and tap water were given ad libitum for 45 days. Animals in group II were given flavonoids from dried peels of mature fruits of *G. cambogia* at a dose of 1 mg 100 g^{-1} body weight (BW) day^{-1} orally by oro-gastric tube. Food intake was recorded daily and BW gain weekly.

2.2. Experiment II

The rats were divided into two groups with 12 rats in each group. A high fat diet containing Gold-Mohur rodent chow (83%), coconut oil (15%) and cholesterol (2%) was fed to animals of both the groups. Group II animals were given flavonoids from *G. cambogia* at a dose of 1 mg 100 g^{-1} BW day^{-1} orally by oro-gastric tube. Group I was treated as control for group II. Diet and tap water were available on an ad libitum basis for 90 days. Both diet intake and BW gain were recorded.

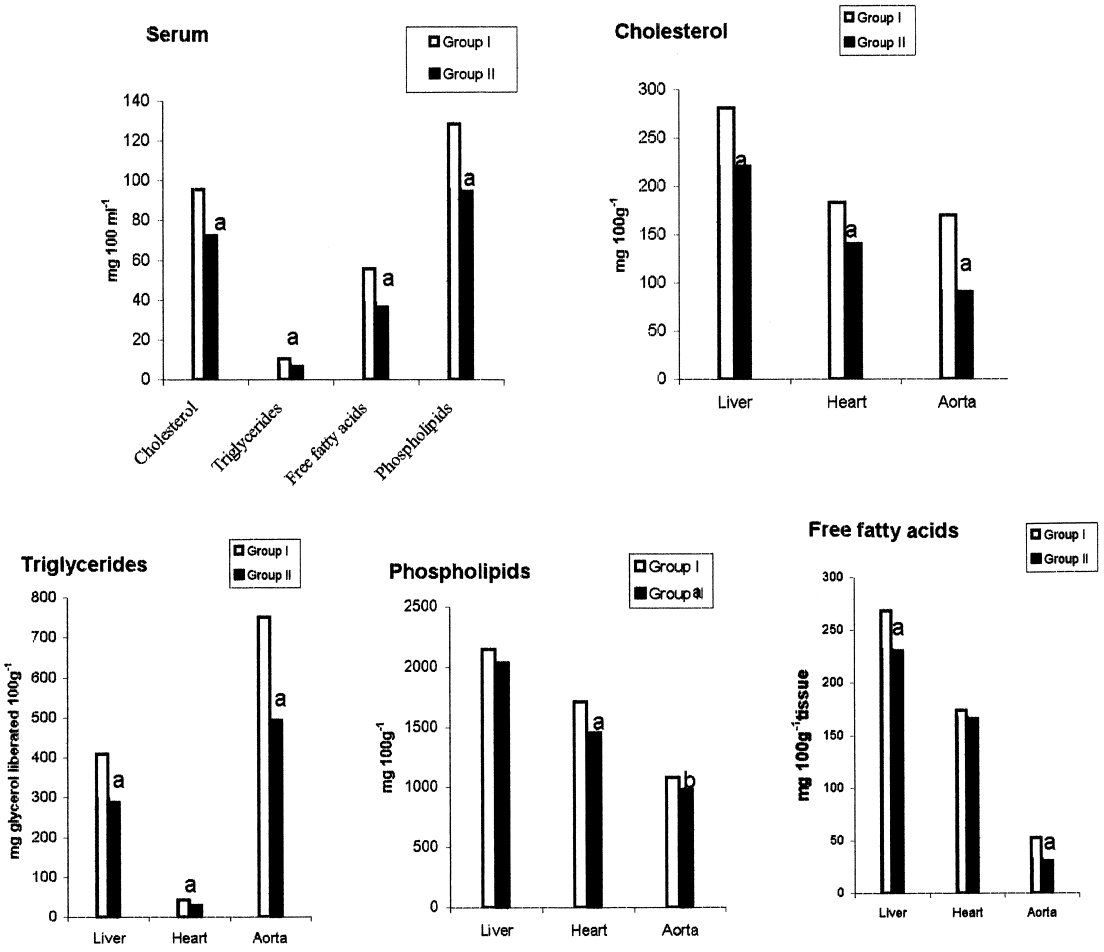

Fig. 1. Effect of flavonoids from *Garcinia cambogia* on concentrations of cholestrol, triglycerides, phospholipids and free fatty acids in serum and tissues of rats fed normal diet. Average of the values of 12 rats in each group ± S.E. Group II is compared with Group I; a = $P < 0.01$, b = $0.01 < P < 0.05$ between groups I and II.

A 24-h sample of feces was collected quantitatively prior to sacrificing the animals housed in metabolic cages. At the end of the respective experimental periods, animals were fasted overnight and sacrificed. Blood was drawn for lipid analysis. The liver, heart, kidney and aorta were removed to ice-cold containers, blotted dry, weighed and frozen until biochemical analysis was carried out.

2.2.1. Biochemical analysis

Lipids in the tissues were extracted by the method of Radin (Radin, 1981). Determination of lipids, β-hydroxy β-methyl glutaryl coenzyme A (HMG CoA) reductase [EC 1.1.1.34], lipoprotein lipase [LPL; EC. 3.1.1.3] and plasma lecithin cholesterol acyl transferase [LCAT; EC 2.3.1.3] were carried out by standard procedures described earlier (Gomathy, Vijayalakshmi & Kurup, 1989). Total bile acids, neutral sterols and serum lipoproteins were estimated by standard procedures given elsewhere (Valsa, Usha Kumari & Vijayalakshmi, 1995). Activity of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was estimated by the method of Kornberg and Horecker (1955), malic enzyme (EC 1.1.1.40) and isocitrate dehydrogenase (EC 1.1.1.41) by the method of Ochoa (1955a,b). The protein in the enzyme extract was determined after trichloro acetic acid (TCA) precipitation by the method of Lowry et al. (Lowry, Rosebrough, Farr & Randall, 1951).

2.3. In vivo studies on lipids

The rats deprived of food overnight, were injected intraperitoneally with 0.5 ml solution of 58.5 mmol (10 μci) of 1,2-^{14}C sodium acetate; 3 h after injection the rats were killed by decapitation. The liver was quickly dissected out. The tissue was gently blotted and homogenised in 80% aqueous ethanol. After saponification with 10 N NaOH for 1 h, lipids were extracted with petroleum ether. Cholesterol was separated by thin layer chromatography (TLC) over silica gel (Silica gel G; solvent system — hexane:ether:acetic acid 80:20:1 v/v/v) and activity counted in a Packard Priya liquid scintillation counter. The scintillant fluid was 2,5 diphenyl oxazole (PPO), 6 g, and 1/4-bis (2-15 phenyl oxazolyl) benzene (POPOP), 0.2 g/l of toluene.

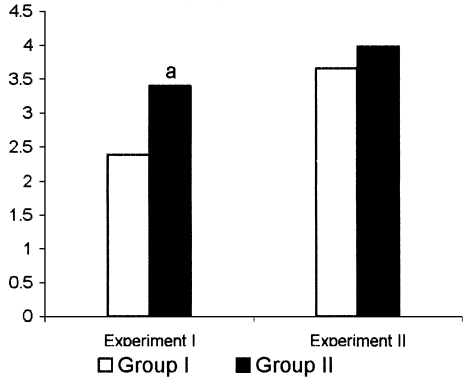

Fig. 2. Effect of flavonoids from *Garcinia cambogia* on β-hydroxy β -methyl glutaryl coenzyme A (HMGCoA) reductase activity in the liver of rats fed normal (Experiment I) and cholestrol-containing diet (Experiment II). Activity is expressed as the ratio of HMG CoA/mevalonate, i.e. the lower the ratio, the higher the activity. Values are mean ± S.E. for 12 rats. Group II is compared with Group I; a = $P < 0.01$ between groups I and II.

2.3.1. Statistical analysis

Statistical significance was calculated using Student's *t* test (Bennet & Franklin, 1967). Significance was accepted at $P \leqslant 0.05$.

3. Results

3.1. Results of experiment I

Food intake and BW gain did not show any significant variation in the two groups. The concentrations of cholesterol and triglycerides were decreased significantly in the serum and tissues of experimental animals when compared to the control group ($P < 0.01$). The concentration of phospholipids and free fatty acids were also reduced significantly ($P < 0.01$) in the serum and tissues of animals fed flavonoids when compared to the control group (Fig. 1).

Activity of HMG CoA reductase was decreased significantly ($P < 0.01$) in the liver of experimental animals (Fig. 2). Glucose-6-phosphate dehydrogenase activity was increased significantly but malic enzyme did not show any stimulation in activity (Table 1). Activity of isocitrate dehydrogenase was lowered significantly ($P < 0.01$) in the liver of experimental animals (Table 1). Activities of LPL and plasma LCAT were enhanced in group II animals (Table 1). Concentration of high density lipoprotein (HDL) cholesterol exhibited significant increase whereas low density lipoprotein + very low density lipoprotein (LDL + VLDL) levels declined significantly ($P < 0.01$) in experimental group when compared to control (Table 2). The lipid components in the liver of experimental rats showed a significantly decreased ($P < 0.01$) incorporation of ^{14}C acetate when compared to the control group (Table 3). Hepatic and fecal bile acids and fecal neutral sterols showed significantly elevated values ($P < 0.01$) in the experimental group when compared to the control (Table 4).

3.2. Results of experiment II

No significant change was observed in diet consumption or weight gain during the 90 day duration of the study in experimental animals compared to control animals. Concentrations of cholesterol and triglyceride showed marked reductions ($P < 0.01$) in the serum, liver,

Table 1
Effect of flavonoid administration on the activities of lipogenic enzymes in liver, lipoprotein lipase in the heart and adipose and plasma lecithin cholesterol acyltransferase (LCAT) in rats fed normal (Experiment I) and cholesterol-containing diets (Experiment II)[a]

Enzymes	Experiment I		Experiment II	
	Group I (control)	Group II (experimental)	Group I (control)	Group II (experimental)
Glucose-6-phosphate dehydrogenase[b] (units mg^{-1} protein)	58.2±1.7	66.4±1.9*	80.4±2.8	62.9±2.2*
Malic enzyme[c] (units mg^{-1} protein)	995±34.8	1063±36.1	958±33.5	924±32.3
Isocitrate dehydrogenase[c] (units mg^{-1} protein)	1.8±0.04	1.19±0.02*	1.9±0.06	1.64±0.05**
Lipoprotein lipase[d]				
Heart	30.6±1.1	38.6±1.2*	23.4±0.8	30.3±0.96*
Adipose	148±5.2	160±6.4	119±4.2	147±4.8*
Plasma LCAT[e]	21.3±0.53	23.9±0.6**	21.8±0.76	24.6±0.86**

[a] The values are mean ± S.E. for 12 rats. Group II is compared with Group I.
[b] One unit is defined as the amount of the enzyme that causes an increase of 1 in optical density min^{-1}.
[c] One unit is defined as the amount of the enzyme that causes an increase of 0.01 in optical density min^{-1}.
[d] Activity expressed as μ moles of glycerol liberated h^{-1} mg^{-1} protein.
[e] Activity expressed as percentage increase in the ratio of ester cholesterol to free cholesterol during incubation.
*$P < 0.01$.
**$0.01 < P < 0.05$.

Table 2
Effect of administration of flavonoids on concentrations of HDL and LDL + VLDL cholesterol in serum (mg/100 ml) of rats fed normal (Experiment I) and cholesterol-containing diets (Experiment II)[a]

	Experiment I		Experiment II	
	Group I (control)	Group II (experimental)	Group I (control)	Group II (experimental)
HDL − cholesterol	56.8±1.7	63.5±1.6**	50.3±1.1	77.4±2.3*
LDL + VLDL cholesterol	30.6±0.9	13.3±0.3*	85.1±2.2	28.2±0.7*

[a] The values are mean ± S.E. for 12 rats. Group II is compared with Group I.
*$P < 0.01$.
**$0.01 < P < 0.05$.

Table 3
Effect of administration of flavonoids on in vivo incorporation of ^{14}C acetate (values expressed as counts/min/g tissue)[a]

Groups	Cholesterol ester	Free cholesterol	Triglycerides	Phospholipids
I (control)	460.4±12.9	1254.2±37.6	908.3±19.9	4085.8±102.1
II (experimental)	319.1±8.0*	1000.1±32.0*	533.72±13.3*	2106.8±58.9*

[a] The values are mean ± S.E. for 12 rats. Group II is compared with Group I.
*$P < 0.01$.

Table 4
Effect of administration of flavonoids on concentrations of bile acids and neutral sterols in rats fed normal (Experiment I) and cholesterol-containing diets (Experiment II)[a]

Bile acids and neutral sterols	Experiment I		Experiment II	
	Group I (control)	Group II (experimental)	Group I (control)	Group II (experimental)
Liver bile acids	27.9±0.8	40.9±1.06*	50.67±1.77	58.72±2.05**
Fecal bile acids	19.3±0.48	28.2±0.68*	33.0±1.15	36.9±1.3**
Fecal neutral sterols	13.0±0.4	22.3±0.67*	25.0±0.88	37.5±2.01*

[a] The values are mean ± S.E. for 12 rats. Group II is compared with Group I.
*$P < 0.01$.
**$0.01 < P < 0.05$.

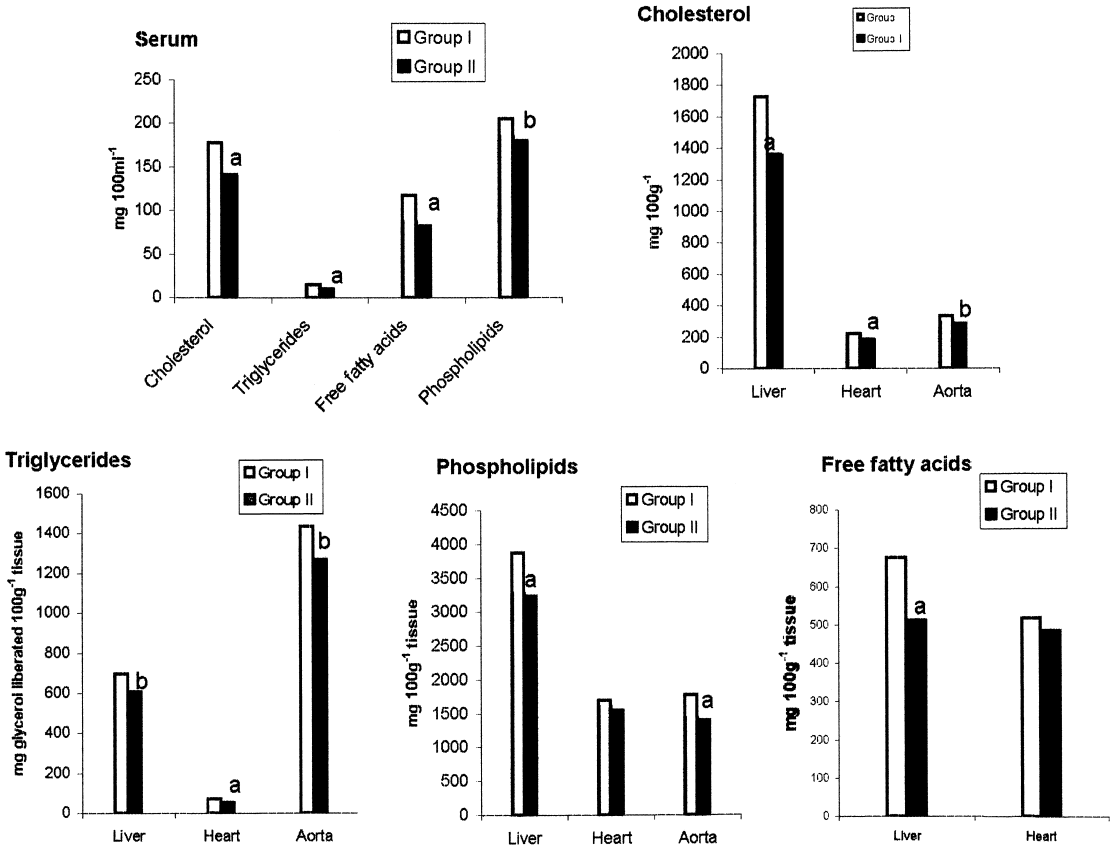

Fig. 3. Effect of flavonoids from *Garcinia cambogia* on concentrations of cholestrol, triglycerides, phospholipids and free fatty acids in serum and tissues of rats fed cholesterol-containing diet. Average of the values of 12 rats in each group ± S.E. Group II is compared with Group I; a = $P < 0.01$, b = $0.01 < P < 0.05$ between groups I and II.

heart and aorta of the experimental group (Fig. 3). Phospholipid concentration in the liver and kidney and free fatty acids in serum and liver showed significant reduction ($P < 0.01$) in the flavonoid-treated group (Fig. 3). HMG CoA reductase activity showed no marked reduction in the experimental group (Fig. 2). Activities of glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase were decreased significantly ($P < 0.01$) while malic enzyme did not show any significant change in group II animals (Table 1). Activities of LPL and plasma LCAT were enhanced significantly ($P < 0.01$) in group II animals (Table 1). Concentration of HDL cholesterol showed a significant increase in the experimental group while the LDL + VLDL fraction showed a significant reduction ($P < 0.01$; Tables 2 and 3). The concentrations of hepatic and fecal bile acids and fecal neutral sterols were elevated significantly ($P < 0.01$) in the experimental group when compared to the control group (Table 4).

4. Discussion

The above studies make it clear that flavonoids from *G. cambogia* effectively lower lipid levels in normal and hypercholesterolemic rats. Diet intake and BW gain are unaltered on flavonoid consumption. The concentrations of cholesterol showed significant reductions in serum and tissues of experimental animals administered flavonoids. Triglycerides, phospholipids and free fatty acids exhibited similar patterns in experimental animals, thus providing a net fall in the level of all lipid components. The action of HMG CoA reductase is inhibitory in normolipidemic rats receiving flavonoids, thus eliciting highly favourable hypocholesterolemic action. However in rats fed a high fat diet, HMG CoA reductase showed no any inhibitory effect, probably due to the high concentration of cholesterol in liver as a result of cholesterol feeding. HDL cholesterol showed elevated levels while the LDL + VLDL fraction showed a significant reduction. In vitro studies using ^{14}C acetate also support the above findings. Atherosclerosis results from the accumulation of LDL in the arterial wall and from the cellular response of wall components to injury. Oxidative modification of LDL is a key early event in the pathogenesis of atherosclerosis (Steinberg, Parthasarathy, Carew, Khoo & Witztum, 1989). Several dietary flavonoids have been shown to lower LDL levels and inhibit the oxidative modification of LDL in vitro (Monforte, Trovato, Kirjavainon, Forestieri, Galati &

Lo Curto, 1995; Kwiterovich, 1997; Catapano, 1997) and hence have the potential to reduce LDL oxidation and atherogenesis in vivo. Leont'eva, Kazakov and Ryzhenkov (1979) claimed inhibition of hyperlipidemia and accumulation of triglycerides in rat liver and blood caused by ethanol administration on flavonoid treatment. Significant reductions of phospholipids and free fatty acids were manifested in the tissues of rats receiving flavonoids. The control of plasma fatty acid concentration, the transport of fatty acids between adipose, liver and muscle and fatty acid disposal (storage vs. oxidation) are potential sites of disruption in obesity, hyperlipidemias and diabetes. Fatty acid uptake into cells is an especially controversial area of research. The activity of LPL which catalyses the hydrolysis of lipoprotein triglycerides in the capillary beds was highly stimulated in the tissues of rats administered flavonoids from *G. cambogia*. Lipogenesis was inhibited, as shown by the reduced activities of the lipogenic enzymes, glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase, although activity of malic enzyme was neutral. On analysing the data on the degradation of cholesterol, it is seen that the route for degradation is more effectively chanelled than that of synthesis. This is evident from the significantly higher levels of hepatic and fecal bile acids and neutral sterols in experimental rats given Garcinia flavonoids. On reviewing the results, it can be summarized that flavonoid compounds from *G. cambogia* offer promising therapeutic value in preventing advancement of atherosclerosis and related cardiovascular anomalies, by inhibiting cholesterol synthesis and alleviating hyperlipidemia.

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