

Cholesterol and Bile Acid Balance in *Macaca fascicularis*

EFFECTS OF ALFALFA SAPONINS

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ABSTRACT We determined the effects of alfalfa top saponins on cholesterol and bile acid balance in eight cynomolgus macaques (*Macaca fascicularis*). The monkeys ate semipurified food containing cholesterol with or without added saponins. The saponins decreased cholesterolemia without changing the levels of high density lipoprotein-cholesterol; hence, they reduced the total cholesterol/high density lipoprotein-cholesterol ratio. Furthermore, they decreased intestinal absorption of cholesterol, increased fecal excretion of endogenous and exogenous neutral steroids and bile acids, and decreased the percent distribution of fecal deoxycholic and lithocholic acids. The fecal excretion of fat was also slightly increased, but steatorrhea did not occur. We saw no signs of toxicity in the monkeys after 6 or 8 wk of saponin ingestion. The data suggest that alfalfa top saponins may be of use in the treatment of patients with hypercholesterolemia, but long-term studies on possible toxicity are needed before this therapy can be recommended for humans.

INTRODUCTION

By mechanisms as yet unknown, alfalfa saponins reduce intestinal absorption of cholesterol in rats (1) and prevent the expected rise in cholesterolemia ob-

served in cholesterol-fed monkeys (2). Coulson and Evans (3) surmised that the formation of insoluble saponin:cholesterol addition products reduced intestinal absorption of cholesterol. In vitro studies also suggest that saponins from several dietary plants bind bile acids (4), but whether alfalfa saponins interfere with the resorption of bile acids has not been established. The data reported here elucidate certain effects of alfalfa saponins on cholesterol metabolism in monkeys.

METHODS

Animals. Eight adult female cynomolgus macaques (*Macaca fascicularis*), laboratory-conditioned and chow-fed (Purina monkey chow, 15% protein; Ralston Purina Co., St. Louis, Mo.), were housed individually in metabolic cages. The room was kept at ~25°C and was lighted from sunrise to 11:00 pm. Food and water were offered *ad lib*. The monkeys had been offered a high-fat, high-cholesterol diet in an earlier study (5), and we used their plasma cholesterol values to rank and randomly assign them to group A and group B (four monkeys per group). The design of the study is depicted in Fig. 1. The animals were first conditioned to a cholesterol-free semipurified diet (SPD)¹ (diet 1, Table I) for 2 wk. Subsequently, group A received cholesterol-containing SPD 2 (Table I); in group B, 0.6% alfalfa top saponins were added to the same food; the food was mixed with 1.5% agar in water to facilitate measurement of dietary intake. At the end of 4 wk, an oral dose of labeled cholesterol and β -sitosterol was given to each monkey after the morning feeding. The isotopes (~20 μ Ci of [¹⁴C]cholesterol and ~50 μ Ci of β -[³H]sitosterol) had been previously dissolved in diethyl ether with 26 mg of cholesterol and 13 mg of β -sitosterol; 13 ml of corn oil was added and mixed,

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¹Abbreviations used in this paper: HDL, high density lipoprotein; SPD, semipurified diet.

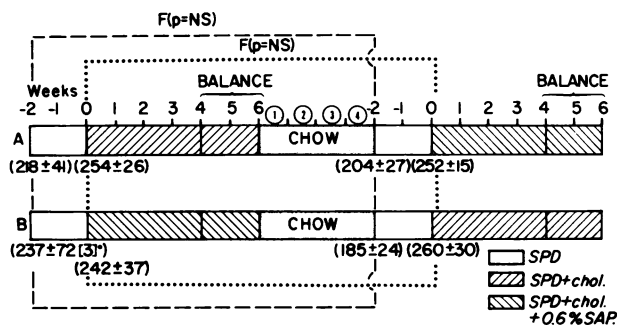


FIGURE 1 Experimental design. Monkeys were assigned randomly to group A or group B (see Methods). Abbreviations and symbols: chol, cholesterol; SAP, alfalfa top saponins; ··· and ---, one-way analysis of variance (F); *, number of animals. Plasma cholesterol concentration in parentheses (mean \pm SE, $n = 4$).

and the ether was evaporated under N_2 overnight. 1 ml of corn oil containing the labeled cholesterol and β -sitosterol was given to each monkey by nasogastric tube (#8 French; Pharmaseal, Toa Alta, Puerto Rico), and the catheter was washed with 6 ml of water. Subsequently, the food intake was carefully measured as the difference between the weights of offered and discarded food; feces were then collected for 12 d in consecutive 3-d pools. A wash-out period of 4 wk on the chow diet followed; during this period, plasma cholesterol returned towards the initial values. The groups were then reversed, and the procedure was repeated. At the end of the experiment, the monkeys were continued on their respective diets for 2 wk, then anesthetized, and full-thickness elliptical samples of the jejunum (about 10 cm distal to the ligament of Treitz) were obtained at laparotomy. Each specimen was immediately pinned out flat under a large drop of buffered 4% formaldehyde-1%

TABLE I
Composition of SPD*

Component	Diet 1	Diet 2
Casein, g/100 g diet	17	15
Sugar, g/100 g diet	33	33
Honey, g/100 g diet	10.5	10.5
Coconut oil, g/100 g diet	11	11
Corn oil, g/100 g diet	1	1
Alphacel, g/100 g diet	17	17
Alfalfa leaf protein, g/100 g diet	2.0	4.0
Banana, g/100 g diet wet wt	10	10
Vitamins (OWP)†, g/100 g diet	2	2
Salts (Hegsted IV)†, g/100 g diet	4	4
Vitamin D-3 (2,000 IU/ml), ml	0.2	0.2
Cholesterol, g/100 g diet	—	0.08
Cholesterol, mg/100 cal	—	22.2
Protein, % cal	21.3	21.3
Fat, % cal	30.0	30.0
Carbohydrates, % cal	48.7	48.7

* Diets contained 360 cal/100 g. Food mixed with 1.5% agar in water.

† ICN Nutritional Biochemicals, Cleveland, Ohio.

glutaraldehyde, and then fixed for 24 h by immersion in the same fixative. Blocks cut in the longitudinal axis of the intestine were embedded in both glycol methacrylate and Spurr's epoxy medium. Glycol methacrylate sections were cut at 2 μ m and stained with hematoxylin-basic fuchsin-methylene blue; epoxy sections were cut at 1 μ m and stained with toluidine blue. The specimens were studied blindly.

Chemicals. Cholesterol and β -sitosterol were obtained from Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif., and Applied Science Labs, Inc., State College, Pa., respectively. Dietary cholesterol (U. S. Pharmacopoeia XV) was obtained from Sigma Chemical Co., St. Louis, Mo. The [$4\text{-}^{14}\text{C}$]cholesterol (New England Nuclear, Boston, Mass.; 54.0 mCi/mmol) and β -[22,23(N)- ^3H]sitosterol (Amersham Corp., Arlington Heights, Ill.; 58 Ci/mmol) were purified by thin-layer chromatography before use.

Saponins obtained from alfalfa aerial plants (alfalfa top saponins) were prepared by a modified version of the method of Walter et al. (6); the biological activity was enhanced by partial acid hydrolysis (1). Alfalfa leaf protein was prepared from a low-saponin cultivar of Ranger alfalfa by the method described by Edwards et al. (7) and Kohler and Knuckles (8); it was incorporated progressively into the diet because it had previously been shown that monkeys would more readily accept the saponin with this protein. The saponin content of the alfalfa leaf protein was 0.11%, as determined by the assay method of Livingston et al. (9). This small content of saponin has been disregarded in the consideration of results.

Analytical methods. Plasma cholesterol was determined with a modified version of the FeCl_3 method of Rudel and Morris (10). High density lipoprotein-cholesterol (HDL-cholesterol) levels were determined after precipitation with Na phosphotungstate and MnCl_2 (11). Radioactivity of the plasma sterols and β -sitosterol was determined in an aliquot of a petroleum ether extract of plasma; plasma had previously been saponified with 33% KOH at 100°C for 1 h. Assays of radioactivity were carried out by liquid scintillation spectrometry after the addition of suitable scintillation fluid. The figures were computed as disintegrations per minute by an automatic external standardization method and by the addition of internal standards when necessary.

The following serum determinations were made with usual laboratory procedures (AutoAnalyzer, SMA-12; Technicon Corp., Ardsley, N. Y.) during weeks 0 and 6 of each experimental period (see figure): glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, CO_2 , uric acid, calcium, phosphorus, total protein, albumin, cholesterol, triglycerides, total bilirubin, direct bilirubin, alkaline phosphatase, lactic dehydrogenase, and serum glutamic oxalacetic transaminase.

The feces collected daily during the balance period were pooled for 3 d, weighed, and stored frozen in tared blender jars. For analysis, feces were thawed, an approximately equal volume of water was added, and the mixture was homogenized in an Oster blender. Approximately half of the fecal homogenate was poured into tared plastic bottles and frozen for subsequent neutral steroid and bile acid analysis. Two aliquots of the homogenate were immediately pipetted into tared 50-ml screw-cap tubes for analysis of the labeled neutral steroids according to methods reported elsewhere (1).

The mass of neutral steroids and bile acids was measured by a procedure described previously (12), based on the methods reported by Meittinen et al. (13) and Grundy et al. (14), involving the separation of fecal steroids into neutral and acidic fractions. These fractions were separately puri-

fied by thin-layer chromatography, and individual components were measured by gas-liquid chromatography. The gas-liquid chromatography was performed on an instrument equipped with a flame ionization detector (HP model 7610A, Hewlett-Packard Co., Palo Alto, Calif.). The column used was a 4-ft glass U-tube, 4 mm i.d., packed with Diatoport-S 80/100 (Hewlett-Packard Co.) coated with 3.8% SE 30. The oven temperature was 230°C, and the carrier gas flow was set at 70 ml/min; 5- α -cholestane was used as an analytical standard for quantification. The amount of recovered β -sitosterol was used to correct the amount of excreted fecal neutral steroids.

The total fat content of the feces was determined gravimetrically by a modified version of the method of Sobel (15). Approximately 3-ml aliquots of fecal homogenates were acidified with HCl to pH 3.0 or less; 5 ml of ethanol and 5 ml of water were added, and the lipids were extracted three times with 20 ml of diethyl ether. The extracts were combined in a tared container, and the solvent was evaporated under N₂. The remaining fecal homogenate was brought to ~pH 8 with ~8 drops of 10 N NaOH, and extraction was performed as described above. The diethyl ether extracts were combined with the acid extract and evaporated under N₂.

Food analysis. The sterol—i.e., cholesterol, β -sitosterol, and campesterol—content of the food was determined by a method similar to that described for steroid analysis in feces.

Estimation of intestinal absorption of cholesterol. The radioactivity received by each animal was calculated from the weight of the syringe before and after injection. The fecal labeled steroids were assayed as described elsewhere by total fecal output of the label (1). Losses caused by degradation of cholesterol to substances not recovered by the method of analysis were estimated from losses of β -[³H]-sitosterol used as a standard; absorption of β -[³H]sitosterol was minimal (see below), and it was disregarded in the calculations. The excreted labeled neutral steroids were considered to represent the nonabsorbed cholesterol, and thus absorption was expressed as “100-feces,” equivalent to the percentage of the injected dose.

Calculation of steroid balance. Steroid balance analysis was based upon the assumption that the input of sterols (oral intake) was equal to the output (fecal excretion) during a metabolic steady state. Therefore, the difference (excretion minus intake) equaled the amount of synthesis. Fecal total steroids indicated the sum of neutral steroids and bile acids. The difference between fecal neutral steroid excretion and the calculated nonabsorbed exogenous cho-

lesterol was assumed to represent the excretion of endogenous neutral steroids. However, since a portion of exogenous cholesterol was reexcreted after having mixed with cholesterol pools in the organism, the “endogenous” neutral steroid values were only approximations.

Analysis of the data. Having been conditioned to a cholesterol-free SPD, the monkeys received a cholesterol-containing SPD with or without alfalfa saponins during a 6-wk period, and the balance study proceeded for the last 12 d of this period. Thus, the only difference during the balance period was the presence of alfalfa top saponins in the diet of one group of monkeys. It was thus possible to disregard any additional effects of the quality of protein and the presence of β -sitosterol in the SPD or other nondefined conditions because all these factors were obviously identical in both groups of monkeys. Moreover, for analysis of the data, corresponding periods were combined. This rearrangement of the data was based on the assumption that the conditions after the wash-out period were similar to those during the initial observation period. Plasma cholesterol levels for weeks -2 and 0 (Fig. 1) justified this approach. In addition, any residual effects after the first set of determinations would have been opposite to those of the second set of determinations because of the crossover design, and thus the possible differences due to saponins would have been reduced. Consequently, the data were considered to consist of two sets of information: data on eight monkeys given a diet with added alfalfa top saponins, and data on the same eight monkeys given a similar diet without added saponins. Results were subsequently contrasted by *t* test.

RESULTS

General. The monkeys appeared healthy and had similar body weights at the beginning of each observation period (Table II); the body weights were maintained throughout. Food intake was similar with both diets during the balance study: 92±5 g/d for controls and 86±8 g/d for monkeys receiving alfalfa top saponins (wet wt; mean±SE). The intake of cholesterol and of plant sterols was consequently similar for both diets. Weights of the feces (not shown in the table) were similar with both diets during the balance study: 20.7±2.2 g/d for controls and 22.9±4.3 g/d for monkeys receiving alfalfa top saponins (*P*, not signifi-

TABLE II
Body Weight and Food Intake in *M. fascicularis*

Diet	Body weight		Food intake (wet) <i>g/d</i>	Balance period			Cholesterol intake <i>mg/d</i>
	Initial (week 0)	Final (week 6)		Sterol content			
				Cholesterol	β -Sitosterol	Campesterol	
	<i>g</i>			<i>mg/100 g food wet wt</i>			
Control (<i>n</i> = 8)	3.10±0.27	3.12±0.27	92±5	54.3±1.3 (5)*	2.9±0.3 (5)	1.6±0.04 (5)	48.8±1.9
Saponin (<i>n</i> = 8)	3.12±0.24	3.07±0.25	86±8	52.3±1.7 (5)	2.8±0.3 (5)	1.5±0.06 (5)	45.7±4.1

P not significant for all categories. Values are means±SE. Number of animals between parentheses.

* Number of diet pools.

TABLE III
Serum Values in *M. fascicularis*

Serum variable	Control diet value		Saponin diet value	
	Week 0	Week 6	Week 0	Week 6
Glucose, mg/dl	62±2	62±5	61±5	64±8
Blood urea nitrogen, mg/dl	20±1*	13±1*	19±2	17±2
Creatinine, mg/dl	1.1±0.02	1.0±0.05	1.1±0.06	1.1±0.06
Sodium, megliter	154±1	154±1	152±2	151±1
Potassium, megliter	6.1±0.3	6.4±0.2	6.2±0.3	6.0±0.1
Chloride, megliter	113±1	112±1	114±1	112±1
CO ₂ , megliter	16±1	17±1	16±1	16±1
Uric acid, mg/dl	1.2±0.2	1.0±0	1.0±0	1.0±0
Calcium, mg/dl	11.4±0.1	11.1±0.1	11.0±0.2	10.8±0.2
Inorganic phosphate, mg/dl	5.0±0.2	4.7±0.4	4.9±0.4	4.3±0.3
Total protein, g/liter	8.9±0.1	8.4±0.2	8.6±0.3	8.4±0.1
Albumin, g/liter	4.2±0.1	4.0±0.1	4.1±0.1	4.0±0.1
Triglycerides, mg/dl	65±14	58±11	69±16	56±11
Total bilirubin, mg/dl	0.2±0.03	0.1±0.02	0.3±0.04	0.2±0.03
Direct bilirubin, mg/dl	0.1±0.00	0.0±0.02	0.2±0.03	0.0±0.02
Alkaline phosphatase, IU/liter	178±14‡	208±21‡	185±20	212±17
Lactic dehydrogenase, IU/liter	309±15*	413±27*	298±22§	400±26§
Serum glutamic oxalacetic transaminase, IU/liter	39±9*	57±7*	31±5*	52±5*

Values are mean±SE.

* $P < 0.01$.

‡ $P < 0.02$.

§ $P < 0.001$.

cant). Although serum enzyme levels were higher at the end of each observation period, multiple serum parameters showed no differences attributable to the ingestion of alfalfa top saponins (Table III).

Villi from a chow-fed cynomolgus macaque, included here for comparison, and villi from the animals maintained on the SPD or SPD plus alfalfa top saponins were indistinguishable by light microscopy; thus, the saponins had no detectable effect on the structure of the jejunal mucosa.

Plasma cholesterol. The observations were conducted in cynomolgus macaques with basal cholesterolemias higher than those usually seen in this

species (5); the animals were selected from a large sample and thus showed plasma cholesterol levels similar to those observed in U. S. adults (16). However, one animal from group B refused the diet at the beginning of the observation period, and another with a somewhat lower cholesterolemia was substituted. Initial plasma cholesterol values were similar for the control and saponin diets: 202±23 and 219±32 mg/dl, respectively (Table IV). The cholesterol-free SPD given during the following 2 wk raised the cholesterol values to 257±18 and 247±18 mg/dl, respectively. Addition of cholesterol to the SPD further raised the cholesterol levels; however, the rise

TABLE IV
Plasma Cholesterol Concentration in *M. fascicularis*

Diet	Plasma cholesterol concentration					HDL-cholesterol concentration Week 6	Total cholesterol/ HDL-cholesterol ratio Week 6
	Week -2	Week 0	Week 4	Week 5	Week 6		
	mg/dl						
Control (n = 8)	202±23	257±18	408±45	393±40	447±49	113±17	4.6±0.9
Saponin (n = 8)	219±32 (7)	247±18	278±30	280±27	288±33	115±13	2.7±0.4
P	NS	NS	<0.01	<0.01	<0.001	NS	<0.01

All values are mean±SE.

TABLE V
Balance Study in *M. fascicularis*

Diet	Cholesterol intestinal absorption	Plasma radioactivity at 48 h*		Fecal excretion				Calculated body steroid synthesis
		¹⁴ C	³ H	Neutral steroids	Endogenous neutral steroids	Bile acids	Total steroids	
	100-feces	% injected dose		mg/d				mg/d
Control (n = 8)	75.6±1.6 (7)	22.1±1.9 (7)	0.35±0.05 (7)	33.0±3.3	18.9±3.2 (7)	16.9±4.1	49.9±6.6	1.1±6.3
Saponin (n = 8)	59.7±3.6 (7)	16.4±2.3 (7)	0.21±0.05 (7)	59.8±7.5	42.4±5.1 (7)	28.6±7.1	88.5±14.0	42.8±10.7
P	<0.01	NS	NS	<0.01	<0.01	<0.05	<0.01	<0.001

All values are mean±SE. Number in parentheses is the number of animals included.

* Assumes a plasma volume equal to 3.5% of body weight (Stahl and Malinow. 1967. *Folia Primatol.* 7: 12).

was not as marked when the diets contained alfalfa top saponins, and the differences between the two diets were significant. At the end of the observation period (week 6), the levels of HDL-cholesterol were similar with both diets; however, the total cholesterol/HDL-cholesterol ratio was significantly less in monkeys receiving alfalfa top saponins.

Steroid balance. The level of radioactivity assayed in the feces of one monkey was inordinately high in the first pool immediately after administration of the isotope. Consequently, the radioactivity data for this animal have been deleted from the following calculations.

Intestinal absorption of [4-¹⁴C]cholesterol was reduced by alfalfa saponins from 75.6±1.6 to 59.7±3.6% of the injected dose (Table V), and this was probably reflected in plasma radiocholesterol concentrations. The amounts of cholesterol radioactivity in the plasma volume 48 h after the pulse dose were 22.1±1.9 and 16.4±2.3% of the injected dose in the control and saponin diet periods, respectively. Plasma β -sitosterol values, which probably indicate the minimal absorption of β -sitosterol, were 0.35±0.05 and 0.21±0.05% of the injected dose, respectively. The diet containing added alfalfa top saponins increased the excretion of neutral steroids (33.0±3.3 to 59.8±7.5 mg/d), endogenous neutral steroids (18.9±3.2 to 42.4±5.1 mg/d), bile acids (16.9±4.1 to 28.6±7.1 mg/d), and total steroids (49.9±6.6 to 88.5±14.0 mg/d). The relative amounts of excreted deoxycholic and lithocholic acids, identified solely by gas chromatography, were lower in the monkeys ingesting saponins (Table VI), but the feces of these animals contained a compound showing a long-retained peak on the gas chromatogram. Feces to which alfalfa top saponins were added in vitro did not demonstrate such a peak. Identification of this compound, which has the solubility and chromatographic

characteristics of a bile acid, was attempted by mass spectrometry with a DuPont 21-491B gas chromatograph/mass spectrometer system (DuPont Instruments, S & P Div., Wilmington, Del.). The mass spectrum had a main peak corresponding to mass equivalent 526, which is not typical of trimethylsilyl ethers of methyl bile acids or bile alcohols with 0-, 1-, 2-, or 3-hydroxy or ketone-substituted groups.

The calculated body synthesis (total steroid fecal excretion minus cholesterol intake) was near zero when the monkeys ingested the cholesterol-containing SPD, and it was 42.8±10.7 mg/d when alfalfa top saponins were included (Table V).

Fat excretion. Fecal fat excretion (not shown in the tables) was 0.6±0.1 g/d when the monkeys ingested the control food and 1.0±0.1 g/d during the ingestion of food containing alfalfa top saponins ($P < 0.05$).

DISCUSSION

The observed diminution in the rising plasma cholesterol levels associated with the ingestion of

TABLE VI
Relative Amounts of Bile Acids Excreted in Feces of *M. fascicularis*

Diet	Excretion		
	Lithocholic acid	Deoxycholic acid	Other
	%		
Control (n = 8)	27.1±2.8	45.5±4.7	27.4±3.9
Saponin (n = 8)	15.2±2.0	21.0±5.9	63.7±7.0*
P	<0.001	<0.01	<0.001

Results are ±SE.

* Includes unidentified compound; see text.

alfalfa top saponins in cholesterol-fed cynomolgus macaques extends our previous results with alfalfa root saponins (2, 17). Similar results have been reported for several species of animals ingesting saponins from different plant sources (18–22). Thus, the data suggest that saponins may be involved in the hypocholesterolemic effects of alfalfa meal in rabbits (23–26), rats (27), and monkeys (28–31), although possible additional effects of other components of alfalfa meal, such as fiber, (27) have not been ruled out. Moreover, the levels of HDL were unchanged in our studies, and hence the plasma total cholesterol/HDL-cholesterol ratio was decreased.

The hypocholesterolemic effects were associated with a decrease in intestinal absorption of cholesterol and with an increase in fecal excretion of neutral steroids, endogenous neutral steroids, and bile acids. Similar findings have been reported in connection with intestinal absorption of cholesterol in rats (1) and fecal excretion of neutral steroids in mice (32). The decrease in absorption of cholesterol may be due to formation of saponin: cholesterol complexes in the intestine (3), a hypothesis supported by studies on the *in vitro* interaction of alfalfa saponins and sterols (33). Moreover, as suggested by the observed small increase in fecal fat excretion, saponins may also affect micellar dispersion of cholesterol; a similar effect of alfalfa saponins on lipid excretion in mice has been reported by Reshef et al. (32). Furthermore, saponins may conceivably interact with membrane cholesterol of intestinal cells and decrease the maximal transport rate or the number of transport sites (21). Finally, it is possible that saponins increase the thickness of the unstirred water layer or its resistance (21).

That alfalfa top saponins increase bile acid excretion has not been reported until now, but a similar phenomenon has been observed in rats fed a commercial saponin (34). The decrease in the distribution of secondary bile acids has also been observed by Oakenfull et al. (34) in rats. These findings are compatible with the increased acidic steroid excretion observed in rats after the addition of fiber to the diet (27, 35), if we assume that saponins are involved in the binding of bile acids to plant fiber, as has been surmised by Oakenfull and Fenwick (4) and Hood et al. (22); adsorption of bile acids by plant fiber may also be mediated by pectic substances, however (36).

The data reported here suggest that alfalfa top saponins increase the synthesis of cholesterol in cholesterol-fed monkeys. Although they agree with the observation that mice given alfalfa saponins incorporate more [¹⁴C]acetate into liver-unsaponifiable material than controls not receiving saponins (32), the occurrence of a negative steroid balance under

these conditions cannot be ruled out in the absence of measurements of cholesterol pool size.

We have paid particular attention to the detection of possible toxic effects. As shown by body weight, food consumption, general appearance, multiple blood determinations, and intestinal biopsy, ingestion of alfalfa top saponins for 6–8 wk is not toxic to monkeys. Similarly, cynomolgus macaques given 1.2% alfalfa top saponins for 12 mo show no anemia or other serologic abnormalities (unpublished observations), and no toxicity has been observed in cynomolgus macaques receiving a diet containing 50% alfalfa meal for 18 mo (31) (equivalent to ingestion of ~1.0% alfalfa saponins [37]). Thus, toxicity is absent at ingestion levels that reduce the hypercholesterolemia associated with a diet rich in saturated fat and cholesterol. Because reduction of hypercholesterolemia occurs simultaneously with a lowering of the total cholesterol/HDL-cholesterol ratio—which is associated with a reduced incidence of atherosclerosis in humans (38, 39)—alfalfa top saponins may eventually be useful for the treatment of patients with hypercholesterolemia. However, further studies on long-term toxicity and tolerance are needed before we can recommend that alfalfa top saponins be used to affect the course of atherosclerosis in humans.

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