THE LOWERING EFFECT OF PANAXYDOL PURIFIED FROM KOREAN RED GINSENG ON BLOOD HIGH CHOLESTEROL LEVELS IN RATS

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ABSTRACT

The lowering effect of cholesterol in Sprague Dawley rats was investigated with panaxydol which was purified from the petroleum ether soluble fraction(PESF) of Korean red ginseng.

The level of total cholesterol(TC), Triglyceride(TG) and low density lipoprotein(LDL) - cholesterol in serum was reduced by 48%, 47% and 41%, respectively while high density lipoprotein(HDL) - cholesterol was in creased up to 29% as compared with their control values when the panaxydol(20 μmoles, 5 mg/kg/day) was administered by intraperitoneal rout for 3 consecutive days along with a 1% cholesterol diet.

The hepatic ester cholesterol content which was increased in proportion to the cholesterol content of the diet in the control, clearly decreased with panaxydol administration to about 40% regardless of the two diet cholesterol contents, 1% or 2%.

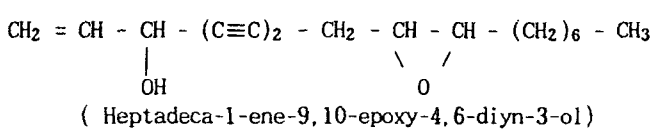
A threshold of supression on the serum lipid levels in both administration routes was observed ; the maximium suppression in i.p. and p.o. administration was observed to be at 5mg/kg b.w. and in the range of 50 - 100 mg/kg b.w., respectively.

Panaxydol may reduce serum lipid and cholesterol levels by inhibiting cholesterol absorption and/or by modulating the cholesterol metabolism, at least in part.

INTRODUCTION

Cholesterol has definitely been implicated in the early development and progression of atheroma plaque. Moreover, many clinical trials have shown that cardiovascular events in hypercholesterolemic patients were decreased after reduction of total and LDL - cholesterol levels(1). This reduction can be partly achieved by a combination of a diet low in saturated fats and cholesterol. However, in many cases drugs must be used to obtain a substantial reduction of cholesterolemia.

Panaxydol, one of 10 polyacetylenes that have been purified from Panax ginseng C.A. Meyer, has been isolated as a petroleum ether extract of ginseng and has been mainly linked with cytotoxicities against some cancer cell lines(2, 3, 4). It is known that the 9, 10 - epoxide and hepta - 1 - ene - 4, 6 - diyne - 3 - ol of the panaxydol molecule(Fig. 1.) can be potent cytotoxins to cancer cells. However, there are no signs of toxicity when panaxydol was administered to normal rat intraperitoneally at doses below 20μmoles/kg, b.w.(5). Besides cytotoxicity to cancer cell lines, this compound has various pharmacological signs such as antioxidant activities on CCl_4 induced lipid peroxidation(6), and alteration of benzo(a)pyrene metabolism(7). we thought that it was important to investigate the main biochemical effects of panaxydol based on the several pharmacological signs.



(Heptadeca-1-ene-9,10-epoxy-4,6-diyn-3-ol)

Fig. 1. Structure of Panaxydol

Recently, we found that panaxydol, purified from PESF, clearly reduced the serum cholesterol levels in rats that were fed a diet containing high cholesterol. Quresh et al.(10) reported that the PESF lowered serum total cholesterol and LDL - cholesterol levels without affecting the HDL cholesterol in chicken. However, there was no evidence showing which of compounds in PESF might play a major role as hypocholesterolemic agents.

The present study, therefore, was undertaken to identify the panaxydol compound that is responsible for lowering cholesterol in blood and to examine changes in the level of plasma lipids in rats following panaxydol administration.

Materials and Methods

Isolation of PESF and panaxydol from Korean red ginseng

PESF and panaxydol were obtained by the procedure of Fig. 2. Dried and pulverized Korean red ginseng roots(500g) were refluxed with 2L of 70% methanol for 3hrs and concentrated after filteration. The methanol extracts were dissolved in 1L of water and then extracted 3 times with 1L of petroleum ether.

Panaxydol(m.w. 260) was purified from petroleum ether faction by silicagel column chromatographey with 50% ether/n - hexane as a solvent system and by preparative HPLC using Altecch NH_2 Semi preparative column.

Purified ginsenoside Rb_2(m.w. 1,078), which was also purified from ginseng, was also used as a comparative agent with panaxydol.

Animal and Diet

Male Sprague Dawley rats (180~200g b.w.) at the time of experiments were bred and maintained in KGTRI Animal Laboratory. The animals were housed in rooms designed to maintain conditions of 22 - 24℃ with 12hr light/dark cycle, 50% humidity. Food and water were fed ad libitum. Prior to the stu-

dies, both rat groups were fed high cholesterol diets containing 1% cholesterol and 0.5% cholic acid or 2% cholesterol and 1% cholic acid for 3 consecutive days. Animals were made to fast for 17hrs before sacrifice.

Administration and Sample Analysis

PESF(800mg/kg diet) was mixed with a high cholesterol diet powder containing 1 or 2% cholesterol and 0.5% or 1% sodium cholate and the pellet was fed to the rats ad libitum for 3 days. Panaxydol(5mg/kg b.w./day) and ginsenoside Rb_2(25 mg/kg b.w./day), which were dissolved in dimethyl sulfoxide(50 μl) and saline, respectively, were administered through the intraperitoneal rout. The control animals were treated with vehicle (DMSO). The rats were killed at 24hrs after the last dose, the blood samples were collected by heart puncture from each animal. The serum was used for analysis of total cholesterol(TC), triglyceride(TG). HDL - cholesterol and LDL - cholesterol levels by employing the Sigma kit. The TC and free cholesterol in the liver tissue was measured by the method of Calson at. al(8). Data was analyzed by observing the differences between the means, and statistical significance based on a student's t - test(9).

Synthesis of sterols from [^{14}C] mevalonate and [^{14}C] acetate

The synthesis of sterols was analyzed by using 20 mg of the homogenate protein in a buffer containing 100mM potassium phosphate, pH 7.4, 3 mM ATP, 3mM glucose - 6 - phosphate, 1mM NADP. [^{14}C]mevalonic acid lactone(0.2μCi/50nmol/assay) obtained from Amersham was used as substrate and the final reaction volume was 1 mℓ. Reactions were started with oxygen flush into the incubation mixture for 10sec and were terminated by the addition of 1mo ethanolic KOH after incubation with shaking at 37℃ for 60 min(or 120min when sodium acetate, 1μCi/6.7 μmol/assay was used as a precursor substrate. The reaction mixture was heated at 75℃ for 2hr. Nonsaponifiable lipids were extracted three times with petroleum ether. The pooled extracts were washed twice with water. samples were dried under nitrogen and analyzed by thin layer chromatography.

Dual isotope plasma ratio method

Male Sprague Dawley rats(190~200g, b.w.) were maintained with standard laboratory rat chow(Samyang Co.) and panaxydol(5mg/kg, b.w.) was injected intraperitoneally once a day for 5 days.

On the 3rd day of panaxydol administration, [4 - ^{14}C] - cholesterol(1㎌ Ci/200g, b.w.) was injected into tail viein while [1, 2 - ^{3}H] - cholesterol(3μCi/200g, b.w.) containing cholesterol(6 mg), triolein(156mg) and cholic acid(7.5mg) was given through a stomach tubing. 3μCi of [4 - ^{14}C] - cholesterol(60mCi/nmol) solution was prepared by dissolving in 25μl of 95% ethanol and 475μl of 0.9% NaCl. The animals were killed at 48hr after the stomach administration allowing the food and water ad. libitum.

Serum was obtained from the blood collected by heart puncutre. The radioactivity in the non - saponifiable lipid extracted from the serum was measured by using liquid scintillation spectrometer.

Percent absorption of cholesterol was calculated by the formula as follows :

$$\% \text{ absorption} = \frac{[\text{plasma } ^{3}\text{H(dpm)}][\text{administered } ^{3}\text{H(dpm)}]}{[\text{plasma } ^{14}\text{C(dpm)}][\text{administered } ^{14}\text{C(dpm)}]} \times 100$$

Result and Discussion

Table 1. shows the effects of PESF on serum lipid content. The serum TC and TG levels of the normal control group increased in about 3 times after fed a 2% cholesterol diet to the rats. These values were reduced by PESF administration 77% and 51% of the control values, respectively. The hepatic cholesterol content(Fig. 3.) reduced to some extent, but there were no significant differences to their control values.

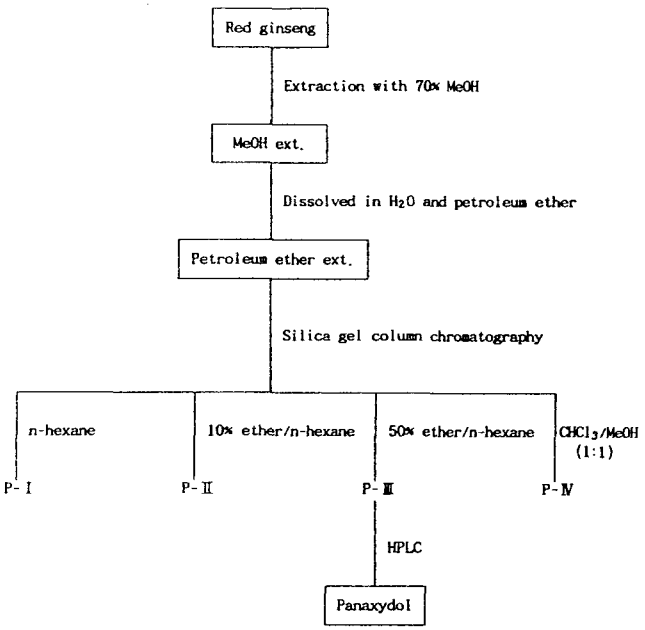


Fig. 2. Effect of PESF on hepatic cholesterol content of high cholesterol fed rats.
- Data are expressed as means± S.E (n=5)
- Test groups were fed diet including PESF (petroleum ether soluble fraction, 800mg/kg diet)

Quresh et al.(10) reported that the PESF lowered serum total cholesterol and LDL - cholesterol levels without affecting the HDL cholesterol in chicken. However, there was no evidence showing which of compounds in PESF might play a major role as hypocholesterolemic agents. In general, neutral lipid fractions of red ginseng consist of TG(32.8%), sterol esters and hydrocarbons (20.3%), diglycerides(14.9%), unidentified lipids III(10.6 %) and other minor compounds(11). The unidentified lipids

Table 1. Effect of PESF on serum lipids of rats fed a high cholesterol.

Diet	Concentration in serum(mg/dl)			
	TC	TG	HDL - chol	LDL - chol
normal diet	64.8± 2.5	36.2± 3.9	25.7± 2.4	32.5± 1.7
2% cholesterol diet				
Cont	185.1± 11.6 (100)	94.2± 5.2 (100)	17.2± 1.1 (100)	147.0± 13.6 (100)
Test	142.4± 11.8* (77)	48.5± 6.0** (51)	18.4± 1.0 (107)	114.6± 12.8 (78)

· Data are expressed as means± S.E (n=5)
· Test groups were fed diet including PESF(petroleum ether soluble fraction, 800mg/kg diet)
· Values in parenthesis represent percentages of control.
* Significantly different from control(P<0.05)
** Significantly different from control(P<0.01)

As shown in Table 3, the dosages of panaxydol in Table 2. were appropriate amounts to be administered to rats. When the rats were made to fast for 17hrs before being fed a high cholesterol diet, the levels of serum cholesterol in pre - fasted rats(Table 3, zero dose) were significantly increased to almost twice as compared with the non - fasted rats before treatment of test compound(Table 2, control). Therefore, in order to amplify the panaxydol effect, data was obtained by using the pre - fasting animals as shown in Table 3. The optimum ranges of action were determined to be 2.5 - 5mg/kg b.w. after intraperitoneal administration of panaxydol at several different dosages. The threshold of panaxydol suppression was always observed to be above a 5mg/kg b.w. dosage during the experiment for the past two years. ; also, the suppressive effect was observed at dosage of about 10 times with oral administration(Table 4.). When comparing panaxydol with clofibrate(m.w. 242.7), which is one of hypolipidemic drugs, within the same range(20μmoles/kg b.w.), panaxydol was more effectively lowered serum cholesterol than III contains polyacetylene compounds which can be purified from PESF. Panaxydol is the most abundant among these polyacetyle-

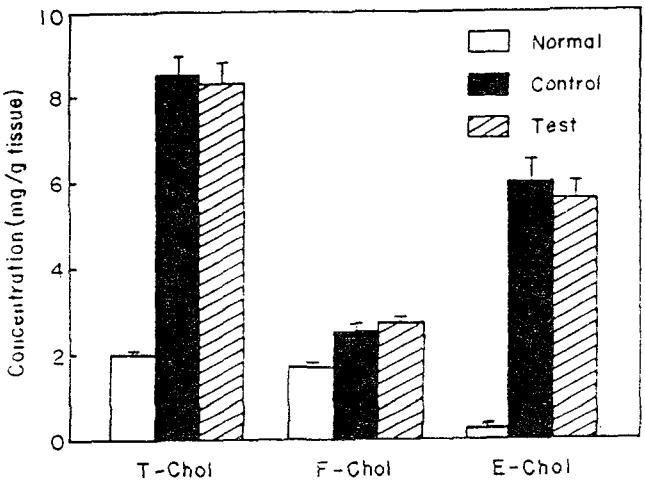


Fig. 3. Effect of panaxydol on hepatic cholesterol content of high cholesterol fed rats.
A ; 1% cholesterol diet. B ; 2% cholesterol diet.
· Data are expressed as means± S.E. (n=5).
· Panaxydol was administered for 3 consecutive days by i.p. injection(5mg/kg b.w.).
* Significantly different from control(P<0.05).

Table 2. Effect of panaxdyol on serum lipids of rats fed a high cholesterol diet.

Diet	Concentration in serum(mg/dl)			
	TC	TG	HDL - C	LDL - C
normal diet	67.7± 4.2	52.1± 5.2	28.6± 1.5	28.7± 3.7
1% cholesterol diet				
Control	128.5± 14.2 (100)	102.6± 15.8 (100)	14.0± 1.5 (100)	94.1± 12.1 (100)
Test	62.3± 4.4** (48)	48.7± 6.0** (47)	16.4± 0.9* (117)	38.9± 4.9** (41)
2% cholesterol diet				
Control	174.3± 22.0 (100)	80.1± 10.3 (100)	22.2± 2.2 (100)	130.3± 21.2 (100)
Test	152.3± 11.5 (87)	72.3± 3.8 (90)	18.2± 2.6 (82)	115.0± 11.5 (88)

· Data are expressed as means± S.E (n=5)
· Values in parenthesis represent percentages of control.
· Panaxydol was administered by i.p. injection(5mg/kg b.w.). for 3 consecutive days.
* Significantly different from control(P<0.05)
** Significantly different from control(P<0.01)

nes(data not shown). The active agents for the suppression of cholesterogenesis and lipogenesis are not ginsenosides(saponins) at least in PESF.

The results in Table 2. showed that panaxydol administration along with a 1% cholesterol diet signicantly lowered levels of serum TC, TG and LDL - cholesterol by 48%, 47% and 41% respectively, as compared with control values. But, these parameters were not significantly reduced in rats fed 2% cholesterol diet. The hepatic ester cholesterol(Fig. 3.) increased in proportion to the cholesterol content of the diet in the control, and was clearly decreased by panaxydol administration to about 60% of the control regardless of cholesterol contents in the diets administered in the experiment. There was also no significant change in food intake or weight gain between the test and control groups during the experiment(Data not shown).

clofibrate regardless of the two administration route. However, unlike intraperitoneal injection, TG was not suppressed while high density lipoprotein (HDL) cholesterol was significantly increased with oral administration. These discrepancies are thought to be the result of different routes of administration

Among the various compounds of Panax ginseng, saponins like ginsenoside Rb_1, Rb_2, have been understood to be as hypocholesterolemic agents(12, 13). Among these saponins, ginsenoside Rb_2 has been observed to be the most effective. Also, it has been reported that action mechanism of the hypocholesterolemic ginsenosides is the stimulation of cholesterol metabolism including synthesis of bile acids and steroid hormones(14). The effects of ginsenoside Rb_2 on serum lipid levels of rats treated with 1% cholesterol diet are shown in Table 5. The TC and TG levels decreased by 33% and 44%, respectively. However, hepatic cholesterol content(Fig. 4.) was not significantly changed in these same animals. Furthermore, the effect of panaxydol via intraperitoneal administration clearly differed from the effect of ginsenoside Rb_2 treatment(Table 5.).

Table 3. Dose response of Panaxydol on serum lipids of rats fed a high cholesterol diet(i.p.).

Treatment	Concentration in Serum(mg/dl)			
	TC	TG	HDL - chol	LDL - chol
Panaxydol(mg/kg)				
0	213.8± 30.1 (100)	124.3± 14.4 (100)	19.3± 1.9 (100)	165.9± 30.5 (100)
5	97.9± 12.1** (45)	83.3± 5.8* (67)	24.9± 1.8 (129)	55.0± 11.0** (33)
10	112.2± 12.5* (52)	59.7± 1.5** (48)	23.5± 0.8 (122)	78.3± 21.4* (47)
Clofibrate(mg/kg)				
10	170.4± 19.0 (80)	101.9± 7.2 (71)	24.3± 1.3 (126)	120.8± 20.4 (73)

· Data are expressed as means± S.E (n=5)
· Values in parenthesis present percentages of control.
· Panaxydol was administered for 3 consecutive days by i.p. injection.
· Rats were fasted for 17hrs before feeding the 1% cholesterol diet.
* Significantly different from control($P<0.05$)
** Significantly different from control($P<0.01$)

Table 4. Dose response of Panaxydol on serum lipids of rats fed a high cholesterol diet(p.o.).

Treatment	Concentration in Serum(mg/dl)			
	TC	TG	HDL - chol	LDL - chol
Panaxydol(mg/kg)				
0	202.9± 4.3 (100)	95.1± 14.4 (100)	10.9± 1.8 (100)	174.8± 5.1 (100)
50	118.4± 9.5* (58)	90.1± 6.1 (95)	18.1± 4.5* (166)	84.0± 10.2** (48)
100	104.6± 17.3* (51.5)	98.1± 13.8 (103)	19.6± 3.0* (180)	67.3± 16.3** (39)
Clofibrate(mg/kg)				
100	154.7± 24.6 (76)	67.1± 2.4* (71)	10.6± 0.7 (97)	131.9± 19.2* (75)

· Data are expressed as means± S.E (n=5)
· Values in parenthesis present percentages of control.
· Panaxydol was administered by p.o. injection for 3 consecutive days.
· Rats were fasted for 17hrs before feeding the 1% cholesterol diet.
* Significantly different from control($P<0.05$)
** Significantly different from control($P<0.01$)

Table 5. Effect of ginsenoside - Rb_2 on serum lipids of rats fed a high cholestrol diet.

Diet	Concentration in serum(mg/dl)			
	TC	TG	HDL - C	LDL - C
normal diet	72.8± 3.2	49.2± 3.2	30.2± 2.0	32.8± 3.0
1% cholesterol diet				
Control	145.3± 12.0	103.8± 8.8	14.0± 1.8	110.7± 11.9
	(100)	(100)	(100)	(100)
Test	115.5± 7.6*	58.4± 5.8**	13.7± 1.5	95.4± 9.4*
	(77)	(56)	(97)	(86)

· Data are expressed as means± S.E (n=5 - 7)
· Values in parenthesis represent percentages of control.
· Ginsenoside - Rb_2 was administered by i.p. injection(25mg/kg b.w.) for 3 consecutive days.
* Significantly different from control($P<0.05$)
** Significantly different from control($P<0.01$)

Table 6. Effect of panaxydol on in vitro incorporation of [u - ^{14}C]acetic acid into sterols.

Panaxydol (uM)	Total Nonsaponfiable Lipids (CPM± S.E.)	% of Nonsaponifiable lipids		
		Cholesterol	Lanosterol	Squalene
Control	352,530± 17,900	93	1.8	1.0
	(100)	(100)	(100)	(100)
100	294,987± 2,701	92	1.3	1.6
	(84)	(99)	(72)	(160)
500	204,150± 151	72	4.3	1.9
	(58)	(77)	(239)	(190)
1000	175,497± 1,046	35	27	4.6
	(50)	(38)	(1500)	(460)

Table 7. Effect of panaxydol on in vitro incorporation of R, S - [2 - ^{14}C]Mevalonolactone into sterols.

Panaxydol (uM)	Total Nonsaponfiable Lipids (CPM± S.E.)	% of Nonsaponifiable lipids		
		Cholesterol	Lanosterol	Squalene
Control	130,585± 2,475	45.8	13.9	14.4
	(100)	(100)	(100)	(100)
100	131,987± 1,413	43.9	18.8	12.6
	(101)	(96)	(135)	(78)
500	135,660± 350	19.7	35.6	16.5
	(104)	(43)	(256)	(115)
1000	150,832± 7,507	8.8	43.3	16.8
	(116)	(19)	(312)	(117)

Table 8. Effect of panaxydol on cholesterol absorption in rats.

Treatment	Radioactivities in serum(dpm/ml)		Cholesterol absorption (%)*
	[^{3}H] - Chol(p.o.)	[^{14}C] - Chol(i.v.)	
Control	5,185± 191	2,729± 176	44± 2.0
	(100)	(100)	(100)
Panaxydol	3,316± 213**	2,387± 496	33± 2.6
	(64)	(87)	(75)

* : Cholesterol absorption was calculated by the ^{3}H/^{14}C ratio in nonsaponifiable lipid and the administration doses.
** : Significantly different from the control by student's t - test($p<0.05$)

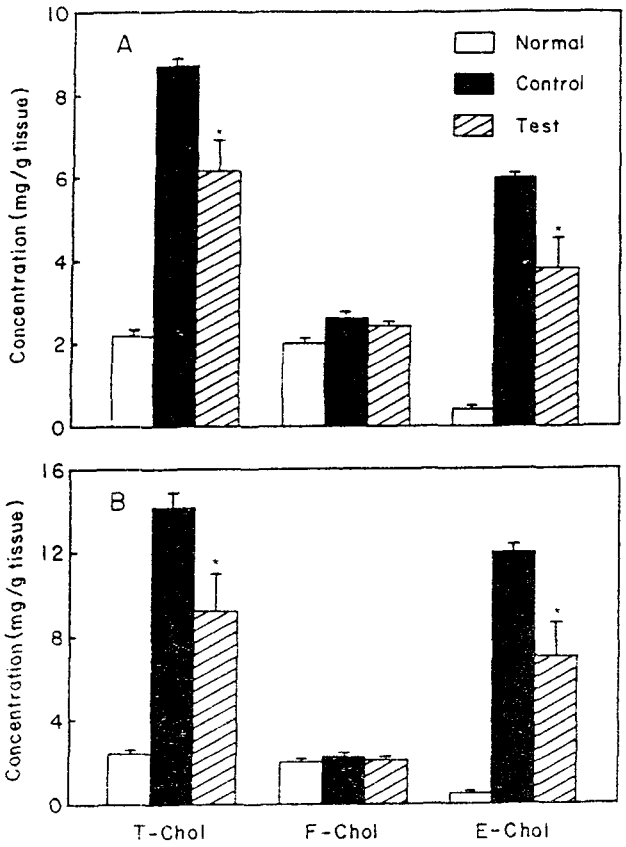


Fig. 4. Effect of ginsenoside Rb_2 on hepatic cholesterol content of high cholesterol fed rats.
- Data are expressed as means ± S.E. ($n = 5 - 7$).
- Ginsenoside - Rb_2 was administered for 3 consecutive days by i.p. injection(25mg/kg b.w.).

These results show that panaxydol has a lowing effect on serum and hepatic cholesterol content in rats fed a high cholesterol diet. It also suggests that panaxydol has a mechanism that acts on serum and hepatic cholesterol metabolism in a somewhat different fashion than ginsenoside Rb_2 or clofibrate.

In order to elucidate the hypolipidemic properties of panaxydol we investigated its effect on cholesterol biosynthesis and absorption. In Table 6, panaxydol showed a dose dependent inhibitory effect on the incorporation of labelled acetate into sterols in the liver. Panaxydol, at a concentration of 0.5mM, significantly inhibited synthesis of sterol from acetete. This inhibition increased with elevation of [^{14}C] lanosterol levels, and with decrease in the non - saponifiable lipids, in a dose dependant manner.

On the other hand, the incorporation of [^{14}C]MVL into cholesterol(Table 7.) was inhibited by addition of panaxydol without changes in total nonsaponifiable lipid levels. The transformation steps of lanosterol into cholesterol seems to be inhibited by panaxydol in the biosynthetic pathway of cholesterol. Table 8. showed a result of cholesterol absorption which was also moderately inhibited by 30% after feeding panaxydol. Panaxydol may reduce serum lipids and cholesterol levels by inhibiting cholesterol absorption at least in part. These studies are currently in progress.

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