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Hypolipidaemic Potentials of *Solanum melongena* and *Solanum gilo* on Hypercholesterolemic Rabbits

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Abstract: The fresh, ripe fruits of *Solanum melongena* and *Solanum gilo* were investigated for their possible hypolidemic potentials in hypercholesterolemia induced in New Zealand white rabbits by feeding the animals with normal diet supplemented with 1% cholesterol and groundnut oil for 3 weeks. Hypercholesterolemic rabbits were thereafter treated with normal diet supplemented with 10% of each fruit for 6 weeks. Rabbits fed with normal diet and hypercholesterolemic diets through out the experiment were used as negative and positive controls respectively. There was a significant increase in the weight of hypercholesterolemic rabbits, both test (Groups D and E) and control (Groups B and C) ($P<0.01$) compared with normal control (Group A). It was observed that the hypercholesterolemic rabbits treated with normal diet elicited a significant reduction in fasting serum cholesterol (11.52%), triglyceride (16.5%) and LDL cholesterol (41.13%) but a significant increase in HDL cholesterol (16.48%) and also in the HDL/LDL ratio (1.10), four weeks after replacement of high cholesterol diet with normal diet. This positive response was however more pronounced in fruits treated rabbits. *Solanum melongena* and *Solanum gilo* significantly reduced serum total cholesterol by 65.40 and 52.69% respectively, triglyceride by 47.7 and 27%, LDL cholesterol by 85 and 83% respectively. They also increased significantly serum HDL by 24.7 and 25% respectively leading to increased HDL/LDL cholesterol ratio (3.37 and 3.25 respectively). This trend was also similar with liver lipid levels. Histopathological examination of the liver and aorta paraffin section stained with Haematoxylin and Eosine showed fewer lesions in the hypercholesterolemic rabbits treated with Solanum fruits compared with control hypercholesterolemic rabbits. These observations demonstrated that *Solanum melongena* and *Solanum gilo* have strong hypolipedemic effect which combined with improved HDL/LDL ratio is an indication of the possible use of this fruit in the treatment of diseases associated with hyperlipidemia such as ishcaemic heart diseases and arteriosclerosis.

Key words: Hypolipidaemic, *solanum melongena*, *solanum gilo,* hypercholesterolemic rabbits

Introduction

The importance of serum lipoprotein disturbances and abnormal lipid metabolism characterized by hyperlipidaemia or hyperlipoproteinmia as etiological factors in the development of coronary heart diseases and potentiating of arteriosclerosis is now supported by a considerable body of evidence amassed from epidemiological and population studies (Arteriosclerosis, 1971; Coronary Drug Project Research Group, 1975; Lipid Research Clinics Programme, 1984; Turpanen, 1979). Infect, it is almost accepted that arteriosclerosis is a disorder of lipid transport and metabolism. Cholesterol by-product would form thick, tough deposit called plague on the inner wall of the arteries, stiffening them and then starving the heart of blood, creating choke point where a clot could stop the flow entirely (Duff and Macmillian, 1951; Goldstein *et al*., 1979). Apart from the lipid from the diet source, the body in turn manufactures its own cholesterol. Inefficient clearance of excess cholesterol for reasons that are largely genetic (Lemonick, 1999), resulting in accumulation of cholesterol in the blood, and deposition of lipid in the minor layer of arterial wall causes arteriosclerosis. Studies have however reported that increased HDL appears to retard or prevent the development of arteriosclerosis while reduced levels are associated with increased risk for coronary artery diseases (Glueck *et al*., 1975). Furthermore, blood lipid levels, particularly total cholesterol and LDL cholesterol are usually related to promoting arteriosclerosis, hence interventions that lower these lipid levels can retard or reverse the progression of this processes.

An indication of possible hypolipidemic potentials of solanum emerged from the study of Sultana *et al*., 1995, who found that a reaction mixture of solanum and cichorium containing calf thymus DNA and free radical generating system protected DNA against oxidative damage of its deoxyribose sugar moiety. This author concluded that this mixture has both hepatoprotective and hypolipidemic potentials.

Several cultivars of Solanum fruits (Garden egg or egg plant) are found throughout Nigeria and cultivated domestically. Solanum contains steroid alkaloid flavonoids. Solanium fruits contain approximately 92.5%

of water, 1% of protein, 0.3% fat and 6% carbohydrate (Bradiosk, 1970). Cytostatic and cholinesterase inhibitory activities have been demonstrated in the fruits (Manske and Holman, 1974). They have been used in traditional medicine in the treatment of various types of diseases (Middleton and Kandaswani, 1993; Kritchersky, 1970; Nadeem and Hussain, 1996).

Since various fruits with antioxidant property have been shown to have ability to lower serum lipids, especially cholesterol, the possible hypocholesterolemic potential of *Solanum melongena* and *Solanum gilo* is hereby investigated for its possible use for prophylaxis and treatment of ischaemic heart disease, a fast growing plague in the world and recently in African.

Materials and Methods

Chemicals and Plant Materials: All chemicals used in this study were of analytical grades purchased from British Drug House (Pool, U.K). Fruits of *Solanum melongena* and *Solanum gilo* were bought in local markets in Ibadan metropolist. The botanical identification of the fruits was done in the department of Botany, University of Ibadan, Ibadan, Nigeria.

Animals and Treatments: Twenty-five male rabbits (New Zealand) weighing 0.72 ± 0.5kg were obtained from a private farm in Ibadan. The animals were fed ad libitum on normal commercial chow and had free access to water. They were randomly divided into five groups with five rabbits in each group.

Rabbits in group A served as negative control and were fed with normal commercial chow throughout the period of the experiment. Rabbits in group B served as positive control and were feed with standard diet supplement with cholesterol and groundnut oil at 1% each, throughout the experimental period. Rabbits in-groups C were fed with lipid supplemented diet (as for group B) for 3 weeks and subsequently with normal diet (as for group A) for 6 weeks. Rabbits in groups D and E were fed with hyperlipidaemic diet for 3 weeks and subsequently with standard diet supplemented with 10% *Solanum melongena* and *Solanum gilo*, respectively for 6 weeks (as for group C). Animals were fasted for 24 hours after last feeding and then sacrificed.

Preparation of Samples (Serum, Liver and Aorta): Sera from blood withdraw from marginal ear vein of the animals in the various groups were used for the determination of the various lipid levels. At the end of 7 days acclamation period, all the animals were fasted for 12 hours and blood samples taken from them for measurement of basal lipid levels of the experimental animals (stage 1). Another blood sample was taken after 3 weeks of feeding Groups B, C, D and E with hypercholesteronenic diet (group A animals were not fed with this diet) (stage II). The animals were then given their various treatments for 6 weeks during which blood was taken at the middle of the treatment (stage 111a) and the end of the treatment (stage 111b).

At the end of the treatment, the animals were fasted for 24hrs and sacrificed by cervical dislocation and dissected. Part of the liver was removed, rinsed in saline solution and homogenized. The liver homogenate and serum were stored at 4°C until analyzed. The aorta and remaining liver was removed and stored in formalin for histology examination.

Lipid determination: The serum and liver {homogenate} total cholesterol, High-density lipoprotein {HDL}, and triglyceride were determined by the High Power Lipid Chromatography {H.P.L.C.} method. The low density lipoprotein {LDL} was calculated from the value of serum HDL cholesterol and triglyceride level as described by Friedewald *et at.*, 1972.

Histology examination: Paraffin sections of liver and aorta were stained with Haematoxylin and Eosin (H and E) and examined microscopically with X119 objective. Microscopical pictures of interesting features were taken.

Statistical analysis: SPSS statistical software was used in all the statistical analyses (Marija, 1988). The results were expressed a mean ± SD. The significant difference in mean value of test and control was detected using paired sample student "t" test.

Results

The effect of normal diet and various foods supplement on body weight and feed intake of the rabbits is presented in Table 1. A significant weight-gain was observed in hypercholesterolemic rabbits compared to rabbits on animal diet. This weight-gain was however reduced in rabbits fed with diet supplemented with solanum fruits without affecting the food consumption of the rabbits. The weight of the rabbits at different stages of the experimented is presented in Fig. 1.

Table 2 shows the comparison of lipid levels in the experimental animals at different stages of the experiment. There was significant increase in the serum lipid level of our experimental animals {B, C, D and E} in stage II {$P < 0.05$}. A constant and progressive significant increase in serum cholesterol, triglyceride and LDL was observed in group B rabbits compared with group A and a reduction in serum HDL cholesterol level. A similar pattern was observed in liver lipid levels of experimental rabbits (Table 3). The serum and liver HDL/LDL cholesterol ratio in different groups of rabbits is presented in Table 4. Both fruits caused significant increase in the serum HDL cholesterol and decrease in LDL cholesterol, which resulted in increased HDL/LDL cholesterol ratio in the test animals.

Table 1: Effect of normal diet and various treatments on body weight and feed intake of the rabbits

Group	Weight {Kg}		Weight Gained	Food Intake {gm}	
	Initial	Final		Initial	Final
A	0.65 ± 0.01	1.45 ± 0.02	0.80 ± 0.02	0.08 ± 0.01	0.10 ± 0.01
B	0.85 ± 0.08	*2.51 ± 0.01	*1.66 ± 0.06	0.08 ± 0.01	0.09 ± 0.01
C	0.80 ± 0.02	1.85 ± 0.05	1.05 ± 0.03	0.08 ± 0.02	0.12 ± 0.05
D	0.74 ± 0.02	1.93 ± 0.20	1.19 ± 0.18	0.10 ± 0.02	0.09 ± 0.01
E	0.58 ± 0.07	1.45 ± 0.66	0.87 ± 0.61	0.10 ± 0.01	0.10 ± 0.01

The results are expressed as mean ± SD for five rabbits in each group.
*Significantly different from controls in group A at $P < 0.05$.

A = Negative control, fed with normal commercial chow.
B = Positive control, fed with standard diet supplemented with cholesterol and groundnut oil at 1% each.
C = Fed with lipid supplemented diet (as for group B) for 3 weeks and Subsequently with normal diet (as for group A) for 6 weeks.
D = Fed with hyperlipidaemic diet for 3 weeks and subsequently with standard diet supplemented with 10% *Solanum melongena* for 6wks.
E = Fed with hyperlipidaemic diet for 3 weeks and subsequently with standard diet supplemented with 10% *Solanum gilo* for 6 weeks.

Table 2: Serum lipid levels of different experimental groups of rabbits at various experimental stages

	A	B	C	D	E
Stage			Total cholesterol		
I	75.87±5.20	123.5±5.07	85.00±11.33	123.80±4.20	85.00±11.33
II	84.05±2.94	188.56±3.55	136.11±8.28	170.86±11.61	132.00±0.30
IIIa	88.17±2.13	130.32±3.35	128.25±2.32	70.00±1.40	68.56±4.87
IIIb	100.98±5.08	346.75±16.78	120.42±5.00	55.36±6.25	55.89±5.51
			Triglyceride		
I	35.94±3.1	39.88±2.4	40.25±2.1	39.91±2.1	46.57±6.1
II	42.83±2.50	66.91±6.1	60.25±3.1	58.09±2.5	53.57±4.2
IIIa	40.40±2.60	123.75±7.2	55.25±3.1	38.16±5.1	38.26±4.1
IIIb	45.40±5.1	125.75±8.2	50.25±2.5	30.36±2.1	30.71±2.5
			LDL		
I	20.61±3.1	58.53±2.5	49.25±3.1	76.68±0.4	43.46±0.81
II	22.65±2.5	104.0±3.5	86.27±6.5	129.88±0.2	84.27±0.65
IIIa	28.11±4.1	203.86±2.5	60.12±4.2	37.67±0.8	19.06±0.21
IIIb	29.38±5.1	242.61±2.5	50.00±5.3	17.54±0.35	10.84±0.22
			HDL		
I	48.00±3.2	85.74±3.2	86.25±6.2	99.15±8.1	53.95±3.5
II	52.69±8.4	52.13±3.5	45.50±8.2	44.50±7.5	32.91±3.4
IIIa	51.98±4.5	54.54±4.5	52.00±3.5	58.70±5.5	42.85±7.5
IIIb	59.49±3.5	57.79±5.2	53.00±3.2	62.14±4.5	57.71±3.2

Table 3: Liver lipid levels of different experimental groups of rabbits

Group	T. Cholesterol	Tricglycerine	IDL	HDL
A	+7.81 ± 2.1	+8.73 ±2.5	+5.14±3.2	+13.25± 4.5
B	*27.12 ± 8.1	*16.80±4.5	*17.11±6.2	*11.23 ± 3.1
C	*25.21 ± 4.5	*14.20±3..5	*16.10±5.1	*+20.32±4.1
D	*+15.87 ± 2.5	+9. 6±2.1	*+10.25±1.5	*+34.61±1.5
E	*+15.14 ± 4.5	+11. 1±2.1	*+10.55±8.1	*+34.46±2.1

The results are expressed as mean ± SD for five rabbits in each group. Comparison between values from treated rabbits {C, D and E} and hypercholesterolemia control +(B) on one hand and normal control *(A) on the other hand are significantly different at $P < 0.05$.

Table 4: Serum And Liver HDL/LDL Cholesterol Ratio In Different Groups Of Rabbits

Group	Serum				Liver
Stages	I	II	IIIa	IIIb	
A	2.32	2.32^{+}	1.85^{+}	1.86^{+}	2.57^{+}
B	2.90	0.47*	0.24*	0.23*	0.70*
C	2.0	0.50*	0.80*$^{+}$	1.10^{+}	1.26^{+}
D	1.20	0.41*	1.35^{+}	2.64*$^{+}$	3.37*$^{+}$
E	1.24	0.39*	1.68^{+}	3.48*$^{+}$	3.25*$^{+}$

*Significantly different from A at $P < 0.05$. +Significantly different from B at $P < 0.05$

Photomicrograph of a paraffin sections of liver and thoracic aorta of rabbits on normal diet, hypercholesterolemic diet and diet supplemented with solanum fruits stained with hematoxylin and eosin were compared (Fig. 2-9). Fatty liver showing hepatic curd cells with several vacuoles in the cytoplasm and some with simple vacuole which displaced the nucleus to one side, and lesions with thickened intima containing form cells and extracellular lipid in the thoracic aorta was observed in rabbits fed with hypercholesterolemic diet. Less lesions was observed in the liver and thoracic aorta of hypercholesterolemic rabbits fed with supplemented diet and little or no lesions on rabbits fed with normal standard diet.

Discussion

The prevalence of atherosclerosis and ischaemic heart disease is on the increase in the world and recently in Africa. The clinical consequences of these conditions are serious and exert major research efforts to improve knowledge of its pathogenesis and thereby provide a more rationed approach to its prophylaxis and therapy (Kritchersky, 1970). The earliest investigations of experimentally induced atherosclerosis were done in rabbit model by feeding with milk, meat and egg (Ignatowski, 1980). Kritcherslay (1970) established that the atherosclerosis causing component of the diet was cholesterol. This has formed the basis for the use of cholesterol and groundnut oil to induced hyperlipidemia in our study. A significant increase ($P < 0.05$) in the serum lipid level of the experimental animals (B, C, D and E) 3 weeks after feeding them with cholesterol supplemented diet (i.e stage II), coupled with fatty liver, extracellular lipid and thickened intima containing foam cells in the aorta of rabbits (Fig. 7) are evidence in support of the observation of Ignatowski (1980) and Kritchersky, 1970.

The weight-gain (1.66 ± 0.06kg) in hyperlipidemic control rabbits (B in Table 1) was significantly higher ($P < 0.05$) than weight-gain (0.80 ± 0.02kg) in control rabbits fed with standard diet (A in Table 1). Excessive weight-gain as in obesity has been implicated as a risk factor for development of hypertension, ischaemic heart diseases

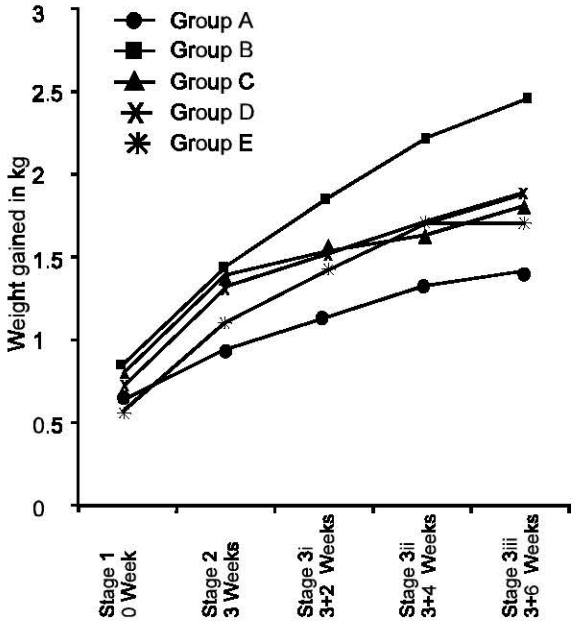

Fig. 1: Weight of the rabbits at different stages of the experiment

Fig. 2: Normal liver showing little lesions (Photomicrograph are of paraffin sections stained with hematoxylin and eosin x 119

and heart failure (Agbedana, 1999). In this study, treatment of hypercholesterlomic rabbits with standard diet {C} and diets supplemented with solanum {D and E}

Fig. 3: Fatty liver from hypercholesterolemic rabbit. Showing some hepatic cord cells with serval vacuoles in the cytoplasm and some with large simple vacuole which has displaced the nucleus to one side

Fig. 4: Liver of rabbits fed with S. melogena supplemented diet showing less lesions compared to hypercholesterolemic rabbit

Fig. 5: Liver of rabbit fed with *S. gilo* showing less lesions compared to hypercholesterolemic rabbit

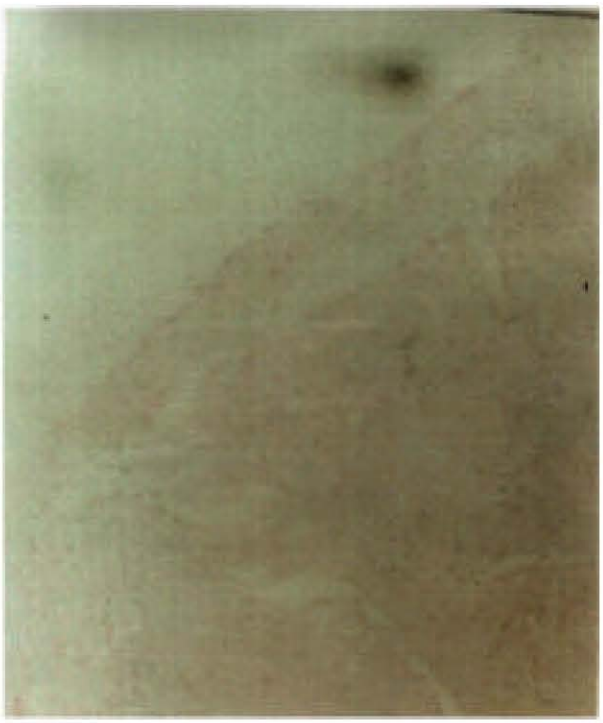

Fig. 6: The thoracic aorta of a rabbit fed normal diet showing little or no lesions. Photomicrographs are of paraffin section stained with Hematoxylin and eosin x 119

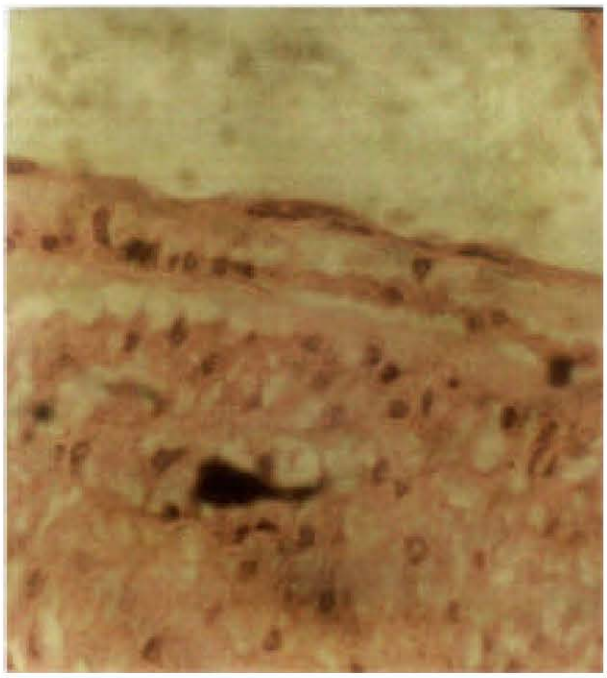

Fig. 7: The thoracic aorta of hypercholesterolemic diet showing lesions, thickened intima contained foam cells and extracellular lipid. A portion of the media is visible at the lower left corner. There is a thin fibromuscular 'cap' at the upper region and a calcified area of necrotic material just above the media

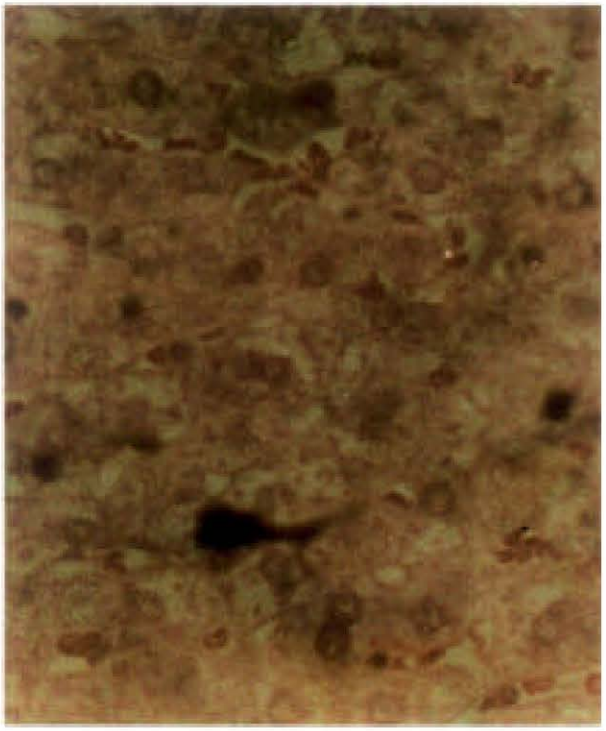

Fig. 8: The thoracic aorta of a hypercholesterolemic rabbit fed with *S. melongena* showing less lesions compared with hypercholesterolemic rabbit

reduced weight gain remarkably from 1.66 ± 0.06Kg in the control {B} to 1.05 ± 0.03, 1.19 ± 0.18kg and 0.87 ± 0.61kg respectively. The reduction was particularly significant in group E animals treated with *S. gilo*. There was however no significant difference in the food consumption of the different groups (Table 1). The fact that treatment of hypercholesterolemic rabbits with standard diet supplemented with solanum fruits, especially *Solanum gilo* reduced weight gained without affecting food consumption suggests that these fruits could be used by obese individuals to control their weight thus confirming earlier reports of the use of low cholesterol diet and various plant dietary supplements for diet weight control (Coulsan and Evans 1960; Igile, 1994; Milgate and Robert, 1995).

Results of the lipids assayed in this investigation (Table 2) showed consistent progressive and significant increases ($P < 0.05$) in serum cholesterol, triglycerides and LDL cholesterol but reduction in serum HDL cholesterol in group B rabbits compared with group A. Replacement of the hypercholesterolemic diet with standard diet for 6 weeks led to a reduction of 11.52, 16.5 and 41.13% in the serum level of cholesterol, triglyceride and LDL cholesterol respectively. This is evidence in support of the view that reduced consumption or complete elimination of cholesterol from diet will lead to a natural reduction in cholesterol level. Feeding hyperlipidemic rabbits with Solanum melongina and *Solanum gilo* for 6 weeks caused significant decline in cholesterol values by 65.40 and 57.69% and LDL cholesterol by 86 and 83% respectively. Similar reductions were observed in the level of triglyceride (Table 2).

In addition, both fruits caused significant increases in the serum level of HDL cholesterol, which is usually termed the "Good cholesterol." The combined effect of increased HDL cholesterol {Good cholesterol} and decreased LDL cholesterol {termed the Bad Cholesterol} resulted in increased HDL/LDL cholesterol ratio in the test animals. This is an additional evidence that consumption of these fruits can lead to reduction in the risk of development of heart diseases since high HDL/LDL ratio has been shown to be beneficial and is indicative of a lower risk of coronary heart diseases (Castelli, 1984).

The decline in levels of cholesterol, triglyceride and LDL cholesterol was progressive with the 6^{th} week values being lower than the 4^{th} week values after treatment commenced. This may be an indication of progressive metabolic control of the test plants on mechanisms involved in elimination of the lipids from the body. Work

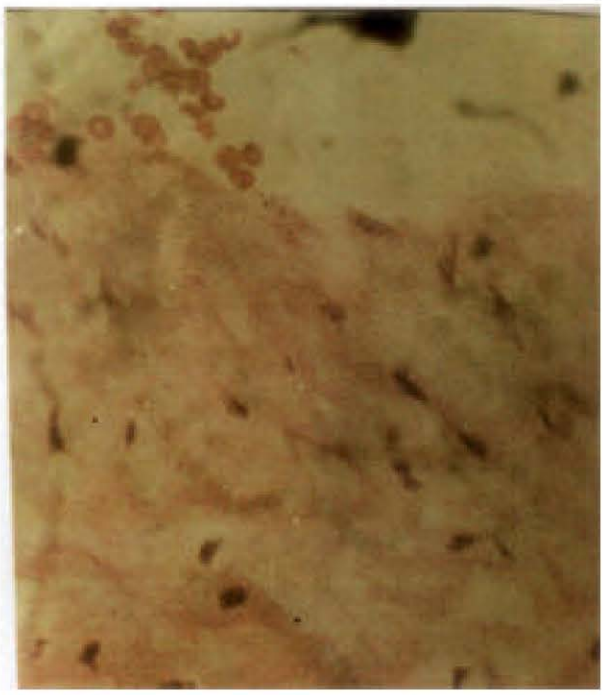

Fig. 9: The thoracic aorta of a hypercholesterolemic rabbit fed with *S. gilo* showing less lesions compared with hypercholesterolemic rabbit

is in progress to elucidate the mechanism for hypolipidemic properties of these plants.

Hypolipidemic properties have been confirmed in many plants and plant products in medicinal use. Polichetti *et al.* (1996) reported that soy bean lecithin was effective in the dietary treatment of mild cholesterolemia by stimulating the ApoA1 (high density lipoprotein) Bhandari and Zater (1998) also found that the ethanolic extract of ginger (200mg/kg) lowered serum triglycerides lipoproteins, phospholipids as well as serum and tissue cholesterol. In addition they showed that animals receiving ginger extract with cholesterol showed a lower degree of atherosclerosis. Flavonoids extracted from the fruits of *Solanum melongena* (Brinjal) at a dose of 1mg/100g BW/day showed significant hypolipidemic action in normal and cholesterol fed rats (Sudheesh *et al.*, 1997).

It may therefore be concluded from the evidences from this study, that *S. melongena* and *S. gilo* possess hypolipidemic potentials and may therefore be useful for prophylaxic and therapeutic treatment of clinical conditions associated with hyperlipidaemia such as atherosclerosis. Further studies are in progress to isolate the active ingredient and elucidate the exact mechanism of action of these fruits.

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