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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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The Effects of Heat Treated Lima Beans (*Phaseolus lunatus*) on Plasma Lipids in Hypercholesterolemic Rats

H.A. Oboh and C.O. Omofoma
Department of Medical Biochemistry, School of Basic Medical Science,
College of Medicine, University of Benin, Benin City, Nigeria

Abstract: The effects of legume consumption on a dietary induced model of hypercholesterol in rats have been determined. Grower's mash from Bendel Feed and Flour Mill (BFFM) Ewu, Nigeria, was fed to two groups of rats: the test and control groups for 30 days. To induce hypercholesterolemia in the test group, 25% Coconut oil and 1% cholesterol was included in the diet of the test group. The hypercholesterolemic rats were divided into two subgroups. The first group was fed only with heat treated Lima beans and the second group with the grower's mash mixed with 0.1% Saponin. This was done for another 30 days. Biochemical analysis was carried out on blood samples of the rats. The results show that there was a significant ($p<0.05$) reduction in the amount of serum lipids in rats fed the lima beans Legume Diet (LD) and Saponin Diet (SD) when compared to the control (CD) and Hypercholestrolemic Diet (HD). The consumption of lima beans could be recommended to also lower cholesterol and promote cardiovascular health due to the presence of saponin in the legume.

Key words: Lima beans (*Phaseolus lunatus*), Saponins, Hypercholesterolemia, Health benefit, Thermal processing

Introduction

Grain legumes are a major source of cheap proteins for humans in West Africa. In Nigeria, Cowpeas and soybeans are the most widely consumed legume seeds. Lima beans, Pigeon peas, African yam beans and jackbeans are popular legumes consumed in the Esan Community of Edo State, Nigeria (Aletor and Aladetimi, 1989; Edem *et al*., 1990). Chemical composition of these grain legumes were evaluated and shown to contain high quantities of proteins, amino acids and minerals (Apata and Ologhobo, 1994a,b). Despite the rich composition of nutrients these legumes are under-utilized because of their hard-to-cook defect, which lead to long cooking periods which require the use of scarce, expensive fuels (Uzogara and Ofuya, 1992).

Saponins are steriodal or triterpenoid glycosides which occur primarily in legumes (Oakenfull, 1981). They could be beneficial or deleterious (Shi *et al*., 2006; Koratkar and Rao, 1997). Several authors (Singh *et al*., 2002; Koulshon *et al*., 2005; Han *et al*., 2002; Brown *et al*., 1999), have reported the significance of plant sterols and fiber in lowering blood cholesterol level in rats and humans. Their high intake has been associated with reducing the risk of developing diabetes, hypertension, cancer and hypercholesterolemia (Gardner *et al*., 2005; Anderson, 2005). Messina (1999) and Erdman (2000), reported that the mechanisms by which legume seed constituents lower plasma lipid could be by LDL receptor up regulation, inhibitory effects on intestinal cholesterol absorption, increased fecal acid bile excretion, bile acid synthesis from cholesterol and cholesterol loss from the body.

The present study was designed to examine the cholesterol lowering ability of lima beans in diet induced hypercholesterolemic rats.

Materials and Methods

Preparation of experimental diets: Legume seed samples of Lima beans (*Phaseolus lunatus*) were purchased in October 2005, from Uromi Market, Uromi, Edo State, Nigeria. The whole seeds that were free from injury and insect invasion were sorted out and washed in distilled water.

The legume seeds were added to boiling distilled water (1:5w/v) at 100°C and cooked on an electric hot plate for 3 h. (Ikatherm HCT, Ika Staufen, Germany). The cooked seeds were drained to remove the cook water and dried at 70°C in an oven (Gallenkamp, UK) for 16 h. The dried seeds were cooled in a dessicator, milled to powder in a warring blender. The bean flour was stored in an air-tight container at-10°C until used as feed for the rats.

The other Diet types were prepared by weighing out the different constituents as presented in Table 1. Fresh diets were prepared daily and utilized as feed for the animals. Stale remnants were discarded after weighing.

Animals experiment: Twenty four male albino rats (3-4 months old) weighing about 200g-250g were obtained from an animal breeder in Benin City. They were marked and randomly assigned to two groups, control rats (n = 6) and experimental rats (n = 18). This was done such

Table 1: Composition of Experimental Diets

Dietary Component (g)	CD	HD	LD	SD
Growers mash (Bendel feed and Flour Mills BFFM Limited, Ewu)	99.0	73.0	-	98.9
Coconut oil	-	25.0	-	-
Cholesterol	-	1.0	-	-
Lima bean flour	-	-	99.0	-
Saponin-soyasapogenin	-	-	-	0.1
*Mineral and vitamin mix (OPIMIX PREMIX)	1.0	1.0	1.0	1.0
Total Composition	100g	100g	100g	100g

*Mineral and vitamin mix, CD: Control Diet, HD: Hypercholesterolemic Diet, LD Legume Diet, SD: Saponin Diet

(OPIMIX PREMIX)

Vitamin A	8,000,000IU	Copper	5gm
Vitamin D	1,600,000IU	Iron	20gm
Vitamin E	5,000IU	Iodine	1.2gm
Vitamin K	2,000mg	Selenium	200mg
ThiamineB_1	1,500mg	Cobalt	200mg
Riboflavin B_2	4,000mg	Cholin chloride	200gm
Pyridoxine-B_6	1,500mg	Anti oxidant	125gm
Niacin	15,000mg	Manganese	80gm
Vitamin B_{12}	10mg	Zinc	50gm
Pathothenic acid	5,000mg	Biotin	20mg
Folic acid	5,000mg		

The constituents are reported, as the composition stated on the feed bag

that the differences in average weight per group did not exceed 2 grams. The rats were housed in wire cages kept in the animal house of the Medical Biochemistry Department, School of Basic Medical Science, College of Medicine, University of Benin. The animals in the two groups were acclimatized on Growers mash (Bendel Feed and Flour Mills (BFFM) Limited, Ewu, Nigeria) for 2 weeks, prior to the study. Food and water was given *ad libitum*.

Control rats (n = 6) were fed throughout the experimental period with grower's mash (Bendel Feed and Flour Mills (BFFM) Limited, Ewu, Nigeria). This is the Control Diet (CD). Experimental rats (n = 18) received grower's mash enriched with 25% coconut oil and 1% cholesterol. After 30 days, blood samples were collected from the rats and serum lipids assayed for total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol and triacylglycerol. Total cholesterol was found to be significantly elevated to 60mg/dl. The rats were assigned to three new subgroups. Group one (n = 6) continued to receive the high fat diet (HD). The group two (n = 6) were fed on the diet containing Lima Beans (LD) only. The group three (n = 6) were fed on grower's mash mixed with 0.1% soyasapogenin (Merck) in the diet (SD). Animal management and experimental procedures were performed in strict accordance with the requirements of the National Research Council's Guide for the use of Laboratory Animals (NRC, 1985).

Collection and analysis of blood samples: Baseline blood samples were collected from the tail region of the rats in all groups, before the feeding of the different diets commenced.

After 30 days of feeding the different experimental and control diets, the animals were fasted for 18h and were anesthetized with pentobarbital (60mg/Kg body weight). Insertion was made into the heart region for collection of blood samples with the use of a needle and heparinized syringe. The blood samples were collected into labeled bijou bottles containing heparin as anticoagulant and centrifuged immediately (3,000×g for 10 min), to obtain the serum. The serum samples were stored in the biofreezer at-10°C until analyzed for, total serum cholesterol (TC), High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL) and triaclyglycerols (TAG) (Anderson *et al*., 1971) using commercial kits (Boehringer Mannheim).

Statistical analysis: Data were expressed as mean±Standard error of the mean (SEM) for each group of rabbits. Comparison between the control and experimental set of data was analyzed by the ANOVA and p values <0.05 were indicative of significance. The statistical analyses were done with INSTAT statistical package.

Results and Discussion

Table 1 shows the Composition of Experimental Diets fed to the rats.

The weight gain, feed and water intake, feed efficiency and dry fecal output of the rats in the control and experimental groups are presented in Table 2.

Statistical analysis showed that there was no significant ($p = 0.05$) decrease in weight gain, feed and water intake and feed efficiency in the experimental diet when compared with the control diet.

Table 3-5 shows the Concentration of the serum lipids in Experimental and control rats. fed the different diet; Control Diet (CD), Hypercholesterolemic Diet (HD) Saponin Diet (SD) and Legume Diet (LD). Statistical analysis showed that there were significant ($p<0.05$) differences between the basal and final values of the serum lipids in all the groups fed the different experimental diets, except the control diet which showed that there were no significant ($p<0.05$) differences between the basal and final values of the lipid profile concentrations.

Table 2: Weight Gain, Feed Intake, Water Intake, Feed Efficiency and Dry Fecal Output of Rats in the Control and Experimental Groups

Group	Control diet	Experimental diet
Weight gain (g/rat)	35±8.0[a]	25±6.0[a]
Feed intake (g/rat/day)	5.8	4.5
Water intake(ml/rat/day)	10.50±2.0[a]	9.80±2.50[a]
Feed efficiency (g/body weight/g feed)	6.03	5.5
Dry fecal output (g/rabbit/day)	2.24±1.14[a]	2.25±1.02[a]

Values are mean (gm) ± SEM of six rats; Means of the same row followed by different letters differ significantly (p<0.05)

Table 3: Effect of changes in serum lipids in rats fed the Control Diet (CD)

Serum lipids (mg/dl)	Basal values	Final values
Total Cholesterol	21.5±1.5[a]	22.3±0.87[a]
HDL-Cholesterol	8.7±2.2[a]	8.6±1.6[a]
LDL-Cholesterol	4.1±1.2[a]	5.2±1.0[a]
VLDL-Cholesterol	15.8±2.3[a]	14.9±1.8[a]
Triacylglycerol	79.4±1.2[a]	82.01±3.0[a]

Values are mean±SEM of six rats; Means of the same row followed by different letters differ significantly (p<0.05)

Table 4: Effect of changes in serum lipids in rats fed the Hypercholesterolemic Diet (HD)

Serum lipids (mg/dl)	Basal values	Final values
Total Cholesterol	20.3±1.20[a]	60.0±2.0[b]
HDL-Cholesterol	9.2±0.87[a]	36.3±1.2[b]
LDL-Cholesterol	4.1±0.6[a]	20.1±0.7[b]
VLDL-Cholesterol	18.5±1.9[a]	49.8±2.3[b]
Triacylg;ycerol	82.7±3.1[a]	218.5±3.9[b]

Values are mean±SEM of six rats; Means of the same row followed by different letters differ significantly (p<0.05)

Table 5: Effect of changes in serum lipids in rats fed the Saponin Diet. (SD)

Serum lipids (mg/dl)	Basal values	Final values
Total Cholesterol	60.0±2.0[b]	39.6±3.7[c]
HDL-Cholesterol	34.0±0.5[b]	23.0±1.7[c]
LDL-Cholesterol	16.0±1.1[b]	10.4±0.5[c]
VLDL-Cholesterol	44.67±1.3[b]	36.0±1.5[c]
Triacylg;ycerol	215.67±3.4[b]	134.0±2.3[c]

Values are mean±SEM of six rats; Means of the same row followed by different letters differ significantly (p<0.05)

Table 6: Effect of changes in serum lipids in rats fed the Legume Diet (LD)

Serum lipids (mg/dl)	Basal values	Final values
Total Cholesterol	62.0±1.56[a]	39.6±3.7[b]
HDL-Cholesterol	36.3±1.2[a]	30.0±1.7[b]
LDL-Cholesterol	20.4±0.7[a]	14.0±0.5[b]
VLDL-Cholesterol	49.8±2.3[a]	26.0±1.5[b]
Triacylg;ycerol	218.5±3.9[a]	104.0±2.3[b]

Values are mean±SEM of six rats; Means of the same row followed by different letters differ significantly (p<0.05)

Table 4 represents the basal and final values of serum lipids of the rats fed the hypercholesterolmic diet. The hypercholesterolemic diet led to a significant increase (p<0.05), in the levels of the serum lipids. This was diet induced by the addition of 25% coconut oil and 1% cholesterol to the growers mash used for the hypercholesterolmic diet.

Table 7: Mean Concentrations of Serum lipids on rats fed the control, hypercholesterolemic and Saponin Diet

Serum lipids (mg/dl)	CD	HD	SD	LD
Total Cholesterol	21.5±1.5[a]	60.0±2.0[b]	39.6±3.7[c]	39.6±3.7[c]
HDL-Cholesterol	8.7±2.2[a]	36.3±1.2[b]	23.0±1.7[c]	30.0±1.7[c]
LDL-Cholesterol	4.1±1.2[a]	20.1±0.7[b]	10.4±0.5[c]	14.0±0.5[c]
VLDL-Cholesterol	15.8±2.3[a]	49.8±2.3[b]	36.0±1.5[c]	26.0±1.5[c]
Triacylg;ycerol	79.4±1.2[a]	218.5±3.9[b]	134.0±2.3[c]	104.0±2.3[d]

Values are mean±SEM of six rats; Means of the same row followed by different letters differ significantly (p<0.05). CD: Control Diet, HD: Hypercholesterolemic Diet, LD Legume Diet, SD: Saponin Diet

The changes in serum lipids as affected by the saponin diet and legume diet are represented in Table 5 and 6 respectively. The values of the serum lipids which includes, total cholesterol, HDL-Cholesterol, LDL-Cholesterol, VLDL and Triacylglycerol, were significantly (p = 0.05) reduced in the rats fed the saponin and legume diet.

A significant reduction in serum lipids has been observed in experimental rats, when heat-treated legumes, were fed to hypercholesterolemic rats (Zulet and Martinez, 1995; Singh *et al.*, 2002). The saponin content of raw and processed lima beans has been determined and found to be 1.26g/Kg for the raw and 732.3g/Kg for the heat treated samples respectively (Oboh *et al.*, 1998; Oboh and Osagie, 2003).

Saponins form strong insoluble complexes with cholesterol and bile making them unavailable for absorption (Oakenfull and sidhu, 1990). This mixture is then removed from the body through the normal elimination process. Increased bile acid excretion may cause compensatory increase in bile acid synthesis from cholesterol in the liver. As the body needs more cholesterol for bile acid production used for digestion, the liver removes cholesterol from the bloodstream and thus lowers serum cholesterol. These findings confirm the role of saponins in lowering blood cholesterol as suggested by Oakenfull and sidhu (1984). The saponins in lima beans can be extracted and used in the synthesis of drugs which can lower blood cholesterol. This will provide a good market for the use of lima beans in the manufacture of cholesterol lowering drugs.

Table 7 Represents a summary of the mean concentration of serum lipids in rats, fed the control and Experimental Diets. Significant (p<0.05) differences were observed in rats fed the Control, Saponin and hypercholesterolemic diet. No significant difference (p<0.05) was observed between the saponin and legume diet for the serum lipids assayed except for the triacylglycerol values, where, the legume diet, had significantly (p<0.05) reduced the values, when compared to the saponin diet. It is notable that, the lima beans were cooked without dehulling. Oshodin and Adeladun (1993), reported that lima beans contain a fiber level of 4.3%. The fiber content of the legume could have a contributory role in the reduction of cholesterol levels. The role of dietary fiber in the reduction of cholesterol has been reported (Glore *et al.*, 1994). This study shows that the legume diet gave lower serum lipid values in dietary induced hypercholesterolemic rats. The presence of saponins and fiber in the heat treated legume is probably responsible for the cholesterol lowering ability of the legume.

Conclusion: The Hypocholesterolemic effect of lima beans could be due to the combined effect of the saponins and fiber present. This study shows that there is a distinct beneficial effect from the consumption of heat treated lima bean; therefore the use of lima beans is shown to likely diminish the risk of artherogenic dyslipidemia.

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