

Hypocholesterolemic Effect of Pedada (*Sonneratia caseolaris*) Fruit Flour in Wistar Rats

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Abstract : Pedada fruit is rich in dietary fiber and phenolic compounds which can reduce cholesterol levels in humans. The aim of this study was to investigate the cholesterol-lowering effect of pedada fruit flour (PFF) in rats. Five normal male Wistar rats were the normal control group (0) were fed AIN-93 M and twenty hypercholesterolemia male Wistar rats were randomly divided into 4 groups with different diets for 4 weeks. Group 1 (the hypercholesterolemia control) was fed an AIN-93M diet, no supplements. Groups 2, 3 and 4 were fed AIN-93M supplemented with 3%, 6%, and 9% PFF, respectively. Blood samples were taken on week 0, 1st, 2nd, 3rd, and 4th for total plasma cholesterol, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and triglyceride analyses. The results showed that the PFF supplement significantly decreased ($P = 0.000$) the total plasma cholesterol levels by 32.45 -58.87 mg/dl; LDL-c by 32.06 - 55.54 mg/dl ($p = 0.000$); triglyceride by 9.20 - 23.93 mg/dl ($P = 0.000$) respectively. HDL-c concentration remained unaltered ($P = 0.997$). These results indicate the beneficial effect of PFF in the treatment of hypercholesterolemia. This effect is possible related to dietary fiber and phenol compounds on PFF.

Keywords: Pedada fruit flour (PFF), Hypercholesterolemia, Total cholesterol, LDL-c, HDL-c, Triglyceride, Rat.

Introduction

Pedada (*Sonneratia caseolaris*) is a mangrove tree species belonging to family *Sonneratiaceae*, which is native to South and South East Asia. The immature fruits are sour but edible, used as a vegetable and flavouring agent. However, mature fruits have a cheese like flavour and are eaten raw or cooked¹. In herbal medicine, the fruit is used as an analgesic and anti-inflammatory agent², possesses hepatoprotective activity³, and also has hypoglycemic effects¹.

Previous research has shown that the pedada fruit flour (PFF) is rich in dietary fiber (63.70%) consisting of soluble (9.80%) and insoluble (53.90%) dietary fiber and also has high levels of phenolic compounds (30.61 mg GAE/g of PFF) that can help to protect cardiovascular disease.

Cardiovascular disease (CVD) is the leading cause of death in the world⁴, and high cholesterol levels are a major risk factor⁵ and dietary fiber helps to protect against cardiovascular disease by improving blood lipid profile, lowering blood pressure, and reducing inflammation⁶. Consumption of dietary fiber lowers the low density lipoprotein cholesterol (LDL-c) plasma levels as has been demonstrated in both human and animal studies⁷⁻⁸. A study observed a decrease of 12.5 mg/dl in total plasma cholesterol levels ($P < 0.05$) for each 10 grams increase in the consumption of dietary fiber over 7 years in a cohort of 316 Japanese-Brazilians subjects⁹. Soluble dietary fiber 2-10 g/d was associated with small but significant decrease in total cholesterol [$-0.045 \text{ mmol.L}^{-1}.\text{g}^{-1}$ (95% CI: $-0.054, -0.035$)] and LDL-c [$-0.057 \text{ mmol.L}^{-1}.\text{g}^{-1}$ (95% CI: $-0.070, -0.044$)]. Various soluble dietary fiber reduce total cholesterol and LDL-c by similar amounts¹⁰. Dietary phenolic compounds, ubiquitous in vegetables and fruits and their juices possess antioxidant activity that may also have a protective effect towards the susceptibility of LDL-c to oxidative modification and ultimately, to atherosclerosis¹¹. Various in vitro studies using different methods of oxidation have shown that phenolic compounds from red wine, green tea, and olive oil can inhibit LDL-c oxidation and reduce risk factor for CVD¹²⁻¹⁴.

The present study was designed to investigate the cholesterol lowering effect of PFF in rats by determining levels of total plasma cholesterol, LDL-c, HDL-c and triglyceride. The rats were considered suitable for investigating the effects of dietary interventions on the parameters of kinetics, including cholesterol absorption and synthesis, because the cholesterol metabolism process is similar in humans and rats¹⁵.

Materials And Methods

Materials

Pedada fruit (50-55 g; ± 2 months) were harvested from mangroves in Wonorejo village, Surabaya, Indonesia. Male Wistar rats (*Rattus norvegicus*) weighing 155-208 grams were purchased from animal physiology laboratory, the Science and Mathematics Faculty, Brawijaya University, Indonesia. Standard diet was AIN-93M [16], Pure cholesterol was obtained from Sigma (Saint Louis, MO), liquid pork fat and egg yolk were purchased from markets in Malang. Plasma lipid profiles were analyzed by using enzymatic methods with kit no. 10 130 021 for total plasma cholesterol, 10 571 021 for triglycerides, and 10 350 022 for HDL-c which were obtained from DiaSys Diagnostic Systems GmbH, Holzheim, Germany.

Methods

Preparation of pedada fruit flour (PFF)

The mature pedada fruit were collected and selected randomly from different parts of pedada trees, and were immediately transferred to the laboratory and freeze dried. After that, the fruit were peeled, and pulped in a blender with distilled water (1:3). Fruit-pulp was filtered to remove the seeds, and dried in a drying cabinet for 15-18 hours at 50-60°C. After that, it was grounded until 80 mesh.

Animals and study design

A 4-week rat trial, approved for ethical clearance by Animal Care and Use Committee, Brawijaya University Indonesia with ethical clearance No. 101-KEP-UB. Twenty male Wistar rats (weighing 155-208 g) were fed with a high calorie and cholesterol (HCC) diet by enriching the diet with 13.3% yellow egg, and 10% liquid pork fat. Each animal received 0.015 g cholesterol (Sigma) by orally and 15 g of HCC diet daily during 3 weeks and had free access to water. As a normal control was used five normal Wistar rats that fed AIN-93M. Animals were maintained individually in the metabolic cage in a temperature-controlled room ($22 \pm 2^\circ\text{C}$) under a light/dark cycle of 12 h.

Once hypercholesterolemia was reached (total plasma cholesterol of ≥ 200 mg/dl), the induction of cholesterol and HCC diet was stopped. The rats were randomly divided into four groups of five animals according to the

treatment received. For the normal and hypercholesterolemia control rats were fed with AIN-93M. Treated groups fed AIN-93M supplemented with (a) 3% PFF; (b) 6% PFF; and (c) 9% PFF. Each rat received 15 g experimental diet daily during 4 weeks and had free access of water. Body weight and blood plasma profile of rats were measured every week. Blood was taken from the tail of rats by intra vena. Plasma samples were separated by centrifuge at 3000 rpm for 20 minutes and stored at -20°C for further analysis (total cholesterol, HDL-c and triglyceride).

Biochemical analysis

Total cholesterol plasma high-density lipoprotein cholesterol (HDL-c) and triglyceride (TG) were measured by enzymatic photometric test¹⁷. Total cholesterol was measured based on the liberation of ester cholesterol lipoproteins by the effect of the detergent. HDL-c in plasma was measured by precipitation of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) by magnesium ion. Plasma levels of triglycerides were assessed by precipitation in the presence of amphiphilic polymers. The results were also expressed as non-HDL-c instead of LDL-c because of the Friedwald equation¹⁸:

$$LDL = total\ cholesterol - \left(HDL + \frac{Triglyceride}{5} \right)$$

Statistical analysis

Results are expressed as mean \pm standard error of the mean (S.E.M). To determine the effect of dietary intervention on the plasma cholesterol levels, data were analysed by one way analysis of variance (ANOVA) using the general linier model procedure of SPSS 16.0 software. Significant differences between dietary treatments were analysed by least significance difference (LSD). $P < 0.05$ values were considered as significantly different.

Table 1: The diet types and composition of rats

Composition	AIN-93M (%)	HCC (%)	3% PFF (%)	6% PFF (%)	9% PFF (%)
Corn starch	46.57	27.27	43.57	40.57	37.57
Casein	14.00	14.00	14.00	14.00	14.00
Dextrin	15.50	15.50	15.50	15.50	15.50
Sucrose	10.43	10.43	10.43	10.43	10.43
Soybean oil	4.00	-	4.00	4.00	4.00
Cellulose	5.00	5.00	5.00	5.00	5.00
Mineral mix	3.50	3.50	3.50	3.50	3.50
Vitamin mix	1.00	1.00	1.00	1.00	1.00
PFF	-	-	3.00	6.00	9.00
Yellow egg	-	13.30	-	-	-
Liquid pork fat	-	10.00	-	-	-
Total	100.00	100.00	100.00	100.00	100.00

HCC = High calorie and cholesterol diet

PFF = Pedada Fruit Flour

Results And Discussion

All the rats were in healthy condition throughout the study. The diet was controlled every day and added when necessary. Cages were cleaned once a week and the replacement of husk as bedding was also carried out at the same time.

Food intake and weighing of rats after supplemented PFF in the diet

Table 2 shown that there were significant differences in food intake and weighing of rats among all the groups (P = 0.000), this fact indicates that PFF can be supplemented into the diet and affecting the rat appetite, and food intake. PFF was given in the diet and not by gavage, so the intake depended on the rat’s appetite, and became the limitation of this study.

Dietary fiber intakes are presented in Figure 1. The addition of PFF in the diet will increase the content of phenand dietary fiber.The higher supplemented PFF, higher of dietary fiber intake, and lower of diet intake and weighing of rats. A diet of food that provide adequate fiber is usually less energy, dense and larger in volume than a low-fiber diet that may limit spontaneous intake of energy¹⁹. Rolls et al.²⁰ recently concluded that fruit and vegetable consumption does play a role in weight management, probably because their consumption decreases energy density, promotes satiety, and decreases energy intake. An additional 14 g/d of fiber resulted in a 10% decrease energy intake and an energy loss of greater than 1.9 kg through approximately 3.8 mo of intervention²¹. In addition, the beneficial effect of dietary fiber on weight regulation was seen for soluble and insoluble dietary fibers. In addition, the beneficial effect of dietary fiber on weight regulation was seen for soluble and insoluble dietary fibers.

Dietary fiber acts as a physiological obstacle to energy intake by at least three mechanisms: 1) fiber displaces available calories and nutrients from the diet; 2) fibers increase chewing, which limits intake by the promoting secretion of saliva and gastric juices, resulting in an expansion of the stomach and increased satiety; and 3) fiber decreases the absorption efficiency of the small intestine²².

Table 2. Average of food intake and weighing in each group

Groups	Food intake (g/day)	Weighing (g)
Normal	13,32 a	240,92 d
Hypercholesterolemia	12,98 a	256,28 a
3% PFF	12,01 b	254,76 a
6% TBP	11,63 bc	251,52 b
9% TBP	11,48 c	245,16 c

*Different code indicated the differences in one column

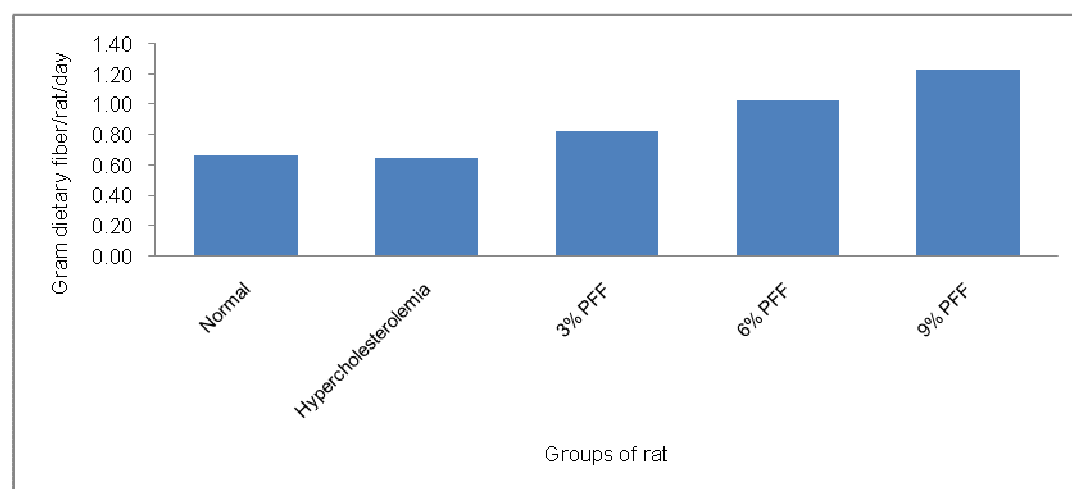


Figure 1. Average dietary fiber intake (gram/rat/day)

The profile lipids of rats during supplemented PFF in the diet for 4 weeks

The results obtained showed an improvement in the lipid profile in rats fed on diets supplemented with 3%, 6% and 9% of PFF. Significant decreases were observed in total plasma cholesterol concentration (32.45 mg/dl, 50.51 mg/dl and 58.87 mg/dl, respectively ($P = 0.000$)), LDL-c (32.06 mg/dl, 48.23 mg/dl, and 55.54 mg/dl, respectively ($P = 0.000$)), and triglyceride (9.20 mg/dl, 17.18 mg/dl, and 23.93 mg/dl, respectively ($P = 0.000$)) (Table 3). However, the increase in HDL-c concentration was not significant. The results obtained showed an improvement in the rat lipid profiles after 4-weeks treatment with PFF in diets (3%, 6% and 9% from total diet/day). No significant differences were observed between normal and hypercholesterolemia control groups after 4-weeks treatment. Although there were improvements in plasma lipids concentrations, they did not reach normal values. This may due to the fact only received PFF for 4 weeks i.e. not long enough. The differences among the rat groups after treatment are presented in Table 4.

Table 3. Profile lipids of rats during the treatment for 4 weeks

Week	Groups of rat				
	Normal	Hypercholesterolemia	3% PFF	6% PFF	9% PFF
Total Cholesterol					
0	70.23 ± 1.28	215.30 ± 1.81	218.43 a	217.14 a	216.40 a
1	70.70 ± 1.06	215.81 ± 2.02	211.90 b	204.65 b	201.49 b
2	70.59 ± 0.91	216.47 ± 2.13	202.71 c	192.40 c	186.97 c
3	71.14 ± 0.79	217.54 ± 1.96	194.09 d	179.12 d	172.42 d
4	71.85 ± 0.82	218.01 ± 2.05	185.98 e	166.63 e	157.53 e
LDL-c					
0	12.03 ± 0.67	142.35 ± 1.34	146.37 a	145.55 a	144.67 a
1	11.96 ± 0.99	142.94 ± 1.26	140.00 b	133.79 b	130.60 b
2	11.41 ± 0.98	143.14 ± 1.55	130.80 c	121.94 c	116.80 c
3	11.63 ± 1.12	143.66 ± 1.44	122.58 d	109.51 d	103.43 d
4	12.05 ± 1.07	143.47 ± 1.52	114.31 e	97.32 e	89.13 e
Triglyceride					
0	51.96 ± 1.16	145.24 ± 0.97	147.60 a	146.27 a	145.98 a
1	52.95 ± 1.01	146.33 ± 1.01	145.31 ab	141.96 b	140.22 b
2	53.73 ± 1.03	147.01 ± 1.03	142.88 bc	137.57 c	134.17 c
3	54.34 ± 1.00	147.81 ± 1.00	140.65 cd	133.05 d	128.03 d
4	55.18 ± 0.88	148.91 ± 0.89	138.40 d	128.47 e	122.05 e
HDL-c					
0	47.80 ± 1.41	43.90 ± 0.69	42.54 ± 0.50	42.34 ± 0.50	42.54 ± 0.50
1	48.15 ± 1.45	43.60 ± 0.67	42.84 ± 0.70	42.47 ± 0.70	42.84 ± 0.70
2	48.43 ± 1.44	43.92 ± 0.57	43.34 ± 0.72	42.94 ± 0.72	43.34 ± 0.72
3	48.64 ± 1.44	44.32 ± 0.69	43.38 ± 0.69	43.00 ± 0.72	43.38 ± 0.69
4	48.76 ± 1.29	44.76 ± 0.67	44.00 ± 0.70	43.62 ± 0.69	44.00 ± 0.70

*Different code indicated the differences in one column.

Table 4. The differences of profile lipids among of all groups after the treatment of PFF for 4 weeks

Groups of rat	Profile Lipid			
	Total Cholesterol (mg/dl)	LDL-c (mg/dl)	Triglyceride (mg/dl)	HDL-c (mg/dl)
Normal	71.85 e	12.05 e	55.18 e	48.76 ± 1.29
Hypercholesterolemia	218.01 a	143.47 a	148.91 a	44.76 ± 0.67
3% PFF	185.98 b	114.31 b	138.40 b	44.00 ± 0.70
6% PFF	166.63 c	97.32 c	128.47 c	43.62 ± 0.69
9% PFF	157.53 d	89.13 d	122.05 d	44.00 ± 0.70

*Different code indicated the differences in one column.

This effect of PFF on cholesterol-lowering effect was engendered by different mechanism. In order to explain the cholesterol-lowering effect of PFF, it is necessary to consider its richness in dietary fiber (63.70%) and total phenol (30.61 mg GAE/g of PFF). This dietary fiber has shown beneficial effects like decreasing cholesterol and improving lipoprotein levels, and phenol compounds are known to have beneficial effects by protecting human (LDL) against lipid peroxidation and promoting HDL-mediated cholesterol efflux to reduced atherosclerosis¹¹.

Most the soluble fiber on PFF is pectin (8.90%). Pectin is one of a category of complex polysaccharides found in the primary cell walls of most plants. The dominant component is galacturonic acid with neutral sugars, primarily galactose, arabinose, rhamnose and xylose²³. In the small intestine, pectin and other gel-forming polysaccharides increase viscosity and affect the process of digestion and absorption. The physiological effects of pectin include a reduction in plasma and liver cholesterol concentrations in rats²⁴⁻²⁵. Pectin in animals, a decreases of the ileum digestibility of organic matter and protein²⁶, and increases of the fecal excretion of nitrogen²⁷. Pectin passes the small intestine as a macromolecule²⁸. In humans, approximately 90% of ingested pectin is recovered in the terminal ileum²⁹. In the lower intestinal tract pectin is fermented into short chain fatty acid³⁰. When pectin is present in the diet of rats, the acetate and propionate contents are increased and that of butyrate decreased³¹. Generally, soluble fiber reduces dietary fat and cholesterol uptake in the intestine in part by thickening the unstirred water layer that cholesterol diffuses³²⁻³³. Furthermore, they interfere with the enterohepatic circulation of bile acids, thereby increasing the fecal loss of bile acids³⁴.

Another study carried out with some soluble fiber, confirmed the above and showed that the addition of dietary fiber was associated with small but significant decrease in total cholesterol as well as LDL-c concentrations. The effects soluble fiber on plasma lipid from oat, psyllium or pectin was significantly different but HDL-c was not significantly influenced by soluble fiber. Lipid changes were independent of study design, treatment length, and background dietary fat content¹⁰. Hypertriglyceridemia have also found in Zucker fatty rats³⁵⁻³⁶. In this study, the addition of 10% soluble fiber to the diet resulted in a significant decrease in triglycerides. However the triglycerides-lowering effects of soluble fiber appeared less consistent and, in some cases, less robust. In barley-derived soluble fiber was found reduced triglycerides³⁷, but for the other soluble fiber, including psyllium, oat bran, and guar gum, the reduction in cholesterol occurred without significant changes in serum triglycerides³⁸.

Other components present in PFF that play a role in reducing atherosclerosis is phenol. The supplementation of PFF in the diet will increase the content of phenolic compounds (Figure 2). Phenolic compounds have been shown to be successfully attenuate hypercholesterolemia³⁹⁻⁴⁰. Phenol has a protective effect towards the susceptibility of LDL-cto oxidative modification and ultimately, to atherosclerosis¹⁷. Few studies have investigated the effect of antioxidant in promoting HDL-mediated cholesterol efflux. Cholesterol efflux capacity increases with the fluidity of HDL, which in turn depends on the length and saturation of fatty acids in the HDL composition⁴¹. Previous studies have suggested that when HDL is oxidized, a process that leads to a loss of polyunsaturated fatty acids, their capacity to remove free cholesterol from cell is decreased, due in part to a reduction in HDL fluidity⁴².

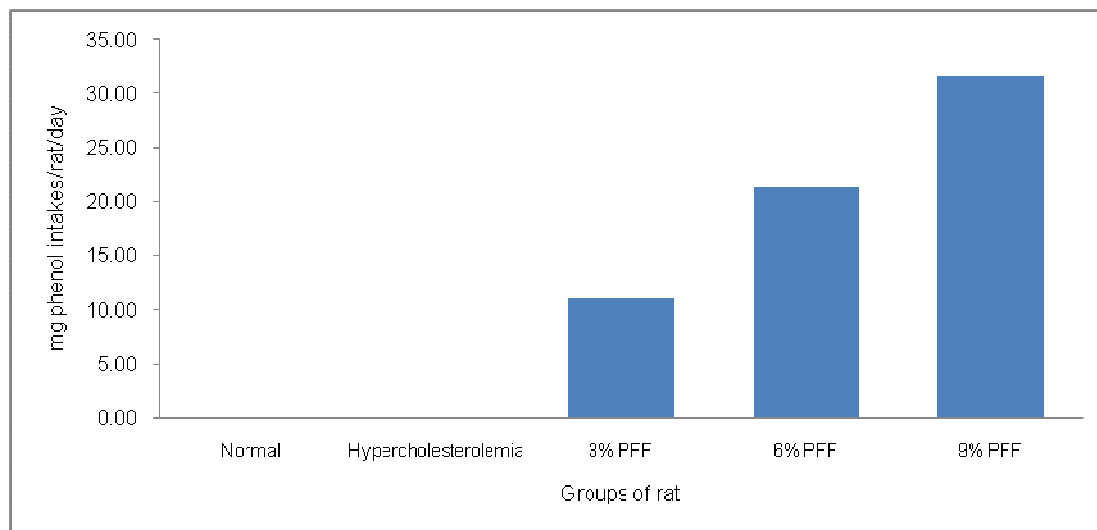


Figure 2. Average phenol intake (mg/rat/day)

In conclusion, supplemented PFF on the diet at the level 3%, 6% and 9% for 4 weeks could improve the profile lipids by decreasing of total plasma cholesterol, LDL-c and triglyceride levels, but not affecting the HDL-c levels. Supplemented PFF on the diet was also affecting the weighing loss of rats.

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