

The Functional Properties of Ledok Added with Red Beans Viewed from in Vivo Hypocholesterolemic Effects

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Abstract

Ledok is a traditional food found in Nusa Penida, Bali, it is porridge made from local food without rice, i.e. yellow cassava, white corn, beans, green vegetables and seasonings. Nuts as one of the main ingredients in the making of *ledok* are the sources of dietary fiber. The problem is whether *ledok* made from red beans have the hypocholesterolemic properties. The objective of this study was to test the hypocholesterolemic properties of *Ledok* added with red beans. To achieve this objective, it was conducted *ledok* hypocholesterolemic properties in vivo experiment by using Wistar rats. The study design is a Post Test Control Group Design. The treatments were four, namely: P0 = standard feed (negative control); P1 = standard feed + oral feeding (feeding tube) of egg yolk (positive control); P2 = standard feed: *Ledok* flour (90: 10) + oral feeding of yolk; P3 = standard feed: *Ledok* flour (80: 20) + oral feeding of yolk. The treatment was repeated six times (6 rats), so totally it was used as many as 24 Wistar rats. The experiment was conducted for 30 days and analysis was conducted on the content of total cholesterol, total triglyceride, LDL and HDL from the blood of rats before and after treatments. The experimental results showed that the addition of 20% *Ledok* resulted in the increase in cholesterol levels, the lowest HDL and triglycerides are consecutively at 9.28%, 10.41% and 4.41%, but on the contrary the decrease in the LDL blood levels was 3.61% or the *Ledok* addition of 20% can inhibit the increase in cholesterol and triglyceride levels as well as lowering the LDL blood levels.

Keywords: *Ledok*, nuts, dietary fiber and hypocholesterolemic.

1. Introduction

Ledok, a kind of porridge, is one of the traditional foods in Nusa Penida, Klungkung, Bali. *Ledok* uses main raw materials of corn, yellow cassava and beans as well as vegetables and spices. The vegetables used are spinach and basil, while the cooking spices consist of garlic, chilli, bay leaves and galangal. The uniqueness of this *ledok* that it does not use rice, so this is considered as an effort to support the national food security.

Ledok is food that could potentially be developed into functional foods not only because of its high nutritional content but also it contains bioactive component such as dietary fiber. *Ledok* which is made with the addition of purple sweet potato contains dietary fiber of 21.42% (Suter, *et al.*, 2013). Dietary fiber is part of carbohydrate that can not be digested by digestive enzymes (Winarno, 1997 and Linder, 2010). According to Kusharto (2006), dietary fiber is not only found in vegetables and fruits, but also found in other foods such as rice, potatoes, beans and tubers. Consumption of enough fiber can prevent the onset of some degenerative diseases such as heart disease, diabetes mellitus and other diseases associated with obesity. Dietary fiber can not be digested and absorbed by the human digestive tract, including indigestible polysaccharides, such as cellulose, hemicellulose, oligosaccharides, pectin, gum, and waxes (Marsono, 2004; Astawan and Wresdiyati, 2004). Some viscous fibers can lower the blood cholesterol such as gum, pectin and products derived from legumes (Rusilanti and Kusharto, 2007). Fibers derived from cereals (grains) are generally water-insoluble, whereas fiber derived from vegetables, fruits and legumes tend to be soluble (Astawan, *et al.*, 2004). Dietary fiber has properties of improving health or preventing degenerative diseases such as diabetes mellitus, coronary heart disease and high blood pressure (Subroto, 2008). Soluble dietary fiber has a hypocholesterolemic properties that can lower the cholesterol levels (Stark and Madar, 1994).

Legumes are one of the foodstuffs potentially as a source of vegetable protein and dietary fiber. Types of legumes commonly eaten include red bean, pigeon pea, cowpea and soybeans. In the first year of research (2015), it has experimented *ledok* made of six types of legume namely peanuts, fava beans, mung bean, pigeon pea, soya beans and red beans. Based on the content of nutrients, dietary fiber and sensory properties, it was obtained the best *ledok* made from red beans (Yusa, *et al.*, 2015), but whether *ledok* made from red beans has hypocholesterolemic properties is not yet known. On the basis of the above description, then it was conducted a research with the objective to determine whether *ledok* made from red beans has hypocholesterolemic properties (it can lower blood cholesterol levels).

2. Materials and Methods

2.1. Ingredients and Equipments

The ingredients used to make *ledok* were: white corn, yellow cassava, cowpea, beans, bay leaf, basil, spinach leaves, galangal, garlic, salt, and lemon. The chemicals used were of reagent kits (cholesterol, triglycerides, LDL

and HDL). Experimental animal used was male Wistar strain rats and the mice feed. The equipments used were equipment for cooking *ledok* such as gas stoves, knives, tool grinder / blender, and pots, rat cage and its equipment.

2.2 Implementation of Research

a. Design of Experiments

The type of research was laboratory experiment on the Wistar rats by using a research design of Post Test Control Group Design (Notoatmojo 2002 in Maligan, *et al.*, 2011). Selection of the object of research for the grouping and the treatment using a group random design. This experiment used four treatments as follows:

P0: standard feed (negative control)

P1: standard feed + oral feeding (feeding tube) of egg yolk (positive control)

P2: standard feed + *ledok* flour (10%) + feeding tube of yolk.

P3: standard feed + *ledok* flour (20%) + feeding tube of yolk.

Each treatment was repeated six times (consisting of 6 Wistar rats), so that the number of rats required was 24 Wistar rats.

b. Ways of Making *ledok*

Ledok was made by using the best formula of *ledok* from the research phase 1 (the first year, 2015), namely white corn (125 g), yellow cassava (125 g), spinach (62.5 g), basil (25 g), bay leaves (5 g), galangal (5 g), water (4 x weight of the total ingredients), seasoning (19 g), cowpea (62.5 g) and red beans (62.5 g). The *ledok* making process was as follows: all of the materials were cleaned, then weighted according to the above formula of *ledok*. Water was heated in a saucepan until boiling (temperature reached 100 ° C). Rice white maize, cowpea, beans, and bay leaves, put and cooked until half cooked (for ± 15 minutes at 100 ° C). Other materials of yellow cassava tubers, ginger, spinach leaves and basil leaves were cleaned, washed and chopped, and spices (garlic, salt, lime skin, and chili with a ratio of consecutively; 4 : 4 : 5 : 6) put into the pot, then continued stirring until evenly blended and boiled until cooked (for 45 minutes), finally *ledok* was ready to eat.

c. The Making of *ledok* Flour

Instant *ledok* was made into flour in the following ways: instant *ledok* was mixed until evenly blended and then dried in the oven at 70 ° C until dry. Furthermore, it was reduced in size by means of a blender, then sieved by 16 mesh sieve to obtain *ledok* flour.

d. The Making of Standard and Treatment Feeds

The making of standard feed was in accordance with the standards according to AIN 1993 (Reeves, *et al.*, 1993). The compositions of the standard feed material can be seen in Table 1. The treatment feed of *ledok* flour (P2) was made by mixing the standard feed with *ledok* flour by a ratio of 90: 10, and the treatment feed (P3) was made by mixing standard feed with *ledok* flour by a ratio of 80: 20.

Table 1. Compositions of standard feed (Reeves, *et al.*, 1993)

Ingredients	Standard feed (g / kg)
Cornflour	620.69
Casein	140
Sucrose	100
Soybean oil	40
CMC	50
Mineral mix	35
Vitamin mix	10
L-cysteine	1,8
Choline bitartrate	2,5
Total	999.99

e. Bioassay

Rats used in this research were Wistar rats with weight ranging between 100-200 grams, totaling of 24 rats. Rats were adapted to the standard feed for a week, after which the rats were given different treatment feeds. The duration of the implementation of the bioassay was 30 days. During the experiment, the feeds and drinks were replaced every day. The observations of the feed consumption were carried out every day. The weighing of the rats, the testing of lipid profiles (total cholesterol, triglycerides, LDL and HDL) were carried out before the treatments (day 0) and 30 days after being given the treatments.

f. Observed Parameters

Analysis of lipid profile of blood serum of rats include total cholesterol by using GOD-PAPmethod (Deeg, *et al.*, 1983 and Artiss, *et al.*, 1997), HDL cholesterol by the CHOD-PAPmethod (Lopes-Virella, *et al.*, 1997), triglycerides by the GPO-PAP method (Fossati and Principe, 1982), LDL cholesterol was calculated by Friedewald equation, *et al.*, 1972).

3. Results and Discussion

On the pretest (prior to treatment feed containing *ledok*), analysis was done on cholesterol, HDL, LDL and triglyceride of blood carried out on six (6) rats taken randomly from 24 male rats. The average value of cholesterol, HDL, LDL and triglyceride blood of rats in succession are 72.05 mg / dL, 61.83 mg / dL, 2.47 mg / dL and 38.74 mg / dL, while the result of the analysis of cholesterol, HDL, LDL and triglyceride blood of rats after 30 days of being given treatment feed, in succession is presented in Tables 2, 3, 4 and 5.

Table 2. The average value of cholesterol (mg / dL) on days 0 and 30.

Treatments *)	Day		The increase in cholesterol levels (%)
	0	30	
P0	72.05	93.12	29.24
P1	72.05	89.75	24.57
P2	72.05	85.01	17.99
P3	72.05	78.74	9.28

*)P0: standard feed (negative control)

P1: standard feed + oral feeding (feeding tube) of egg yolk (positive control)

P2: standard feed + *ledok* flour (10%) + feeding tube of yolk.

P3: standard feed + *ledok*flour (20%) + feeding tube of yolk.

It can be seen from Table 2 the increase in blood cholesterol levels in all treatments after the administration of treatment feeds for 30 days. The highest increase in cholesterol levels occurred on the control namely at 29.24%, while the lowest occurred in the treatment of *ledok* addition of 20% i.e. in the amount of 9.28% or the use of *ledok* can inhibit an increase in blood cholesterol the most.

Table 3. The average value of the levels of HDL (mg / dL) on day 0 and day 30.

Treatment *)	Day		The increase in HDL levels (%)
	0	30	
P0	61.83	70.69	14.33
P1	61.83	74.24	20.07
P2	61.83	68.96	11.53
P3	61.83	68.27	10.41

*) P0: standard feed (negative control)

P1: standard feed + oral feeding (feeding tube) of egg yolk (positive control)

P2: standard feed + *ledok* flour (10%) + feeding tube of yolk.

P3: standard feed + *ledok*flour (20%) + feeding tube of yolk.

It can be seen from Table 3 the increase in the HDL blood levels in all treatments after the administration of treatment feeds for 30 days. The highest increase occurred in the positive control at 20.07%, while the lowest at the treatment of *ledok* addition of 20%, which amounted to 10.41%.

Table 4. The average value of the levels of LDL (mg / dL) on day 0 and day 30.

Treatment *)	Day		Increase/decreaseof LDL levels (%)
	0	30	
P0	2.47	8.56	246.56
P1	2.47	7.22	192.31
P2	2.47	5.96	141.29
P3	2.47	2.38	-3.64

*) P0: standard feed (negative control)

P1: standard feed + oral feeding (feeding tube) of egg yolk (positive control)

P2: standard feed + *ledok* flour (10%) + feeding tube of yolk.

P3: standard feed + *ledok*flour (20%) + feeding tube of yolk.

It can be seen from Table 4 the increase in the LDL blood levels in the negative control, the positive control and the *ledok* treatment of 10% after the administration of treatment feeds for 30 days, while in *ledok* treatment of 20% occurred decrease in LDL blood levels.

Table 5. The average value of the levels of triglyceride (mg / dL) on day 0 and day 30.

Treatment *)	Day		The increase levels of triglycerides (%)
	0	30	
P0	38.74	69.37	79.06
P1	38.74	41.45	6.99
P2	38.74	50.43	30.17
P3	38.74	40.45	4.41

*) P0: standard feed (negative control)

P1: standard feed + oral feeding (feeding tube) of egg yolk (positive control)

P2: standard feed + *ledok* flour (10%) + feeding tube of yolk.

P3: standard feed + *ledok* flour (20%) + feeding tube of yolk.

It can be seen from Table 5 the increase in triglyceride blood levels in all treatments after the administration of treatment feeds for 30 days. The highest increase of triglycerides occurred in the negative control i.e. at 79.06%, while the lowest occurred at the *ledok* addition of 20% increase in the amount of 4.41% .

4. Conclusion

The experimental results showed that there was the effect of treatment of *ledok* addition to lower cholesterol, HDL, LDL and triglyceride of the rats' blood. *Ledok* addition of 20% led to increased levels of cholesterol, HDL and triglycerides to its lowest in a row 9.28%, 10.41% and 4.41%, but on the contrary, it occurred the decrease of blood LDL levels by 3.61% or the *ledok* addition of 20% is able to inhibit the increase in cholesterol and triglyceride levels as well as to lower the LDL blood levels.

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