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Testing of Hypocholesterolemic Effects on Instant Ledok In Vivo

I Ketut Suter* Ni Made Yusa
Study Program of Food Science and Technology, Faculty of Agriculture Technology, Udayana University, Bukit Jimbaran, 80361,Badung, Bali, Indonesia, Phone/Fax: 0361 701801

Abstract
Ledok is a traditional food in Nusa Penida, in form of porridge made from corn, cassava, beans, green leaves vegetables and seasoning without rice. *Ledok* instant is development of *ledok* which requires preparation times around five minutes. *Ledok* instant has the potentials to be developed as a functional food, because it contents sufficient nutrients and bioactive component which is dietary fiber. The objectives of this research is to discover the hypocholesterolemic effect of instant *ledok* in vivo. The research begins with producing instant *ledok* added with purple sweet potato, production of standard feed and treatment feed (standard feed + instant *ledok*) and continued with bioassay testing using experiment rats. The experiment is conducted by Completely Randomized Design. There are four treatments i.e : P0 (rat group without hypercholesterol + standard feed = negative control), P1 (rat group with hypercholesterol + standard feed = positive control), P2 (rat group with hypercholesterol + standard feed + instan *ledok* 15 %) and P3 (rat group with hypercholesterol + instant*ledok*of 30 %). Every group consists of seven rats. Analysis are taken before (pretest) and after (post test) on cholesteroltotal,cholesterol HDL, cholesterol LDL, triglyceride of rat blood and rat body weight. The feed treatment is given for 28 days. The research shows that there is a lowering of cholesterol total level, triglyceride and cholesterol LDL of rats blood. In the other hand there is raise in rats blood cholesterol HDL level take place by giving mix of standard feed and instant *ledok*. Treatment of *ledok* instant by 30 % can lower cholesterol total level, triglyceride and cholesterol LDL in the following order : 42.48 %, 25.29 % and 53.04 % from pretest level which are : 208.15 mg/dl, 136.50 mg/dl and 74.07 mg/dl. Meanwhile cholesterol HDL level of rat blood has a raise of 150 % from pretest level 26.40 mg/dl. Rats body weight during the experiment raises about 14.5 % - 16.6 % from the original weight 218.1 g – 226.1 g.
Keywords : instant *ledok*, hypocholesterolemic, dietary fiber, cholesterol, triglyceride

1. Introduction

Ledok is a kind of porridge, is one of the traditional foods in Nusa Penida, Klungkung Regency, Bali. *Ledok* is made from white corn, yellow cassava, nuts, vegetables and spices, without using rice (Suter, et al., 2007). The non-use of rice in the making of *ledok* means contributing to reducing the need for rice as a staple food and enhancing the role of non-rice foods such as corn and tubers as a source of carbohydrates. To ease the preparation, increasing shelf life and extending the distribution reach, *ledok*has been developed into an instant *ledok*(Suter, et al., 2007 and Suter, et al., 2009). Instant *ledok* takes up to 17.5 minutes of ready-to-serve time, whereas for traditional *ledok* takes up to 48 minutes (Suter, et al., 2007). In addition, by reducing the size of the ingredients, the length of cooking can be shortened from 17.5 minutes to five minutes (Suter, et al., 2009).

Consumer demand for functional food is increasing, because functional food has the properties to prevent certain diseases beyond its nutritional function (Subroto, 2008). Instant *ledok* is potentially developed into functional food because in addition to its relatively high nutritional content, it also contains bioactive components such as dietary fiber and antioxidants. Dietary fiber is a component of plant tissue, is part of a carbohydrate that is resistant to hydrolysis process by the digestive enzymes in the stomach and small intestine (Winarno, 1997 and Linder, 2010). Dietary fibers include non-digestible polysaccharides such as cellulose, hemicellulose, oligosaccharides, pectin, gum, and waxes (Marsono, 2004; Astawan and Wresdiyati, 2004).Dietary fibers are not only found in vegetables and fruits but also in rice, potatoes, beans and tubers (Kusharto, 2006). Fiber derived from cereals (grains) is generally water-insoluble, while fiber derived from vegetables, fruits and nuts tends to dissolve (Astawan et al., 2004). Dissolved food fiber has hypocholesterolemic properties that can lower cholesterol (Stark and Madar, 1994). Some viscous fibers can lower blood cholesterol such as gum, pectin and products derived from beans (Rusilanti and Kusharto, 2007). Plasma cholesterol levels are positively correlated with the formation of atherosclerosis, a degenerative disorder of blood vessels characterized by thickening of vascular wall tissue filled with lipids, complex carbohydrates, various blood products and fibrous tissues (Khomsan 2006).

The nutrient content of the instant *Ledok* (porridge of *Ledok*) is 80.68% of water, 0.91% of ash, 7.32% of protein, 1.81% of fat and 9.29% of carbohydrates. (Suter, et al., 2009), whereas the instant *Ledok* made by the addition of 50% purple sweet potato (the percentage is calculated on the number of composite flour or a mixture of yellow cassava flour and purple sweet potato flour) contains a total dietary fiber of 21.42% consisting of 2.08% soluble dietary fiber and 19.34% of insoluble dietary fiber, and 0.10% of GAEAC antioxidant capacity (Suter, et al, 2013).

The American Diabetes Association recommends that diabetics consume a total of 20-35 g / day of fibers derived from soluble or insoluble fibers. It was also reported that a diet high in fibers can lower blood glucose and lipid levels in patients with type 2 diabetes mellitus (McIntos, et al., 2001, Kim, et al., 2008). Instant *ledok* hypoglycemic effect testing has been done by Suter and Yusa (2016) and the results show that 15% of instant *ledok* feeding of the standard feed and instant *ledok* mixture can lower the blood serum glucose levels in rats by 66.96%.

On the basis of the above description, then it was conducted further research in order to determine the hypocholesterolemic effect of instant *Ledok* diet in rats(to lower the blood serum total cholesterol level).

2. Materials and Methods

2.1. Materials and Equipments

The ingredients used to make instant *ledok* are: white corn, yellow cassava, peanuts, red beans, purple sweet potatoes, bay leaves, basil leaves, spinach leaves, galangal, garlic, salt, red pepper and lemon. Reagent kits (cholesterol, triglycerides and HDL). The mice feed used refers to the standards established by the American Institute of Nutrition (AIN, 1993) including corn starch, CMC, soybean oil, sucrose, casein (Sigma, AS), a mixture of vitamins and mineral mixtures (ICN Biomedical, Inc. Aurora, Ohio, United States).

The equipments used are utensils for making instant *ledok* such as gas stove, knife, blender (Philips), and pans. Equipment used include rat cages and equipment, ovens, rough scales (Sartorius), analytical balance (Sartorius), syringe injection, micro-hematocrite tube (Becton Dickinson & Company).

2.2. Research Methods

a. The experimental design

This type of research is an experimental laboratory at Wistar rats by using Post Test Control Group Design (Notoatmodjo 2002 in Maligan, et al., 2011). The selection of research object and treatment was conducted by using Completely Randomized Design method. This experiment uses the following four treatments :

P0: Rat group of without hypercholesterol + standard feed (negative control)
P1: Rat group of hypercholesterol + standard feed (positive control)
P2: Rat group of hypercholesterol + standard feed + instant *ledok* (15%)
P3: Rat group of hypercholesterol + standard feed + instant *ledok* (30%)

Each treatment consisted of seven rats, so the total number of samples was 28 Wistar rats.

b. Instant *ledok* preparation of materials

Instant *ledok* making is based on the way reported by Suter, et al. (2013). Preparation of instant *Ledok* ingredients: (1) The yellow cassava tubers are prepared as follows: tubers peeled, the flesh is washed, drained and then cut into pieces with a size of 0.5 x 0.5 x 0.2 cm, then 500 g of it is steamed at 100 ° C for 35 minutes (until cooked), cooled, and then put in the oven at 70 ° C until it dries. (2) 500 g of dried peanut seeds are boil at 100 ° C in a pot containing water with the ratio of ingredients and water is 1: 3 until it is cooked, and then drained.Then cooked peanut dried in an oven at 70 ° C until dry. (3) Red beans are prepared just like preparing peanut kernels. (4) Dry white corn rice of 500 g is boiled (maize and water ratio is 1: 3) at 100 ° C for 37 minutes (until cooked) and then drained. Corn rice is dried in an oven at 70 ° C (4 hours). (5) Spinach leaves, bay leaves and basil leaves are filtered at 85 ° C for five minutes, then dried in a 70 ° C oven to dry. (6) Galangal is finely chopped then dried in an oven at 70 ° C and (7) Purple sweet potato is prepared just like preparing the yellow cassava tubers. Preparation of spices: ingredients such as garlic, red pepper, salt and lime skin with a ratio of 4,0: 6,0: 5,0: 4,0 is weighed, then the ingredients are blended until smooth. Next the spices that have been finely dried in an oven at the temperature of 70^{o}C until it dries.

c. How to make instant *ledok*

The ingredients and spice that have been prepared as above blended, then sifted. Spinach leaves, bay leaves and basil leaves are sieved with a sieve number 10 (9 mesh), while other ingredients sieved with size 18 (16 mesh) sieve. Composite flour is made by weighing and purifying purple sweet potato flour and yellow cassava flour with ratio of 1: 1. All the materials are weighed according to instant *ledok* formula as shown in Table 1. Furthermore all the ingredients are mixed evenly, so that it gets instant *ledok*.

Table 1. Instant *Ledok* Formula (Suter, et al., 2013).

Ingredients of instant *Ledok*	Total (g)
White corn kernels	110,0
Composite flour	55,0
Red beans	55,0
Peanut beans	55,0
Spinach leaves	9,0
Basil leaves	3,0
Bay leaf	5,0
Galangal	1,0
Seasoning	19,0

d. Making of standard and treatment feeds

Making standard feed refers to the manufacture of standard feed according to AIN 1993 (Reeves, et al., 1993). The composition of the ingredients for the standard feed can be seen in Table 2. Instant *ledok* treatment feed is made with the addition of 15% instant *ledok* on standard feed (P2) and the addition of 30% of instant *ledok* on the standard feed (P3).

Table 2. Standard feed composition (Reeves, et al., 1993)

Ingredients	Standard feedd (g/kg)
Corn starch	620,69
Casein	140,00
Sucrose	100,00
Soy oil	40,00
CMC	50,00
Mineral mix.	35,00
Vitamin mix.	10,00
L-sistein	1,80
Colin bitartrates	2,50
Total	999,99

e.Bioassay

The rats used in this study were Wistar rats weighing between 100-200 g, 28 in total. Rats are placed in individual cages and adapted to standard feed for 4 (four) days. On the 5th day it was fed hypercholesterol (given duck egg yolk) and 0.01% propyl tio urasil for 5 weeks, except the negative control group (P0) was not given hypercholesterol feed. After five weeks of feeding of hypercholesterolemia, a total serum cholesterol analysis was performed to confirm that the rats had been positive for hypercholesterolemia.In addition, it was also performed the Pre-test analysis of triglycerides, LDL cholesterol and HDL cholesterol and weighing rats. Rats were divided into three groups, each group consisting of seven rats. Each group was fed according to the treatment of P1, P2 and P3. The treatment test was conducted for four weeks (28 days). Four weeks after feeding the treatment feeds, it was carried out the lipid profile analysis or post-test (total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride). Observation of feed consumption was carried out every day, while weighing the weight of rats was performed on the pre test and post test.

f. Observed parameters

Parameters observed/measured in experimental animals were rat body weight, amount of feed consumed and lipid profile. Analysis of lipid profile includes total cholesterol in serum by the method of CHOD-PAP (Deeg et al., 1983 and Artiss, et al., 1997), HDL cholesterol by the method of CHOD-PAP (Lopes-Virella, et al, 1997), triglycerides by methods of GPO-PAP (Fossati and Principe, 1982), LDL cholesterol was calculated by the Friedewald equation, et al., 1972). The observational data were analyzed statistically (Gomes and Gomes, 1995).

3. Results and Discussion

3.1. Total Cholesterol Levels

The result of variance analysis showed that on pretest, the feeding of hypercholesterol diet was very significant influenced (P <0,01) to total cholesterol level of rat blood. The mean data on the total cholesterol levels of the rats' blood pretest and posttest mice were presented in Table 3. In pretest, the provision of hypercholesterolemia diet significantly increased total cholesterol level of rats blood, i.e. from the total blood cholesterol of rats of 81.39 mg / dl in the rats without hypercholesterol diet, and it increased ranged from 207.16 mg / dl - 211.49 mg / dl in the rats group that were given the hypercholesterol diet. At posttest, feeding treatment that is standard feed mixed with instant *Ledok* was highly significant (P <0.01) on the total blood cholesterol levels of rats. Based on the data in Table 3, the posttest showed that the total cholesterol level of rats' blood in the group of rats treated with the treatment feed was significantly different than that of the rats group without treatment (positive control).

When compared between pretest and posttest, there was a decrease in total blood cholesterol level of rats in the group of rats fed with standard feed mixed with 15% and 30% instant *ledok* respectively 30.37% and 42.48%, whereas in the group of rats without given feed treatment (positive control) there was a slight increase in total cholesterol level of 1.02%.

Table 3. Mean value of total cholesterol level of rat blood

Treatments*	Pretest (mg/dl)	Posttest (mg/dl)	Increase / decrease (mg/dl)	Percentage (%)
P0	81,39 c **	82,47 d	1,08	1,33
P1	211,49 a	213,65 a	2,16	1,02
P2	207,16 b	144,24 b	- 62,92	- 30,37
P3	208,15 b	119,72 c	- 88,43	- 42,48

*P0: Rat group of without hypercholesterol + standard feed (negative control)
P1 : Rat group of hypercholesterol + standard feed (positive control)
P2 : Rat group of hypercholesterol + standard feed + instant *ledok*(15 %)
P3 : Rat group of hypercholesterol + standard feed + instant *ledok* (30%)
** The same letter behind the mean value in the same column shows no significant difference at P> 0.05.

The reduction in the total cholesterol levels of rat blood in the rat groups given instant *ledok* may be due to dietary fibers in instant *ledok*. Dietary fiber can lower blood cholesterol levels (Rusilanti and Kusharto, 2007). Instant *Ledok* contains 21.42% of total dietary fiber which includes 2.08% of soluble dietary fiber and 19.34% of insoluble dietary fiber (Suter, et al., 2013). In high-fiber food there was an increase in fat excretion, bile acids and cholesterol (Anderson, et al., 1994). Dietary fiber can bind blood cholesterol and is removed with feces so that the blood cholesterol decreases (Wisaniyasa, 2017).

3.2. Triglycerides levels

The results of variance analysis showed that in pretest, the provision of hypercholesterol diet significantly influenced (P <0,01) on the triglyceride level. The group of rats fed with hypercholesterol diet were higher in blood triglyceride levels than in the group without being given a high cholesterol diet. In posttest, feeding with the standard mixed feed mixture with instant *ledok* had a very real effect (P <0.01) on rat blood triglyceride level. In the hypercholesterol rat group, the triglyceride blood level of the rats given instant *ledok* was significantly different, i.e. it was lower than the blood triglyceride level of the rats without being given instant *ledok* (positive control). Composite feeding of standard feed and instant *ledok* of 30 percent was significantly lower than those fed with a 15 percent instant *ledok* (Table 4).

Table 4. Mean blood triglyceride levels of rats

Treatments*	Pretest (mg/dl)	Posttest (mg/dl)	Increase / decrease (mg/dl)	Percentage (%)
P0	82,48 c **	83,37 d	0,89	1,08
P1	131,49 b	133,30 a	1,81	1,38
P2	133,47 ab	118,35 b	- 15,12	- 11,33
P3	136,50 a	101,98 c	- 34,52	- 25,29

*P0: Rat group of without hypercholesterol + standard feed (negative control)
P1 : Rat group of hypercholesterol + standard feed (positive control)
P2 : Rat group of hypercholesterol + standard feed + instant *ledok*(15 %)
P3 : Rat group of hypercholesterol + standard feed + instant *ledok* (30%)
** The same letter behind the mean value in the same column shows no significant difference at P> 0.05.

When compared between pretest and posttest, in the group of hypercholesterol rats without being given instant *ledok* (positive control) there was an increase of triglyceride level of 1.38 percent, in contrast to the group of rats given instant *ledok* of 15 percent and 30 percent,triglyceride levels decreased as much as 11.33 percent and 25.29 percent. This decrease in triglyceride levels may be due to the instantaneous effects of dietary fiber. Consumption of dietary fiber that meets the needs can increase the excretion of fat through the feces (Brown, et al., 1999). Fiber can bind fat, so the fat absorption is getting lower, which means lower blood triglyceride levels.

3.3. HDL Cholesterol levels

Dietary hypercholesterolemia on pretest significantly influenced (P <0.01) on HDL cholesterol of rat blood. The group of rats fed with hypercholesterol diet of HDL cholesterol was significantly different (P <0.05), which was lower than the HDL cholesterol level of the rat blood group without being given a hypercholesterol diet (Table 5). In posttest of feeding mixture namely the standard feed and instant *ledok* significantly influenced (P <0,01) on the HDL cholesterol. Instant *ledok* feeding led to HDL blood cholesterol levels significantly different (P <0.05) that is higher than the HDL cholesterol levels in the blood group of rats without instant *ledok* (positive

control). When it is compared between pretest and posttest, there was an increase in HDL cholesterol levels of blood in the group of rats given a 15 percent and 30 percent of instant *ledok* respectively of 71.09 percent and 150 percent during the 28-day experiment (Table 5).

Table 5. Mean value of HDL level of rat blood

Treatments*	Pretest (mg/dl)	Posttest (mg/dl)	Increase (mg/dl)	Percentage (%)
P0	74,18 a **	78,24 a	4,06	5,47
P1	25,60 b	26,69 d	1,09	4,26
P2	25,60 b	43,80 c	18,2	71,09
P3	26,40 b	66,00 b	39,6	150

*P0: Rat group of without hypercholesterol + standard feed (negative control)
P1 : Rat group of hypercholesterol + standard feed (positive control)
P2 : Rat group of hypercholesterol + standard feed + instant *ledok*(15 %)
P3 : Rat group of hypercholesterol + standard feed + instant *ledok* (30%)
** The same letter behind the mean value in the same column shows no significant difference at P> 0.05.

Increases in HDL cholesterol levels in groups of rats given instant *ledok* may be related to decreased triglycerides.

3.4. LDL levels

Dietary hypercholesterolemia on pretest had a very real effect (P <0.01) on LDL cholesterol level of rat blood. The group of rats fed with hypercholesterolemia of LDL cholesterol was significantly different (P <0.05), which was higher than the LDL cholesterol level in the rat group without the hypercholesterol diet (Table 6). The posttest feeding mixture, that is standard feed and instant *ledok* have a very real effect (P <0.01) on LDL cholesterol levels. Instant *ledok* led to significantly lower levels of LDL cholesterol (P <0.05) lower than LDL cholesterol in the blood group of rats without instant *ledok* (positive control). When it is compared between pretest and posttest, there was a decrease in LDL cholesterol levels in the group of rats given instant *ledok* of 15 percent and 30 percent respectively by 38.17 percent and 53.04 percent for 28-day experiment (Table 6).

Table 6. Mean average of LDL level of rat blood

Treatments*	Pretest (mg/dl)	Posttest (mg/dl)	Increase / decrease (mg/dl)	Percentage (%)
P0	28,96 c **	29,96 d	1,00	3,45
P1	71,47 b	72,01 a	0,54	0,75
P2	70,61 b	43,66 b	- 26,95	- 38,17
P3	74,07 a	34,78 c	- 39,29	- 53,04

*P0: Rat group of without hypercholesterol + standard feed (negative control)
P1 : Rat group of hypercholesterol + standard feed (positive control)
P2 : Rat group of hypercholesterol + standard feed + instant *ledok*(15 %)
P3 : Rat group of hypercholesterol + standard feed + instant *ledok* (30%)
** The same letter behind the mean value in the same column shows no significant difference at P> 0.05.

A decrease in LDL cholesterol levels in a group of rats given a mixed instant *ledok* diet may be due to the effects of dietary fiber found in instant *ledok*. As already discussed before, the provision of instant *ledok* can lower the total blood cholesterol. A decrease in total cholesterol levels also means a decrease in LDL cholesterol (Indra dan Panunggal, 2015).

3.5. Weight of Feed Consumed

Data on average weight of feed consumed by rats per week during the experiment of 28 days (four weeks) of feeding treatment is presented in Table 7. In Table 7, it can be seen that there is an increase in the amount of feed consumed when compared between week I and week IV i.e. ranged from 12.15 percent to 19.73 percent for the amount of feed consumed in week I. The increasing amount of feed consumed by all groups of rats may be due to better health conditions of rats so that the rats' appetite increases.

Table 7. Average feed weight values consumed by rats per week (g)

Treatments*	Week I (mg/dl)	Week IV (mg/dl)	Increase (mg/dl)	Percentage (%)
P0	15,35	17,51	2,16	14,07
P1	15,24	17,37	2,13	13,97
P2	15,96	17,90	1,94	12,15
P3	15,41	18,45	3,04	19,73

*P0: Rat group of without hypercholesterol + standard feed (negative control)
P1 : Rat group of hypercholesterol + standard feed (positive control)
P2 : Rat group of hypercholesterol + standard feed + instant *ledok*(15 %)
P3 : Rat group of hypercholesterol + standard feed + instant *ledok* (30%)

5.6. Rat Body Weight

Early mouse weight before being fed with hypercholesterol diet ranged from 169 g to 200 g. In the pretest, the provision of hypercholesterol diet was not significant (P> 0.05) to rat body weight. Among the groups of rats given hypercholesterol diet and no hypercholesterol diet, there was no significant difference in the body weights i.e. between 218.1 g - 226.1 g (Table 8). In the posttest, the feeding mixture, that is standard feed and instant *ledok* have no significant effect (P> 0,05) on the rat body weight. Instant *ledok* caused rat body weight was not significantly different (P <0.05) which ranged between 254.3 g to 260.9 g (Table 8). When it is compared between pretest and posttest, there was an increase in body weight of rats around 14.50 percent to 16.60 percent for 28 days of experiment (Table 8). The increase in body weight of these rats is due to the increasing amount of feed consumed.

Table 8. Mean value of rat body weight

Treatments*	Pretest (mg/dl)	Posttest (mg/dl)	Increase (mg/dl)	Percentage (%)
P0	223,0 a **	257,1 a	34,1	15,29
P1	226,1 a	258,9 a	32,8	14,50
P2	225,4 a	260,9 a	35,5	15,75
P3	218,1 a	254,3 a	36,2	16,60

*P0: Rat group of without hypercholesterol + standard feed (negative control)
P1 : Rat group of hypercholesterol + standard feed (positive control)
P2 : Rat group of hypercholesterol + standard feed + instant *ledok*(15 %)
P3 : Rat group of hypercholesterol + standard feed + instant *ledok* (30%)
** The same letter behind the mean value in the same column shows no significant difference at P> 0.05.

4. Conclusions

It can be concluded from this research as follows:

1. The mixed feeding of standard feed and instant *ledok* has a very significant effect on total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol.
2. There was a decrease in total cholesterol, triglyceride and LDL cholesterol of the blood, while the HDL cholesterol of rat blood increased with the provision of a mixture of standard feed and instant *ledok*.
3. Feeding containing 30% of instant *ledok* can reduce the total cholesterol, triglyceride and cholesterol LDL respectively of 42,48%, 25,29% and 53,04% from pretest level i.e.: 208,15 mg / dl, 136, 50 mg / dl and 74.07 mg / dl., While HDL cholesterol of rat blood increased 150% from the pretest of 26,40 mg / dl.
4. Mixed feeding did not result in a significant difference in body weight of the rats, but the weight of the rats during the experiment increased approximately 14.5% to 16.6% from the initial weight of 218.1 g to 226.1 g.

It can be recommended from the results of this study that instant *ledok* can be consumed as a diet for people with hypercholesterolemia disorder.

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