Food Science and Quality Management www.iiste.org
ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online)
Vol.81, 2018

Functional Characteristics of Ledok Added with Red Bean Observed from Hypoglicemic Effect in Vivo

Ni Made Yusa* Ni Wayan Wisaniyasa
Study Program of Food Science and Technology, Faculty of Agriculture Technology, Udayana University, Bukit Jimbaran, 80361,Badung, Bali, Indonesia

Abstract
Ledok is a tradisional food in Nusa Penida, Bali, in form of porridge made by local ingredients without rice, which are yellow cassava, white corn, beans, green leaves vegetables and seasoning. *Ledok*is made with additional red bean, is proven to have hypocholesterolemic effect, that impede the raise of cholesterol level of rats'blood serum. However, it is not learnt yet whether *ledok* added with red beans has hypoglycemic characteristic (lower blood glucose). The obyectives of this research is to test hypoglicemic characteristics of *ledok* added with red beans. Study of hypoglycemic characteristic of *ledok* made by red bean is conducted in vivo by using wistar rats. Research design is Control Group Test Design, and the treatments given by using Completely Randomize Design. There is four treatments used. PO = without alloxan injection + standard feed (negatif control); P1 = alloxan injection + standard feed (positive cntrol); P2 = alloxan injection + standard feed + *ledok* flour 15 % and P3 = alloxan injection + standard feed + *ledok* flour 30 %. The treatment is repeated by seven times (seven rats). The experiments is conducted for five weeks and glucose analysis on rats'blood is taken prior the treatment (pretest) and after five weeks give the treatment feed (posttest). The research shows that *ledok* made by adding red beans has hypoglicemic characteristics (able to lower rats'blood glucose level). Additional of *ledok* by 15 % in rats standard feed can lower rats'blood glucose level from 236,7 mg/dl to 168,0 mg/dl or by 29.0 %.
Keywords : *ledok*, red bean, hypoglicemic, dietary fiber, glucose

1. Introduction

Ledok is a kind of porridge, is one of the traditional foods in Nusa Penida, Klungkung regency, Bali. *Ledok* uses the main raw materials of corn, yellow cassava, beans, vegetables and spices. Vegetables used are spinach and basil, while the spices consist of garlic, chili, bay leaf and galangal. *Ledok* is unique as it does not use rice, so it can support the national food security. *Ledok* is potentially developed into functional food because it contains nutrients and bioactive components of dietary fiber that plays a role in the physiological processes of the body. *Ledok* contains the following nutrients: water (71.92%), ash (0.98%), protein (3.15%), fat (4.71%), crude fiber (3.18%) and carbohydrates 16.05%) Suter, et al. (2007). Instant *ledok* is made with 50% purple sweet potato addition (percentage is calculated against the amount of composite flour which is mixture of yam cassava flour and purple sweet potato flour) contains total dietary fiber of 21.42% consisting of 2.08% soluble dietary fiber and insoluble dietary fiber of 19.34% (Suter et al. 2013), while *Ledok* added with red beans contains 25.11% of total dietary fiber (bk) (Yusa and Suter, 2015).Dietary fiber has the properties to prevent degenerative diseases such as diabetes mellitus (DM), coronary heart disease and high blood pressure (Subroto, 2008). DM disease has now become an epidemic disease. Various surveys show that DM patients always increase from year to year. It is estimated that in 2030 the number of DM patients in the world will reach 366 million (Hartati, 2007). In Indonesia alone, the number of DM patients also have increased and by 2025 is estimated to reach 12.4 million patients. The American Diabetes Association (2006) recommends that diabetics should consume a total of 20 to 35 g / day fiber derived from soluble and insoluble dietary fiber. It was also reported that a diet high in dietary fiber can lower blood glucose and lipid levels in patients with type 2 DM (McIntos, et al., 2001, Kim, et al., 2008). Water soluble dietary fiber has hypocholesterolemic properties that can lower cholesterol (Stark and Madar, 1994).

Other ingredients which make up *ledok* such as galangal contain bioactive compounds (galangol, galangin, alpinen, camphor, methyl-cinnamate) are good for increasing appetite, and anti-bacterial. Basil leaves contain bioactives such as citral, eugenol, tannin and flavonoid efficacious for curing uric acid, lowering cholesterol, and high blood pressure and to cure diarrhea and ulcers (Wijayakusuma, 2002). Basil contains camphor, d-limonene, mirsen, etilkavikol, and eugenol which are efficacious for curing digestive disorders such as gastritis, vomiting, flatulence, treat fever, runny nose, headache, lowering uric acid. Alisin bioactive in garlic is efficacious for lowering cholesterol, high blood pressure, preventing cancer and retarding aging, improving insulin and relieving gastric ulcers (Subroto, 2008).

Nuts are one of the potential food ingredients as a source of vegetable protein and dietary fiber. Common types of nuts consumed include red beans, gude nuts, cowpea and soybeans. In the year 2015 research has been carried out to make *ledok* from six types of beans namely peanuts, *kara* nuts, green beans, *gude* beans, soybeans and red beans. Based on the content of nutrients, dietary fiber and sensory properties obtained the best *ledok* produced from red beans with characteristics: water content 78.70%, ash 1.05%, fat 0.13%, protein 6.40%, carbohydrate 13.71%, total dietary fiber 25.11% (bk), soluble dietary fiber 3.13% bk), insoluble dietary fiber 21.96% (bk) (Yusa and Suter, 2015). The red bean *ledok* has been tested for its hypocholesterolemic effect on rats and it is proven that by adding a 20% *ledok* on standard feed can inhibit elevated cholesterol and triglyceride levels and lower the LDL cholesterol serum blood of rats (Yusa, et al., 2017). Whether the *ledok* made of red beans also has a hypoglycemic nature (that it can lower blood sugar levels) is unknown. On the basis of the above description, then it was conducted a study with the aim to determine whether *ledok* that is made from red beans has the property of lowering the blood glucose (hypoglycemic).

2. Materials and Methods

2.1. Materials and Equipment

The materials used for making *ledok*are: white corn, yellow cassava, cowpea, red beans, bay leaves, basil leaves, spinach leaves, galangal, garlic, salt, and lemon. The chemicals used are:alloxan monohydrate (sigma) and glucose kit. The rats feed used refers to the standards set by the American Institute of Nutrition (AIN) including corn starch, CMC, soybean oil, sucrose, casein (Sigma, AS), a mixture of vitamins and mineral mixtures (ICN Biomedical, Inc. Aurora, Ohio, America).

The equipment used is cooking utility equipment such as gas stove, knife, blender (Philips), and pot. Equipment used for chemical analysis are: homogenizer, vortex, small centrifugation (Hettich EBA III), mouse cage and equipment, muffle furnance (Heraeus Instrument), oven, dryer cabinet, weighing scales (Sartorius), analytical balance (Sartorius), syringe injection, micro-hematocrite tube (Becton Dickinson & Company), micro pipette.

2.2. Research Methods

a. The experimental design

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

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

Each treatment consisted of 7 rats (7 replicates), so the total number of samples was 28 rats Wistar.

b. Ways of making *ledok*

Instant *ledok* is made by using a formula of white maize (125 g), yellow cassava (125 g), spinach leaves (62.5 g), basil leaves (25 g), bay leaves (5 g), galangal (5 g), water (4 x total material weight), spices (19 g), cowpea (62.5 g) and red beans (62.5 g) (Yusa and Suter, 2015).The process of making *ledok* is as follows: all materials are cleaned, then weighed in accordance with the above-mentioned formula. Water is heated in a pan until boiling (temperature reaches 100 ° C). White corn, cowpea, red beans, and bay leaves are added and cooked until half cooked (for ± 15 minutes at 100 ° C). Other ingredients are yellow cassava tuber, galangal, spinach leaves and basil leaves that have been cleaned, washed and chopped, and spices (garlic, salt, lime peel and pepper in a row proportion of 4: 4: 5: 6) put in a pan, then stirred evenly and boiled until cooked (for 45 minutes), finally it is produced an instant *ledok*.

c. *Ledok* powder making

Instant *ledok* is made into powder in the following way: instant *ledok* mixed evenly then dried in oven at 70^{o}C until it dries. Then it is reduced in size by blender, then sieved with a sieve of 16 mesh so as to obtain *ledok* powder.

d. Making standard and treatment feed

Making standard feed refers to the making of standard feed according to AIN 1993 (Reeves, et al., 1993). The ingredient composition for the standard feed can be seen in Table 1. The *ledok* treatment feed is made by the addition of 15% *ledok* powder in the standard feed (P2) and the addition of 30% *ledok* powder on the standard feed (P3).

Table 1. Composition of standard feed (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 bioassay test was performed following a procedure reported by Suter, et al., (2014) with modification. The rats used in this study were male Wistar rats aged ± 3 months with body weight ranging from 100 to 200 g, amounting to 28 rats. Rats are placed in individual cages and adapted to standard feed for 4 days. The rats were then fasted overnight and divided into 4 groups (7 rats per group). Group 1 rats were used as controls, whereas groups 2, 3 and 4 rats were injected with alloxan 125 mg/kg body weight. (rats were purposively made to be hyperglycemic). After the rats experienced hyperglycemic (blood glucose level of at least 135 mg /dl) then rats were given treatment. Group 1 (P0) and group 2 (P1) were given standard feed, group 3 (P2) were fed standard + 15% *ledok* and group 4 (P3) were fed standard + 30% *ledok*. The testing was done for 35 days.

f. Observed Parameters

The parameters observed / measured in the experimental animals were: (1) the amount of feed consumed, (2) body weight of the rats, and (3) blood glucose levels. Observation of feed intake was done every day, weighing rat weight and blood glucose level analysis were done before the feeding treatment (pretest) and after the feeding treatment (posttest). Blood glucose levels were determined by the GOD-PAP method. The observational data were analyzed statistically (Gomes and Gomes, 1995).

3. Results and Discussion

3.1. Feed Weight Consumed By Rats

The feed weight data consumed by pretest and posttest rats (five weeks) is presented in Table 2. Data in Table 2 shows that there is an increase in the amount of feed consumed by rats ranging from 17.5 percent to 97.5 percent. The highest increase in the amount of feed consumed of 97.5% was found in the group of negative control rats (P0) and the lowest (17.5%) was in the group of alloxan injected rats + standard feed (positive control = P1). This is because the rats in the positive control were still sick so that they lost their appetites.

Table 2. Average feed weight values consumed by rats (g)

Treatment*	Pretest (g)	Posttest (g)	Increase (g)	Percentage (%)
P0	8,0	15,8	7,8	97,5
P1	8,0	9,4	1,4	17,5
P2	8,0	14,1	6,1	76,2
P3	8,0	15,0	7,0	87,5

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

3.2. Rat Body Weight

Pretest and posttest (five weeks) body weight data are presented in Table 3. Based on the pretest analysis of rats' body weight varieties were not significantly affected by standard feeding treatment mixed with *ledok*, but after standard feeding was mixed with *ledok* (posttest), it very significantly effected on body weight of rats. The weight of the rats before treatment ranged between 160.4 g to 166.7 g, whereas after treatment was ranging from 150.8 g to 187.7 g. After administration of injected alloxan the body weight of rats was significantly lower than the weight of the rats without injection of alloxan (negative control). In the alloxan-injected group of rats, the highest weight of rats fed with the standard feed mixed with 30% *ledok* was 175.8 g, while the lowest weight of the rats fed with standard feed without the addition of *ledok* was 150.8 g. This situation is due to the group of rats whose feed without *ledok*,consumed less feed than those in the group of rats whose feed was mixed with *ledok*. Increased body weight of rats, especially in rats that did not injected with alloxan (negative control = P0) equal to 12.6 percent, followed by body weight of rats injected with alloxan and fed with treatment feed with the addition of 30 percent *ledok* (P3) i.e. 7.4 percent . In alloxan-injected rats fed with the standard feed (P1) and alloxan-injected rats and fed with feed treatment with 15 percent *ledok* led to the weight loss of 9.5 percent and 0.7 percent respectively.

Table 3. Mean value of rat body weight

Treatment*	Pretest (g)	Posttest (g)	Increase/Decrease (g)	Percentage (%)
P0	166,7 a**	187,7 a	21,0	12,6
P1	166,7 a	150,8 d	- 15,9	- 9,5
P2	160,4 a	159,2 c	- 1,2	- 0.7
P3	163,7 a	175,8 b	12,1	7,4

*P0 : Rat group of without alloxan injection + standard feed (negative control)
P1 : Rat group of alloxan injection + standard feed (positive control)
P2 : Rat group of alloxan injection + standard feed + instant *ledok* (15%)
P3 : Rat group of alloxan injection + standard feed + instant *ledok* (30%)
** The same letter behind the average value in the same column shows the difference was not significant (P> 0.05)

3.3. Rat Blood Glucose Levels

Blood glucose data of pretest and posttest rats (five weeks) are presented in Table 4. Based on the analysis of variations prior to feeding treatment (pretest) alloxan injection led to a very significant increase in blood glucose levels. Blood glucose levels of alloxan injected rats were significantly higher (ranging between 197.1 mg / dl to 251.4 mg / dl) compared with blood glucose levels of mice without injection of alloxan of 107.8 mg / dl. This indicates that the group of alloxan injected rats had diabetes (fasting blood glucose level ≥ 126 mg / dl) (Soegono, 2006 in FKUI, 2015). The provision of feed mixture of standard feed and *ledok* (posttest) had a very significant effect on blood sugar levels of rats. In the alloxan-injected rat group the standard feeding mixed with *ledok*, the blood glucose was significantly lower than the blood glucose level of the rats fed without *ledok* (Table 4).

Tabel 4. The avarage value of rat blood glucose levels (mg/dl)

Treatment*	Pretest (mg/dl)	Posttest (mg/dl)	Increase/Decrease (mg/dl)	Percentage (%)
P0	107,8 c **	104,0 c	- 3,8	- 3,5
P1	251,4 a	255,8 a	4,4	1,7
P2	236,7 a	168,0 b	- 68,7	- 29,0
P3	197,1 b	142,8 b	-54,3	- 27,5

*P0 : Rat group of without alloxan injection + standard feed (negative control)
P1 : Rat group of alloxan injection + standard feed (positive control)
P2 : Rat group of alloxan injection + standard feed + instant *ledok* (15%)
P3 : Rat group of alloxan injection + standard feed + instant *ledok* (30%)
** The difference letter behind the average value in the same column shows signficant difference (P<0,05)

The data in Table 4 indicate that there is a decrease in blood glucose levels of rats in alloxan injected rats and the feeding with *ledok*. Decreased glucose levels by 29.0 percent was in alloxan injected rats with 15 percent *ledok* (P2) and 27.5 percent in alloxan-injected rats and fed with 30 percent of *ledok* (P3). Decrease in blood glucose levels of rats fed with standard feed mixed with*ledok* may be caused by the presence of dietary fiber in *ledok* that may contribute to lower glucose levels. *Ledok* made with red bean addition contains total food fiber of 25.11% (bk) (Yusa and Suter, 2015). Diet high in dietary fiber can lower blood sugar levels in patients with type 2 diabetes (McIntos, et al., 2001 and Kim, et al., 2008). In rats injected with alloxan and without the feeding of *ledok* (P1) there was an increase in glucose levels of 1.7 percent (sick rats). whereas in rats without injection of alloxan (P0) a slight decrease in blood glucose levels occurred (rats looked normal / healthy).

4. Conclusions

Ledok made by adding red beans turned out to be hypoglycemic that can lower blood glucose levels. The addition of 15 percent *ledok* on standard feed can lower blood glucose levels of rats from 236.7 mg/dl to 168.0 mg/dl or decreased by 29.0 percent. Based on the results of this study, it can be recommended that *ledok* can be used as a diet for people with diabetes.

Acknowledgment

The researchers would like to thank the Dean of the Faculty of Agricultural Technology Udayana University who has provided funding for this research.

References

American Diabetes Association. (2006), "Nutrition Recommendation and Principles for People With Diabetes Mellitus", *Diabetes Care* **23**, S43 – S46.

Astawan, M & Wresdiyati, T. (2004), "Diet Sehat dengan Makanan Berserat", Tiga Serangkai Pustaka Mandiri, Solo.

Departemen Kesehatan R.I. (1995), "Daftar Komposisi Zat Gizi Pangan Indonesia", Direktorat Bina Gizi Masyarakat. Pusat Penelitian dan pengembangan Gizi, Jakarta.

FKUI. (2015), "Penatalaksanaan Diabetes Mellitus Terpadu", Balai Penerbit FKUI, Jakarta.

Goldberg, I. (1994), "Introduction", *In* Functional Foods. Designer Foods, Pharmafoods, Nutraceuticals. Goldberg, I. (Ed.). Chapman & Hall, New York, 3-16.

Gomes, K.A. & Gomes, A.T. (1995), "Prosedur Statistik Untuk Penelitian Pertanian", Terjemahan Sjamsudin, E. & Baharsyah, J.S., UI Press, Jakarta.

Hartati, R. (2007), "Indeks Glikemik dan Efek Hipoglikemik Kue Kering dan Roti Tawar Berbasis Tepung Garut (*Maranta arundinacea* LINN) dan Tepung Ubi Jalar (*Ipomea batatas*)", *Tesis*, Fakultas Teknologi Pertanian, Universitas Gajah Mada, Yogyakarta.

Kim, M.S., Kim, J.Y., Choi, W.H., & lee, S.S. (2008), "Effect of Seaweed Suplementation on Blood Glucose Concentration, Lipid profile and antioxidant Enzyme Activities in Patient with Type 2 Diabetes Mellitus", *Nutrition Research and Practice* 292, 62 – 67

Kusharto, C. M. (2006). Dietary Fiber and Its Role for Health. *J. Gizi dan Pangan* **1**(2), 45-54.

Linder, M.C. (2010), "Biokimia Nutrisi dan Metabolisme Dengan Pemakaian Secara Klinis", (Terjemahan). UI-Press. Jakarta, 27-33.

Maligan, J.M., Estiasih, T., Sunarharum, W.B. & Rianto, T., (2011), "Efek Hipokolesterolemik Tepung Umbi Gadung (*Dioscorea hispida* Dennst) pada Tikus Wistar Jantan Yang Diberi Diet Hiperkolesterol", *J. Tekno. Pertanian* **12** (2), 91-99.

MacIntosh, M. & Clara, M. (2001), "A Diet Containing Food Rich in Soluble and Insoluble Fiber Improves Glycemic Control and Reduce Hyperlipidemia among Patiens with Type 2 Diabetes Mellitus". *Nutrition Review* **59** (2), 52 – 55

Reeves, P.G., Nielsen, F.H. & Fahey, G.C. (1993), "AIN-93, Purified Diets for Laboratory Rodents : Final Report of the American Institute of Nutrition Ad Hoc writing Committee on the Reformulation of AIN-76 Rodent Diet", *J. Nutr*. 123, 1939-1953.

Rusilanti & Kusharto, C. M. (2007), "Sehat Dengan Makanan Berserat", Agromedia, Jakarta.

Stark, A. & Madar, Z. (1994), "Dietary Fiber", *In* Functional Foods. Designer Foods, Pharmafoods, Nutraceuticals..Goldberg, I (Ed.). Chapman & Hall, New York, 183-201.

Subroto, M.A.(2008), "Real Food, True Health, Makanan Sehat Untuk Hidup lebih Sehat", PT AgroMedia Pustaka, Jakarta.

Suter, I K., Wijaya, I M. A. S., Agung, I G.N., Yusa, Ni M. & Suryawantha, I B. K. (2007), " Studi Pengembangan Produk Olahan Dari Umbi-umbian Dan Jagung Dalam Rangka Diversifikasi Pangan", Kerjasama Dinas Pertanian Tanaman Pangan Provinsi Bali dengan Pusat Kajian Makanan Tradisional Lembaga Penelitian Universitas Udayana, Denpasar.

Suter, I K., Yusa, Ni M., Ari Yusasrini, N.L. & Nocianitri, K.A. (2013), "Peningkatan Sifat Sensorik, Zat Gizi dan Daya Anti Oksidan *Ledok* Instan Dengan Penambahan Ubi Jalar Ungu. *Proseding Seminar Nasional* PS.Teknologi Industri Pertanian Bekerja sama dengan Assosiasi Profesi Teknologi Agro Industri (APTA).

Suter, I K., Kencana Putra, I N., Ari Yusasrini, N.L. & Yusa, Ni M. (2014), "Sifat Fungsional Campuran Kedelai dan Rumput Laut Ditinjau dari Efek Hipoglikemik Secara In Vivo", *Proseding Seminar Nasional Sains dan Teknologi (SENASTEK)*, Denpasar, Bali.

Yusa, Ni M. & Suter, I K. (2015), "Sifat Fungsional *Ledok* Yang Dibuat Dari Beberapa Jenis Kacang-kacangan Ditinjau Dari Efek Hipokolesterolemik Secara In Vivo", *Prosiding SENASTEK,* Denpasar, Bali.

Yusa, Ni M., Suter, I K. & Ari Yusasrini, N.L. (2017), "The Functional Properties of Ledok Added with Red Beans Viewed from In Vivo Hypocholesterolemic Effects. Food Science and Quality Management 61, 62-66.

Wijayakusuma, H. (2002),"Tumbuhan Berkhasiat Obat Indonesia: Rempah, Rimpang dan Umbi", Milenia Populer, Jakarta.

Winarno, F.G. (1997), "Kimia Pangan dan Gizi", P.T. Gramedia Pustaka Utama, Jakarta.