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By Bayu Kanetro

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Potency of High Protein Oyek for Decreasing Cholesterol of Diabetic Rats

Bayu Kanetro1, Sri Luwihana2, Dwiyati Pujimulyani3

1Food Technology Department Mercu Buana Yogyakarta University (bayu_kanetro@yahoo.co.id)
2Food Technology Department Mercu Buana Yogyakarta University (sri_luwihana@yahoo.co.id)
3Food Technology Department Mercu Buana Yogyakarta University (dwiyati2002@yahoo.com)

ABSTRACT

High protein oyek is traditional food from Kulon Progo Special Region of Yogykarta that was made of cassava and developed by addition of cowpeas sprout for increasing the protein of oyek This research was aimed to determine the potency of hypocholesterolemic of oyek through in vivo bioassay by using Sprague Dawley male rats. There were two treatments of the research, the first treatment were normal rats and diabetic rats which was induced by aloxan injection, and the second treatment were standard feed and oyek feed. The blood triglyceride, cholesterol total, High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) cholesterol were analysed on 3^{th}, and 18^{th} days for the treatment and before the treatment as control (0^{th}). The result of this research showed that the total cholesterol of normal and diabetic rats increased during the feed treatment but the increase of cholesterol of diabetic rats were higher than normal rats. The potency of hypocholesterolemic were shown by decreasing of blood triglyceride, cholesterol total, LDL, and increasing of blood HDL in diabetics rats with oyek feed treatment. Keywords: oyek, protein, cassava, cowpeas, hypocholesterolemic

INTRODUCTION

Oyek is traditional food from Kulonprogo Yogyakarta Indonesia. Oyek is staple food providing energy especially in dried climate condition that is high price of rice. Oyek is produced by spontaneous fermentation of cassava in water for 5 days, and then the fermented cassava are pressed to remove water, formed, steamed, and dried (Kanetro and Luwihana, 2015). The same product as oyek in the other country is *gari* or *rale* that is fermented cassava using lactic acid bacteria (Eduardo et al., 2013). Oyek had been developed into artificial rice that its glycemic index was lower than original rice, and the artificial rice was more preferably than oyek according to the sensory testing by Kalirejo community (Kanetro et al., 2015)

The protein of oyek was lower than rice, so the addition of legumes flour into oyek is important to increase protein. There are many local legumes in Indonesia, such as cowpeas. Cowpeas may be potential for increashing protein of traditional staple food, such as oyek. In the preliminary research was known that oyek with addition of cowpeas sprout flour 30% could increased protein, that was the same as rice (Kanetro and Luwihana, 2015).

The potency of legumes as functional food could be increased by germination. Germination of soybean increased arginine (Kanetro et al, 2008) that was known as hypocholesterolemic amino acid (Damasceno etal, 2000).The protein of cowpeas sprout contained high of arginine (Arg) that was the same as protein soybean (Kanetro and Dewi, 2013). In the preliminary research also showed that the arginine /lisine ratio of cowpeas sprout protein was no significant different with protein of soybean (Kanetro and Dewi, 2013). The ratio of arginine/lysine was important to controle cholesterol level (Damasceno et al.,2000). Protein isolate of soybean had been known as functional food due to hypocholesterolemic effect (Damasceno et al., 2000). Protein isolate of cowpeas sprout had hypocholesterolemic properties based on in vivo bioassay by using Sprague Dawley male (Kanetro, 2015). Therefore oyek with addition of cowpeas sprout might has hypocholesterolemic effect, so it was potential as functional food. This research was aimed to determine hypocholesterolemic properties of the oyek in normal and diabetic rats through in vivo bioassay..

MATERIALS AND METHOD

Material

The cowpea (*Vigna unguiculata*) were obtained from Beringharjo market in Yogyakarta. Chemical agents, such as aloxan, corn starch, casein, vitamin mix, mineral mix, sucrose, choline bitartat, soy oil, and kholesterol kit (*DiaSys Diagnostic System GmBH & Co*), were purchased from Sigma Chemical Co. The other material was rats that were obtained from Animal Experiment Development Unit, Gadjah Mada University, Yogyakarta.The methods of experimental activities were performed as follows:

Preparation of Cowpeas Sprout Flour and Oyek

Cowpea seeds were soaked for 8h, and then germinated for 36h. The germinated cowpeas were dried and milled to make flour (Kanetro and Dewi, 2013). The cowpeas sprout flour were prepared for increashing protein of oyek. Oyek was made of cassava through many step process according to Kanetro and Luwihana (2015). The first step, cassava was peeled, washed, and size reduced. After that cassava was fermented by soaking in water for 5 days. Then the fermented cassava are pressed to remove water, mixed with 30% cowpeas sprout flour, formed, steamed, and dried. The dried product was called oyek that was prepared to in vivo bioassay.

In Vivo Bioassay

The *in vivo* bioassay was done to determine the potency of hypocholesterolemic of oyek by using 24 *Sprague Dawley* male rats. The experiment sequences of the steps were adaptation of rats for 3 days, diveded rats into 4 groups, treated rats for 18 days with the condition of rat and feed treatments, and analysed the blood triglyceride, cholesterol total, High Density Lipoprotein (HDL) cholesterol , and Low Density Lipoprotein (LDL) cholesterol for the treatment of rats on 3^{th}, 18^{th} days and before treatment as control (0^{th}). The experimental design of this research was randomized complete design with 2 factors. The first factors were rat condition treatments, that were normal rats and diabetic rats which was induced by aloxan injection. The second factors were feed treatments, that were standart feed according to AIN–93 (Reeves et al, 1993) and oyek feed which was prepared by subtitution of corn starch in standard feed with the oyek. The data of this experiments was statistical analysed by Anova (analysis of varian) and DMRT (Duncan Multiple Range Test). The in vivo bioassay of this research had passed ethical clearance that was approved by Center Research Laboratorium of Gadjah Mada University, Yogyakarta, Indonesia.

RESULTS AND DISCUSSION

Trygliceride

Table 1 showed that the trygliceride of normal rats treatment were no significant differences between standart feed and oyek feed treatment. While the trygliseride of diabetic rats teratment showed significantly differences, especially for the treatment of rats on 18^{th}. The trygliseride of diabetic rats with protein isolate feed treatment increased after injection of aloxan at 3^{th} days of the tratment, and then decreased until below the standart of normal for the treatment of rats on 18^{th}.

The normal trygliceride of human according to US National Cholesterol Education Program (NCEP) was < 150 mg/dl (Anonim, 2007). While the normal trygliceride of rat was < 120 mg/dl (Herlina et al., 2013). Based on this data indicated that oyek could inhibit increashing trygliceride that was ussual happen in complication of diabetic.

Table 1. The effect of oyek feed treatment on blood tryglyceride of normal and diabetic rats (mg/dL) *)

Rats condition	Feed treatment	0th days	3th days	18th days
Normal	Standard	72,79a	72,92a	79,33a
	Oyek	67,15a	69,07a	75,82a
Diabetic	Standard	69,52a	117,45b	120,67c
	Oyek	69,33a	113,60b	90,30b

*) The same notation of statistic in the table showed not significantly differences at the same column

Total cholesterol

The total cholesterol of normal and diabetic rats increased during the feed trealment but the increase of cholesterol of diabetic rats were higher than normal rats, that was seen at Table 2.

Table 2. The effect of oyek feed treatment on blood total cholesterol of normal and diabetic rats (mg/dL)*)

Rats condition	Feed treatment	0th days	3th days	18th days
Normal	Standard	106,28a	112,15a	112,59a
	Oyek	106,46a	108,66a	112,53a
Diabetic	Standard	104,11a	152,49b	154,31c
	Oyek	103,40a	156,54b	133,45b

*) The same notation of statistic in the table showed not significantly differences at the same column

Data of the table showed that the increase of cholesterol was inhibited by oyek feed treatment. The cholesterol of diabetic rats reduced 20.5% after oyek feed treatment for 3th days until 18th days. The data indicated that protein isolate of oyek was potential to controle cholesterol of diabetic patient. However the cholesterol level of all rats were still normal, that were < 200mg/dl (Anonim, 2007; Herlina et al., 2013).

HDL cholesterol

Decreashing HDL of diabetic rats could be avoided by oyek treatment, that was seen at Table 3. The HDL of diabetic rats after oyek treatment for18th days increased significantly. Although the HDL of all rat was abnormal. The normal HDL cholesterol of human according to US National Cholesterol Education Program (NCEP) was > 60mg/dl (Anonim, 2007). While the normal HDL cholesterol of rat was > 45mg/dl (Herlina et al., 2013). This fact indicated that oyek with additionof cowpeas sprout could induce the formation of HDL, so the complication of diabetic could be prevented. The result conformed with Airliss and Biermann (2002) who showed that protein isolate of soybean feed treatment could increase 50% HDL and decrease 30-40% total cholesterol. Kanetro (2015) also showed that protein isolate of cowpeas sprout could increase HDL through in vivo bioassay.

Table 3. The effect of oyek feed treatment on blood HDL cholesterol of normal and diabetic rats (mg/dL)*)

Rats condition	Feed treatment	0th days	3th days	18th days
Normal	Standard	44,16b	45,08b	40,12c
	Oyek	41,69b	40,84b	38,50bc
Diabetic	Standard	35,91a	16,16a	14,76a
	Oyek	39,34ab	19,49a	30,86b

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LDL cholesterol

The LDL of all rats were normal that was seen at Table 4, The normal LDL cholesterol of human according to US National Cholesterol Education Program (NCEP) was < 100mg/dl (Anonim, 2007). While the normal LDL cholesterol of rat was < 135mg/dl (Herlina *et al.*, 2013). The increase of LDL after injection of aloxan at 3th conformed with the increase of total cholesterol (Table 2).

The trend was same as the diabetic rats after oyek feed treatment. The LDL of this treatment increased at 3th days and then decreased until treatment for 18th days. The LDL of diabetic rats after standard feed treatment for 18th days increased 84,4% compared with control treatment for 0th days. While The LDL of diabetics rats after oyek feed treatment increased 36% compared with control treatment for 0th days. The result indicated that oyek with addition of cowpeas sprout could inhibit the increase of LDL cholesterol.

Table 4. The effect of oyek feed treatment on blood LDL cholesterol of normal and diabetic rats (mg/dL)*)

Rats condition	Feed treatment	0th days	3th days	18th days
Normal	Standard	57,54b	62,91a	59,59a
	Oyek	58,09b	58,19a	61,59a
Diabetic	Standard	46,75a	86,20b	87,18
	Oyek	49,01a	82,78b	66,67ab

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The Ratio of Total Cholesterol/HDL and LDL/HDL

The ratio was computed from the data of Table 2 and 3 for total cholesterol/HDL ratio and the data of Table 3 and 4 for LDL/HDL ratio, that was seen at Table 5 and 6 respectively. The ratio indicated the risk of coronary heart disease (Fernandez and Webb. 2008). The normal level of the ratio of total cholesterol/HDL was < 5 and LDL/HDL was <3.2 for women and < 3.5 for men (Chandler and Zamora, 2011). Based on the ratio of cholesterol total/HDL and LDL/HDL were known that the ratio of all the rats including diabetic rats with oyek feed treatment for 18 days were normal, exception diabetic rats with standard feed treatment.

Table 5. The effect of oyek feed treatment for 0thand 18th days on the ratio of total cholesterol/HDL *)

Rats condition	Feed treatment	0th days	18th days
Normal	Standard	2.41	2.81

	Oyek	2.55	2.92
Diabetic	Standard	2.90	10.45
	Oyek	2.63	4.32

*computed according to Table 2 and 3.

Table 6. The effect of oyek feed treatment for 0^{th} and 18^{th} days on the ratio of LDL/HDL cholesterol *)

Rats condition	Feed treatment	0^{th} days	18^{th} days
Normal	Standard	1.30	1.49
	Oyek	1.39	1.60
Diabetic	Standard	1.30	5.91
	Oyek	1.25	2.16

*computed according to Table 3 and 4.

CONCLUSIONS

The potency of hypocholesterolemic of oyek were shown by decreasing of blood triglyceride, cholesterol total, LDL, and increasing of blood HDL in all rats treatments, especially diabetics rats with oyek feed treatment. Based on the ratio of cholesterol total/HDL and LDL/HDL showed that the ratio of all the rats including diabetic rats with oyek feed treatment for18 days were normal. This result indicated that oyek with addition of cowpeas sprout flour had the potency of hypocholesterolemic and might be used to prevent diabetic complication. Oyek with addition of cowpeas sprout flour could be potential as functional food.

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