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Hypocholesterolemic Properties of Protein Isolate from Cowpeas (*Vigna unguiculata*) Sprout in Normal and Diabetic Rats

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Abstract

This research was aimed to determine the potency of hypocholestrolemic properties of protein isolate from cowpeas sprout through in vivo bioassay by using Sprague Dawley male rats. The treatments of the research were rat conditions (normal and diabeic rats) and feed treatments (standard and protein isolate feed). Blood triglyceride, cholesterol total, High Density Lipoprotein (HDL), and Low Density Lipoprotein (LDL), of rats were analysed on 3th, 6 th, 9 th, 12 th, 15 th days for the treatment and before treatment as control. The result of this research showed that the blood triglyceride, cholesterol total, LDL decreased and the blood HDL increased in diabetics rats with protein isolate treatment. The potency of hypocholestrolemic activity were shown by the ratio of cholesterol total/HDL and LDL/HDL. The ratio of the rats were normal, exception diabetic rats with standart feed.

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Key words: cowpeas, sprout, hypocholesterolemic, protein isolate

INTRODUCTION

Soy protein has been known as functional food due to its composition of amino acid especially arginine. Soy protein had hypocholesterolemic effect.The ratio of arginine/lysine was important to control cholesterol level [4]. Soybean prices in the world market increased recently. Its prices in Indonesia would also increase because soybean was still imported.

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Therefore many researches efforted to find the materialfor substituting soybean as a functional food .

There are many local legumes in Indonesia, such as cowpeas. The local legumes may be potential for protein isolate production which substitute soybean. The protein isolate from cowpeas sprout contained arginine and its ratio of arginie/lysine was no significant different with protein isolate of soybean [7]. Therefore the protein isolate of cowpea sprout was potential as functional food for decreasing cholesterol. However the potency of protein isolate form cowpea sprout as hypocholesterolemic component base on in vivo bioassay has not been known. This research was aimed to determine hypocholesterolemic properties of protein isolate from cowpea sprout in normal and diabetic rats through in vivo bioassay.

MATERIAL AND METHODS

The main materials of this research were cowpea seed (*Vigna unguiculata*) from Beringharjo market in Yogyakarta, and Sprague-Dawley rats were obtained from Animal Experiment Development Unit UGM, Yogyakarta for in vivo biological testing. The other materials were bioassay's materials, including alloxan (Sigma), corn starch, casein, vitamin mix, mineral mix, sucrose, choline bitartat, soy oil, cholesterol kit (DiaSys Diagnostic System GmBH & Co.), and chemicals for protein isolation ie HCl (Merck), and NaOH (Merck). Chemical agents, such as aloxan, glukosa kit (*DiaSys Diagnostic System GmBH & Co*), dan kholesterol kit (*DiaSys Diagnostic System GmBH & Co*) were purchased from Sigma Chemical Co.

Isolation of Protein from Cowpea Sprout

Cowpea seeds were soaked for 8h, and then germinated for 36h. Proteins of cowpea sprout were isolated along with Yusniardi [9]. The protein were extracted at pH 9 and then precipitated at pH 4. The precipitates of protein were dried by oven at 50^{o}C before stored and analyzed.

In Vivo Bioassay

The *in vivo* bioassay was done to determine the potency of hypocholesterolemic of protein isolate from cowpeas sprout by using 20 *Sprague Dawley*male rats. The experiment sequences of the step were adaptation of rats for 3 days, diveded rats into 4 groups, treated rats for 15 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}, 6^{th}, 9^{th}, 12^{th}, 15^{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 feedaccording to AIN–93 [8] and protein isolate feed which was prepared by subtitution of casein protein in standart feed with the protein isolate from cowpeas sprout.The data of this experiments was statistical analysed by Anova (analysis of varian) and DMRT (Duncan Multiple Range Test).

RESULT AND DISCUSSION

Trygliceride

The trygliceride of normal rats treatment were no significant differences between standart feed and protein isolate feed treatment. While the trygliseride of diabetic rats teratment showed significantly differences, especially for the treatment of rats on 12^{th} and 15^{th} days, that was seen at Table 1. The trygliseride of diabetic rats with protein isolate feed treatment increased in the beginning treatment until 6^{th} days, and then decreased until below the standart of normal cholesterol. The normal trygliceride of human according to *US National Cholesterol Education Program* (NCEP) was < 150 mg/dl [1]. While the normal trygliceride of rat was < 120 mg/dl [6].

Table1. The effect of protein isolate from cowpea sprout feed treatment on tryglyceride of normal and diabetic rats (mg/dL) *)

Rats condition	Feed treatment	0th days	3th days	6thdays	9thdays	12th days	15th days
Normal	Standard	72.4b	74.8a	73.5a	75.4a	75.9a	77.0a
	Protein isolate	77.9b	79.3b	78.7a	76.7a	75.0a	76.6a
Diabetic	Standard	75.2b	154.3c	152.2b	156.9b	158.1c	160.9c
	Protein isolate	67.2a	157.3c	155.3b	141.1b	135.1b	92.5b

*) The same notation of statistic in the table showed 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. Data of the table showed that the increase of cholesterol was inhibited by protein isolate feed treatment. The cholesterol of diabetic rats reduced 40.4% after protein isolate feed treatment for 6th days until 15th days. The data indicated that protein isolate of cowpea sprout was potential to controle cholesterol of diabetic patient. However the cholesterol level of all rats were still normal (< 200 mg/dl) [1; 6].

Table 2. The effect of protein isolate from cowpea sprout feed treatment on total cholesterol of normal and diabetic rats (mg/dL) *)

Rats condition	Feed treatment	0th days	3th days	6thdays	9thdays	12th days	15th days
Normal	Standard	104.5ab	105.7a	105.1a	107.8a	108.5a	111.8b
	Protein isolate	111.5b	112.4b	111.1a	109.3a	107.6a	100.0a
Diabetic	Standard	106.4ab	192.4c	189.7b	193.3c	195.5c	200.8c
	Protein isolate	99.3a	193.3c	189.7b	165.3b	153.5b	106.3ab

*) The same notation of statistic in the table showed significantly differences at the same column

HDLCholesterol

The HDL of rats during treatment were stable except standard feed treatment for diabetic rats. The HDL of the diabetic rats decreased 53.6% after standard feed treatment for 15th days, that was seen at Table 3. The normal HDL cholesterol of human according to *US National Cholesterol Education Program* (NCEP) was < 60mg/dl [1]. While the normal HDL cholesterol of rat was < 45mg/dl [6].

Table 3. The effect of protein isolate from cowpea sprout feed treatment on HDL cholesterol of normal and diabetic rats (mg/dL) *)

Rats condition	Feed treatment	0^{th} days	3^{th} days	6^{th}days	9^{th}days	12^{th} days	15^{th} days
Normal	Standard	67.3a	62.3b	63.7b	62.7b	61.3b	61.0b
	Protein isolate	71.8a	69.7b	71.0b	73.3b	70.2b	72.1b
Diabetic	Standard	69.2a	40.8a	41.8a	40.4a	39.4a	38.2a
	Protein isolate	63.0a	40.3a	41.4a	43.6a	47.7a	62.2b

*) The same notation of statistic in the table showed significantly differences at the same column

Data in Table 3 showed that the decrease HDL of diabetic rats after protein isolate feed treatment could be avoided. Even the HDL of diabetic rats after protein isolate feed treatment for 15^{th} days increased significantly. This fact indicated that protein isolate of cowpea sprout could induce the formation of HDL, so the complication of diabetic could be prevented. While casein protein in standard feed treatment might decrease HDL. The result conformed with Airliss and Biermann [2] who showed that protein isolate of soybean feed treatment could increase 50% HDL and decrease 30-40% total cholesterol.

LDL Cholesterol

The increase of LDL, that was seen at Table 4, conformed with the increase of total cholesterol (Table 2). The trend was same as the diabetic rats after protein isolate feed treatment. The LDL of this treatment increased before treatment for 6^{th} days, and then decreased until treatment for 15^{th} days at normal level of LDL. The normal LDL cholesterol of human according to *US National Cholesterol Education Program* (NCEP) was < 100mg/dl [1]. While the normal HDL cholesterol of rat was < 135mg/dl [6]. The LDL of diabetic rats after standard feed treatment for 15^{th} days increased 4.87 times compared with control treatment for 0^{th} days. The decrease of LDL of normal rats after protein isolate feed treatment was 47.82%.

Table 4. The effect of protein isolate from cowpea sprout feed treatment on LDL cholesterol of normal and diabetic rats (mg/dL) *)

Rats condition	Feed treatment	0th days	3th days	6thdays	9thdays	12th days	15th days
Normal	Standard	22.8a	28.4a	26.7a	30.1a	32.0a	35.4a
	Protein isolate	24.1a	26.8a	24.4a	20.6a	22.3a	12.6a
Diabetic	Standard	22.2a	120.8b	117.4b	121.5b	124.6b	130.4b
	Protein isolate	22.8a	121.6b	117.2b	93.4b	78.7ab	25.5a

*) The same notation of statistic in the table showed significantly differences at the same column

The Ratio of Total Cholesterol/HDL and LDL/HDL

The ratio indicated coronary risk disease [5]. 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 [3]. 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. Based on the ratio of cholesterol total/HDL and LDL/HDL, the ratio of all the rats including diabetic rats with protein isolate feed treatment for 15 days were normal except diabetic rats with standart feed treatment.

Table 5. The effect of protein isolate from cowpea sprout feed treatment for 0thand 15th days on the ratio of total cholesterol/HDL *

Rats condition	Feed treatment	0th days	15th days
Normal	Standard	1.6	1.8
	Protein isolate	1.6	1.4
Diabetic	Standard	1.5	5.3
	Protein isolate	1.6	1.7

*computed according to Table 2 and 3.

Table 6. The effect of protein isolate from cowpea sprout feed treatment for 0thand 15th days on the ratio of LDL/HDL *

Rats condition	Feed treatment	0th days	15th days
Normal	Standard	0.3	0.6
	Protein isolate	0.3	0.2
Diabetic	Standard	0.3	3.4
	Protein isolate	0.4	0.4

*computed according to Table 2 and 3.

CONCLUSION

The potency of hypocholesterolemic were shown by decreasing of blood triglyceride, cholesterol total, LDL, and increasing of blood HDL in all rats treatments especially diabetics rats with protein isolate feed treatment. Based on the ratio of cholesterol total/HDL and LDL/HDL, the ratio of all the rats including diabetic rats with protein isolate feed treatment for 15 days were normal. This result indicated that cowpeas sprout protein had the potency of hypocholesterolemic and might be used to prevent diabetic complication.

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