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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 hypocholesterolemic 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 diabetic 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 3<sup>th</sup>, 6<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup>, 15<sup>th</sup> 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 diabetic rats with protein isolate treatment. The potency of hypocholesterolemic 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°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, divided 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<sup>th</sup>, 6<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup>, 15<sup>th</sup> days and before treatment as control (0<sup>th</sup>). 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 [8] and protein isolate feed which was prepared by substitution 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<sup>th</sup> and 15<sup>th</sup> days, that was seen at Table 1. The trygliseride of diabetic rats with protein isolate feed treatment increased in the beginning treatment until 6<sup>th</sup> 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	0 <sup>th</sup> days	3 <sup>th</sup> days	6 <sup>th</sup> days	9 <sup>th</sup> days	12 <sup>th</sup> days	15 <sup>th</sup> 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 treatment 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 6<sup>th</sup> days until 15<sup>th</sup> days. The data indicated that protein isolate of cowpea sprout was potential to control 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	0 <sup>th</sup> days	3 <sup>th</sup> days	6 <sup>th</sup> days	9 <sup>th</sup> days	12 <sup>th</sup> days	15 <sup>th</sup> 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

### HDL Cholesterol

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 15<sup>th</sup> 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 <sup>th</sup> days	3 <sup>th</sup> days	6 <sup>th</sup> days	9 <sup>th</sup> days	12 <sup>th</sup> days	15 <sup>th</sup> 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<sup>th</sup> 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<sup>th</sup> days, and then decreased until treatment for 15<sup>th</sup> 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<sup>th</sup> days increased 4.87 times compared with control treatment for 0<sup>th</sup> 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	0 <sup>th</sup> days	3 <sup>th</sup> days	6 <sup>th</sup> days	9 <sup>th</sup> days	12 <sup>th</sup> days	15 <sup>th</sup> 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 0<sup>th</sup> and 15<sup>th</sup> days on the ratio of total cholesterol/HDL \*

Rats condition	Feed treatment	0 <sup>th</sup> days	15 <sup>th</sup> 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 0<sup>th</sup> and 15<sup>th</sup> days on the ratio of LDL/HDL \*

Rats condition	Feed treatment	0 <sup>th</sup> days	15 <sup>th</sup> 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.

## REFERENCE

- [1].Anonim, 2007. Normal and High Cholesterol Value.<http://www.prolipid.com/cholesterol-reference-levels/normal-and-high-cholesterol-values.html> 25/9/2012.
- [2].Arliss, RM. and Biermann, CA. 2002. Do Soy Isoflavon Lower Cholesterol. Inhibit Atherosclerosis.and Play a Role in Cancer Prevention. *Holistic Nursing Practice* 16(5):40-48.
- [3].Chandler ,S.and Zamora, A. 2011. Cholesterol-LDL-HDL-Ratio. <http://www.scientificpsychic.com/health/cholesterol-LDL-HDL.html> 25/9/2012.
- [4].Damasceno, NR. Goto,H. Fernada,MDR. Dias,CTS. Okawabata,FS. Dulcinela,SP. Abdalia, DS. and Gidlund,MA. 2000. Soy Protein Isolate Reduces the Oxidizability of LDL and Generation of Oxidized LDL Autoantibodies in Rabbit with Diet-Induced Atherosclerosis. *J of Nutr.* 130:2641-2647.
- [5].Fernandez,ML. and Webb, D. 2008. The LDL to HDL Cholesterol Ratio As a Valuable Tool toEvaluate Coronary Heart Disease Risk. *Journal of the American College of Nutrition*27:1–5.
- [6].Herlina. Harijono. Subagio, A. and Estiasih, T. 2013. Hipolipidemic Potential of Water Soluble Polysaccharides from Gembili Tuber (*Dioscoreaesculenta* L.) in Hiperlipidemia Rats. *Agritech J Teknol Pertanian* 33:8-15.
- [7].Kanetro, B and Dewi,SHC. 2013.Effect of Various Local Legume Sprouts as Raw Materials of Meat Analog on The Physical (Texture), Preference and Arginine/Lysine Ratio Characteristics. *Agritech J Teknologi Pertanian* 33:1-7.
- [8].Reeves,PG. Nielsen,FH. and Fahey, GC. 1993. AIN-93 Purified Diets for Laboratory Rodents: Final Report of The American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. *J of Nutr.* Vol. 123:1939-1951.
- [9].Yusniardi ,E. Kanetro, B.and Slamet, A. 2010.The Effect of Fat Content on Physical and Sensory Properties of Meat Analog from Germinated Cowpeas (*Vigna unguiculata*) . *Agritech J Teknologi Pertanian* 30: 148-151.

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