Hypoglycemic In Vivo Bioassay of Protein Isolate from Cowpeas (Vigna unguiculata) Sprout

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Abstract

This research was aimed to determine the potency of hypoglycemic activity 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 glucose 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 potency of hypoglycemic activity were shown by decreasing of blood glucose level in diabetic rats with protein isolate treatment. While the blood glucose of diabetic rats with protein isolate feed reduced to normal level on 12th and 15th days, that was indicated that protein isolate from cowpeas sprout could normalize blood glucose.

Keywords: cowpeas, sprout, hypoglycemic, protein isolate

1. INTRODUCTION

The development of protein isolate consumption or vegetable-based meat alternatives in the future will increase in the future along with increasing in vegetarian group, due to their potency prevent the onset of many degenerative diseases. Soybean seed has popularly known as functional food for preventing degenerative diseases, esspecially diabetes. it is due to its ability to reduce blood glucose. Soybean protein had hypoglicemic effect due to its potency for stimulation of insulin secretion, and its ability to reduce blood glucose. The potency of soybean sprout protein stimulated insulin secretion was higher than soybean protein (Kanetro *etal*, 2008). The other reseacher had shown that the germination of soybean (Pathak, 2005), rice (Usuki *et al.*, 2007) could increase the potency for decreasing blood glucose. Soybean sprout protein also showed the role as *insulin-like protein* (Pathak dan Martirosyan, 2011).

Hypoglicemic property of soybean related to the composition of amino acids, in particular arginine (Kanetro *etal,* 2008). Amino acids may influence insulin secretion via a number of possible mechanisms, including generation of metabolic coupling factor, depolarization of the plasma membrane, or enhancement of mitochondrial function (Newsholme *et al.*, 2006). The specifics amino acids that are known as insulin stimulation can activate mitochondrial metabolism in pancreatic β-cell via the tricarboxylic acid (TCA) cycle, resulting in the formation of ATP. The rise in ATP levels leads to closure of ATP-dependent K^+ channels, which in turn depolarizes the cell membrane, thus opening of voltage-dependent Ca^{2+} channels and increasing

intracellular Ca^{2+} concentration, which triggers insulin exocytosis and hence facilitating insulin secretion from pancreatic β-cell (Argmann and Auwerx, 2006; Newsholme *et al*., 2007).

The increasingly high price of soybean in Indonesia encourage research to replace soybean as a functional food. Based on previous research it was known that the protein isolate from cowpea sprout contained arginine (Kanetro dan Dewi, 2013). But the hypoglycemic ability of protein isolate from cowpea sprouts has not been known. The purpose of this research was to study the hypoglycemic ability of protein isolate from cowpea sprouts through in vivo bioassay

2. METHODS

2.1 Chemicals

The main materials of this research were cowpea seed (*Vigna unguiculata*) from Beringharjo market in Yogyakarta, and Sprague-Dawley rats obtained from Animal Experiment Development Unit UGM, Yogyakarta for in vivo biological testing. The other materials were chemicals to the feed and bioassay, 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.

2.2. Procedures

2.2.1 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 *et al.,* (2010). 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.

2.2.2 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 glucose 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 feed according to AIN–93 (Reeves *etal,* 1993) 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).

3. RESULTS AND DISCUSSION

The body weight of rats during the experiment shown in Table 1 The weight of

normal rats increased, while the body weight of diabetic rats decreased despite the decline in the treatment of diabetic rats with fed protein isolates occur at the beginning of the experiment or until 12th day and subsequent the weight increased. The percentage change in body weight of rats on day 15 (end of treatment) compared to day 0 (before treatment) shown in Table 1 showed that the treatment of diabetic rats fed protein isolate was relatively stable or declining as the standard feed treatment. This indicated that feeding protein isolate could be expected to improve the condition of diabetic rat to normal, because of the potential for protein isolates of cowpeas sprout as functional food described in further discussion.

Tabel 1. Body weight of rats during the experiments (g)*

Rats conditions	Feed treatments	0th day	3th days	6th days	9th days	12th days	15th days	% icrease (+)/decrease (-) in weight
Normal	Standar	201.4b	204.4b	207,b	209.6b	214.4c	220.8b	+9,6
	Protein isolate	202.0b	205.4b	208.4b	212.6b	218.2c	224.2b	+11,0
Diabetic	Standar	195.2a	189.2a	187.8a	185.4a	183.0a	179.8a	-7,9
	Protein isolate	199.9b	193.2a	194.6ab	197.2ab	199.2b	203.2b	+1,7

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

Table 2 showed that blood glucose level of all rats before treatment were 72.2 – 73.1 mg/dL. This indicated that all rats were normal or not diabetic. The glucose level of human diabetic condition was higher than 180mg/dL (Burtis *et al*., 1988), while The glucose level of rat diabetic condition was higher than 109mg/dL (Garrison, 2013). The glucose level after aloxan injection at 3th days increased significantly and these rats were diabetics. The potency of hypoglycemic were shown by decreasing of blood glucose level in diabetic rats with protein isolate oyek. On 15th days treatment, The blood glucose of the diabetic rats with standard feed increased and they were still diabetec. While the blood glucose of diabetic rats with protein isolate feed treatment reduced 52.78% on 15th days after the treatment. This indicated that protein isolate of cowpeas sprout was potential to normalize blood glucose.

Table 2. The effect feed treatment of protein isolate of germinated cowpeas on glucose level of normal and diabetic rats (mg/dL)

Rat conditions	Feed treatment	0th day	3thdays	6th days	9th days	12th days	15th days
Normal	Standar	72.2a	72.5a	72.4a	73.6a	73.3a	74.6a
	Protein isolate	73.1a	73.1a	72.8a	72.6a	71.3a	71.8b
Diabetic	Standar	72.4a	223.2b	223.6b	226.2b	225.2b	228.3b
	Protein isolate	72.5a	221.9b	221.4b	189.7b	160.3b	104.8ab

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

4. CONCLUSIONS

The potency of hypoglycemic were shown by decreasing of blood glucose level in diabetic rats with the treatment of protein isolate of germinated cowpeas. This result indicated that protein isolate might be used to prevent and cure diabetic. Protein isolate of cowpeas sprout could be potential to be added to the product as functional food.

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