The effective dose and pattern of soybean extract administration to regulate body weight of laboratory rats

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Abstrak

Latar Belakang: *Protein dalam kedelai, β conglycinin mempunyai efek menekan nafsu makan melalui stimulasi Kolesistokinin. Kolesistokinin adalah hormon yang dilepaskan di saluran pencernaan dipicu oleh asupan protein dan berefek menekan nafsu makan untuk jangka pendek. Detam 1 adalah jenis kedelai berkualitas tinggi berdasarkan dengan Menteri Pertanian Indonesia. Ekstrak protein kedelai Detam 1 oleh Deak metode mengandung kadar β conglycinin yang tinggi. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh ekstrak protein kedelai Detam 1 metode Deak dalam menurunkan asupan makanan, berat badan, dan kadar CCK plasma selama 14 dan 28 hari pada berbagai dosis dan pola pemberian pada tikus Wistar jantan.*

Metode: *Terdapat sebelas kelompok perlakuan (n=3), kelompok dosis (5mg/1x//hari, 10mg/1x/hari, 20mg/1x/hari), (2,5mg/2x/hari, 5mg/2x/hari, 10mg/2x/hari) dan (1,7mg/3x/hari, 3,4mg/3x/ hari, 6,7mg/3x/ hari), kelompok kontrol negatif (akuades) dan kelompok positif kontrol (Sibutramine). asupan makanan (g), berat badan (g) dan pengukuran tingkat Cholecystokinin plasma dengan metode ELISA (ng / ml).*

Hasil: *Hasil penelitian menunjukkan bahwa persentase penurunan asupan makanan yang terbaik adalah: kelompok 3,4mg/3x/hari (p <0,05), penghambatan berat badan selama 14 hari: kelompok 10 mg/1x/hari, selama 28 hari: kelompok 1,7mg/3x/hari (p <0,05), peningkatan kadar Kolesistokinin plasma: kelompok 20 mg / 1 x / hari (p <0,05).*

Simpulan: *Dosis dan pola pemberian terbaik untuk menghambat berat badan selama 14 hari adalah ekstrak 10 mg sekali sehari di pagi hari, selama 28 hari adalah 1,7 mg tiga kali sehari.* ***(Health Science Journal of Indonesia 2016;7:17-26)***

Kata kunci: *Kedelai Detam 1 -dosis efektif - berat badan - kolesistokinin*

Abstract

Background: Protein in soybean, β conglycinin is responsible for anti-obesity effects by suppressing appetite via stimulation of Cholecystokinin. Cholecystokinin is a hormone released in the digestive tract in response to the intake of protein and suppress appetite for short-term. *Detam* 1 variety is a high-quality soybean according to the Minister of Agriculture of Indonesia. Soybean protein extract Detam 1 by Deak method contains high levels of β conglycinin. The purpose of this study was to determine the effective dose of protein extract Detam 1 soybean Deak Method (PEDSDM) in reducing food intake, regulate body weight, and plasma CCK level for 14 and 28 days at various dosage and pattern of treatment on male Wistar rats.

Methods: There were eleven groups of treatment (n = 3), administrated with extracts at 5 mg/1x/day, 10 mg/1x/day, 20 mg/1x/day, 2.5 mg/2x/day, 5 mg/2x/day, 10 mg/2x/day and 1.7mg/3x/day, 3.4mg/3x/day, 6.7 mg/3x/day, negative control group (distilled water) and positive control group (Sibutramine). Food intake (g), weight loss (g) and measurement of plasma Cholecystokinin levels by ELISA (ng /ml)

Results: The results showed that the highest percentage decrease in food intake is: group 3.4mg /3x/ day ($p < 0.05$), inhibition weight gain for 14 days: group 10 mg /1x/ day, for 28 days: group 1.7 mg/3x/day ($p < 0.05$), increased plasma Cholecystokinin levels: group 20 mg /1x/day ($p < 0.05$).

Conclusions: The effective dose and pattern administrating the rats for 14 days is extract of 10 mg once a day in the morning, for 28 days is 1.7 mg three times a day. ***(Health Science Journal of Indonesia 2016;7:17-26)***

Keywords: Soybean var Detam 1 -effective dose - body weight - Cholecystokinin

The worldwide prevalence of obesity has nearly doubled between 1980 and 2008. In 2008, 10% of men and 14% of women in the world were obese (BMI ≥30 kg/m2), compared with 5% for men and 8% for women in 1980. An estimated 205 million men and 297 million women over the age of 20 were obese – a total of more than half a billion adults worldwide.[1] This condition should be overcome because obesity can cause many side effects. The solution is to discover good nutrition sources that can reduce body weight, like soybean. Soybean (*Glycine max* L. merr) is known for the health benefits, good dietary supplements for both humans and animals. Nutritional study showed that soybean protein produced higher thermogenic and satiety effect than that of carbohydrate. This fact made soybean as a good nutrition source for reducing obesity.[2]

Elvira de Mejia, assistant professor of food science and human nutrition in University of Illinois at Urbana Champaign, found that soy did have an effect on mechanisms which similar with Leptin.[3,4] Leptin is a hormone which is produced in our adipose tissue that interacts with receptors in the brain and signals us that we're full so we stop eating and hormones that are induced in the body to help us degrade lipids and reduce body weight, by boosting metabolism and not by reducing food intake.[3,4] According to Liu study, six-month supplementation of soy protein with isoflavones had a mild favorable effect on body composition in postmenopausal women.[5]

Previous studies reported that, β-conglycinin peptone which contains abundantly in soybean, stimulating endogenous Cholecystokinin (CCK) to transduction signal in intestinal cells that suppressed appetite and food intakes.[6,7] The fragment from 51 to 63 of the β-subunit (β51-63) of β-conglycinin had the strongest binding activity to stimulate releasing CCK and appetite suppression.[7] CCK is an important hormonal regulator of the digestive process. CCK is released in the digestive tract in response to the intake of protein and fat and suppress appetite for short-term. The physiological actions of CCK include stimulation of pancreatic secretion and gallbladder contraction, regulation of gastric emptying, and induction of satiety. Therefore, the ability to stimulate CCK has made β-conglycinin in soybean as one of the important therapy in obesity treatment.[8]

Protein extracts of Detam 1 soybean *(Glycine max* L.Merr) Deak method (PEDSDM) contained protein level of 41,82% from its dry weight, measured by *Kjeldahl* methods; alkaloids (Wagner) and triterpenoids by qualitative phytochemical test. The PEDSDM was reported for the best in decreasing the food intake compared to other extracts on male Wistar rats, which administered PEDSDM 80 mg/kgBW~20 mg/250g/rat (the food intake and bodyweight at baseline were (21,77 + 2,82)g and (238,33 + 21,38)g, and at endline were (12,6 + 0,89) g and (189,33 + 27,30)g, p = 0,022), and (p = 0,020), respectively.[9,10] But we don't have any data about the effective dose and the best pattern of PEDSDM administration, to complete these data, therefore this study need to be done.

There are several kinds of extraction methods to produce β-conglycinin. Deak method is the procedure to produce protein fractions (> 90% protein) which is rich in either glycinin or β-conglycinin by employing CaCl2 and NaHSO3.[11] Deak method was proved to produce β-conglycinin better than other methods, measured by SDS PAGE and CBB staining. The proportion of β-*conglycinin* level (7 S *Globulin*) in *Detam* 1 soybean seed is 27% while β-*conglycinin* sub unit β (54 kDa) in PEDSDM is 25 : 31,7 DU= 78,86 % from total β-*conglycinin*.[12]

The duration effect of CCK in suppressing appetite is short, begin in 15 minutes after induction by peptone, reached the peak level in 45 minutes then sustained for 2 hours.[6,7] So that we want to know the best administered of PEDSDM to induce CCK secretion due to regulating body weight.

The aim of this study is to determine the effective dose and pattern of the PEDSDM administration on food intake, body weight and CCK level in male Wistar rats.

METHODS

Soybean seed variety *Detam* 1, a high quality of black soybean approved by the Agricultural Ministry of Republic of Indonesia were cultivated on Research and Development Unit of Legumes and Tubers' in Malang, East Java Indonesia.[9] Its protein content is higher than other soybean varieties 45.36%. Then *Detam* 1 soybean were extracted to be PEDSDM using Deak's method.[11]

The procedures of Deak's method is as explained below. Defatted soy flour of 100 g was extracted using deionized water with ratio of 1:15 (w/v) at pH 8.5 adjusted with 2 N NaOH. The slurry was stirred for 1 h and centrifuged at 14,000 g and 15 oC for 30 min. The protein extract (first protein extract) was

decanted. The extract was then added with sufficient $NaHSO_3$ and $CaCl_2$ to obtain concentration of either SO2 and Ca2+ to 5 mM and the pH was adjusted to 6.4 with 2 N HCl. The slurry was stored at 4 °C for 12-16 h, then centrifuged at 14,000 g for 30 min at 4 °C. A glycinin-rich fraction was obtained as the precipitated curd, which was neutralized and treated as described on Wu procedure. The supernatant (second protein extract) was adjusted to pH 4.8 with HCl. Then it was stirred for 1 hour and centrifuged at 14,000 g and 4 °C for 30 min. A β - conglycinin-rich fraction was obtained as the precipitated curd and treated as described previously. The amount of supernatant (whey) was determined and sampled. Freeze-drying steps were modified to evaporation process in cycling evaporator at 30 °C until the solution extracts become thick liquid. Samples were placed in sealed containers and stored at 4 °C up to analysis. There are two steps in Deak's procedure i.e. D4C (in 4 °C) and DRT (in 25 °C).[11] The Deak β -conglycinin-rich fraction D4C (the methods which performed in this study) comprised 23.1% of the solids, 37.1% of the protein and 37.5% of the isoflavones in the starting soy flour. PDSDM protein purity was > 80%. Their D4C method produced 85.6% β-conglycinin and 14.4% glycinin. The β–conglycinin subunit consisted of 27.3% subunit α', 38.0% subunit α'' and 34.7% subunit α.[12]

Animal Research Procedure

The number of experimental animals were calculated by Ali Hanafiah formula,[13] that 3 rats each groups, with 11 different group of treatments is enough for this experimental. This study has approved by University Christian of Maranatha Ethics Commission, SKEP no 148/KEP FK UKM-RSI/V/2009. Thirty three male Wistar rats, aged 8-10 weeks, weighing between 220-250 g, after aclimatization for 7 days, they were randomly divided into 11 groups, then administrated with protein extract of Detam 1 soybean for 28 days.

Group **A**, Negative control **B.** PEDSDM 5 mg/rat/day, once a day, **C.** PEDSDM 2.5 mg/rat /day, twice a day, **D.** PEDSDM 1.7 mg/rat/day three times a day, **E**. PEDSDM 10 mg/rat/day, once a day, **F.** PEDSDM 5 mg/rat/day, twice a day, **G.** PEDSDM 3.4 mg/rat/day three times a day, **H.** PEDSDM 20 mg/rat/day, once a day, **I.** PEDSDM 10 mg/rat/day, twice a day, **J.** PEDSDM 6.7 mg/rat/day three times a day, **K.** Sibutramine 0.18 mg/rat/day, as a positive control. All rats and the rest of fed were weighed once in two days. The parameters measured were daily food intake, body weight, once in 2 days and CCK plasma level after 28 days of treatments.

The food intake which were given *ad libitum* was Complete Feed Grains CP 551, which is produced by PT Charoen Pokphand Indonesia; contains 310 - 320 kkal/per 100g, with protein contents: 18.5 - 20.5 %. The treatment dose in this study, PEDSDM were calculated based on Nishy's formula[6] (10 mg/ kg or 2.5 mg / 250g), the rats were administered 5, 10 and 20 mg once daily at 08.00 a.m, twice at 08.00 a.m and 08.00 p.m, 3x daily at 08.00 a.m, 16.00 p.m and 24.00 p.m via oral gastric sonde.

Statistical Analysis

Data of food intake, body weight and CCK were analysed using ANOVA test, followed by Duncan HSD test.

Ethical approval from Ethical Review Board of Faculty of Medicine, Maranatha Christian University with No. 148/KEP FK UKM-RSI/V/2009.

RESULTS

After analysed distribution normality with Shapiro - Wilk test, it was found that most of the data were normally distributed ($p > 0.05$), then parametric statistical tests can be used

Effect of Soy Protein Extract Variety Detam 1 on Male Wistar rats feed intake

Table 1 showed the feed intake on male Wistar rats at baseline (day 0) and endline (day 28).

As seen in Figure 1, the result of Negative control group along 28 days of treatment, the food intake was between 19.5g/day-25g/day, baseline 22.00 and endline 21.5 with $p>0.05$. Group B, the least food intake was 15 g/day, the highest was 24g/day and decreased after 14 days, baseline 23.25 and endline 16.5 with $p>0.05$. Group D food intake was between 15g/day-22.9g/day and decreased after 7 days, baseline 21.33 and endline 16.0, $p>0.05$. The highest food intake of group G was 24.9g/day while the least food intake was 12 g/day, it was very low and tend to decrease after 7 days, baseline 24.00 and endline 17.0, $p>0.05$. Group K was between 14g/day-24g/day and decreased after 7 days, baseline 20.33 and endline 17.33 with $p>0.05$.

In general, all groups of treatment showed that the feed intake at endline tended to be decreased, however, the best was group B (PEDSDM 5 mg/rat 1x day), the second was D (PEDSDM 1.7 mg/rat 3 x day) and the third was G (PEDSDM 3.4 mg/rat 3 x day) ($p<0.05$).

Table 1. Description and Food Intake Paired t test on Male Wistar rats

Group No	Treatment	Animal Feed Baseline	Animal Feed Endline	Changes (Δ %)	p Value
		$\bar{x}$ (sd)	$\bar{x}$ (sd)		
A	NC, 2 ml aquadest	22.00 (2.16)	21.50 (2.38)	2.27 %	0.182
B	5 mg/rat/day, once a day	23.25 (1.71)	16.50 (1.29)	**29.3 %**	**0. 4**
C	2.5 mg/rat/day, twice a day	21.00 (1.73)	18.67 (0.58)	11.9 %	0.346
D	1.7 mg/rat/day three times a day	21.33 (3.21)	16.00 (6.24)	**24.98 %**	**0.18**
E	10 mg/rat/day, once a day	23.00 (2.45)	19.25 (3.50)	16.3 %	0.83
F	5 mg/rat/day, twice a day	22.00 (1.00)	21.00 (2.65)	4.54 %	0.796
G	3.4 mg/rat/day three times a day	24.00 (1.00)	17.00 (4.36)	**29.16 %**	**0 . 2**
H	20 mg/rat/day, once a day	24.50 (1.00)	22.25 (0.96)	9.18 %	0.281
I	10 mg/rat/day, twice a day	23.67 (2.31)	22.33 (3.06)	5.66 %	0.666
J	6.7 mg/rat/day three times a day	20.00 (1.00)	16.67 (4.93)	16.65 %	0 .15
K	PC, Sibutramine 0.18 mg/rat/day	20.33 (5.03)	17.33 (4.04)	**14.75 %**	**0.162**
	p Value	0.229	0.112		

Description : NC = Negative Control PC = Positive Control
% Change (Δ)= (The average and standard deviation sd feed intake at endline - feed intake at baseline) / baseline feed intake x 100 %

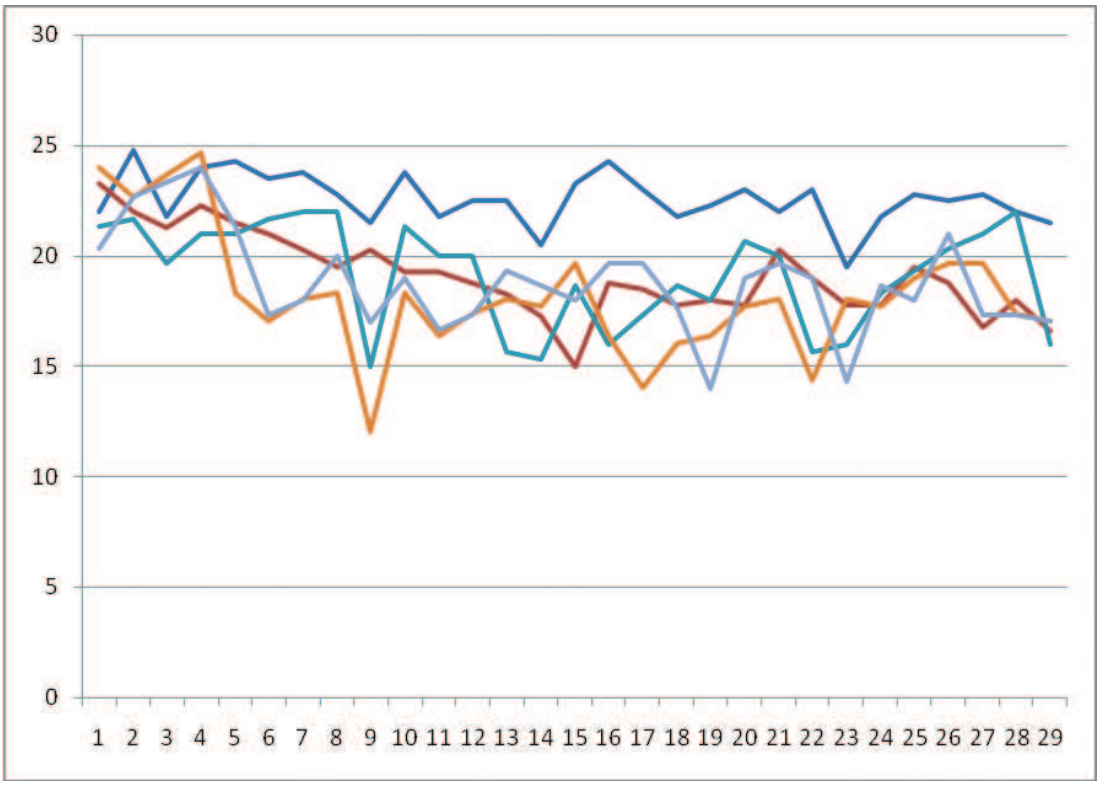

Figure 1. Profile of Food Intake in each group during 28 days

Legend:

Dark blue line : Group A: Negative Control
Red : Group B: PEDSDM 5 mg/rat 1x day that gives the best result in suppressing food intake after 28 days.
Light Blue Line : Group D: PEDSDM 1.7 mg/rat 3 x day that gives the third best result in suppressing food intake after 28 days.
Orange Line : Group G: PEDSDM 3.4 mg/rat 3 x day that gives the second best result in suppressing food intake after 28 days.
Pale Blue Line : Group K: Positive Control, Sibutramine dosage 0.18 mg/rat 1 x day

Effect of PEDSDM on Body Weight of Male Wistar rats

This study was conducted to find out the effect of PEDSDM on the inhibition of weight gain in Male Wistar rats during 28 days. The description of body weight in Male Wistar rats after 14 days, 28 days of treatments can be seen in table 2.

Table 2 showed that between the treatment groups there was no significant difference in average of bodyweight at end line, 0 days and 14 days ($p > 0.05$). Statistical analysis showed F count 14 days was 2.774 ($p = 0.018$), while F count 28 days was 5.870 ($p < 0.001$), which mean there was an effect of the treatment. There was significant changes of percentage between 14 days and 28 days ($p< 0.05$).

The results of the positive control group K Sibutramin were not in the same column with group A, negative control, so it conclude that the research methods used are considered valid. Most of the treatment group were not in the same column with group A, this suggests that the group treatment had different effects with Negative control, and was assumed that the treatment groups had potencies to control weight gain. Treatment Group E, H, I, F and B were in the same column with the comparison group K, indicated that this treatment groups had a similar effect with sibutramine, after 14 days of treatment (Table 3).

Table 2. Body Weight of Male Wistar rats in each group of treatment every two weeks

Group of Treatments	BW 0 d (g)	BW 14 d (g)	Percentage Increased	BW 28 d (g)	Percentage Increased
	$\bar{x}$ (sd)	$\bar{x}$ (sd)		$\bar{x}$ (sd)	
A	237.8 (20.3)	273.0 (21.7)	14.874%	305.3 (24.2)	28.450%
B	227.5 (10.4)	250.3 (20.1)	9.903%	270.8 (17.8)	18.929%
C	232.7 (8.1)	261.0 (1.0)	12.259%	292.3 (9.5)	25.762%
D	249.3 (32.9)	287.0 (54.8)	14.467%	293.3 (36.7)	**17.781%**
E	244.0 (19.6)	261.8 (22.8)	**7.248%**	289.0 (27.8)	**18.331%**
F	241.0 (17.8)	263.7 (23.3)	**9.325%**	302.0 (29.5)	25.167%
G	229.3 (19.7)	262.3 (30.0)	14.212%	275.0 (29.5)	19.783%
H	251.8 (24.1)	270.8 (25.3)	**7.575%**	298.3 (28.5)	**18.474%**
I	251.0 (34.9)	272.3 (37.5)	8.521%	317.3 (46.2)	26.356%
J	249.3 (21.4)	281.0 (19.0)	12.897%	308.7 (28.4)	23.768%
K	244.3 (18.0)	257.7 (22.6)	**5.388%**	284.3 (21.0)	**16.371%**
F test (ANOVA)	F = 0.562 p = 0.829	F = 0.502 p = 0.873	F = 2.774 p = 0.018	F = 0.844 p = 0.593	F = 5.870 p < 0.001

Description : NC = Negative Control PC = Positive Control

% Increased (Δ)= (The average and standard deviation sd feed intake at 14 d or 28 d - feed intake at 0 d or 14 d) / 0 d or 14 d feed intake x 100 %

$\bar{x}$ = average sd= standard deviation

Table 3. Duncan Test Results of Percentage Increased Weight for 14 days on Male Wistar rats

	Group of Treatments	Average Percentage Collumn (%)		
		1	2	3
K	Sibutramine 0.18mg/rat. 1x day	5.388*)		
E	Dosage 10mg/rat. 1x day	7.248	7.248	
H	Dosage 20mg/rat. 1x day	7.575	7.575	
I	Dosage 10mg/rat. 2x day	8.521	8.521	8.521
F	Dosage 5mg/rat. 2x day	9.325	9.325	9.325
B	Dosage 5mg/rat. 1x day	9.903	9.903	9.903
C	Dosage 2.5mg/rat. 2x day		12.259	12.259
J	Dosage 6.7mg/rat. 3x day		12.897	12.897
G	Dosage 3.4mg/rat. 3x day			14.212
D	Dosage 1.7mg/rat. 3x day			14.467
A	Control Neg 0mg/rat. 1x day			14.874

Notes: Treatment results in the same collumn were assumed showed same effects

Table 4 showed changes in percentage of bodyweight in each treatment after 28 days, And positive control showed significant reduction of bodyweight as compared to negative control. Most of the treatment groups showed significant reduction of bodyweight as compared to group negative control. Group D, E, H, B and G showed no significant differences with positive control, group K, indicated that those treatment groups had similar effect with sibutramine, after 28 days of treatment. Group D, PEDSDM 1.7 mg/rat 3x day (17.781%) and group E, PEDSDM dosage 10 mg / rat 1x day (18.331%) showed the least increase of percentage of bodyweight gain, mean the highest bodyweight loss after 28 days of treatment.

Effect of PEDSDM towards Plasma CCK Level on Male Wistar rats

The CCK level showed significant different effects (p=0.001) between negative control and all treatments after 28 days of administration (Table 5).

Table 4. Duncan Test Results of Percentage Increase Weight for 28 days on Male Wistar rats

	Group of Treatments	**Analysed Collumn of Average Percentage of Increasing Body Weight (in %)**			
		a	b	c	d
K	Sibutramine 0.18mg/rat. 1x day	16.371*)			
D	PEDSDM Dosage 1.7mg/rat. 3x day	17.781*)			
E	PEDSDM Dosage 10mg/rat. 1x day	18.331*)	18.331*)		
H	PEDSDM Dosage 20mg/rat. 1x day	18.474*)	18.474*)		
B	PEDSDM Dosage 5mg/rat. 1x day	18.929*)	18.929*)		
G	PEDSDM Dosage 3.4mg/rat. 3xday	19.783*)	19.783*)	19.783*)	
J	PEDSDM Dosage 6.7mg/rat. 3xday		23.768	23.768	
F	PEDSDM Dosage 5mg/rat. 2x day			25.167	25.167
C	PEDSDM Dosage 2.5mg/rat. 2x day				25.762
I	PEDSDM Dosage 10mg/rat. 2x day				26.356
A	Neg Control 0mg/rat. 1x day				28.450

Notes: Treatment results in the same collumn were assumed showed same effects

Table 5. Analysed of Average Plasma CCK level in male Wistar rats after 28 days of treatment

	Group of Treatments	CCK (ng/ml)		F	p value
		X	(sd)		
A	Neg Control 0mg/rat. 1x day	30.13	(1.6)	61.342	<0.001
B	PEDSDM Dosage 5mg/rat. 1x day	63.83	(1.6)		
C	PEDSDM Dosage 2.5mg/rat. 2x day	63.10	(2.3)		
D	PEDSDM Dosage 1.7mg/rat. 3x day	38.80	(2.7)		
E	PEDSDM Dosage 10mg/rat. 1x day	69.07	(1.5)		
F	PEDSDM Dosage 5mg/rat. 2x day	55.23	(1.4)		
G	PEDSDM Dosage 3.4mg/rat. 3x day	61.93	(2.8)		
H	PEDSDM Dosage 20mg/rat. 1x day	73.90	(5.1)		
I	PEDSDM Dosage 10mg/rat. 2x day	62.57	(4.5)		
J	PEDSDM Dosage 6.7mg/rat. 3x day	60.47	(1.8)		
K	Sibutramine 0.18mg/rat. 1x day	67.47	(3.7)		

Table 6. Duncan Test results Plasma CCK Level in Male Wistar rats

	Group of Treatments	Analysed Collumn of Average of CCK Plasma (ng/ml)						
		a	b	c	d	e	f	G
A	Negative Control	30.13						
D	PEDSDM Dosage 1.7mg/rat. 3x day		38.80					
F	PEDSDM Dosage 5mg/rat. 2x day			55.23				
J	PEDSDM Dosage 6.7mg/rat. 3x day				60.47			
G	PEDSDM Dosage 3.4mg/rat. 3x day				61.93			
I	PEDSDM Dosage 10mg/rat. 2x day				62.57	62.57		
C	PEDSDM Dosage 2.5mg/rat. 2x day				63.10	63.10		
B	PEDSDM Dosage 5mg/rat. 1x day				63.83	63.83		
K	Sibutramine 0.18mg/rat. 1x day					67.47	67.47	
E	PEDSDM Dosage 10mg/rat. 1x day						69.07	69.07
H	PEDSDM Dosage 20mg/rat. 1x day							73.90

Notes: Treatment results in the same collumn were assumed showed same effects

Result of treatment groups there were not in the same column with group A, mean there were highly significant differences (Table 6). This suggests that PEDSDM had effects in increased the plasma CCK levels. Group H, PEDSDM dosage 20 mg / rat 1x day and group E, PEDSDM dosage of 10 mg / rat 1x day are in the different column compared with the positive control group K, showed better of CCK plasma levels, 73.90 ng / ml and 69.07 ng / ml compared to group K, 67.47 ng / ml, indicated greater potential than Sibutramine.

DISCUSSION

The group that consume the least food intake 14 days were group B and G, PEDSDM dose 5 mg/rat once daily, and dose 3.4 mg/rat three times daily, as shown in table 1. The least result between groups once daily for food intake 28 days was group B, PEDSDM dosage 5mg/rat/day. Food intake of the 3 rats in group B decreased as much as 8g, 6g and 6g concomitant with increased CCK plasma level 62.6 ng/ml, 63.2 ng/ml, and 65.7 ng/ml (respectively), so high level of CCK (65.7ng/ml) did not cause better decreased food intake than lower level of CCK (62.6 ng/ml) (8g), because rats food intake were influenced not only by CCK but several factors and individual variation as well, and also there was limitation of subject sample (n=3).

PEDSDM contained high protein level (74.99%). Jean *et al*, stated that daily food intake will decrease at condition of high intake of protein diet.[14] High intake of protein was proved can suppressed appetite and followed by decreased total food intake. A metaanalysis study about energy density reports that the highest effect to satiety, is protein, second is carbohydrate then followed with fat.[15] Hydrolysate protein that comes from soybean showed the best result in inducing CCK secretion.[16] CCK will suppress hunger and short time appetite, so that animal study will lost hunger and appetite for a mean time after being given PEDSDM.

In herbal medicine, higher dosage did not cause better effect. This condition often occured, the response did not equal with assumption, dosage and predicted effects. In herbs, besides main active substance, there were another substances, although in a small amount, could influence their potency or effect. In PEDSDM, besides the main active substances, β-*conglycinin* sub unit β, there were another sub-unit like β-*conglycinin* sub unit α and α', *Glycinin* fraction which has potential to influence their satiety effect. In low dose, these substances still in small amount and not yet influence the effects. In therapeutic dose, these byproduct substances still not caused any effects, but in higher dose, it will begin ruin the effects of satiety so if we raise the dosage, we can't expect better result.

The results of average body weight male Wistar rats did not show a significant decrease and result of statistical analyses did not show a clear significant difference among groups of treatment 14 days and 28 days ($p > 0,05$). This is because the rats as experimental animals are living creatures, were well maintained, appropriate with ethical aspects of research on experimental animals, fed and watered ad libitum so that the rats aged 8-10 weeks will grow and develop, with an average growth of 5g / day and

the adult rat will grow continuously throughout their life (2-4 years) but very slow.[17] In this study, we choose rats aged 8-10 weeks because at that age they give optimum physiological responses.

In this study, the phenomena that happened was inhibition of weight gain and after the percentage result of average body weight changes were calculated, among treatment groups showed highly significant difference on 14 days of treatments ($p = 0,018$), 28 days of treatments ($p < 0,001$). If we compared the body weight graphic between NC group and treatments group, there was a difference of slope α tangensial, that the graphic of treatment groups had a smaller angle (graph more sloping) which indicates an increase in body weight that occurs is smaller than the negative control group. Effect of PEDSDM that happens in this study is inhibit the increasing weight gain or controlled body weight in male Wistar rats.

In once daily treatment, the group that showed the least result was group E, dosage 10mg/rat once daily, food intake of 3 rats in group E was 4g, 7g and 7g with CCK level 68,1 ng/ml, 70,8 ng/ml, and 68,3 ng/ml (average: 69,07 ng/ml). PEDSM dose 10 mg/rat/day showed good effects to control body weight in 14 days, and caused high level of CCK plasma mean the inhibition of weight gain was caused by CCK that suppress appetite. In this study, the once daily treatment were given at 08.00 a.m so it can be concluded that administration pattern once daily is good and also means CCK were good given not in divided dose and best given in the morning (08.00 a.m). This result was appropriate with Merino's study, which stated that CCK primary effects is in the morning, assumed that circadian had effects towards CCK. Aoyama study stated that rats which administered with soybean protein isolated for 14 days showed dramatically decrease fat and body weight compared with rats which given diet casein base.[18]

The best result for 28 days treatment that showed the least percentage changes of body weight was group D (17,781%) (administration pattern of three times daily), had an equal potency with positive control sibutramine, as seen in table 3 dan 4. This pattern was good in reducing food intake, (24,98%; $p = 0,018$) as seen in table 1. Treatment for longer time (28 days), divided and low dose feeding pattern gave good results. The mechanism of weight loss that occurs due to PEDSDM involves at least three factors. The first is a result of the effects of CCK. At low protein dose, it will induce low plasma CCK levels, in this study group D showed plasma CCK level at 38.80 ng/ml was only slightly above the negative controls (30.13 ng/ml) but give good result in weight control during 28 days. A research states that the effect of CCK appears on minimum dosage at 10 mg / kg (~ 100 ng / kg or 25 ng / rat 250g).[19]

Second, weight loss may caused by another active components that found in soybean, although the number is limited, like Anti Nutrition Factors (ANF). Soybean contains many sort of anti nutrition factors (**ANF**), like trypsin inhibitor, lectin, poliphenol, phitic acid, saponin and antivitamin which have negative affects to the absorbtion of food in gastro intestinal tract[20], although we have not measured their contents in the PEDSDM. Previous studies mentioned that treatment of PEDSDM during the 14 days will caused weight loss accompanied by atrophy of the villi and significant shortening of Lieberkuhn crypt on the jejunum of Wistar rat, while the treatments for 28 days symptoms of weight loss greater tendency accompanied with jejunal mucosa hyperplasia, indicate there is role of ANF. ANF role is supported by the fact that plasma CCK levels showed no significant difference ($p = 0.182$) between the treatment 14 days and 28 days.[21] Third, protein in soybean has thermogenic effects and energy expenditure larger than carbohydrates, although the mechanism is still not clear entirely.[22] One of the hypotheses is soy stimulates quite high release of the hormone glucagon. The results of Bensaid study showed that administration of high-protein diet including soy protein that not restricted (*ad libitum*) for long term significantly decrease food intake and white fat mass or white adipose tissue (WAT), and this makes soy a good source of nutrients for the treatment of obesity.[22]

Results of treatment of PEDSDM against plasma CCK levels on male Wistar rats, the comparison group (sibutramine) and all treatment groups showed highly significant differences with the negative control group (Table 5). This suggests that the group treatment had effect towards increasing plasma CCK levels. Group H which given PEDSDM dose 20 mg / rat once a day and group E dosage of 10 mg /rat once a day showed plasma CCK levels greater (73.90 ng / ml and 69.07 ng / ml) than the positive control group K, sibutramine (67. 47 ng / ml) and signifies its potential better in increasing plasma CCK levels. This is because sibutramine is not include protein or fat, which has the ability to trigger the secretion of CCK.

In this study, treatment of PEDSDM dosage of 20 mg /rat once a day, caused the highest levels of CCK plasma (73.90 ng / ml) (Table 5 and Figure 5) but showed only good for weight control on 14 days treatment, while treatment for 28 days just showed moderate effect. This showed that the CCK mainly plays a role in weight loss for the treatment of short time period (14 days) and better administered in a single dosage (not divided pattern) in the morning. Effect of CCK lowered portions (meal size) in rats that ate as much as they like (feed *ad libitum*) is better than the size of overall food in fasted rats.[23,24] However CCK in small level have already shown effect, referring to another study that says that the minimum level of CCK effect was 25 ng/rat, even on lower dosage of CCK can show effects in experimental animals are fasted or at the time of the morning, it is estimated that circadian factors also affect the effect of CCK.[20,24] In rats that fed *ad libitum,* given treatment dosage CCK 5-6 (1 μ / rat per dosage) for 5 days, CCK lowered portions but no effect on the overall food intake. Treatment of CCK dose 10 mg/kgBW can increase levels of CCK (20-40 ng / ml) in blood better compared to the average level of CCK after daily eating. CCK-8 in dosage 10 mg /kgBW showed the effect of towards motility and gall bladder contraction accompanied by weight loss.[23] Nakajima study showed that administration of soy peptide β-conglycinin 51-63 high arginine levels in intraduodenal shown to decrease food intake through increased secretion of CCK on rats.[25]

Inconclusion. Administration of PEDSDM at 10 mg/day once daily in the morning (08.00 a.m), for 14 days, showed significant mean best dose and pattern of administration in regulate the bodyweight of the rats. While administration of PEDSDM at 1.7 mg three times a day, significantly regulate the bodyweight of the rats for 28 days.

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