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Rom J Diabetes Nutr Metab Dis. **25**(1):091-097
doi: 10.2478/rjdnmd-2018-0010

THE EFFECT OF TEMPEH GEMBUS VARIATIONS TO SERUM LEVELS OF HIGH SENSITIVITY C-REACTIVE PROTEIN (hsCRP) AND SERUM LEVELS OF FIBRINOGEN OF SPRAGUE DAWLEY RATS WITH ATEROGENIC DIET

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received: November 21, 2017 *accepted*: February 10, 2018
available online: March 15, 2018

Abstract

Background and aims: *Cardiovascular diseases are widespread and causes many deaths in the world. The concentration of acute phase protein: C-reactive protein (CRP) and fibrinogen will rise dramatically when inflammation happens, which that can be used as an early marker of cardiovascular disease risk. Tempeh gembus contains fiber, unsaturated fatty acids and isoflavones are believed to reduce the inflammatory reaction. The aim of the study was to determinate the effect of tempeh gembus variations to levels of hcCRP and levels of fibrinogen of Sprague Dawley rats with atherogenic diet.* ***Material and methods:*** *This study was quasi-experimental with posttest only randomized control group design using 35 Sprague Dawley mice. The rats were randomized into 5 groups: negative control group given the standard diet, the positive control group given standard diet and atherogenic diet, and three treatment groups were given the standard diet, atherogenic diet and variation of tempeh gembus (tempeh gembus, heated tempeh gembus and tempeh gembus with bromelain enzyme) for 28 days. Serum levels of hsCRP and fibrinogen examined using ELISA (Enzyme-linked Immunosorbent Assay).* ***Results and conclusions:*** *The administration of tempeh gembus with bromelain enzyme is the most effective treatment for hsCRP serum level indicated a significant difference (p=0.028) between the negative control group, positive control group and first group with the third group. Fibrinogen serum levels showed significant differences in all treatment groups (p =0.042), administration of tempeh gembus with bromelain enzyme is the most effective treatment is shown by a significant difference between the negative control group and the positive control group with third group. The administration of tempeh gembus with bromelain enzyme for 28 days can reduce the serum levels of hsCRP and fibrinogen on rats significantly.*

key words: *Tempeh Gembus, Serum levels of hsCRP, Fibrinogen, Aterosclerosis, Bromelain Enzyme*

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Background and aims

Cardiovascular disease is caused by a disturbance in the function of the heart and blood vessels. WHO data show that cardiovascular disease is expected to continue to increase to 23.3 million in 2030 [1]. WHO mortality record in 2014 shows 7.4 million deaths in the world due to atherosclerosis [1]. Atherosclerosis sufferers are associated with inflammation [2-5]. Acute phase proteins in the form of C-Reactive Protein (CRP) and fibrinogen are synthesized during inflammation. CRP and fibrinogen concentrations will increase during inflammation and tissue destruction therefore can be used as early markers of inflammation [4-6].

Tempeh gembus is a fermented food made from basic ingredients of fermented waste tofu. Tempeh gembus is selected because it contains fiber, unsaturated fatty acids and isoflavones believed to reduce the inflammatory reaction [7-12].

The main purpose of the heating method is to increase digestibility, taste, killing pathogenic microorganisms and affect nutrient content of food especially increase antioxidant activity [13-14]. The bromelain enzyme is a proteolytic enzyme contained in the pineapple. The addition of bromelain enzyme to tempeh gembus plays a role in coagulation and fibrinolysis and can hydrolyze protein in tempeh gembus [15-16].

The purpose of this study was to prove the effect of variations of tempeh gembus on the decrease in serum levels of hsCRP and fibrinogen in Sprague Dawley mice with atherogenic diet.

The benefit of this research is to provide scientific information about the effect of variation of tempeh gembus on serum levels of hsCRP and fibrinogen in Sprague Dawley mice with atherogenic diet and the results of the research are expected to be used as food source alternative of fiber, isoflavone and pufa which is safe and useful for protection In cardiovascular disease.

Material and methods

The study was experimental quasi with posttest only, randomized control group design. The research was conducted at Laboratorium Hewan Coba UNDIP Semarang in November - December 2016 for 60 days.

The samples used were rats with inclusion criteria of male rat with Sprague Dawley strain, 8 weeks old with body weight 150-180 gram and rat exclusion criteria had weight loss 10% from initial weight, died at the time of the research and mice being sick and lost appetite.

The sample is divided into 5 groups that are (K-), (K+), (P1), (P2) and (P3) groups. The negative control group was fed standard, the positive control group was fed standard feed and the atherogenic diet, the treatment group 1 was fed standard, atherogenic diet, and unfit tempeh without treatment, the standard 2 treatment group, the atherogenic diet and the heated tempeh gembus while the treatment group 3 were fed standard, atherogenic diets, non-treated tempehh and bromelin enzymes. All subjects were given standard feed as much as 20 grams per day and drink water ad libitum. Prior to the intervention, treatments were made to establish atherosclerotic conditions in experimental animals of positive control group and treatment group with atherogenic diet for 4 weeks.

Study objectives: The results analyzed in this study were serum levels of hsCRP, serum levels of fibrinogen and body weight of mice. Blood collection and serum production were performed on day 60. Rat blood serum was studied using kit from Bioassay Technology Laboratory with ELISA method in LPPT laboratory of Gajah Mada University Yogjakarta.

Statistical analyses: The data obtained is processed by SPPS program. Normality is tested

using the Shapiro-Wilk test. Differences in hsCRP levels between the analytical groups were tested with one-way ANOVAand showed different test of hsCRP levels between groups using post hoc test. Differences of fibrinogen levels between the analysis groups were tested with kruskal-wallis and showed different test of fibrinogen levels between groups using the mann whitney test.

Sample size calculation: The minimum subject is determined by the formula of Federer (t-1) (n-1)> 15, where t is the number of treatment groups, while n is the subject of each treatment group. This study used a sample of 35 rats obtained from Peternakan Hewan Uji UD. Tiput Abadi Jaya.

The Ethical Clearance application of the research was submitted to the Komisi Etik Penelitian Kesehatan (KEPK) of the Faculty of Medicine, Diponegoro University, Semarang and has been reviewed and approved by the issuance of the ethical clearance certificates No.1.033 / EC / FK-RSDK / XII / 2016.

Results

Sample Characteristics

Changes in body weight of mice during the study are presented in Table 1.

The highest increase in body weight was the K + group. The post hoc trials were tested after the results of the one-way ANOVA statistical test showed that the change in body weight obtained significant results (p = 0.011) and showed significant difference test in the K- group (negative control) with group P1 (tempeh gembus without treatment) (p-value 0.016).

hsCRP

Examination of hsCRP serum levels was performed at the end of the study. The levels of hsCRP serum in each group can be seen in Table 2.

Table 1. Mean Weight Rat Analysis.

Groups	n	Rats Weight		Δ (g)	*p*
		Start (g) Mean±SD	After (g) Mean±SD		
K-	7	208.57±20.6	252.29±24.1	43.71	0.011[a]
K+	7	213.57±25.1	266.43±39.7	52.86	
P1	7	244.71±22.7	270.00±27.6	25.29	
P2	7	227.50±26.3	263.14±26.0	35.64	
P3	7	234.86±37.8	263.71±36.2	28.86	

[a] *Anova test*
K- = Negative control group
K+ = Positive control group (atherogenic diet)
P1 = Treatment group 1 (atherogenic diet and tempeh gembus without threatment)
P2 =Treatment group 2 (atherogenic diet and heated tempeh gembus)
P3=Treatment group 3 (atherogenic diet and tempeh gembus with bromelain enzyme)

Table 2. The Serum Levels of hsCRP.

Groups	n	hsCRP levels (mg/L) Mean±SD	*p*
K-	7	319.98 $x10^{-6}$± 36.6	0.028 [a] *
K+	7	305.60$x10^{-6}$±36.4	
P1	7	322.08$x10^{-6}$±22.9	
P2	7	283.79$x10^{-6}$±61.5	
P3	7	253.60$x10^{-6}$±45.9	

The P1 group had the highest levels of hsCRP compared to the other treatment groups. This is probably due to the P1 group given tempeh gembus without treatment so that no additional treatment or addition of substances that support the increase in nutrient activity in tempeh gembus. The P2 group were given heated tempeh gembus had a lower hsCRP level than P1 given untreated tempehh without treatment. Methods of heating used in this study because heating can increase the antioxidant activity in tempeh gembus [7-9]. The P3 group had the lowest levels of serum hsCRP from the P1 and P2 groups. This suggests that administration of tempeh gembus with bromelin enzyme may have an influence in the decrease of hsCRP serum levels in Sprague Dawley rat.

The K- group and P1group are expected to have lower hsCRP levels than the K + group but there's an opposite result in this study. High levels of hsCRP are likely due to both groups had a disorder or certain conditions such as excessive weight gain, inflammation, systemic vasculitis, pancreatitis, myocardial infarction, bacterial infections, parasites or fungi or chronic disease [4,6,9].

The analysis of serum hsCRP mean levels showed that there was a difference of hsCRP levels between K (-), K (+), P1, P2 and P3 groups (p = 0.028) which means that variation of tempeh gembus can decrease level of serum hsCRP.

The result of hoc post test showed significant difference test in K- group (negative control) with P3 group (tempeh gembus with bromelin enzyme) (p 0.007); K+ group (positive control) with P3 group (tempeh gembus with bromelin enzyme) (p 0.030), and P1 group (tempeh gembus without additional treatment) with P3 group (tempeh gembus with bromelin enzyme) (p 0.005).

Fibrinogen

The levels of serum fibrinogen were examined at the end of the study. Fibrinogen serum levels in each group can be seen in Table 3 below:

Table 3. The Serum Levels of Fibrinogen

Groups	n	Fibrinogen Levels (mg/dl) Median (min-max)	*p*
K-	7	0.032 (0.021-0.035)	0.042 [a]*
K+	7	0.028 (0.023-0.029)	
P1	7	0.025 (0.019-0.036)	
P2	7	0.027 (0.006-0.032)	
P3	7	0.022 (0.006-0.026)	

The mean levels of serum fibrinogen content of the samples in the K (-), K (+), P1, P2 and P3 groups were respectively 0.032 (mg / dl), 0.028 (mg / dl), 0.025 (mg / dl), 0.027 (mg / dl) and 0.022 (mg / dl). The result of Kruskall-Wallis test in Table 3, obtained p value = 0.042 indicates that there is significant difference of fibrinogen level in five group.

Descriptively P2 group have the highest fibrinogen levels and P3 groups have the lowest fibrinogen levels amongst all groups. The P1 group has lower fibrinogen levels than the P2 group, this is likely because P2 groups are given heating treatment which may interfere with fibrinolytic activity in tempeh gembus. P3 groups have lower fibrinogen levels than P1 and P2 because the addition of bromelin enzymes can increase fibrinolytic activity in tempeh gembus so it gives a positive effect on low levels of fibrinogen in group P3.

The K- group is expected to have lower fibrinogen levels than the K + group, but there's an opposite result in this study. This possibility occurs because of heart problems or circulatory system in rats, stomach cancer, breast and kidney system and inflammatory diseases such as rheumatoid arthritis [4].

Discussion

The nutritional content of Tempeh Gembus

Table 4. Nutritional value in 100 g tempeh gembus [8]

Nutrition	Gross weight	Dry weight
Energy (kkal)	65.10	77.70
Protein (g)	3.41	4.07
Fat (g)	0.20	0.23
Carbohydrate(g)	11.94	14.25
Fiber (g)	3.93	4.69
Ash (g)	0.69	0.82
Calcium (mg)	143.00	159.98
Phosphor-P(mg)	50.00	59.69
Iron – Fe (mg)	0.40	0.48
Water (%b.d.d.)	83.76	6.00
b.d.d. (%)	100.00	100.00

Tempeh gembus has three times of fiber content from soybean tempeh however, the energy, protein and fat content of the tempeh is lower than the soybean tempeh [7-8]. The amino acid content in tempeh gembus is lower than soybean tempeh. Soy contains isoflavones between 0.6-03.89 / kg fresh weight [10]. Tempeh gembus with soybeans as the basic ingredients contain isoflavones daidzein (33.1 mg / g) and genistein (57.1 mg / g) and unsaturated fatty acids in the form of oleic fatty acids, linoleic and linolenic fatty acids which including essential fatty acids [7-8].

Atherogenic Diet

Table 5. The composition of normal diet and atherogenic diet [17]

The composition of a normal diet	The composition of a atherogenic diet
Comfeed PAR-S 67%	Comfeed PAR-S 55%
Wheat flour 33%	Wheat flour 28%
Enough water	Egg yolk 6%
	Kolat acid 0,2%
	Pig oil 10%
	Coconut oil 1%
	Enough water

Sample Weight

The highest weight gain in rats was K + group, it possibly because the group received an atherogenic diet during the study. Treatment groups (P1, P2 and P3) who received intervention of tempeh gembus had a weight gain in all groups. The K- group had higher body weight gain than the treatment group (P1, P2 and P3). This was probably because the treatment group was given tempeh gembus. Tempeh gembus has fiber content which can contribute to low weight gain in the treatment group. Fiber is known to reduce daily energy intake by 20-35% and can provide a longer satiety to prevent excessive food consumption.[21]

The Serum Levels of hsCRP

It can be concluded that the provision of tempeh gembus with bromelin enzyme is the most effective treatment, it is shown in the existence of significant difference between group giving tempeh gembus without treatment and tempeh gembus with enzyme bromelin. This suggests that the administration of tempeh gembus with bromelin enzyme may be able to decrease the serum levels of hsCRP in Sprague Dawley's rat. The bromelin enzyme was added to this study to increase the antioxidant activity of tempeh gembus. The tempeh gembus is added with bromelin enzyme because it has the ability to hydrolyze protein peptide bonds into smaller molecules (amino acids). Protein preparations in the form of amino acids allow the tempeh to become more easily digested by the body [18].

The low levels of hsCRP in P3 groups may occur due to the content of the tempeh gembus such as isoflavones (daidzein and genistein) as well as unsaturated fatty acids (fatty acids oleate, linoleate and linolenic) which include essential fatty acids [7-8]. The anti-inflammatory mechanism of isoflavones is done by lowering the production of pro-inflammatory contributors by inhibiting the transcriptional system of NF-κB and modulating arachidonic acid metabolism (AA) and NO production by inhibiting protein levels and prophylactic enzyme activity

(phospholipase A2 (PLA2), lipoxygenase (LOX), COX-2, and iNOS [21]. Many studies show that high intake of PUFAs, especially n-6, is associated with lower levels of C-Reactive Protein to decrease chronic inflammation [11]. PUFA n-3 and n-6 worked to inhibit the activities of δ-6 desaturase, δ-5 desaturase, and cyclooxygenase (three of them are involved in the regulation of fatty acids that affect the pro-mediator and anti-inflammatory) [11].

The Serum Levels of Fibrinogen

It can be concluded that the provision of tempeh gembus with bromelin enzyme is the most effective treatment with the lowest fibrinogen level, this is shown in the existence of significant difference between K- and K + with P3 group. The addition of bromelin enzyme was carried out in this study in the hope of increasing fibrinolytic activity acting as thrombolotics by activating plasminogen to form plasmin which then degrades fibrin to break the thrombus [18-19].

The content of isoflavones (daidzein and genistein) may play a role in low fibrinogen levels in the P3 group. In vivo studies in 2012 showed isoflavones genistein capable of inhibiting thrombin formation and platelet activity resulting in decreased fibrinogen. There was a 5.9% reduction in fibrinogen in the group taking 11 mg of isoflavones and 4.6% in the group taking 96 mg of isoflavones [12].

Conclusions

The administration of tempeh gembus with bromelain enzyme for 28 days can reduce the serum levels of hsCRP and fibrinogen on rats significantly.

Acknowledgements: This research was funded by Diponegoro University Research Founding.

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