The Effect of Tempe Gembus on High-Sensitivity C-Reactive Protein and Adiponectin Levels in Rats with Metabolic Syndrome

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Summary Metabolic syndrome can affect the inflammatory state which results in increased high-sensitivity C-reactive protein (hs CRP) and decreased adiponectin levels. Tempe gembus is a functional food that can reduce the risk of metabolic syndrome through the inflammatory pathway. This study applied a quasi experimental method, with a post-test only control group design. Sprague Dawley rats (n=30) were divided into 2 control groups (K− and K+) and 3 treatment groups (P1, P2, P3) which were given a 4-wk diet that included 2.5 g (P1), 5 g (P2), and 7.5 g (P3) of tempe gembus. Adiponectin and hs CRP levels were measured with ELISA. Statistical analysis was done with a one-way ANOVA test and a Kruskal Wallis test. It appears that administering tempe gembus in these amounts can reduce the hs CRP levels (p=0.037) and increase adiponectin levels in rats with metabolic syndrome (p=0.008). This research has shown that a 2.5 g of tempe gembus can have a strong effect on hs CRP and 5 g of tempe gembus have a strong effect on adiponectin.

Key Words metabolic syndrome, tempe gembus, hs CRP, adiponectin

Metabolic syndrome is a complex disorder that affects 1 in 5 people in the world characterized by obesity, dyslipidemia, high blood pressure, insulin resistance, and proinflammation. These signs are additionally associated with an increased risk of heart disease, atherosclerosis and type 2 diabetes mellitus (1). Adiponectin and hs CRP are associated with several metabolic syndrome markers such as body mass index, waist circumference, triglycerides, cholesterol, HDL cholesterol, glucose, and insulin (2, 3). Additionally, hs CRP has a positive correlation with fasting glucose levels and has a negative correlation with HDL cholesterol. Some studies have shown an association of hs CRP with several components of the metabolic syndrome (2, 4–6). The prevalence of metabolic syndrome in Jakarta was 28.4% among 1,591 subjects, using a modified ATP III Guideline that confirms to Asian criteria (7). The prevalence of metabolic syndrome in Bali was 18.2% among 1,840 subjects (8).

One of the causes of metabolic syndrome is consumption of foods high in carbohydrates (sucrose) and high in fat (9). It can lead to insulin resistance, dyslipidemia, hypertension, increased signs of inflammation, and decreased antioxidants (10–12). A study where fat with 21% dan 34% sucrose levels of total energy was given to human subjects for duration of 2 to 4 wk resulted in hepatosteatosis, adipose tissue hypertrophy and hyperinsulinemia (13).

Tempe gembus is a functional food made from fermented tofu waste and it contains flavonoids, fiber, amino acids, and polyunsaturated fatty acids which can reduce the risk of metabolic syndrome through inflammatory pathways (14). Okara, which is another name for soybean pulp in Japan, is effective in preventing obesity, hepatic steatosis, and disorders of fat metabolism (15).

This study analyzes the effect varying doses of tempe gembus on hs CRP and adiponectin levels in rats with metabolic syndrome.

MATERIALS AND METHODS

Experiment. This study was set up as a quasi-experimental study with a post-test only control group design. We used 30 Sprague Dawley 2-mo-old male rats with a weight of ±150–200 g. Maintenance and care of the rats was carried out in the Animal Laboratory of the Faculty of Medicine, Diponegoro University. The acclimatization process was carried out for 7 d by providing standard food and drinking water ad libitum. The 30 rats were divided into 5 groups, namely 2 control groups (K− and K+) and 3 treatment groups (P1, P2, and P3). The K− group was given standard feed, whereas the K+ group was given standard feed and a high-fat and high sucrose diet (20% pork oil, 20% quail egg yolk, 60% sucrose). The P1 group received the same diet with K+ group and with 2.5 g tempe gembus included. The P2 5 g tempe gembus included and finally, the P3 group had 7.5 g tempe gembus included. The study was conducted with 2 wk of administering a high fat, high sucrose diet and 4 wk of tempe gembus. The experimental protocol was considered and approved by the Ethical Clearance from the Health Research Ethics Commission of the Medical Faculty of Diponegoro University under the ethical clearance number of 13/CH/H/
Biomarker analysis of metabolic syndrome. Biomarkers of metabolic syndrome, such as fasting blood sugar, HDL cholesterol, triglycerides, and measuring body weight and body length to obtain Lee index were carried out after giving a high fat high sucrose diet for 2 wk. The examination was carried out by the Central Java Health Laboratory.

Analysis of hs CRP and adiponectin. After the administration of tempe gembus for 4 wk, the examination of hs CRP and adiponectin levels was done using a serum with the Enzyme Links Immunosorbent Assay (ELISA) method at the Integrated Research and Testing Laboratory of Gajah Mada University.

Statistical analysis. Statistical analysis was done with SPPS Statistics software. The normality of the data was tested using the Shapiro Wilk test. Analysis of differences in hs CRP and adiponectin after treatment in each group was done using a one-way ANOVA test followed by a test using Post Hoc LSD. A Kruskal-Wallis non-parametric test followed by a different test using the Mann-Whitney test for the data was distributed abnormally.

RESULTS

Characteristics of experimental animals

Acclimatization-induction

Changes in rat body weight during acclimatization and induction are shown in Table 1. The results of the Post Hoc LSD test showed differences in body weight between groups K– and those given a high fat, high sucrose diet. Insignificant differences in weight gain were in the K+, P1, P2, P3 groups. The K– group also showed the lowest increase in body weight compared to groups K+, P1, P2, P3 which were given a high fat, high sucrose diet. This shows that high fat, high sucrose diets affect the rat’s body weight.

Induction-intervention

Fasting glucose levels, HDL levels, triglyceride levels, and the Lee index after induction are shown in Table 2. This data shows the rats experienced hyperglycemia, decreased HDL cholesterol levels, hypertriglycerides and obesity.

Data on rat body weight after induction and after administration of tempe gembus are shown in Table 3. The results of the statistical test showed that there was an effect of 28 d tempe gembus on rat body weight between groups (p=0.000). Rats in the control group experienced a larger increase in body weight compared to the treatment group (P1, P2, P3). The provision of standard feed in the control group during the intervention was due to higher body weight in the control group. The Post Hoc LSD test results show significant differences between the control and treatment groups (p<0.05). Giving tempe gembus between treatment groups (P1) there were significant differences between treatment groups (P1 and P2) in suppressing weight gain.

The effect of tempe gembus on hs CRP levels

The levels of hs CRP in each treatment group are shown in Table 4. The results of the Post Hoc LSD test showed differences in hs CRP levels between the K– and P3 groups. Giving tempe gembus to rats with metabolic syndrome for 28 d significantly affected hs CRP levels, based on the test results (p=0.037). The purpose
Tempe gembus on hs-CRP and Adiponectin Levels in Rats

Effect of tempe gembus on adiponectin levels

The results show adiponectin levels were lowest in the K− group (0.77 ± 0.08 mg/L) while the highest in the P2 group (1.06 ± 0.15 mg/L). Adiponectin levels in each group are shown in Table 5. The results of statistical tests showed that administration of tempe gembus for 28 d significantly affected adiponectin levels in rats with metabolic syndrome (p=0.008). The treatment groups (P1, P2, P3) with 2.5 g, 5 g and 7.5 g of tempe gembus had higher adiponectin levels compared to the control groups (K− and K+). Treatment group (P2) with 5 g tempe gembus had higher adiponectin levels than treatment group (P1 and P3).

Correlation between hs CRP and adiponectin levels

Based on the Pearson correlation test, no noticeable correlation between hs CRP levels and adiponectin levels was found. Pearson’s correlation value of 0.301 is defined as a weak positive correlation.

DISCUSSION

The subject’s body weight changes before and after administering high fat and high sucrose diet. The K− control group experienced the smallest increase in body weight compared to the other groups. Obesity is related to consumption of sweet drinks and high fat diets. Fructose contained in sucrose cannot stimulate insulin secretion or increase leptin production which increases food intake and weight gain (16, 17). The lard based on high fat diet contains higher calories and thus results in weight gain (18). Saturated fats contained in lard and quail eggs cause damage to lipid metabolism resulting in obesity (13, 19). Sucrose given to rats causes an increase in body weight due to increased food intake and changes in production and sensitivity to leptin (20). A high fat diet with sucrose additions of around 10% to 40% can increase weight, abdominal fat, hyperinsulinemia, hyperglycemia and hyperlipidemia (21).

Induction of the metabolic syndrome is giving by a high fat, high sucrose diet. High fat, high sucrose diets causes hyperglycemia, decreased HDL cholesterol levels, and hypertriglycerides. Diets high in saturated fat contained in lard and sucrose administration are associated with the development of metabolic syndrome, especially affecting plasma triacylcerol and LDL cholesterol (22–24). Diet high in fructose and glucose can increase plasma triglyceride concentrations in animals and humans (25).

Tempe gembus administered for 28 d can inhibit weight gain in the treatment group (P1, P2, P3). Tempe gembus with a dose of 7.5 g has the lowest body weight compared to the other groups. It contains fibers to con-

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Table 3. The mean data of induction weight and intervention weight.

| Groups of Treatment | Weight (g) ± SD | p     |
|---------------------|---------------|-------|
| K− (n=6)            | 198.77 ± 8.67 |       |
| K+ (n=6)            | 215.67 ± 12.80|       |
| P1 (n=5)            | 224.60 ± 23.56|       |
| P2 (n=5)            | 237.44 ± 7.63 |       |
| P3 (n=7)            | 216.90 ± 27.64|       |

* uji one way anova.
  a,b,c uji post hoc Mann-Whitney, berbeda bermakna <0.05.

Table 4. The mean data of hs CRP levels.

| Group of Treatment | hs CRP Levels (mg/L) | p     |
|-------------------|----------------------|-------|
| K− (n=6)          | 0.50 ± 0.02a         |       |
| K+ (n=6)          | 0.54 ± 0.45ab        |       |
| P1 (n=6)          | 0.53 ± 0.05a         | 0.037*|
| P2 (n=5)          | 0.54 ± 0.05a         |       |
| P3 (n=7)          | 0.59 ± 0.06b         |       |

* Oneway ANOVA test.
  a,b,c Post hoc LSD test, different mean <0.05.

Table 5. The mean data of adiponectin levels.

| Groups of Treatment | Adiponectin Levels (mg/L) | p     |
|---------------------|---------------------------|-------|
| K− (n=6)            | 0.77 ± 0.08a              |       |
| K+ (n=6)            | 0.87 ± 0.07b              | 0.008*|
| P1 (n=6)            | 0.89 ± 0.05b              |       |
| P2 (n=5)            | 1.06 ± 0.15c              |       |
| P3 (n=7)            | 0.89 ± 0.07abc            |       |

* Kruskal-Wallis test.
  a,b,c Post hoc Mann-Whitney test, different mean <0.05.
trol body weight by increasing satiety by retention of fluids from food and drinks, and increasing glucose and insulin metabolism by increasing food transit time in the intestines (26, 27). Supplementing of basal diet with okara on the basal diet with a dose of 10, 20, or 40% helps prevents obesity (15).

Tempe gembus given to rats with metabolic syndrome for 28 d significantly affected hs CRP levels caused by several factors. It contains polyunsaturated fatty acid which plays a role in modulating the concentration of hs CRP and other inflammatory markers (28). A fiber diet can significantly reduce CRP levels (29). Fibers contained in tempe gembus have anti-inflammatory effects by reducing lipid oxidation, normalizing intestinal flora and inhibiting hyperglycemia (30).

Treatment group (P2) received 5 g tempe gembus and had higher adiponectin levels than treatment groups (P1 and P3). Tempe gembus contains fiber, unsaturated fatty acids and isoflavones which also affect adiponectin levels. Diets that contain unsaturated fiber and fatty acids have a beneficial effect on adiponectin circulation and increase adiponectin levels (31, 32). In obesity and type 2 diabetes, adiponectin causes insulin resistance. Provision of dietary intervention containing isoflavones is predicted to reduce insulin resistance (33, 34).

Correlation test results showed there was no significant correlation between hs CRP levels and adiponectin levels. a non-specific inflammatory cytokine that can increase infiltration of inflammatory cells, increase oxidative stress, interfere with endothelial function and reduce nitric oxide production (35, 36). Conversely, adiponectin is a cardio-protective cytokine that can inhibit inflammatory cytokines such as interleukin-6, expression of TNF-α and hs CRP and increase the formation of nitric oxide.

Tabara et al. showed the effect of CRP and adiponectin as a prognostic metabolic syndrome on the population (37). Fam et al. states that a decrease in serum adiponectin and an increase in hs CRP levels can be used as an inflammatory marker and lipotoxin but cannot be used to diagnose metabolic syndrome (38). Based on the results of statistical tests, CRP hs levels are not related to adiponectin. The duration of the course of the disease affects the role of systematic inflammation causing atherosclerosis which is associated with a decrease in adiponectin (39). Research for 28 d was predicted not to cause atherosclerosis which causes an increase in hs CRP levels and decreases adiponectin levels. The body mass index correlates positively with hs CRP and negatively on adiponectin levels associated with metabolic syndrome but no correlation between hs CRP and adiponectin is found (40).

Our study has some limitations. The levels of isoflavones in tempe gembus used for intervention were not determined. The levels of isoflavone in tempe gembus are based on previous studies in which okara used, which is similar to tempe gembus. Additionally, examination of the parameters of metabolic syndrome (fasting glucose levels, HDL cholesterol and triglycerides) was only carried out before intervention, but was not carried out after wards and thus the condition of metabolic syndrome prior and after the administration of tempe gembus was not compared.

In conclusion, tempe gembus 2.5 g has been seen to reduce hs CRP levels in rats with metabolic syndrome. Giving 5 g of tempe gembus can increase adiponectin levels in rats with metabolic syndrome ($p=0.008$).

Disclosure of state of COI
No Conflicts of interest to be declared.

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