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Original Research Article

The Effect of *Tempeh gembus* on Malondialdehyde and Superoxide Dismutase Enzyme Levels in Rats with Diet-Induced Metabolic Syndrome

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Abstract

Background: High level of malondialdehyde (MDA) and low level of superoxide dismutase (SOD) are markers of oxidative stress and indicate enhanced metabolic syndrome. *Tempeh gembus* is a food that contains isoflavones as an antioxidant which can reduce oxidative stress levels in metabolic syndrome.

Objective: The aim of this research was to determine the effect of *Tempeh gembus* on plasma MDA and serum SOD enzyme levels in metabolic syndrome rats.

Methods: A post-test only experimental design was used in which 25 Sprague Dawley rats were divided into 5 equal groups of 2 control groups K(-) and K(+) and 3 treatment groups (P1, P2, P3). Group K(+), P1, P2, P3 were given high fat and sucrose diet (20% lard, 20% egg yolk, and 60% sucrose). The control groups only received standard food without *tempeh* gembus or pure isoflavones addition. Group K(-) were treated as healthy rats. Group K(+) were presented as metabolic syndrome rats without any treatment. *Tempeh gembus* was given for 4 weeks with the doses of 2.5g (P1), 5g (P2), and 7.5g (P3). Plasma MDA levels were measured by the Thiobarbituric Acid Reactive Substance (TBARS) method and serum SOD enzyme levels were measured by the Enzyme Linked Immunosorbent Assay (ELISA) method. The statistical analysis used One Way Anova test.

Results: *Tempeh gembus* significantly decreased plasma MDA levels of metabolic syndrome rats ($p<0.05$) but had no significant effect on serum SOD enzyme levels in each treatment group ($p>0.05$).

Conclusion: The dose of 7.5g raw *Tempeh gembus* was the most effective dose in reducing the plasma MDA level. *Tempeh gembus in* the 3 treatment doses significantly decreased plasma MDA levels but had no significant effect on serum SOD enzyme levels.

Keywords: *malondialdehyde; metabolic syndrome; superoxide dismutase; tempeh gembus*
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INTRODUCTION

Metabolic syndrome is a clinical condition affecting approximately 20% of the adult population and is marked by metabolic disruptions including hyperglycemia, obesity, dyslipidemia, and hypertension. Metabolic syndrome can lead to the development of type 2 diabetes and cardiovascular disease.[39] The risk of metabolic syndrome increased with the raising of body mass index (BMI) and age.

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Table 1. The feeding regimen for each of the 5 group animal model

Group / Periode	K-	K+	P1	P2	P3
Acclimatization	CP-594	CP-594	CP-594	CP-594	CP-594
Induction	CP-594	• 60% of CP-594 • high fat diet • sucrose solution	• 60% of CP-594 • high fat diet • sucrose solution	• 60% of CP-594 • high fat diet • sucrose solution	• 60% of CP-594 • high fat diet • sucrose solution
Intervention	CP-594	CP-594	CP-594 and 2.5 g *tempeh gembus*	CP-594 and 5 g *tempeh gembus*	CP-594 and 5 g *tempeh gembus*

Table 2. The Mean Data of Initial Weight and Weight After Induction

Weight [g]	Treatment Group					*P*
	K(-) (n = 5)	K(+) (n = 5)	P1 (n = 5)	P2 (n = 5)	P3 (n = 5)	
Initial Induction	174,24 ± 9,101	169,06 ± 7,621	169,36 ± 10,765	181,00 ± 15,996	166,54 ± 7,041	
After Induction	200,82 ± 7,897	217,40 ± 13,506	229,52 ± 22,634	237,44 ± 7,625	212,16 ± 14,744	0,006*
Δ	26,58 ± 2,564[a]	48,34 ± 10,322[b]	60,16 ± 15,074[b]	56,44 ± 21,203[b]	45,62 ± 9,023[b]	

* *Oneway Anova Test*
[a,b] *Post Hoc LSD Test*, significantly different $p<0,05$

The National Health and Nutrition Examination Survey (NHANES) of United States reported that metabolic syndrome occurred in 5% of subjects with normal weight, 22% of subjects with overweight, and 60% of subjects with obesity.[24] Research in Bali indicated the prevalence of metabolic syndrome of 18.2% among 1,840 subjects.[40] Another study in Jakarta reported that grossly obese children (5~9 years old) of whom 52% had dyslipidemia and 35.6% in insulin resistance.[36]

One of the causal factors of metabolic syndrome is frequent and excessive consumption of foods and beverages that contained high carbohydrates (especially sucrose and fructose) and high fats (especially saturated fatty acid and trans fatty acid).[40] Metabolic syndrome and oxidative stress are interlinked and are marked by high levels of malondialdehyde (MDA) and low levels of superoxide dismutase (SOD) enzyme activity. The high levels of MDA are due to Reactive Oxygen Species (ROS) acting on the lipid cell membranes by peroxidation of membrane fatty acids.[8] The elevation of free radicals is also affected by low levels of superoxide dismutase (SOD) enzyme activity which act as an antioxidant.[15] In metabolic syndrome endogenous antioxidants are unable to control the high level of oxidative stress hence it may be necessary to supplement the diet with exogenous antioxidants derived from natural products.

There are many kinds of Indonesian fermented foods originated from tofu waste such as black oncom, red oncom, and *tempeh gembus*. These kinds of food have been studied to explore source of functional food like red oncom that can be used as potential media for fibrinogenolytic protease production.[2] Like red oncom, *tempeh gembus* is an Indonesian fermented food derived as a by-product of tofu manufacture from soybeans. As a by-product of tofu manufactures, *tempeh gembus* is inexpensive, but it contains several nutrients including the isoflavones genistein (57.1 µg/g) and daidzein (33.1 µg/g) which are antioxidants. A study on rats found that giving antioxidants, especially isoflavone can prevent free radicals from oxidizing lipid. Because isoflavone genistein has a role in inducing genetics that control the manufacture of the SOD enzyme, it is thought that isoflavone is also able to protect the SOD enzyme activity. Isoflavone also helps superoxide dismutase in its function to separate free radicals.[27]

Additionally, *tempeh gembus* has a fiber content (4.69%) compared to soybean *tempeh* (1.40%), and it contains essential fatty acid such as linoleic acid (26.83%), oleic acid (36.53%), and linolenic acid (2.46%).[13] In rat studies, *Tempeh gembus* can reduce hypercholesterolemia and reduced lipid levels for three weeks, reduce serum levels of high sensitivity C-Reactive Protein (hsCRP) and fibrinogen significantly and reduce homocystein and malondialdehyde.[27,14,31]

Another benefit of the consumption of *tempeh gembus* may be related to the occurrence of a fibrinolytic enzyme and this thrombolytic agent could prevent the development of cardiovascular disease.[2,3] Further study of *tempeh gembus* showed that there are five new protein fractions that may have benefit to health and its hydrolyzate can be used as antimicrobial and functional food to prevent the cardiovascular disease.[5,33,6]

Table 3. The mean Data of FBG Levels, HDL-c Levels and TG Levels

Parameter	Treatment Group				
	K (-) (n=5)	K (+) (n-5)	P1 (n=5)	P2 (n=5)	P3 (n=5)
GDP [mg/dL]	82,72 ± 7,672	170,16±13,623	178,04±46,094	136,70±41,445	177,58±57,044
HDL-c[mg/dL]	62,40 ± 6,348	33,80± 3,271	35,40 ± 6,427	36,80 ± 3,899	34,60 ±3,782
TG [mg/dL]	86,06±10,005	153,66±16,464	146,36 ±5,876	157,74±21,489	151,54±8,943

Based on the above background, this research was conducted to analyze the effect of giving *tempeh gembus* in various doses on plasma MDA levels and serum SOD enzyme activity in metabolic syndrome rats. The aim of this research is to determine if *tempeh gembus* acts as a functional local food with antioxidant properties that can help in the prevention of the metabolic syndrome.

MATERIALS AND METHODS

Research Design

As an experimental post-test only, control group design was used with ethical approval from the Ethical Commission of Health Research, Medical Faculty, Diponegoro University (Ethical clearance number: 08/EC/H/FK-RSDK/III/2017).

Study Subjects

Twenty-five male Sprague Dawley rats (8~10 weeks of age) with the weights from 150 to 200 grams were randomly assigned into 5 groups, 2 control and 3 treatment groups. The *tempeh gembus* used was specially ordered from a local manufacturer in Semarang using local soybean as the basic material. Each of the 25 rats were acclimatized for 7 days in individual cages at room temperature and each rat was given standard food (CP-594) and water, ad libitum. After the acclimatization period rats weight was recorded as the initial weight.

Intervention Period

The doses of *tempeh gembus* were determined by isoflavone needs per day for human as recommended by Food and Drug Administration (FDA), which was 25 mg/day and converted for rats with the weight of 200 g.[11,30] The isoflavone content of *tempeh gembus* was not examined in this study; rather, it was relied on previous research using similar *tempeh gembus*. The various doses of *tempeh gembus* were 2.5 g, 5 g, and 7.5 g that given to metabolic syndrome rats in treatment groups (P1, P2, P3). The control groups only received standard food without *tempeh* gembus or pure isoflavones addition. Group K(-) were treated as healthy rats. Group K(+) were presented as metabolic syndrome rats without any treatment. The feeding regimen for each of the 5-group animal model could be seen in **Table 1.**

Biomarkers Analysis of Metabolic Syndrome

After induction period for 14 days, metabolic syndrome biomarkers were measured include the level of fasting blood glucose (FBG) >100 mg/dL, High Density Lipoprotein cholesterol (HDL-c) <40 mg/dL, and TG >100 mg/dL, also the body weight and body length of rats to get Lee index (>0.300) as obesity biomarkers.[24,25] During the intervention period, the rats were fed with each group, namely K(-) (a group of rats that were considered healthy were only given standard feed and distilled water), K(+)(a metabolic syndrome group feed only standard feed and aquadest), K+ was considered to be a group with pathological conditions without any intervention because it was conditioned in metabolic syndrome without any intervention, whether pure isoflavone or *tempeh gembus*. P1 (a metabolic syndrome group received standard diet, 5 g of *tempeh gembus*, and distilled water), P2 (a metabolic syndrome group received standard diet, 2.5 g of *tempeh gembus*, and distilled water), and P3 (a metabolic syndrome group was given standard feed and *tempeh gembus* 7.5 g and distilled water). The biomarkers test of metabolic syndrome (level of FBG, HDL-c, and TG) was done in Laboratory of Health, Central Java Province.

Analysis of MDA and SOD Enzyme Levels

After intervention period for 28 days, the body weight of rats was measured. The plasma MDA level was measured using Thiobarbituric Acid Reactive Substance (TBARS) method that was done in Center for Food and Nutrition Studies, Gajah Mada University, Yogyakarta. The serum SOD enzyme activity was measured using Enzyme Linked Immunosorbent Assay (ELISA) method that was done in Integrated Research and Testing Laboratory, Gajah Mada University, Yogyakarta.

Table 4. The mean Data of Lee Index

Treatment Group	n	MDA Plasma Level [nmol/mL] (Mean ± SD)
K(-)	5	0,292 ± 0,006
K(+)	5	0,305 ± 0,004
P1	5	0,307 ± 0,005
P2	5	0,310 ± 0,004
P3	5	0,307 ± 0,004

Table 5. The Mean Data of Weigth after Induction and Weight After Intervention by giving Tempeh gembus

Weight [g]	Treatment Group					*P*
	K(-) (n = 5)	K(+) (n = 5)	P1 (n = 5)	P2 (n = 5)	P3 (n = 5)	
After Induction	200,82 ± 7,897	217,40 ± 13,506	229,52 ± 22,634	237,44 ± 7,625	212,16 ± 14,744	
After Intervention	246,58 ± 10,451	258,52 ± 15,188	259,98 ± 22,665	257,80 ± 7,437	231,54 ± 14,980	0,000*
Δ	45,76 ± 9,699[a]	41,12 ± 8,412[a]	30,46 ± 5,078[b]	20,36 ± 3,693[c]	19,38 ± 4,531[c]	

* *Oneway Anova Test*
[a,b,c] *Post Hoc LSD Test*, significantly different *P<0,05*

Statistical Analysis

Statistical analysis used statistic program. The research data was normality tested using Shapiro-Wilk test. If the data were distributed normally so it was analyzed by One Way Anova and followed by a different test of each group using Post Hoc Least Significance Different (LSD) test. If the distributed data was abnormal so it was analyzed using non parametric Kruskal-Wallis test and different test of each group by Mann-Whitney test. Correlation between MDA levels and SOD enzyme activity was analyzed using Spearman test.

RESULTS

Acclimatization Period – Induction Period

The initial weight was done after acclimatization (8th day). The induction weight was done after induction period on the 22nd day. The mean data of rat weights was shown in **Table 2.** There was the difference in mean weight between initial weight and weight after induction was statistically significant ($p<0.05$). Meaningful differences were in group K (-) with other groups that were given induction. Between groups that given induction, there were no meaningful differences.

Induction Period – Intervention Period

The metabolic syndrome condition was got on induced rats. The result of blood serum test was shown in **Table 3** and Lee index counting was shown in **Table 4**. Giving *tempeh gembus* for 28 days had a significant effect on rats' weight and showed a significant difference in the significance value ($p<0.05$). The mean data of rat weights after induction by high fat-sucrose food and after intervention with *tempeh gembus* could be seen in **Table 5**. Rats in control group had higher weight gain compared to treatment group. The control group of rats gained more weight than the treated rats (P1, P2, P3). The difference in body weight between the treatment group and the control group was statistically significant ($p<0.05$). In terms of preventing weight gain, there was no discernible difference between the administration of *tempeh gembus* at a dose of 5 g and a dose of 7.5 g ($p>0.05$).

Effect of Tempeh gembus *on MDA Levels*

Group K(-) had the lowest plasma MDA level (1.584±0.2064 nmol/ml), while group K(+) had the highest level (6.770±0.4268 nmol/ml). Plasma MDA levels in metabolic syndrome rats were significantly affected ($p<0.05$) after receiving *tempeh gembus* at varying doses for 28 days. Meanwhile, There was a significant difference in plasma MDA levels in each treatment group ($p<0.05$), except for the K(-) group with P3.

Effect of Tempeh gembus *on SOD Enzyme Levels*

The lowest level of serum SOD enzyme (4.07±0.317 ng/mL) existed on group K (-) and the highest (4.46±0.311 ng/mL) existed on group K (+). Statistically, there were no meaningful differences in the level of serum SOD enzyme on each group ($p>0.05$). The mean data level of serum SOD enzyme of each group could be seen in **Table 7**.

Correlation between MDA Levels and SOD Enzyme Activity

According to the correlation test of Spearman, there were no significant correlation between the plasma MDA levels and serum SOD enzyme activity ($p>0.05$). The plasma MDA levels and serum SOD enzyme levels on this research also had a positive correlation ($r=0.272$).

DISCUSSION

Acclimatization Period – Induction Period

During the induction period, group K(+), P1, P2, P3 were given high fat and sucrose diet (20% lard, 20% egg yolk, and 60% sucrose). They had greater mean of weight and higher mean of weight gain compared to group K(-). Group K (+) (metabolic syndrome group, which received only conventional feed and aquadest), for the *tempeh gembus* used, raw *tempeh gembus* that had not been processed in any way. There is an increase in body weight in the groups given induction related to high-fat and sucrose foods. Fat given from lard contained saturated fat, higher calories and easier to absorb, so it triggered the faster weight gain compared to standard food.[26] Excessive sucrose consumption or fructose also gave a similar effect to development of obesity and its consequences, such as metabolic syndrome.[23,34] According to research by Malafaia, the diet contained high sucrose using (30%) from the second week there was a greater weight gain in the sucrose group compared to control (standard diet).[29] Given a diet that contained fat from 20% to 40% and sucrose from 10% to 30 % could cause metabolic disorders such as increased body weight.[28]

Induction Period – Intervention Period

The giving of high fat and high carbohydrate food on rats were able to induce the same condition of metabolic

syndrome as human. Vary compositions on fat and sucrose additional on the diet about 10% to 40% was able to gain the body weight, abdominal fat, hyperinsulinemia, hyperglycemia, and hyperleptinemia.[40] High fat diet derived from lard and quail egg yolks were able to increase body weight, level of plasma triglyceride, concentration of free fatty acid, and concentration of plasma insulin, also decreased the concentration of plasma adiponectin. It was due to the high content of trans fatty acid from lard and high cholesterol from the egg yolks.[12] According to a recent study, white rats fed a high fat diet for 28 days (3 g/200gBW pork oil and 2 g/200gBW duck egg yolk) had a significant increase in total cholesterol and triglycerides.[22] Besides, sucrose giving with high concentration in 2 weeks could turn up the phenotype resistance insulin and hypertriglyceridemia on Sprague Dawley and Wistar rats.[40]

Tempeh gembus giving for 28 days inhibit the weight gain on treatment group. *Tempeh gembus* with doses of 7.5 g had the lowest of mean body weight and mean weight gain than other groups. *Tempeh gembus*' high fiber content has been proven to reduce daily energy intake by 20-35%.[14] This is due to fiber's influence on the lower gastrointestinal tract, which helps regulate stomach emptying and create a prolonged feeling of fullness while also avoiding weight gain. Meanwhile, fibre has been proven to help with obesity-related health problems, potentially by altering the gut-derived metabolites SCFAs.[7] Previous research has showed that increasing dietary fiber consumption can help with weight loss.[17,20]

Effect of Tempeh gembus *on MDA Levels*

The high level of plasma MDA on group K (+) was related to the induction of high fat and high sucrose food for 2 weeks. Lomba et al got the high level of hepatic MDA in rats that had been induced with high fat and high sucrose food as ad libitum compared to control group (Chang, Lin and Chang, 2012). The high blood glucose level also would increase the result of lipid peroxidation, so the higher level of plasma MDA also could be seen on individual who had metabolic syndrome.[8]

Rats with metabolic syndrome that were given *tempeh gembus* with the doses of 2.5 g, 5 g, and 7.5 g had a lower plasma MDA level compared to group K(+). Group P3 had the lowest plasma MDA level compared to group P1 and P2. The doses 7.5 g of *tempeh gembus* were the most influential doses to the plasma MDA level. The plasma MDA level on group P3 was almost close to the plasma MDA level on group K(-) that was considered as the normal level of plasma MDA on health condition.

Tempeh gembus has higher levels of isoflavone antioxidants (genistein and daidzein).[27] As many as 99% of isoflavones in soybeans are in the form of glycosides, which consist of 64% genistin, 23% daidzin, and 13% glycitin in small amounts so for this study only isoflavones (genistein and daidzein) were discussed.[35] Genistein could reduce the lipid peroxide formation by inhibiting Fe^{2+}-ADP-NADPH system and also would increase the level of MnSOD that was able to decrease the level of peroxide.[10,38] Genistein is an antioxidant that helps to protect cells from lipid peroxidation by lowering the generation of free radicals and reactive oxygen. In addition, genestein isoflavones are able to increase the expression of MnSOD through interactions with estrogen receptors in cells.[18] Same as genistein, the daidzein giving for long term also could decrease the MDA level and increase the antioxidant enzyme activity on hyperglycemic rats. The daidzein giving also had hypoglycemia effect and could improve the endotel function.[37] MDA levels will also decrease as a result of isoflavone therapy since isoflavones have the ability to neutralize free radicals and stop lipid peroxidation events.

Effect of Tempeh gembus *on SOD Enzyme Levels*

Groups that were given *tempeh gembus* had the lower level of serum SOD enzyme from group K(+), but higher than group K(-). Group P3 with the giving of highest doses of *tempeh gembus* had higher level of serum SOD enzyme compared to group P1 and P2. The giving induction of high-fat food derived from quail egg yolks possibly affected the high serum SOD enzyme activity on group K(+). The quail egg yolks had high enough Zn ion (70.6 mg L^{-1}), while Zn was anion that had an important role for the structural function of CuZnSOD (SOD1).[15,41] In addition, the stability of SOD enzyme activity is affected by Zn ions.[15]

Related to isoflavones content on *tempeh gembus* as an antioxidant, it is possible if the isoflavone levels in *tempeh* gembus used in this study are low. Additionally, the intervention period was brief, making it impossible for the intervention of feeding *tempeh gembus* to metabolic syndrome rats for 4 weeks to show a significant increase in serum SOD enzyme activity.

According to research from Taipei, the activity of the liver, lung, and kidney SOD enzymes in the treatment and control groups did not differ significantly after receiving soy isoflavone supplements in 3 different doses for 24 days.[21] Several studies have shown that feeding a soybean-based diet containing 27.4 mg/kg BW/day of isoflavones (genistein and daidzein) or a linseed-based diet comprising 28.1 mg/kg BW/day had no effect on the antioxidant enzymes SOD and GSH (made up of secoisolariciresinol and daidzein).[19] However, rats fed diets containing high levels of SBI (150 and 200 ppm) showed significant increases in SOD and catalase activity in multiple organs. Accordingly, it's possible that the mechanism by which *tempeh gembus* exhibit antioxidant activity is influenced by the isoflavone type and/or dose. Large dosages of *tempeh gembus* may be necessary to increase the activity of antioxidant enzymes, but modest or moderate levels may only be enough to scavenge free radicals.[1]

Another factor that influenced the high serum SOD enzyme level on group K(+) compared to other groups was related to cell function to respond SOD enzyme. Previous research has shown that a dose of 3 g/200 g body weight of *tempeh* intervention can successfully increase plasma SOD enzyme activity levels.[18] Dietary feeding of the *tempeh* at a dose of 20 mg/kg BW to sprague dawley rats for 16 weeks can increase serum SOD levels considerably.[9] This is because the isoflavone component of Genistein, which is prevalent in soybeans, serves as a direct antioxidant by donating hydrogen atoms from the hydroxyl group connected to the benzene ring, which can protect against oxidative damage.

Experimental animals given daidzein exhibited a considerable increase in catalase activity and SOD, whereas genistein administration boosted SOD activity but not as much as daidzein administration.[42] Meanwhile, Kimura et al. stated that the activity of serum SOD enzyme on the sufferers of Diabetes type 2 was higher than healthy control group. The high activity of serum SOD enzyme was possibly due to the formation of free radical as long as hyperglycemia that affected the endothelial cell response to bound the EC-SOD enzyme.[16]

Correlation between MDA Levels and SOD Enzyme Activity

The increasing activity of serum SOD enzyme along with the high level of plasma MDA on this research could be affected by some factors. One of them was the usage of quail egg yolks to induce the metabolic syndrome condition. Quail egg yolk contained high enough levels of ions, i.e. iron (Fe) (116.0 mg/L) and Zn (70.6 mg/L) (Tunsaringkarn, Tungjaroenchai and Siriwong, 2013). The existence of Fe catalyst affected to the imbalance of hydroperoxides (ROOH) that leads the formation of reactive alkoxy radical by Fenton reaction. The existence of Fe would strengthen the chain reaction that would degrade ROOH to be an aldehyde, such as MDA and hydrocarbon, ethane and ethylene.[15] While the Zn ion that was contained in the quail egg yolks had an important role for structural function and stability of SOD enzyme.[15,41]

The high level of plasma MDA and activity of serum SOD enzyme on this research was inversely proportional to previous studies which proved that isoflavone supplementation in rats could increase the activity of the enzyme SOD, GSH-px and decrease MDA levels in rats (Yoon and Park, 2014). Another study found that elderly people who were given SOD supplementation for 8 weeks had decreased MDA levels, although the difference was not significant. This could be due to the volatile nature of MDA, which means that if the sample is not tested right away, the real MDA levels will fluctuate.[32]

CONCLUSION

In conclusion, giving *tempeh gembus* with doses of 2.5 g, 5 g, and 7.5 g significantly could decrease the plasma MDA levels, but unable yet to increase the serum SOD enzyme activity in metabolic syndrome rats. *Tempeh gembus* dose of 7.5 g was the most influential to the plasma MDA levels and serum SOD enzyme activity compared to other doses. Correlation between MDA levels and SOD enzyme levels statistically was not significant.

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