

The effect of Sorghum Tempeh (*Sorghum bicolor L. Moench*) on low-density lipoprotein (LDL) and malondialdehyde (MDA) levels in atherogenic diet-induced rats

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ABSTRACT

An atherogenic diet induces oxidative stress leading to hypercholesterolemia. This condition causes atherosclerosis followed by increased LDL and MDA. Sorghum tempeh contains fiber and antioxidants that can protectively improve LDL and MDA levels. Therefore, this research aims to determine the effect of sorghum tempeh on LDL and MDA levels in atherogenic diet-induced rats compared to sorghum flour. It used a randomized pre-post test with a control group design. The test subjects were 30 male *Sprague Dawley* rats, consisting of 6 normal conditioned rats (C1), and 24 that were induced by an atherogenic diet (C2, T1, T2, T3) for 2 weeks. Sorghum flour was administered at a dose of 4.095 g (T1) and the sorghum tempeh at 3.041 g (T2) and 6.081 g (T3) for 4 weeks. Furthermore, C2 was constantly induced through an atherogenic diet. Total cholesterol and LDL levels were then analyzed using the CHOD-PAP method, and MDA levels, using the ELISA method. Meanwhile, statistical analysis for these variables was carried out using IBM SPSS Statistics 21 software. The results showed that the administration of sorghum flour and tempeh significantly reduced total cholesterol, LDL, MDA levels in each group ($p = 0.001$). Furthermore, it showed that there was a significantly strong correlation between LDL and MDA levels before and after treatment ($r = 0.610$, $r = 0.805$, and $p = 0.001$). The administration of sorghum tempeh at a dose of 6.081 g caused the greatest reduction (Δ) in LDL levels at $-44.19 \pm 2.58 \text{ mg.dL}^{-1}$, although, it was not the same as normal control. Meanwhile, sorghum flour at a dose of 4.095 g was the most influential in reducing MDA levels to the same as normal control with delta (Δ) at $-7.67 \pm 0.37 \text{ ng.mL}^{-1}$. In conclusion, sorghum tempeh and flour were the most effective at reducing LDL and MDA levels, respectively.

Keywords: sorghum tempeh; sorghum flour; LDL; MDA; atherosclerosis

INTRODUCTION

Atherosclerosis occurs due to a progressive plaque growth process and is characterized by hypercholesterolemia, abnormalities in the shape of the arterial walls, and increased inflammatory mediators such as IL-6, IL-10, and MCP-1 (Estruch et al., 2016). Moreover, the induction of an inflammatory process and a simultaneous increase in LDL in the tunica intima causes the formation of reactive oxygen species (ROS) (Salvayre, Negre-Salvayre and Camaré, 2016; Marchio et al., 2019). High intracellular levels of ROS are involved in the process of lipid peroxidation which produces secondary reactive products such as MDA (Papac-Milicevic, Busch and Binder, 2016).

Increased reactivity of MDA causes endothelial damage and modifies LDL, which leads to changes in cellular response (Papac-Milicevic, Busch, and Binder, 2016). Meanwhile, an increase in the levels of LDL in blood plasma causes the formation of atheroma plaque, which would develop into cardiovascular disease (Ratnasari, Santosa and Rachmawati, 2018). Various studies have used mice induced by an atherogenic diet as models

of atherosclerosis. These mice experience an increase in the accumulation of fat in their blood vessels leading to abnormal signal transduction in organs, oxidative stress, lipotoxicity, and organ dysfunction. This affects the activity of transcription factors in lipid metabolism in the heart (Lee et al., 2017).

One of the treatments of atherosclerosis is through the provision of fermented food (Şanlıer, Gökçen and Sezgin, 2019). Moreover, Indonesia is known to produce tempeh, the fermented food made from soybeans (Hartanti, Rahayu and Hidayat, 2015). Sorghum is a cereal that contains high fiber and antioxidants, however, it still has low food value (Espitia-Hernández et al., 2020). Furthermore, this cereal has been used as a raw material for the production of tempeh which was tested in atherogenic diet-induced rats. Sorghum tempeh (100 g) contains 134.49% energy, 15.20% carbohydrates, 6.75% protein, 5.21% fat, 66.40% water, 0.15% ash, 68.57% starch digestibility, 63.32% protein digestibility, 52.21% antioxidants, 5.92% total dietary fiber, 0.56% soluble dietary fiber, and 5.63% insoluble fiber.

Sorghum tempeh could be produced through the following process. First, sorghum is soaked for 12 hours, boiled until it reaches a softer texture, and then left to cool. Furthermore, tempeh yeast which contains *Rizhopus oligosporus* is added to start the fermentation process (Hartanti, Rahayu and Hidayat, 2015). The resultant mold produces various protease enzymes with proteolytic activity, which makes sorghum tempeh easier to digest (Endrawati and Kusumaningtyas, 2017). Moreover, the fermentation process lasts for 60 hours and leads to the formation of β -glucosidase enzymes, which produce flavanone, flavonols, and aglycone isoflavones (Salazar-López et al., 2018; Verni, Verardo and Rizzello, 2019). The final stage involves blanching for 5 minutes to stop the microbial activity (Tian et al., 2018).

The advantages of products fermented by *Rhizopus oligosporus* were proven in other similar studies. Quinoa seed extract fermented by this fungi had increased antioxidant enzymes, such as *superoxide dismutase* (SOD) and *glutathione peroxidase* (GSHPx) which help to prevent peroxidative damage (Matsuo, 2005; Hur et al., 2018). Furthermore, in line with the research on soybean and gembus tempeh, the results of yeast fermentation by *Rhizopus spp* was an increase in SOD enzyme which could reduce MDA levels (Harun, Susanto and Rosidi, 2017; Kurniasari et al., 2017).

Sorghum tempeh has the potential as an antioxidant and anti-cholesterol agent, which could repair oxidative stress damage (Pisoschi and Pop, 2015; Dykes, 2019). Moreover, fermented sorghum could reduce oxidative stress through the fermentation effect of its fiber. This fiber modulates the intestinal microbiota to improve immune function through the production of *short-chain fatty acid* (SCFA) (Ohira, Tsutsui and Fujioka, 2017). SCFA reduces inflammation that triggers oxidative stress by suppressing T helper cells, neutrophils, macrophages, and cytotoxic activity in natural killer cells (Tan, Norhaizan and Liew, 2018; Hu et al., 2020).

Sorghum tempeh also plays a role in controlling serum cholesterol. This is because the *cellulose synthase-like* (Csl) gene in the soluble fiber, (1,3;1,4)- β -glucan has the potential to increase fiber levels, which would reduce apoB-100 and LDL (Ermawar et al., 2015; Ho et al., 2016). Additionally, fiber fermented by the intestinal microbiota produces SCFA, which influences lipid metabolism through transport and *de novo* synthesis to lower plasma cholesterol levels (Chen et al., 2018).

From the above, the aim of the research, which is to prove the protective ability of sorghum tempeh to reduce MDA and LDL levels. Sorghum flour which was used as a comparison is sorghum seeds that were processed into flour without fermentation. These seeds were mashed with a grinder to achieve a powdery texture then sifted until they became soft. The previous study has been shown that sorghum flour improving lipid profile and MDA levels in rats (Silva et al., 2020; Zakaria et al., 2011). Besides, the flour (100 g) contains 315.11% energy, 69.75% carbohydrates, 8.12% protein, 1.31% fat, 10.55% water, 1.47% ash, 80.50% starch digestibility, 78.70% protein digestibility, 75.18% antioxidants, 8.79% total dietary fiber, 0.94% soluble dietary fiber, and 7.85% insoluble dietary fiber.

Scientific Hypothesis

Sorghum tempeh decreases LDL and MDA levels in atherogenic diet-induced rats more effect than sorghum flour. Moreover, the levels of these variables were strong correlations before and after treatment.

MATERIAL AND METHODOLOGY

Samples

This study used white sorghum (Figure 1). The grain was soaked in water at a ratio of 1:3 for 12 hours and then boiled for 10 minutes. After that, the addition of the tempeh yeast at 0.25 g for every 100 g of white sorghum was carried out after the sorghum had cooled. The mixture was then incubated at room temperature (29 ± 1 °C) for 60 hours. Subsequently, the sorghum tempeh was blanched at 90 °C for 5 minutes (Figure 2).

Sorghum flour was used as a comparison for sorghum tempeh and was produced by processing sorghum seeds without fermentation. The white sorghum was purred using a grinder to achieve a powdery texture and subsequently sieved with 70 mesh (Figure 3). Moreover, the dosage of sorghum tempeh and flour was based on human fiber needs, which is 20 g.d⁻¹. This amount was then converted to 200 g of body weight for the rats. Finally, the dosage for T1, T2, T3-groups at 4.095 g, 3.041 g, and 6.081 g, respectively.

Chemicals

Two milliliters of blood were obtained from the *retro-orbital plexus* of the rats for total cholesterol (mg.dL⁻¹), LDL (mg.dL⁻¹), and MDA (ng.mL⁻¹) serum were assessed in PSPG UGM Yogyakarta Laboratory, Indonesia.

Animal and Biological Material

This study used male *Sprague Dawley* rats supplied by PSPG UGM Yogyakarta Animal Laboratory, Indonesia. The white sorghum was produced by Sedana Panen Sejahtera Ltd., Jombang, Indonesia. The tempeh yeast was produced by Aneka Fermentasi Industri Ltd., Bandung, Indonesia.

Instrument

The weights and diet consumed of the rats were measured by Digital Camry EK5055 Max.5 kg d = 1 g. Total cholesterol levels were tested using the Cholesterol FS (10') 5 x 25 mL.1 x 3 mL⁻¹ STD. Meanwhile, LDL levels were tested using the LDL Precipitant 250 mL Kit. Finally, MDA levels were ascertained using the General Malondialdehyde ELISA Kit (MDA) 96 wells (IP). The kits of these tests were supplied by Khairos Jaya Sejahtera Ltd., Yogyakarta, Indonesia.

Laboratory Methods

Total cholesterol and LDL levels were tested according to the CHOD-PAP method (WHO/IFCC). MDA levels were tested according to the ELISA method (ISO 9001:2008; ISO 13485:2003).

Description of the Experiment

This study used a true experimental design with a randomized pre-post test control group design. Furthermore, it was conducted at the PSPG UGM Yogyakarta Animal Laboratory from October-December 2020.



Figure 1 Preparation of Sorghum Tempeh and Flour.



Figure 2 Sorghum Tempeh.



Figure 3 Sorghum Flour.

Sample preparation:

The inclusion criteria were male *Sprague Dawley* rats aged 2 months and weighing 150 – 200 g, that were in a healthy condition during the acclimatization period. Moreover, the acclimatization period was 1 week and aimed to get the animals acquainted with the type of feed and new way of life. The atherogenic criteria were based on the hypercholesterolemic condition of the rats (total cholesterol levels more than 100 mg.dL⁻¹). Meanwhile, the dropout criteria were rats that their body weight decreased to <150 g during the adaptation period and those that died during the study.

Number of samples analyzed:

There were 30 male *Sprague Dawley* rats. Additionally, they were divided by simple random sampling into five groups, namely C1, C2, T1, T2, and T3-groups, and were housed individually. The cage room had an ambient temperature of 28 – 32 °C with a lighting cycle of 12 hours dark and 12 hours light. Furthermore, the rat feeds used were standard (Comfeed AD II) and atherogenic feeds (Table 1). Assessment of diet consumed was based on the weight of feed given minus the weight of the remaining feed.

The rats in C2, T1, T2, and T3-groups were induced using an atherogenic diet for 2 weeks. Moreover, this feed was given at 20 g.d⁻¹ and drinking water ad libitum. C1-group was in a normal condition as only the standard feed at 20 g.d⁻¹ and drinking water ad libitum was given therein. For the remaining groups, they received interventions for 4 weeks in a sonde, where the T1 group received sorghum flour at a dose of 4.095 g, while T2 and T3-groups received the tempeh at doses of 3.041 g and 6.081 g, respectively. The treatment groups were also given standard feed at 15 g.d⁻¹ and drinking water ad libitum. Finally, C1-group was maintained at normal conditions, while C2-group was continuously induced using an atherogenic diet until the end of the research.

Number of repeated analyses:

The blood was obtained from the *retro-orbital plexus* of the rats for total cholesterol, LDL, and MDA serum was carried out two assessments namely before and after the treatment period.

Statistical Analysis

Data analysis was carried out using IBM SPSS Statistics 21 software. Furthermore, data normality was tested using the Shapiro-Wilk test. Bivariate analysis was used to analyze the levels of total cholesterol, LDL, and MDA through Paired t-test when they were normally distributed. Meanwhile, when the data was not normal, the Wilcoxon Signed Ranks test was used. The differences in the influence of the conditions of C1, C2, T1, T2, T3-groups on bodyweight, weights of rat feed consumption, and total cholesterol, LDL, and MDA levels were analyzed using the One Way Anova test when the data were normally distributed and homogeneous. However, in a condition where the data were not normally distributed or not homogeneous, used the Kruskal Wallis test. The correlation test used for the levels of LDL and MDA was the Pearson test when the data were normally distributed, however, when not normally distributed the Rank Spearman test was

used. Finally, the value of significance in the study was $p < 0.05$.

RESULTS AND DISCUSSION

The bodyweight of the rats at the beginning and end of the study did not differ significantly between the groups ($p = 0.383$ and $p = 0.136$). After the provision of the treatment products in T1, T2, and T3-groups, there was a significant difference between the groups ($p = 0.010$), that the treatment groups were the same as the normal control ($p = 0.793$, $p = 0.189$, and $p = 0.057$), and significant difference compared to the atherogenic diet induction control ($p = 0.006$ and $p = 0.001$) except for T1-group ($p = 0.091$). Additionally, the mean body weight in T1-group was lower compared to that of the atherogenic diet induction control. Table 2 shows the significant difference in delta (Δ) between the groups after treatment ($p = 0.001$), where the biggest change was in the T1-group at $+18.83 \pm 13.00$ g.

The mean weight percentage of the feed consumed in the groups during the induction period with an atherogenic diet was not significantly different ($p = 0.836$), while during the treatment period there was a significant difference ($p = 0.009$). Moreover, based on further tests, T1, T2, T3-groups had no significant difference in the weights percentage of feed consumed ($p > 0.05$). Table 3 shows that the T3-group from the induction to treatment period had a higher average percentage of feed consumed compared to the other two treatment groups, at 90.65% and 97.81%.

Table 4 shows that the levels of total cholesterol before treatment in T1, T2, and T3-groups were significantly different from the normal control (C1) ($p = 0.001$) and the same as the atherogenic diet induction control group (C2) ($p = 0.852$, $p = 0.729$, and $p = 0.498$). There was a significant increase in cholesterol total levels in the C2-group ($p = 0.001$), while in C1-group, there was the least change (Δ), at $+2.01 \pm 1.36$ mg.dL⁻¹ among all groups. Furthermore, the three treatments with their respective doses significantly reduced atherogenic parameters by lowering total cholesterol ($p = 0.001$) and were significantly different from the atherogenic diet induction control ($p = 0.001$), but still not the same as the normal control ($p = 0.001$). Meanwhile, the biggest change (Δ) occurred in T3-group at -80.55 ± 5.79 mg.dL⁻¹.

LDL levels in T1, T2, and T3-groups after induction with the atherogenic diet was significantly different from normal control (C1) ($p = 0.001$), however, not significantly different from atherogenic diet induction control (C2) ($p = 0.947$, $p = 1.000$, $p = 0.833$). Table 5 shows that after the administration of the treatments, there was a significant decrease in the LDL levels ($p = 0.001$) across the groups, differ to the atherogenic diet induction control ($p = 0.001$) although, they were still significantly different from normal control ($p = 0.001$, $p = 0.001$, $p = 0.008$). When viewed from delta (Δ), it shows that the T3-group (sorghum tempeh a dose of 6.081 g) had the greatest influence, namely -44.19 ± 2.58 mg.dL⁻¹ when compared to the other two treatments. The treatment in this group had the same effect as in the T1-group ($p = 0.265$), but a significantly different effect compared to the T2-group ($p = 0.020$). Meanwhile, the treatment in T1 and T2-groups had the same effect ($p = 0.225$).

Table 1 Composition of Standard and Atherogenic Feed.

Standard Feed	Atherogenic Feed
12% water, 51% carbohydrates, 15% crude protein, 3-7% crude fat, 6% crude fiber, 7% ash, 0.55% phosphorus, 0.9 – 1.1 % calcium.	12% water, 51% carbohydrates, 15% crude protein, 3-7% crude fat, 6% crude fiber, 7% ash, 0.55% phosphorus, 0.9 – 1.1 % calcium, cholic acid, pure cholesterol.

Table 2 Bodyweight of Rats.

Groups	Mean ± SD (g)				
	The Beginning of The Study	After Atherogenic Diet Induction	Δ1	The End of The Study	Δ2
C1 (normal control)	189 ±10.81	215.83 ±9.37	26.83 ±7.94	252.33 ±19.56	36.50 ±17.23
C2 (atherogenic diet induction control)	192 ±13.67	229.83 ±18.57	37.83 ±10.63	288.17 ±38.65	58.33 ±24.79
T1 (sorghum flour a dose of 4.095 g)	192.83 ±12.90	229.33 ±11.86	36.50 ±8.41	248.17 ±13.83 ^{a,c}	18.83 ±13.00
T2 (sorghum tempeh a dose of 3.041 g)	191.17 ±6.37	231 ±11.95	39.83 ±9.45	237.50 ±15,29 ^{a,b}	6.50 ±8.46
T3 (sorghum tempeh a dose of 6.081 g)	181.50 ±8.34	219.17 ±6.74	37.67 ±3.45	230.50 ±8.74 ^{a,b}	11.33 ±8.73
<i>p</i>	0.383 ¹	0.136 ¹	0.091 ¹	0.010 ^{2*}	0.001 ^{1*}

Note: Δ1 = Bodyweight of after atherogenic diet induction – Bodyweight of the beginning of the study; Δ2 = Bodyweight of the end of the study – Bodyweight of after atherogenic diet induction; ¹One Way ANOVA Test; ²Kruskal Wallis Test; ^aNot significantly different from normal control; ^bSignificantly different from atherogenic diet induction control; ^cNot significantly different from atherogenic diet induction control. *Significant ($p < 0.05$).

Table 3 Weights of Rat Feed Consumption.

Groups	Mean ± SD (g)	
	Atherogenic Diet Induction Period	Treatment Period
C1 (normal control)	17.94 ±1.43 (89.71%)	17.82 ±0.71 (89.11%)
C2 (atherogenic diet induction control)	17.49 ±1.87 (87.45%)	16.66 ±2.07 (83.31%)
T1 (sorghum flour a dose of 4.095 g)	17.56 ±1.36 (87.79%)	14.19 ±0.63 (94.61%) ^a
T2 (sorghum tempeh a dose of 3.041 g)	17.18 ±1.47 (85.90%)	14.24 ±0.39 (94.95%) ^a
T3 (sorghum tempeh a dose of 6.081 g)	18.13 ±1.55 (90.65%)	14.67 ±0.40 (97.81%) ^a
<i>p</i>	0.836 ¹	0.009 ^{2*}

Note: ¹One Way ANOVA Test; ²Kruskal Wallis Test; ^aThe comparison between T1, T2, and T3-groups was not significantly different. *Significant ($p < 0.05$).

MDA levels in the T1, T2, T3-groups after induction were also significantly different compared to normal control (C1) ($p = 0.001$, $p = 0.006$, $p = 0.004$) and not significantly different from atherogenic diet induction control (C2) ($p = 0.818$, $p = 0.818$, $p = 0.896$). Additionally, after the administration of the treatment, there was a significant decrease in MDA levels ($p < 0.05$) across the groups and the levels were also significantly different from the atherogenic diet induction control ($p = 0.001$, $p = 0.018$) except for T3-group ($p = 0.238$). The administration of sorghum tempeh at the dose of 6.081 g caused the smallest effect (Δ) among the three treatments at $-3.97 \pm 0.33 \text{ ng.mL}^{-1}$, which was still the same as the atherogenic diet induction control ($p = 0.238$), however, significantly different from normal control ($p = 0.001$). Likewise, sorghum tempeh at a dose of 3.041 g did not have the same effect as normal control ($p = 0.018$). The administration of sorghum flour at a dose of 4.095 g had the most effect on improving MDA

levels to a similar level as normal control ($p = 0.238$) with the largest delta (Δ), namely $-7.67 \pm 0.37 \text{ ng.mL}^{-1}$. Statistically, the treatment in T1-group had a significantly different effect compared to the T3-group ($p = 0.018$) and the same effect as in the T2-group ($p = 0.237$). Meanwhile, the treatment in T2 and T3-groups had the same effect ($p = 0.237$) (Table 6).

Table 7 shows that LDL and MDA levels had a strong positive correlation before treatment, meaning that the higher the LDL levels, the higher the MDA levels ($p = 0.001$, $r = 0.610$). After the treatment period, LDL and MDA levels had a strong positive correlation, because the lower the LDL levels, the lower the MDA levels ($p = 0.001$, $r = 0.805$).

The rats induced with an atherogenic diet for 2 weeks had significantly increased total cholesterol levels, which could lead to atherosclerosis, with levels more than 100 mg.dL^{-1} in rats (Zárate et al., 2016).

Table 4 Changes in Blood Cholesterol Total Levels Before and After Treatment.

Groups	Mean ± SD (mg.dL ⁻¹)			
	Before Treatment	After Treatment	Δ	p
C1 (normal control)	83.22 ±2.36	85.23 ±2.61	+2.01 ±1.36	0.015 ^{1*}
C2 (atherogenic diet induction control)	192.01 ±4.78	222.75 ±3.91	+30.74 ±6.28	0.001 ^{1*}
T1 (sorghum flour a dose of 4.095 g)	189.61 ±4.19 ^{a,c}	122.88 ±3.50 ^{a,b}	-66.74 ±5.50	0.001 ^{1*}
T2 (sorghum tempeh a dose of 3.041 g)	189.04 ±4.84 ^{a,c}	133.60 ±3.28 ^{a,b}	-55.45 ±6.44	0.001 ^{1*}
T3 (sorghum tempeh a dose of 6.081 g)	188.13 ±4.96 ^{a,c}	107.58 ±2.69 ^{a,b}	-80.55 ±5.79	0.001 ^{1*}
<i>p</i>	0.001 ^{2*}	0.001 ^{2*}	0.001 ²	

Note: ¹Paired t Test; ²One Way ANOVA Test; ^aSignificantly different from normal control; ^bSignificantly different from atherogenic diet induction control; ^cNot significantly different from atherogenic diet induction control. *Significant (*p* <0.05).

Table 5 Changes in Blood LDL Levels Before and After Treatment.

Groups	Mean ± SD (mg.dL ⁻¹)			
	Before Treatment	After Treatment	Δ	p
C1 (normal control)	23.88 ±2.50	25.44 ±3.20	+1.56 ±1.18	0.023 ^{1*}
C2 (atherogenic diet induction control)	76.12 ±1.38	85.37 ±1.30	+9.24 ±1.69	0.001 ^{1*}
T1 (sorghum flour a dose of 4.095 g)	76.93 ±1.27 ^{a,c}	39.95 ±2.48 ^{a,b}	-36.98 ±3.16 ^d	0.001 ^{1*}
T2 (sorghum tempeh a dose of 3.041 g)	76.36 ±2.04 ^{a,c}	52.27 ±2.89 ^{a,b}	-24.09 ±3.70 ^d	0.001 ^{1*}
T3 (sorghum tempeh a dose of 6.081 g)	74.97 ±2.08 ^{a,c}	30.78 ±2.04 ^{a,b}	-44.19 ±2.58 ^d	0.001 ^{1*}
<i>p</i>	0.001 ^{2*}	0.001 ^{2*}	0.001 ^{3*}	

Note: ¹Paired t Test; ²One Way ANOVA Test; ³Kruskal Wallis Test; ^aSignificantly different from normal control; ^bSignificantly different from atherogenic diet induction control; ^cNot significantly different from atherogenic diet induction control. ^dThe comparison was significant (T2 and T3-groups), was not significant (T1 and T2-groups, T1 and T3-groups). *Significant (*p* <0.05).

Table 6 Changes in Blood MDA Levels Before and After Treatment.

Groups	Mean ± SD (ng.mL ⁻¹)			
	Before Treatment	After Treatment	Δ	p
C1 (normal control)	1.00 ±0.22	1.03 ±0.02	+0.02 ±0.01	0.001 ^{1*}
C2 (atherogenic diet induction control)	9.36 ±0.51	9.65 ±0.40	+0.29 ±0.14	0.004 ^{2*}
T1 (sorghum flour a dose of 4.095 g)	9.36 ±0.28 ^{a,d}	1.69 ±0.18 ^{b,c}	-7.67 ±0.37 ^c	0.001 ^{1*}
T2 (sorghum tempeh a dose of 3.041 g)	9.29 ±0.21 ^{a,d}	2.95 ±0.18 ^{a,c}	-6.34 ±0.32 ^c	0.028 ^{1*}
T3 (sorghum tempeh a dose of 6.081 g)	9.30 ±0.29 ^{a,d}	5.33 ±0.47 ^{a,d}	-3.97 ±0.33 ^c	0.001 ^{1*}
<i>P</i>	0.007 ^{3*}	0.001 ^{3*}	0.001 ^{3*}	

Note: ¹Paired t Test; ²Wilcoxon Signed Ranks Test; ³Kruskal Wallis Test; ^aSignificantly different from normal control; ^bNot significantly different from normal control; ^cSignificantly different from atherogenic diet induction control; ^dNot significantly different from atherogenic diet induction control; ^eThe comparison was significant (T1 and T3-groups), was not significant (T1 and T2-groups, T2 and T3-groups). *Significant (*p* <0.05).

Table 7 Correlation between LDL and MDA Levels.

LDL and MDA	<i>p</i>	<i>r</i>
Before Treatment ¹	0.001 [*]	0.610 ^a
After Treatment ²	0.001 [*]	0.805 ^a

Note: ¹Rank Spearman; ²Pearson; ^aStrong Correlation. *Significant (*p* <0.05).

Moreover, these characteristics describe atherosclerotic conditions and require intervention (NCEP, 2001). Induction with the atherogenic diet contains high cholesterol and cholic acid continued for up to 6 weeks in C2-group and caused the increase of cholesterol total, LDL, and MDA levels (Yamada et al., 2017).

Cholesterol abnormalities lead to changes in cholesterol content, including LDL (Pirillo et al., 2018). Moreover, LDL levels have a strong positive correlation with MDA levels and this proves that LDL contributes to the oxidative stress process, which is marked by increased MDA (Tsikas, 2017). Other studies stated that increased LDL and MDA have a positive correlation to vascular inflammation which is a clinical marker of atherosclerotic plaque, evaluated with ¹⁸F-FDG PET/CT (Kaida et al., 2014). LDL accumulates in artery walls causing monocytes to differentiate into macrophages and secrete inflammatory mediators such as Interferon- γ (IFN- γ) and tumor necrosis factor (TNF). This inflammation triggers oxidative stress through the activity of myeloperoxidase, lipoxygenase, and ROS (Gisterá and Hansson, 2017).

In this research, to tackle the impact of the development of atherosclerosis, sorghum tempeh and flour were used at different doses calculated based on the needs of human fiber and converted to 200 g of body weight of rats. The results showed a significant decrease in LDL levels followed by total cholesterol and MDA levels.

When traced from fiber content, the cellulose synthase-like (Csl) gene insoluble fiber (1,3; 1,4) - β -glucan in sorghum tempeh and flour has the potential to increase sorghum fiber content (Ermawar et al., 2015; Ho et al., 2016). The digested fiber in the colon, fermented by the intestinal microbiota produces SCFA. SCFA is divided into three substrates regulating plasma cholesterol reduction, namely acetate, propionate, and butyrate. Cholesterol synthesis is reduced in the liver due to decreased acetyl CoA by the inhibition of 3-hydroxyl-3-methylglutaryl-CoA (HMG-CoA) reductase. This increases the activity of the LDL receptor to reduce LDL levels (Feingold, 2000; Chen et al., 2018).

The group treated with sorghum tempeh a dose of 6.081 g (T3) had the most reduced LDL levels, although it was not maximal until it was close to normal control. However, it was lower than the atherogenic diet induction control. This is because at this dose, the fiber needs were satisfied and the percentage of group feed consumption was greater than the two other treatments >90%. Other studies also suggested that food fermented by *Rhizopus oligosporus* has the potential as an anti-hyperlipidemia agent to inhibit cholesterol through SCFA in animals fed with high-fat diets (Huang et al., 2018; Dimidi et al., 2019).

MDA is a parameter of oxidative stress in atherosclerosis (Czerska et al., 2015). The results showed that its levels significantly decreased with treatment using sorghum tempeh and flour. When viewed from the antioxidant content in sorghum, 3-deoxyanthocyanidin is a phenolic that affects reducing oxidative stress (Afify et al., 2012; Ramatoulaye et al., 2016). Sorghum tempeh and flour both contain antioxidants at 52.21% and 75.18%. Meanwhile, high antioxidant activity reduces oxidative stress by increasing the antioxidant enzymes such as SOD and GSHPx (Akyol et al., 2017; Hasanuzzaman et al., 2020).

Sorghum flour at a dose of 4.095 g (T1) was the most effective at reducing MDA levels to the same as normal

control. This is possible because the antioxidant content of this flour is greater than that of sorghum tempeh. Moreover, the flour does not go through immersion or heating processes such as in the process of producing sorghum tempeh, thus it is more likely to maintain greater amounts of antioxidants (Liu et al., 2020). In line with similar studies tested on rats, it was stated that sorghum flour increased lymphocyte proliferation activity leading to higher SOD enzyme, CAT, and GSHPx activity. Therefore, the balance of antioxidants could be improved and MDA levels decreased (Zakaria et al., 2011).

LDL and MDA levels had a strong positive correlation after treatment. This shows that efforts to reduce LDL levels also caused a reduction in MDA levels. Similar studies where sorghum was administered showed similar results. The administration of sorghum kafirin inhibited the increase in serum LDL in hyperlipidemic rats (Cruz et al., 2015). Likewise, fermented sorghum reduced LDL levels in hypercholesterolemic rats (Laleye et al., 2016). Another study where sorghum rice was administered along with black rice to rats with induced hypercholesterolemia in their liver for 12 weeks showed a significant reduction in hepatic LDL (Liu, Huang and Pei., 2021). The same was true for interventions with fermented foods against MDA. There was a decrease in serum LDL and MDA in the liver with the intervention using fermented tea in rats induced by high fat (Sun et al., 2019). Additionally, fermented tempeh also reduced MDA levels in rats induced by an atherogenic diet for 4 weeks (Kurniasari et al., 2017).

The changes in body weight of the rats at the end of the study in T2 and T3-groups were not different from the normal control, although, they were lower than those of the atherogenic diet-induced control. Therefore, sorghum tempeh in the treatment of atherosclerosis following the method used in this study had the protective ability to repair molecules in blood circulation and the advantage of slowing weight gains.

CONCLUSION

The rats induced with the atherogenic diet that received the intervention of sorghum tempeh and flour had decreased LDL and MDA levels. Furthermore, sorghum tempeh a dose of 6.081 g was the most effect at reducing LDL levels, although not the same effect as sorghum flour at reducing MDA levels. Sorghum tempeh and flour could be functional food for atherosclerosis treatment.

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The authors declare no conflict of interest.

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This study obtained ethical clearance from the Health Research Ethics Commission (KEPK), Faculty of Medicine, Diponegoro University Semarang No.94/EC/H/FK-UNDIP/IX/2020.

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