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Effect of seven consecutive days of tempeh milk feeding on low density lipoprotein levels in women over 45 years of age

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Abstract: The premenopausal phase is characterized by a gradual decline in the body's ability to regenerate, repair, and maintain normal physiological functions. This functional decline may contribute to the onset of various health conditions, including hypercholesterolemia. Hypercholesterolemia refers to an elevation in total blood cholesterol levels, often resulting from atherosclerosisplaque accumulation on arterial walls. The buildup of Low-Density Lipoprotein (LDL) cholesterol within arterial walls can lead to the formation of fatty streaks, which, upon oxidation, may progress to atherosclerosis. Dietary modifications have been shown to reduce the risk of cardiovascular disease. The consumption of legumes, such as soybeans, has been associated with improved cardiovascular management. Accordingly, the objective of this study was to examine the effect of consuming tempeh milk for seven consecutive days on LDL cholesterol levels in women over the age of 45. This experimental study involved a total sample of 25 participants, selected through purposive sampling from a population of premenopausal women aged over 45 years. Blood samples were analyzed at the Pratama Clinical Laboratory, Universitas Muhammadiyah Purwokerto, before and after participants consumed 250 grams of tempeh milk daily for seven consecutive days. Statistical analysis using a paired t-test revealed a significant reduction in LDL levels ($p = 0.001$), indicating a meaningful difference in mean LDL concentrations following the intervention.

Keywords: Premenopause; LDL; Tempeh milk.

INTRODUCTION

The premenopausal period generally begins at the end of 40 years of age, which is a change in a woman's menstrual cycle to become irregular from previously regular. There is a decrease in estrogen production during perimenopause, which is a sign of the end of the reproductive period[1]. Women over 45 years old are naturally unable to produce the hormones progesterone and estrogen, which control cholesterol levels in the body and maintain the menstrual cycle[2].

The premenopausal period is also a time when tissues slowly begin to lose their ability to replace, repair and maintain their normal function. This loss of function can lead to a variety of health problems. Hypercholesterolemia is one of the most common diseases in premenopausal women[3].

According to the Ministry of Health in 2022, the normal LDL cholesterol level is below 100 mg/dL. An elevated level of total cholesterol in the blood due to atherosclerosis (plaque buildup on artery walls) is called hypercholesterolemia[4]. Hypercholesterolemia, especially low-density lipoprotein (LDL) cholesterol, is an

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important cause of cardiovascular disease[5]. As a result of the accumulation of LDL cholesterol in the artery wall, it will cause fatty streaks and if oxidized, it will become atherosclerosis[1].

The main cause is an increase in cholesterol levels, namely LDL cholesterol[6]. To reduce these risk factors, dietary changes are needed that can reduce the risk of cardiovascular disease. Consumption of legumes has been associated with improved cardiovascular disease management as has soy[7].

According to epidemiological studies in Thailand, soy consumption with potential benefits can reduce the risk of long-term (chronic) diseases, especially cardiovascular disease[5]. Proteins contained in soybeans, especially daidzein and genistein, are believed to be able to affect lipid collection which can reduce LDL[8]. Unsaturated fatty acids have the effect of lowering the cholesterol content in the serum, so as to stop the adverse effects of sterols on the body[9].

The content of saponins, unsaturated fatty acids or Polyunsaturated Fatty Acids (PUFA), fiber and niacin contained in tempeh is proven effective in reducing LDL levels in the blood[10]. In addition, tempeh also contains lecithin and isoflavones. Lecithin serves to emulsify or dissolve cholesterol in the blood so that blood vessels are not narrowed and clogged. Isoflavones, on the other hand, act as antioxidants, anti-atherosclerosis and estrogenics that are effective in lowering LDL cholesterol[2].

In line with research conducted by Mulyani *et al* (2018), it is proven that tempeh milk given as much as 430 ml for 7 consecutive days can make LDL cholesterol decrease by 13 mg/dL. Another study from Nurpalah & Kusmiati (2021) also proved that the consumption of 200 ml of tempeh milk for 2 weeks showed a significant difference with a P value of 0,022.

The content of tempeh milk is very potential to control LDL levels but not many studies have explored it, which is the reason for researchers to carry out research related to the effect of giving 250 grams of tempeh milk per day for seven consecutive days on LDL levels in women aged more than 45 years.

MATERIAL AND METHOD

Research design

This study was conducted in February 2022 after obtaining ethical approval from the Research Ethics Commission of Muhammadiyah Purwokerto University with registration number KEPK/UMP/124/V/2022, then completed in March 2022. LDL examination took place at the Primary Clinical Laboratory of Muhammadiyah Purwokerto University, while for making tempe milk and sampling was carried out in Ledug Village, Banyumas District, Banyumas Regency.

The method used for this study was experimental with *pre-post test only group design*, then LDL levels were measured before and after giving tempe milk. The purpose is to determine the effect of tempe milk for 7 consecutive days on LDL levels in women aged more than 45 years. The independent variable in this study is the administration of tempe milk, while the dependent variable is LDL level.

Materials and equipment

The equipment used in this study included a 500 mL measuring cup, plastic funnel, vegetable spoon, stove, gas, filter, digital scale, blender, refrigerator, 250 mL volume bottle, a photometer, a micropipette, test tubes, a test tube rack, a centrifuge, blue type, yellow type, red-capped vacutainer tubes, a 3 cc syringe, and a tourniquet.

The materials used in this study were 100 grams of tempeh, 1 piece of ginger, 250 mL of water, 50 grams of granulated sugar, alcohol swabs, dry cotton swabs, LDL reagent, tissue, venous blood serum, and distilled water.

Making Tempeh Milk

Weigh 100 grams of tempeh and cut it into small cubes. Next, blend the tempeh with 250 mL of water. Strain the mixture using a strainer to obtain tempe milk. The strained tempeh milk is then placed in a pot and heated for 15 minutes at a temperature of 80-90 degrees Celsius while stirring to ensure even heating. To enhance the flavor and remove the pungent aroma, 1 piece of ginger and 50

grams of granulated sugar can be added. The tempeh milk is then poured into bottles and sealed. The bottles are then cooled naturally in the refrigerator.

Venipuncture

Prepare the equipment and materials, then check the needle and rubber, instruct the patient to clench their fist and apply a tourniquet, then clean the skin area to be injected using an alcohol swab, insert the needle tip into the vein with the needle side facing upward at a 15-30 degree angle until the needle tip enters the vein and blood is visible at the base of the needle, slowly pull the syringe plunger until the desired volume of blood is obtained, release the hand grip and remove the tourniquet, place a cotton ball soaked in 70% alcohol over the needle and remove the needle by pressing the cotton ball over the injection site with the right hand for several minutes, remove the needle and allow the blood to flow into the container without touching the walls to avoid hemolysis, the blood is then poured into the collection tube according to the required volume and type of test being performed.

Measuring LDL (Low Density Lipoprotein) Levels

The tube containing blood and buffer is placed in the centrifuge, the centrifuge is closed, then the ON button is pressed, the centrifugation process is carried out for 15 minutes, after centrifugation, the sample is removed from the centrifuge, serum and plasma are separated using a micropipette, then placed in an empty tube.

Precipitate preparation for HDL:

Pipette 500 µL of sample and place it in an empty test tube, add 1000 µL of HDL reagent, homogenize the solution until it is thoroughly mixed, the sample is then centrifuged for 10 minutes.

Total Cholesterol Test:

Pipette 10 µL of sample into an empty test tube, add 1000 µL of cholesterol reagent, homogenize the mixture and incubate for 10 minutes, after incubation, check the results using a photometer.

Triglyceride Test:

Pipette 10 µL of sample into an empty test tube, add 1000 µL of cholesterol reagent, homogenize the mixture and incubate for 10 minutes, after incubation, the results are examined using a photometer.

Triglyceride Test:

Pipette 10 µL of sample into an empty test tube, add 1000 µL of triglyceride reagent, homogenize the mixture and incubate for 10 minutes, after incubation, the results are examined using a photometer.

HDL Test:

100 µL of the supernatant from the precipitation is pipetted into an empty tube, 1000 µL of cholesterol reagent is added, the mixture is homogenized and incubated for 10 minutes, after incubation, the results are examined using a photometer.

LDL calculation:

The LDL level is calculated using a formula based on the results of total cholesterol, triglyceride, and HDL tests.

Formula: $Cholesterol\ Total = LDL + HDL + \left(\frac{TG}{5}\right)$

The research techniques carried out are (1) checking LDL levels in women aged more than 45 years before being given tempe milk, (2) giving tempe milk to women aged more than 45 years for 7 consecutive days, (3) re-examining LDL levels in women aged more than 45 years at the TLM UMP Clinical Chemistry Laboratory after giving tempe milk. Data on LDL levels were then analyzed using the Dependent T test to determine the difference in LDL levels before and after the administration of tempe milk.

Statistical analysis

The research data used primary data in the form of results (LDL level tests) before and after receiving tempeh milk treatment, measured using an enzymatic method. The data was analyzed using a dependent t-test.

RESULTS AND DISCUSSION

The test results of the effect of giving tempeh milk for seven consecutive days on LDL levels in women over 45 years of age were analyzed using the Dependent T test which can be seen in Table 1 below.

Table 1. Results of Statistical Analysis of Dependent Tests

No	Category	N	Mean ± SD (mg/dL)	Relative change (%)	p-value
1.	LDL levels before	25	106.12 ± 16.379		
				5.88	0.001
2.	LDL levels after	25	99.88 ± 14.532		

Figure 1, shows that the average decrease in LDL levels was 6.24 mg/dL after 7 days of tempeh milk administration. It is known that the administration of tempeh milk for 7 consecutive days has been proven to significantly lower LDL levels from an average of 106.12 mg/dL with a standard deviation of 16.379 mg/dL to 99.88 mg/dL with a standard deviation of 14.532 mg/dL. Statistical analysis using the dependent t-test yielded a p-value <0.05. This indicates that there is a significant effect of tempeh milk administration for 7 consecutive days on LDL levels. This aligns with the study by Mulyani *et a*l., (2018), which demonstrated that administering 430 ml of tempeh extract for 7 days can lower LDL levels.

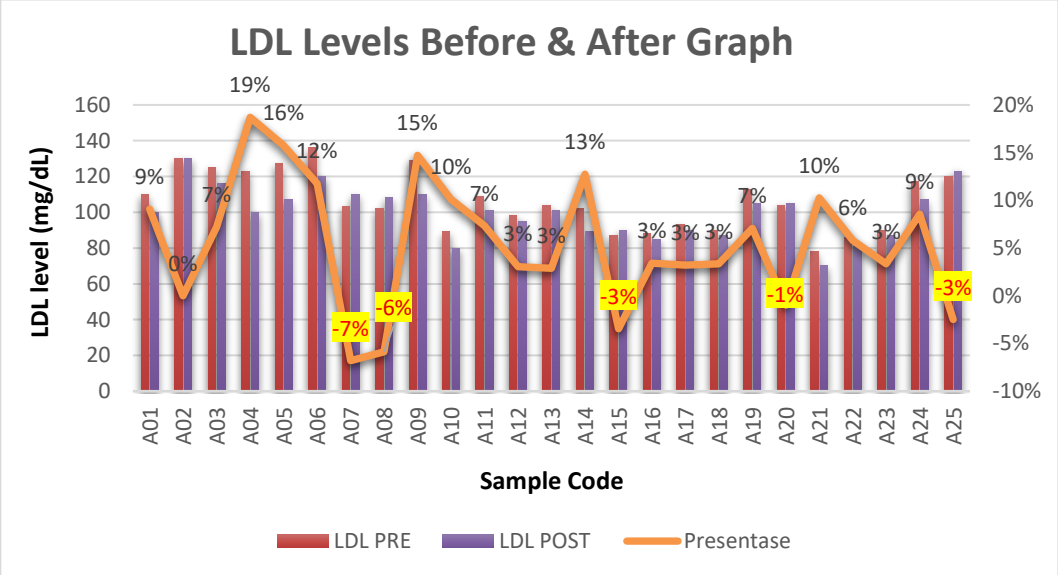

Figure 1. Results of LDL Level Measurement Before and After

Based on Figure 1., the results of LDL level measurements before and after treatment in 25 respondents can be seen. The results showed a significant decrease; however, among the 25 samples, there were 5 samples that did not experience a decrease in LDL levels, namely samples A07, A08, A15, A20, and A25. This aligns with the findings of Wirawanti *et al* (2017), which were attributed to the short duration of the intervention, daily activities, and daily food consumption.

Of the 25 samples tested, there were 5 samples that had the most significant effect on LDL levels, namely samples A04, A05, A06, A09, and A014. The least significant samples were A07, A08, A15, A20, and A25. These five samples were considered insignificant because they showed an increase in LDL levels. These five insignificant samples are attributed to the short intervention period of only 7 days, daily activities, and daily food consumption. This aligns with the findings of Wirawanti *et al* (2017). A longer intervention period with an average duration of six to eight weeks has been shown to reduce total cholesterol, LDL, and triglycerides (TG) while increasing HDL levels.

According to research by Afifah et al (2018), most women with high cholesterol are aged 30-49 years, accounting for 50.3%. This is consistent with research by Kuntarti et al (2017) in the Nurse Health Study Cohort, which states that women aged 40-60 years begin to experience an increase in LDL cholesterol of 2 mg/dL per year. The implementation of medical nutrition therapy (nutrient absorption and dietary changes) has a greater impact on regulating lipid profiles. This can be achieved by increasing dietary fiber sources such as soybeans, which can lower total cholesterol levels in individuals with hypercholesterolemia[11].

Fermented soy products such as tempeh have been shown to have a positive effect on blood cholesterol levels by consistently inhibiting the enzyme HMG-CoA reductase (3-hydroxy-3-methylglutaryl coenzyme A reductase), which is responsible for cholesterol biosynthesis and prevents LDL oxidation[11]. The fermentation process in tempeh production can improve sensory quality and nutritional value compared to other soy products. During fermentation, isoflavones undergo structural changes from glycosides to aglycones, and proteins are broken down into peptides and amino acids[12]. Fermentation improves the functional quality of soybeans by increasing food digestibility and the bioavailability of isoflavones[13]. Fermentation can also improve the nutritional profile of foods, increasing the bioavailability of polyunsaturated fatty acids (PUFAs) and reducing anti-nutritional factors[8].

Compared to whole soybeans, tempeh has higher fat content, protein digestibility, carbohydrates, and vitamins, better mineral bioavailability, and contains various bioactive components that are not found in soybeans[14]. Tempe fermentation produces bioactive compounds such as isoflavone aglycones, gamma aminobutyric acid, superoxide dismutase (SOD), and antimicrobials that function as antioxidants[15].

Tempeh contains many substances, including protein, polyunsaturated fats, dietary fiber, niacin, vitamin E, carotenoids, isoflavones, and calcium, which are believed to have hypercholesterolemic properties that can lower blood fat levels[10]. Isoflavones act as antioxidants and have biological effects similar to estrogen, which can prevent LD oxidation[16]. The active ingredient isoflavone, especially daidzein, genistein, and isoflavone compounds, has antioxidant properties that can help reduce hydroxyl radicals, superoxide radicals, and lipid peroxyl radicals, which can lower blood cholesterol levels, especially LDL[17]. Tempeh also contains antioxidants, one of which is flavonoids, which can reduce the formation of statins, namely the enzyme HMG-CoA, and can reduce apolipoprotein B, thereby reducing the formation of LDL[18].

Several biological explanations have been proposed to support the alleged effects of soy and its components on cardiovascular disease risk. Soy foods are low in saturated fat and are a source of omega-3 (n-3) fatty acids, fiber, and bioactive compounds, and can be used as an alternative to animal protein foods that are high in saturated fat and low in fiber. One of the cholesterol-lowering mechanisms in soybeans is related to the main soy protein (7S globulin), which is known to inhibit hepatic ApoB synthesis[13].

This is in line with research conducted by Nurpalah and Kusmiati (2020), which showed that after 14 days of testing, soy milk can lower LDL levels. This study showed that consuming 200 ml of soy milk every morning and evening for two weeks can lower LDL levels. Another study also showed that consuming soy milk powder twice daily at 30 grams each time for eight weeks can lower LDL levels[1]. Another study conducted by Mulyani (2018) also showed that consuming tempeh extract for 7 days can also

lower LDL levels. This study showed that consuming 430 ml of tempeh extract every afternoon can lower LDL levels.

Foods containing soy protein have been approved by the Food and Drug Administration (FDA) as a protective measure against coronary heart disease (CHD). This decision is based on clinical studies showing that consuming 25 grams of soy protein daily can lower total cholesterol and LDL levels[19].

This study has several limitations that should be noted. First, the intervention period, which lasted only seven days, may not have been sufficient to determine the maximum effect of tempeh milk on significantly and sustainably reducing LDL levels. Second, this study was unable to fully control for other external factors that could influence LDL levels, such as daily dietary patterns and physical activity levels. Subject compliance in consistently consuming tempeh milk over the seven-day period also posed a challenge that could affect the validity of the results.

CONCLUSION

The findings of this study reveal that there is a difference in the average LDL level in women over the age of 45, before and after being given tempeh milk for 7 consecutive days at a dose of 250 ml per day. However, it can be concluded that tempeh milk is effective in lowering LDL in women over the age of 45 for 7 consecutive days.

Future researchers are encouraged to investigate factors that influence the effects of tempeh milk on LDL levels in seniors over 60 years of age, such as diet and daily activity levels, taking into account the amount of tempeh milk consumed and the duration of the intervention.

AUTHORS' CONTRIBUTIONS

Dewi Gita Cahyani: Conceptualization, Writing – Original Draft, Writing – Review & Editing; **Retno Sulistiyowati**: Validation, Supervision; **Kurnia Ritma Dhanti**: Data Curation, Supervision; **Uli Mas'Uliyah Indarwati**: Data Curation, Supervision

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DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT

The authors have no competing interests to declare.

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