



Lowering Cholesterol through Ethanol Extract and Nano-Symplesia of Takokak Fruit (*Solanum torvum* Swartz.): An *In vivo* Study

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Abstract

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BACKGROUND: The human body needs antioxidants that can help protect the body from free radical attacks by reducing the negative impact of these compounds. Antioxidants function to overcome or neutralize free radicals so that it is hoped that by giving these antioxidants, the aging process is inhibited or at least slowed down and can prevent damage to the body from the emergence of degenerative diseases.

AIM: The aim is to prove whether giving Takokak fruit ethanol extract, and nano-simplicia reduces blood cholesterol in hypercholesterolemic mice.

METHODS: This research design is experimental with the randomized post-test-only control group approach. The benefits of Takokak as alternative medicine in preventing hypercholesterolemia by consuming Takokak fruit can be a daily food menu.

RESULTS: The administration of nano Takokak fruit reduced cholesterol, Low-density lipoprotein (LDL), and increased high-density lipoprotein (HDL) levels in mice. Administration of nano is very effective at a dose of 450 mg. At the same time, the ethanol extract of Takokak fruit gave a significant difference in reducing cholesterol and LDL levels at a dose of 900 mg/kg BW and increasing HDL at a dose of 450 mg/kg BW.

CONCLUSION: Giving ethanol extract and nano-simplicia Takokak fruit are effective in lowering cholesterol.

Introduction

The human body requires antioxidants that can help protect the body from free radical attack [1]. Antioxidants function to overcome or neutralize free radicals and prevent damage to the body from degenerative diseases [2]. Oxidative stress and free radicals may play a role in the pathophysiology of diabetes, cardiovascular disease, and cholesterol [3]. Cholesterol is an essential element in the body needed to regulate chemical processes, but cholesterol in high amounts can cause atherosclerosis, eventually impacting coronary heart disease [4]. Cholesterol is also a constituent of bile salts used in digestion to facilitate the absorption of fat-soluble Vitamins A, D, E, and K. [5] Cholesterol is a metabolite containing fat sterols (waxy steroids) found in cell membranes and circulated in blood plasma. Everyone has cholesterol in their blood, of which the body itself produces 80%, and 20% comes from food. Cholesterol produced consists of two types, namely, high-density lipoprotein (HDL) cholesterol is good cholesterol, and low-density lipoprotein (LDL) cholesterol is bad cholesterol; besides that, there are also triglycerides. When LDL cholesterol levels are too

high, a condition referred to as hypercholesterolemia, the risk of premature atherosclerotic cardiovascular disease increases [6]. In several studies, blood cholesterol levels have an inverse relationship with HCC [7]. However, few studies on the efficacy of statins have controlled cholesterol concentrations [8]. The standard limit for cholesterol in the body is 98–122 mg/dl. Hypercholesterolemia is a condition in which blood cholesterol levels exceed 250 mg/dl [9]. High cholesterol levels in the body trigger the emergence of various diseases. Hypercholesterolemia can increase the risk of cardiovascular disease. The factors that cause increased cholesterol levels in the blood include genetic and food. Several studies have shown that fruit is a good source of antioxidants. At present, cholesterol treatment has been developed using medicinal plants. Medicinal plants, which mostly have many phytochemicals with antioxidant properties, have recently become the focus of researchers and scientists to treat and prevent various complications related to oxidative stress [10], [11]. This plant has antioxidant activity due to phytochemicals including phenolic compounds and carotenoids and can reduce the risk of several chronic and degenerative complications [12], [13].

One of the natural ingredients that can lower cholesterol levels in the blood is Takokak (*Solanum torvum* Swartz.). These plants are consumed as leafy vegetables and fruit [14]. Takaoka is a cure for diabetes that helps treat various diseases such as diabetes, acne, skin diseases, and fever. The antimicrobial content in Takokak leaves is believed to treat various diseases effectively. Takaoka has a high superoxide cleaning activity, which is above 70%. The chemical content in Takokak can act as an antioxidant and protect body tissues from the harmful effects of free radicals. For this reason, the purpose of this study was to determine the antioxidant effect of ethanol extract and Takokak fruit nano-simplicia on reducing hypercholesterolemia in mice blood cholesterol.

Methods

The research used a proper experimental design with the randomized post-test-only control group design approach (Figure 1). This study used *Rattus norvegicus* strain Wistar rats as experimental animals. They tested the effect of hypocholesterolemia *in vivo* on experimental rats by first determining the test dose. The preparation of experimental animals was given at a dose of 450 mg and 900 mg. The animals used in this study were white rats approximately 2 months old with a weight of approximately 200–250 g as many as 30 tails and were divided into five groups. Rats must be healthy, visually showing normal behavior, and adapted for 1 month.

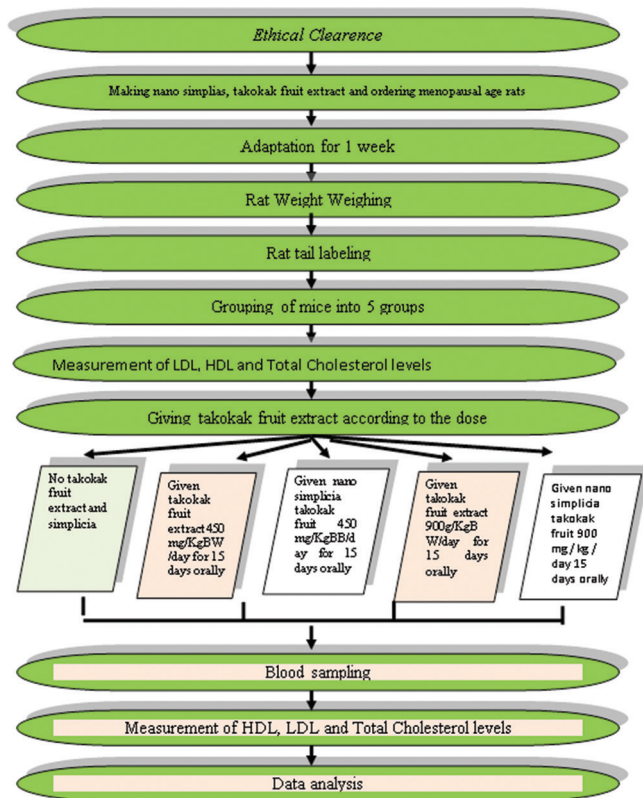


Figure 1: Stages of this research

Furthermore, the measurement of body weight is taken with 200–250 g. Then, the mice were fed pellets mixed with quail eggs and drank *ad libitum* for 1 week, then, the total cholesterol, HDL, and LDL levels in the blood were analyzed. This initial level is used as an average blood cholesterol level. The extract nano Takokak fruit according to the dose of each group for 1 week, then the blood cholesterol levels were measured again.

Examination of blood glucose levels is carried out using the Easy Touch Glucose, Cholesterol, and Uric Acid tool with green test strips and chips. Blood is dripped on the test strip and the results will be visible on the measuring device. This research has been through ethical approval (No. 0494/KEPH-FMIPA/2021).

Results

Test paired t-test

Giving Takokak fruit extract and nano to mice are expected to reduce cholesterol, HDL, and LDL levels. cholesterol level test due to the administration of takokak fruit extract and nano using independent t-test.

Table 1 shows that the group before treatment showed an average cholesterol level of 17.56061, HDL -468,540, and LDL 1,686,147, while after treatment, the average cholesterol level was 13.07551, HDL 5.51451, and LDL 12.03491. Descriptively, each group showed that the group average was due to the treatment, showing that the p-value of each group was 0.000 ($p < 0.005$). The test results showed a significant difference between the groups before and after taking takoyaki. Takaoka plants can lower cholesterol levels, increase HDL, and lower LDL.

Table 1: Paired sample test

S. No.	Variable	Mean	Std. deviation	T	Sig (p < 0.05)
1.	P1-P2 cholesterol	17.56061	13.07551	7.356	0.000
2.	P1-P2 HDL	-468540	5.51451	-4.654	0.000
3.	P1-P2 LDL	1686147	12.03491	7.674	0.000

LDL: Low-density lipoprotein, HDL: High-density lipoprotein.

Description: On average \pm SD, if it contains different letters, it means that there is a significant difference ($p < 0.05$), and if it contains the same letters, it means that there is no significant difference ($p > 0.05$).

Based on the results of the ANOVA analysis in Table 2, the results of the multiple comparison test with the LSD test were obtained, namely, the $p = 0.148$

Table 2: Comparison of average cholesterol levels

S. No.	Treatment group	Average \pm booth deviation (mg/ml)	p-value
1.	Control	34.0657 \pm 15.71422	0.148
2.	P1 (450 mg/Kg BW)	33.3333 \pm 6.83133	
3.	P2 (900 mg/Kg BW)	29.3182 \pm 8.80339	
4.	P3 (450 mg/Kg BW)	25.4798 \pm 4.90255	
5.	P4 (900 mg/Kg BW)	42.2980 \pm 15.49326	

was more significant than = ($p > 0.05$). These results show the difference in average cholesterol levels with the administration of Takokak with different doses. The average difference in cholesterol levels between the control and treatment groups experienced a significant difference (34.0657 ± 15.71422). Based on the average value of cholesterol levels in the control group, the value was higher than the average cholesterol levels in the p1 group (given extract dose of 450 mg/kg BW/day), which was 34.0657 ± 15.71422 , and Group P2 (administration of the extract). Dose of 900 mg/kg BW/ a day which is 29.3182 ± 8.80339 . There was a decrease in cholesterol levels when the extract was given at a dose of 450 mg/kg BW/day, and the extract was given at a dose of 900 mg/kg BW/day. There was an average difference between the control group and the treatment group at a dose of 450 mg/kg BW/day and extract at a 900 mg/kg dose BW/day. Furthermore, Table 2 also shows a significant difference in the average cholesterol level in mice between the control group and the p3 group (administration of nano doses of 450 mg/kg BW/day) that is (25.4798 ± 4.90255). Distinct differences in the treatment group P4 (administration of nano doses of 900 mg/kg BW/day), namely (42.2980 ± 15.49326), showed no decrease in the average value of cholesterol levels. These results showed a decrease in cholesterol levels in the administration of nano at a dose of 450 mg/kg BW/day, and there was no decrease in the administration of nano at a dose of 900 mg/kg BW/day to the control group. It can be concluded that the P1, P2, and P3 treatments can both reduce cholesterol levels. It should be explained that the P3 treatment was very effective in lowering cholesterol compared to the P1, P2, and P4 treatments. The P3 (nano) treatment with a dose of 450 mg/kg, BW/day was very effective in reducing cholesterol levels in hypercholesterolemic rats seen from the lower average cholesterol value than in the P4 treatment and the control group. At the same time, the P2 (extract), which is very effective in lowering cholesterol levels, is a dose of 900 mg/kg BW/day.

In Table 3, there is a difference in the average HDL level between the control group (27.66165 ± 4.15258) and the P1 treatment group (extract dose 450 mg/kg BW/day) (29.3930 ± 4.15258). Hence, HDL levels in the control group were lower than those in the P1 treatment group (extract dose 450 mg/kg BW/day). In the P2 treatment group (extract dose of 900 mg/Kg BW/day) (27.4930 ± 3.87056), there was no increase where the average HDL levels in the control group were higher than the average HDL levels in the P2 group (administration of extract dose 900 mg/kg BW/day). This means no increase in HDL levels at a 900 mg/kg dose BW/day. Takokak extract is effective in the P1 treatment group.

Table 3: Comparison of average HDL levels

No.	Treatment group	Average \pm booth deviation (mg/ml)	p-value
1	Control	27,6165 \pm 2.90750	0.078>
2	P1 (450 mg/Kg BW)	29,3930 \pm 4.15258	
3	P2 (900 mg/Kg BW)	27,4930 \pm 3.87058	
4	P3 (450 mg/Kg BW)	27,3600 \pm 1.95835	
5	P4 (900 mg/Kg BW)	32,9555 \pm 5.17646	

HDL: High-density lipoprotein.

Furthermore, Table 3 also shows no significant difference in the average HDL levels in rats between the control group and the P3 group (administration of nano doses of 450 mg/kg BW/day) was -27.3600 ± 1.95835 , but there was a difference in the average value in the treatment group. P4 (administration of nano dose of 900 mg/kg BW/day) (32.9555 ± 5.17646) experienced an increase in HDL. Administration of Nano at a dose of 450 mg/kg BW/day was not the same as administering a nano dose of 900 mg/kg BW/day to the control group, while the P1 treatment (administration of nano doses of 450 mg/kg BW/day) showed a significant difference between treatment P2, treatment P3, and P4, likewise, for the treatment of P2 in regards to P3 and P4. Next is also between treatments P3 and P4, which experienced differences in the average value. The P1 and P4 treatments had the same ability to increase HDL. At the same time, P2 and P3 did not increase HDL levels. The P4 treatment was very effective in causing differences in HDL levels compared to P1, P2, and P3. Administration of P4 (nano at a dose of 900 mg/kg BW/day) was very effective in increasing HDL in hypercholesterolemic rats seen from the higher HDL value after treatment than the control group.

Based on Table 4, it was found the difference in the average value of LDL levels between the control group (-4.4026 ± 14.19641) and the P1 group (extract dose 450 mg/kg BW/day) ($=9.090 \pm 3.27496$). On average, the LDL level in the control group was higher than the LDL level in the P1 group (administration of extract dose of 450 mg/kg BW/day). This means a decrease in LDL levels when the extract dose is 450 mg/kg BW/day. Likewise, the difference in the average value of LDL levels between the control group (-4.4026 ± 14.19641) and the group given the extract dose of 900 mg/kg BW/day (-12.9951 ± 8.13608). In the control group, the value was higher than the LDL level in the P2 group (administration of extract dose of 900 mg/kg BW/day). This means a decrease in LDL level with the administration of Takokak extract at a dose of 900 mg/kg BW/day and also a difference which is very significant compared to treatment P1 (administration of extract dose 450 mg/kg BW/day). Table 4 also shows that there is a significant difference in the mean value of LDL levels between the control group and the P3 group (administration of nano dose of 450 mg/kg BW/days) (13.9432 ± 5.70927) and in the P4 treatment group (administration of nano doses of 900 mg/kg BW/day) (8.1366 ± 8.04303) both experienced a decrease in LDL. Nano at a dose of 450 mg/kg BW/day and a dose of 900 mg/kg BW/day both caused a decrease in LDL levels compared to the control group. In addition, treatment P1 showed a significant difference between

treatment P2 and treatment P3 and P4. It was likewise for P2 against P3 and P4, as well as between P3 and P4, they all experienced a difference. It was concluded that the P1, P2, and P3 and P4 treatments had the same ability to reduce LDL. It should be explained that P3 treatment was very effective in lowering LDL compared to P1, P2, and P4. This means that the administration of nano at a dose of 450 mg/kg BW/day effectively reduces LDL in hypercholesterolemic rats, seen from the lower LDL value after treatment than the control group. In comparison, the P2 (extract), which is very effective in reducing LDL levels, is a dose of 900 mg/kg BW/day.

Table 4: Comparison of average LDL Levels

S. No.	Treatment group	Average \pm stan. deviation (mg/ml)	p-value
1.	Control	-4.4026 \pm 14.19641	0.338 >
2.	P1 (450 mg/kg BW)	-9.0090 \pm 3.27496	
3.	P2 (900 mg/kg BW)	-12.9951 \pm 8.13608	
4.	P3 (450 mg/kg BW)	-13.9432 \pm 5.70927	
5.	P4 (900 mg/kg BW)	-8.1366 \pm 8.04303	

LDL: Low-density lipoprotein.

Discussion

This study proves that Takokak fruit can lower cholesterol levels in the blood. Treatment with Takokak fruit extract effectively reduced blood cholesterol levels in rats at a 900 mg/kg BW dose compared to 450 mg/kg BW. The bioactive compounds contained in Takokak fruit come from steroids, alkaloids, isoflavonoids, triterpenoids, xanthenes, tannins, and flavonoids. Epidemiologically, consuming foods that contain lots of flavonoids can protect humans from diseases related to oxidation damage caused by the influence of free radicals (Xu and Chang, 2007) [15]. The results also proved that the flavonoid extract showed a hypolipidemic effect that reduced cholesterol levels by 86.45%, Vitamin C, which plays a key role in preventing cholesterol. Vitamin C deficiency causes an increase in cholesterol synthesis. The role of Vitamin C in cholesterol metabolism is through increasing the disposal of cholesterol in the form of bile acids and increasing HDL levels. Takokak fruit is known to contain antioxidants, one of which is anthocyanins. Anthocyanin is a type of flavonoid that can inhibit cholesterol absorption in the gastrointestinal tract or inhibit cholesterol synthesis in the liver. Antioxidants function to overcome or neutralize free radicals, so it is hoped that by giving these antioxidants, the aging process is inhibited or at least slowed down and can prevent damage to the body from the emergence of degenerative diseases [16], [17]. This study proves that Takokak fruit extract at a dose of 900 mg/kg BW can reduce LDL levels in the blood of hypercholesterolemic rats. This is thought to be due to the antioxidant compounds contained in Takokak.

The extracts at dose levels of 125 and 250 mg/kg inhibited the increase in serum cholesterol and

triglyceride levels on the administration of Triton WR 1339 in rats. The results of other studies show a relationship between antioxidant consumption and blood lipid profiles. The antioxidants tested were beta-carotene, Vitamin C, and flavonoids [18]. Natural antioxidants such as flavonoids inhibit the oxidation of LDL *ex vivo*. Oxidative products of LDL can cause narrowing of coronary arteries. Other studies have also proven that flavonol compounds act as potent antioxidants. The results of a study conducted by Ravishankar showed that natural flavonoid compounds such as kaempferol, morin, myricetin, and quercetin have various protective activities against LDL reduction [19].

Conclusion

Giving Takokak fruit extract reduces cholesterol and LDL levels, increasing HDL levels in mice. Takokak fruit extract is very effective in reducing cholesterol and LDL levels with 900 mg. The administration of nano Takokak fruit reduces cholesterol levels and increases HDL levels in mice. Nano Takokak fruit effectively reduces LDL and cholesterol levels with 450 mg. Need to do research continued to increase knowledge and insight more about the benefits of fruits Takokak. It is necessary to consume and cultivate Takokak fruit plants in the community because of the many benefits and ease of growing in various places. Making Takokak plants as plants cultivated on agriculture, not just as wild plants.

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