

Effects Combination of Purple Sweet Potato Tub Ethanol Extract (*Ipomoea batatas L.*) And Atorvastatin on Levels Low-Density Lipoprotein and Intima-Media Thickness of The Aorta in Male Wistar Rats (*Rattus norvegicus*) Exposed to A High Fat Diet

Putu Dian Pratita Lestari*, Anak Agung Ayu Dwi Adelia Yasmin, and I Nyoman Wiryawan

Department of Cardiology and Vascular Medicine, Faculty of Medicine, Udayana University/ Prof. Dr. IGNG Ngoerah Central General Hospital, Denpasar, Bali, Indonesia, 80113

E-mail: dianpl93@gmail.com; adeliajasmine@gmail.com; dr_wiryawan@yahoo.com

*Corresponding author details: Putu Dian Pratita Lestari; dianpl93@gmail.com

ABSTRACT

Background: Coronary heart disease (CHD) is still the leading cause of morbidity and mortality. Preventive measures are very important-density lipoprotein (LDL) and aortic intimal-media thickness (IMT) are associated with cardiovascular events. Statins are not optimal even though their efficacy has been proven. Purple sweet potato extract has been shown to have the potential to lower LDL and have antioxidant effects. **Objective:** Assessing the effect of the combination of ethanol extract of purple sweet potato tubers and atorvastatin on LDL levels and aortic IMT in male Wistar rats exposed to a high-fat diet. Method: An experimental study with a posttest-only posttest control group design. The total sample was 25 mice, all receiving a high-fat diet. Rats were divided into control groups and 3 treatments, namely P(1) atorvastatin 1.4 mg/day, P(2) ethanol extract of purple sweet potato tubers 3 ml/day, P(3) atorvastatin 1.4 mg/day + ethanol extract purple sweet potato tubers 3 ml/day. At week 9, LDL levels and aortic IMT were checked. Results: There is a significant difference in LDL between the treatment and control groups. The combination group had the lowest mean LDL compared to the other groups $P1:20.10\pm1.03\mu g/ml$; $CI95\%22.81-23.77\mu g/ml$; p<0.001; P2:23.29±0.57μg/ml CI95%22.81-23.77μg/ml; p<0.001; P3: 15.93±0.76μg/ml; 95%CI15.30-6.57μg/ml; p<0.001). The ability of single therapy of atorvastatin and purple sweet potato extract was not significantly different in reducing the mean aortic IMT P1: 46.39±3.81µm; CI95% 43.20–39.58µm; p <0.001; P2: 49.93±4.3μm; CI 95% 46.33–53.53μm, p<0.001 The combination of atorvastatin and ethanol extract of purple sweet potato tubers can reduce the mean aortic IMT compared to the single therapy group (P3: 41.22±1.99µm; CI95% 39.55–42.88µm; p<0.001). *Conclusion:* The combination of atorvastatin 1.4 mg/day and ethanol extract of purple sweet potato tubers 3 ml/day significantly reduced LDL levels and aortic IMT compared to the control group and single therapy.

Keywords: a combination of atorvastatin and ethanol extract of purple sweet potato tubers; high-fat diet; LDL cholesterol levels; aortic IMT

INTRODUCTION

Cardiovascular disease remains a significant cause of morbidity and mortality worldwide [1]. In Indonesia, the prevalence of heart disease tends to increase [2,3]. Obesity is a risk factor for non-communicable diseases and contributes to the cause of death due to cardiovascular disease, which is 5.87% of total deaths [4]. An Unhealthy diet is a risk factor that can be modified to control obesity [5]. Saturated fat consumption of up to 18% of total energy is associated with poor cardiovascular disease outcomes [6].

Atherosclerosis is known to be associated with LDL retention in the intima. The formation of foam cells causes atherosclerotic lesions to protrude into the lumen, causing reduced blood flow due to blockages

caused by atherosclerotic plaque and causing complaints of angina in coronary heart disease [7]. Arterial wall thickening is the primary marker of atherosclerosis. Measurement of carotid artery intimal media thickness (IMT) can predict cardiovascular disease through this mechanism [8]. LDL cholesterol is related to the risk of CHD, and reducing LDL cholesterol can reduce the risk of CHD proportionally for each absolute reduction in LDL achieved [9–11].

Despite the large amount of evidence showing benefits in lowering LDL cholesterol, statins have yet to be used [12]. This is caused by clinical inertia, lack of knowledge, perception of side effects, and cost [13].

So, efforts are needed to reduce LDL levels more aggressively. One of the recommended healthy lifestyle efforts is maintaining a nutritional intake low in fat and rich in antioxidants.

Balinese purple sweet potatoes have been shown to have high levels of anthocyanins and have been shown to have antioxidant effects in animal models. Purple sweet potato tuber extract can improve lipid profiles and increase MDA and SOD antioxidant levels in mice fed high-fat diets [14–16]

Based on these things, this study determined the effect of administering a combination of ethanol extract of purple sweet potato tubers (Ipomoea batatas L.) and atorvastatin on LDL levels and aortic IMT in mice on a high-fat diet.

METHODS

This research was conducted experimentally with a randomized post-test-only post-test control group design. This study consisted of three treatment groups and 1 control group of male Wistar rats. Each group was exposed to a high-fat diet. One group was the control group, which was only given a high-fat diet, and the other three groups were differentiated in terms of treatment consisting of atorvastatin only (P1), ethanol extract of purple sweet potato tubers (P2), and a combination of ethanol extract of purple sweet potato tubers and atorvastatin(P3). After exposure and treatment for 8 weeks, LDL levels and aortic IMT were measured.

The Laboratory Animal Unit, Pharmacology Section, Faculty of Medicine, Udayana University, cares for research animals and provides a diet high in blood fats. LDL level examination was conducted at the Biochemistry Laboratory Unit, Faculty of Medicine, Udayana University. Aortic IMT examination was conducted at the Veterinary Pathology Laboratory Unit, Faculty of Veterinary Medicine, Udayana University. The research was carried out for 16 weeks, from January to May 2024.

The population of this study was male Wistar rats (Rattus norvegicus) exposed to a high-fat diet. Inclusion Criteria: a) Male Wistar Rat (Rattus norvegicus); b) Age 12-16 weeks; c) Body weight 150-200 grams; d) The condition of the mice is healthy (active and not disabled). Exclusion Criteria: Deformed mice. Dropout criteria: Mice died during the research.

Procedure for Providing a High-Fat Diet

The high-fat diet consists of standard feed, 2% cholesterol, 0.2% cholic acid, and 5% lard, made according to needs, 30 grams/head/day, and drinking water ad libitum. This dose is the same as previous research Murwani (2006) [20]

Atorvastatin Administration Procedure

Calculating the oral dose of atorvastatin for mice, the dose of atorvastatin for humans is 40-80 mg. Using Laurence and Bacharach's (1964) conversion formula, the daily requirement is 0.72 - 1.44 mg/day, in this study, the dose given to mice is 1.4 mg/day carried out for 8 weeks via sonde (force-feeding) with an administration volume of 1 mL of suspension.

Procedure for Giving Ethanol Extract of Purple Sweet Potato Tubers

Per standard protocol, the Biopesticide Laboratory, Faculty of Agriculture, Udayana University, Postgraduate Building, 3rd Floor, Sudirman Campus, carried out the purple sweet potato tuber extract. The currently available concentration is 147 mg/ml, with a total volume of 150 ml. The ethanol extract of purple sweet potato tubers was given via sonde for 8 weeks with a volume of 3 mL of extract once a day [21].

Procedure for measuring Low-Density Lipoprotein (LDL) level

Animal blood samples were taken intravenously at the orbital cantus as much as 2 ml. LDL levels are examined using the Enzyme-Linked Immunosorbent Assay (ELISA) procedure. In the ELISA procedure, the homogenate is spun in a centrifuge for 15 minutes at a speed of 12,000 rpm at a temperature of 4oC to obtain the supernatant and then analyzed using the Rat ELISA LDL Bioassay Technology Laboratory, which has a value range of 0.1 – 40μ mg/ml with a sensitivity of 0.052 µg/ml.

Procedure for making Aortic Intima-Media Thickness Preparation

Making histology preparations is divided into four (4) stages: fixation, dehydration, transparency, and embedding. Then, the thickness of the epidermis and the number of fibroblasts on the slide are checked, and general staining is performed using hematoxylin and eosin (HE).

Statistical Analysis

All data collected in each group was then analyzed using the SPSS program. Data analysis was carried out using the following steps: descriptive statistical analysis, normality test, homogeneity test, interobserver variability test, and mean comparison test. The confidence level in this study was 95%. Ho is rejected if the p-value <0.05.

RESULTS

The control LDL cholesterol level was found to be 36.22μ g/ml. Aortic IMT in controls was found to be 58.81μ m. The mean LDL cholesterol level in treatment group 3 was 15.9 ± 0.26 µg/ml, with a range of values $14.95 - 17.07\mu$ g/ml. The mean aortic IMT in treatment group 3 was 41.22 ± 0.70 µm, with a value range of 38.94μ m to 44.15μ m. The results of descriptive analysis of LDL cholesterol levels and aortic IMT in each group are presented in Table 1.

Variable	Group	Ν	Average	Standard deviation	Minimum	Maximum
LDL*	К	1	36.22	-	-	-
	P1	8	20.10	0.37	18.02	21.10
	P2	8	23.29	0.20	22.62	24.12
	Р3	8	15.93	0.26	14.95	17.07
IMT # aorta	К	1	58.81	-	-	
	P1	8	46.39	1.34	40.59	53.61
	P2	8	49.33	1.52	42.05	55.50
	Р3	8	41.22	0.70	38.94	44.15

TABLE 1: Descriptive analysis results.

*unit µg/ml,#unit µm.

LDL cholesterol levels and aortic IMT in each group were tested for normality using the Shapiro-Wilk test and homogeneity of variance using Levene's test. The results show that the data is normally distributed (p>0.05) and homogeneous (p>0.05)

Table 2 shows a significant difference in mean LDL cholesterol levels with controls and aortic IMT with controls with a p-value <0.001. Figure 1 shows the mean LDL cholesterol levels and aortic IMT, which show a significant difference from controls.

TABLE 2: Results of Differences in Mean LDL Cholesterol Levels and Aortic IMT with Controls.

Variable	Group	Ν	Average	Standard deviation	р
LDL*	К	1	36.22	-	References
	P1	8	20.10	0.37	< 0.001
	P2	8	23.29	0.20	< 0.001
	Р3	8	15.93	0.26	< 0.001
aortic IMT#	К	1	58.81	-	References
	P1	8	46.39	1.34	< 0.001
	P2	8	49.33	1.52	< 0.001
	Р3	8	41.22	0.70	< 0.001

*unitµg/ml,#unit μm.





FIGURE 1: Graph of mean LDL cholesterol levels and BMI shows a significant difference from controls.

Differences in mean LDL cholesterol levels between treatment groups were measured using the one-way ANOVA test to compare the ANOVA test in Table 3.

478

TABLE 3: Test the difference in mean LDL cholesterol levels between treatment groups.

Table able	Group	Mean ± SB (μg/ml)	CI 95%	р
LDL	P1	20.10 ± 1.03	19.21 - 20.99	< 0.001
	P2	23.29 ± 0.57	22.81 - 23.77	< 0.001
	Р3	15.93 ± 0.76	15.30 - 16.57	< 0.001

The test continued with the difference in mean LDL cholesterol levels between treatment groups, which was carried out using the least significant difference (LSD) post hoc test. Table 6 shows the comparison of mean LDL between treatment groups.

Treatment group 3 had a mean LDL cholesterol of 7.35 μ g/ml lower than the single therapy group of purple sweet potato tuber extract. This difference is statistically significant. The differences in mean LDL cholesterol levels can be seen in Table 4 and Figure 2.

TABLE 4: Post hoc LSD test for average LDL cholesterol levels between treatment groups.

Group		Mean difference (µg/ml)	CI 95%	р
P1	P2	-3.19	-4.052.33	< 0.001
	Р3	4.16	3.30- 5.02	< 0.001
Р3	P2	-7.35	-8.826.49	< 0.001



FIGURE 2: Graph of mean LDL cholesterol levels showing significant differences between study groups.

Aortic IMT was measured blinded by one observer through two measurements at different times, and good reliability was obtained between the two examination results. Statistical tests using One-Way ANOVA were conducted to see the mean differences between groups. Treatment group 1 had a mean aortic IMT46.39 \pm 3.81µm. Treatment group 3 had a mean aortic IMT of 41.22 \pm 1.99 µm. The mean difference for each treatment group was significant. The test results for differences in mean LDL cholesterol levels and aortic IMT can be seen in Table 5.

TABLE 5: Test for differences in mean aortic IMT between treatme	ent groups
--	------------

Variable	Group	Mean ± SB (µm)	CI 95%	р
aortic IMT#	P1	46.39 ± 3.81	43.20 - 39.58	< 0.001
	P2	49.93 ± 4.3	46.33 - 53.53	< 0.001
	Р3	41.22 ± 1.99	39.55 - 42.88	< 0.001

After the analysis, it was found that the P value was <0.001, meaning there were significant differences between the study groups, so this had to be

continued to the next stage using the LSD test to determine the differences between the treatment groups (Table 6).

TABLE 6: Post hoc LSD test for differences in aortic IMT between treatment groups.

Group		Mean difference (µm)	CI 95%	р
P1	P2	-3.53	-7.19 –22	0.057
	Р3	5.17	1.51 - 8.82	0.008
Р3	P2	-8.71	-12.365.05	< 0.001

Table 6 compares mean aortic IMT between treatment groups. There was no difference in mean aortic IMT in treatment group 1 compared to treatment group 2. However, the mean IMT of the aorta in treatment group 3 was $8.71 \,\mu$ m thinner than

in treatment group 2, which was only given purple sweet potato extract. This difference is statistically significant. The graph of the average difference in Aortic IMT based on the One Way Anova test with post hoc LSD can be seen in graph 3.



FIGURE 3: Graph of mean difference in Aortic IMT based on One Way Anova test with post hoc LSD.

DISCUSSION

Atherosclerosis has been known to be associated with CHD. In atherosclerosis, LDL retention occurs in the intima, which forms foam cells. These foam cells cause atherosclerotic lesions to protrude into the lumen, causing reduced blood flow due to blockages caused by atherosclerotic plaque and causing complaints of angina in coronary heart disease. Thickening due to atherosclerotic lesions can be measured by IMT, which measures the thickness of the intima and media, the two most profound layers of the artery wall. This measurement is usually performed with an external ultrasound. Increased carotid IMT is associated with arterial wall stiffness, cardiovascular events, and severe atherosclerosis. Based on this, reducing LDL levels can reduce the progression of intimal thickening, as measured by the thickness of the aortic IMT, thereby reducing the incidence of coronary heart disease.

Purple sweet potato is a variety of sweet potato often found in Bali, and the price is relatively cheap. Purple sweet potatoes are superior to other sweet potatoes because they have high levels of anthocyanins, which give them their purple color. Anthocyanin is an antioxidant widely studied for its benefits in the medical field, including atherosclerosis. So, using purple sweet potato tubers in managing dyslipidemia can expand the use of the Balinese cultivar purple potato tubers. In this study, the control group, which was only given a high-fat diet of 2% cholesterol, 0.2% cholic acid, and 5% lard for 8 weeks, had significantly higher LDL levels, namely 36.22.µg/ml compared with other treatment groups. Similar things were found in research conducted by Setiawan et al., who provided a high-fat diet in the form of standard feed flour, 5% duck egg yolk, cholic acid, lard, and 10% goat fat for 8 weeks; it was found that there was a significant increase in LDL levels $(23,400 \pm 2,302 \text{ mg/dL})$ compared to the group given standard feed (11,400 ± 1,140 mg/dL)[22]. In research conducted by Murwani et al., mice were given an atherogenic diet in the form of 2% cholesterol, 0.2% cholic acid, and 5% pork oil for 8 weeks can increase blood cholesterol levels with an average of 186.78 ± 61.06 mg/dL[20]. Another study that induced an increase in LDL with a high-fat diet in the form of 2% cholesterol, 0.2% cholic acid for 60 days could increase LDL levels to 127 ± 6.5 mg/dL [23]. The difference in mean LDL from the results of this study and the studies mentioned may be due to the method used to measure LDL cholesterol levels. In this study, LDL examination using the Rat ELISA LDL Bioassay Technology Laboratory kit has a detection range of $0.1-40.\mu g/mL$, with a sensitivity of $0.052 \mu g/mL$.

In this study, LDL cholesterol levels were significantly lower in the group of mice that received a combination of ethanol extract of purple sweet potato tubers and atorvastatin with an average of $15.93 \pm 0.76 \,\mu g/ml$, compared to the group of mice that received a single therapy of purple sweet potato tubers and atorvastatin. The group with atorvastatin monotherapy had significantly lower LDL cholesterol levels, is $20.10 \pm 1.03 \,\mu\text{g/ml}$ compared to the single therapy group of purple sweet potato tuber ethanol extract, mean $23.29 \pm 0.57 \mu g/ml$. In the group given single therapy, ethanol extract of purple sweet potato tubers and atorvastatin had significantly lower LDL levels than controls. Still, the group with combination therapy appeared superior to a single treatment.

Purple sweet potato tubers have antioxidant activity and are free radical scavengers. Sweet potatoes have high anthocyanin content and beneficial and protective effects against diseases such as atherosclerosis.[24,25]Anthocyanins can stimulate LXR-dependent expression, namely ABCA1 and CETP, which play a role in cholesterol excretion. One of the first identified LXR-responsive factors was ABCA1 and ABCG1, which play a role in cholesterol ABCA1 excretion. The gene encodes а transmembrane transporter protein that initiates the release of cholesterol from cells to the circulating apo AI with a small fat content (synthesized by the liver and intestines), then forms immature HDL particles. ACG1 facilitates cholesterol efflux to create more mature HDL particles. When free cholesterol is captured by circulating HDL, it undergoes esterification by LCAT, an enzyme activated by apo AI [26,27].

Atorvastatin reduces LDL cholesterol levels by being a competitive selective HMG-CoA reductase inhibitor. This enzyme plays a role in converting HMG-CoA to mevalonate in cholesterol synthesis in the liver. By reducing hepatic cholesterol synthesis, there is an increase in LDL receptor expression and an increase in circulating LDL uptake to the liver[28]. So, the combination of atorvastatin and purple sweet potato extract can suppress LDL production lower than a single therapy because it can inhibit the progression of atherosclerosis in a broader pathway.

This study found that the group given 3 cc/day of ethanol extract of purple sweet potato tubers (Ipomoea batatas L.) had lower LDL cholesterol levels than the control group. The results of this study are supported by research conducted by Jawi et al., water extract of purple sweet potato tubers 3 ccs per day, containing 146 mg/ml anthocyanin, can reduce LDL cholesterol, total cholesterol, and triglyceride levels and increase HDL cholesterol levels in mice. Who was exposed to a high-fat diet for one month? In addition, purple sweet potato tuber extracts also reduced the concentration of MDA, a marker of oxidative stress, in the group of mice given the treatment [29]. Similar results were obtained from research conducted by Setiawan et al. in mice given a high-fat diet for 8 weeks and purple sweet potato tuber extract, compared with the group of mice given purple sweet potato tuber extract; there was no significant difference compared to the rat group. Normal with standard feed. This shows that administration of purple sweet potato extract is effective in reducing LDL levels comparable to normal conditions [22].

Anthocyanins have also been proven to reduce LDL levels in humans, although the number of samples is still limited. A meta-analysis conducted by Jang et al. of 41 studies that meJang et al. conducted a metaanalysisvolved less than 100 samples. Anthocyanin supplementation can significantly improve LDL levels, with the duration of each study varying from 2 to 24 weeks. The average dose given was 238.5 mg/day, ranging from 20 mg/day to 742 mg/day. This study also evaluated the side effects of providing anthocyanin supplements. Almost all studies reported no severe side effects that would cause discontinuation of anthocyanin consumption. Only four studies reported mild side effects such as blackish stools, headaches, insomnia, and diarrhea [30].

This research found that the control group, which was only given a high-fat diet of 2% cholesterol, 0.2% cholic acid, and 5% pork oil for 8 weeks, had a significantly higher aortic IMT than the treatment group. This diet is based on research conducted by Murwani et al. It was found that white mice given an atherogenic diet of 2% cholesterol, 0.2% cholic acid, and 5% pork oil for 8 weeks could increase total blood cholesterol levels and form foam cells. meaningfully[20].

In this study, aortic IMT was significantly thinner in the group of mice that received a combination of ethanol extract of purple sweet potato tubers and atorvastatin, with a mean of $41.22 \pm 1.99 \mu$ m, when compared with the group of rats that received purple sweet potato tubers, mean $49.93 \pm 4.3 \mu$ m, and with the group given atorvastatin, with a mean of $46.39 \pm 3.81 \mu$ m. These results are new findings because, to the author's knowledge, no one has previously studied the potential of this combination therapy for IMT.

Purple sweet potato tubers contain anthocyanins, which act as antioxidants by increasing SOD. SOD has a vital role in catalyzing the degradation of superoxide, one of the dangerous ROS that plays a role in the atherosclerosis process. Anthocyanins can also increase catalase, which breaks down hydrogen peroxide into hydrogen and water. Hydrogen peroxide can increase adhesion molecules through the expression of VCAM1 and MCP1. So that administration of anthocyanins can reduce the expression of VCAM1 and MCP1 by increasing SOD and CAT levels [31].

Anthocyanins can also increase NO levels through the mechanism of reducing NF-kB activation which plays a role in the transcription of inflammatory response factors such as eNOS, iNOS which are proatherogenic, and pro-inflammatory cytokines such as TNF-a, IL-1b and IL-6[31,32] Apart from that, NFkB also plays a role in the transcription factors MCP1 and MCSF which play a role in controlling the migration and differentiation of monocytes to the endothelium, causing phagocytosis of oxLDL, and turning them into foam cells.[19]This entire process caused a decrease in aortic IMT in the group given purple sweet potato tuber extract.

Atorvastatin can reduce BMI indirectly by lowering LDL levels. This is due to the pathophysiology of atherosclerosis progression, starting with the accumulation of LDL in the intima to form foam cells and fatty streaks [33]. Atorvastatin also has pleitropic effects by inhibiting the synthesis of isoprenoids required to activate intracellular signaling processes such as Ras, Rho, Rab, Rac, Ral, or Rap. This causes statins to have antiinflammatory, antioxidant, anti-proliferative, and anti-thrombotic effects, improve endothelial function, and immunomodulatory effects, which play a role in the pathogenesis of atherosclerosis [28,34]. Combining atorvastatin and ethanol extract from purple sweet potato tubers can be reduced better than single therapy.

This study found that administration of ethanol extract of purple sweet potato tubers at 3 cc/day and atorvastatin could reduce aortic IMT in mice exposed to a high-fat diet compared to controls. Similar things were obtained from previous research conducted by Setiawan et al., who gave purple sweet potato tuber extract to mice given an atherogenic diet for 8 weeks. It was found that the aortic IMT in the group given purple sweet potato tuber extract was thinner compared to the group that was only given an atherogenic diet, correlated with an increase in the dose of extract administered [19].

Research conducted by Jawi found that purple sweet potato tuber extract could reduce MDA levels in the blood, liver, and heart in mouse models of oxidative stress. MDA can be used as a biomarker of lipid peroxidation and a marker of the degree of oxidative stress. Under conditions of oxidative stress, MDA levels increase. Oxidative stress is a condition of an imbalance of free radicals and antioxidants. If free radicals are high and meet unsaturated fatty acids, lipid peroxidation will produce aldehydes, one of which is MDA[21,35]

This study found that the group with single therapy of atorvastatin and ethanol extract of purple sweet potato tubers did not significantly differ in mean aortic IMT. However, atorvastatin had a considerably lower mean LDL than purple sweet potato tuber extract. This may be because the time required to reduce aortic IMT is more extended than necessary to reduce LDL cholesterol levels if only a single therapy is used. Research conducted by Nakashima et al., which assessed the effect of administering anthocyanins from blackcurrant extract (Ribes nigrum) on the thickness of the aortic intima in an ovariectomized rat model, found that the thickness of the tunica media was reduced compared to the control group after treatment for 9 weeks [36]. Compared with controls, the group with a single therapy of atorvastatin and ethanol extract of purple sweet potato tubers had thinner aortic IMT. This shows that atorvastatin and ethanol extract from purple sweet potato tubers have the same ability to reduce IMT progression and, when combined, have a potentiating synergistic effect on aortic IMT.

The mechanism of action of anthocyanins is more in the anti-inflammatory and anti-atherosclerosis pathways than atorvastatin. This may be why the ability of anthocyanins to reduce aortic IMT is equivalent to atorvastatin compared to the ability to lower LDL cholesterol levels. This is supported by research that giving 300 mg/day of anthocyanin for 3 weeks can inhibit the expression of NF-kB and adhesion molecules such as VCAM-1 and ICAM-1 and can inhibit the proliferation of vascular smooth muscle cells [37]. Anthocyanins can also reduce MCP-1 protein levels, which play a role in the pathogenesis of atherosclerosis [38]. Anthocyanins also have anti-proliferative effects on vascular smooth muscle cells induced by $TNF\alpha[39]$. This entire process plays a role in the pathophysiology of atherosclerosis.

synergistic effect of atorvastatin and The anthocyanins is supported by in vitro studies conducted by Pantan et al. The study assessed the combined effect of atorvastatin and Cyanidin-3glucoside (C3G) on inflammation in vascular smooth muscle induced by angiotensin II in human aortic smooth muscle. The results show that the combination of atorvastatin and C3G produces a synergistic effect on inflammation and oxidative stress. The mechanism underlying the synergistic effect is the suppression of translocation of the p65 NF-kB subunit from the cytosol to the nucleus and the attenuation of the expression of proteins, including iNOS, ICAM-1, and VCAM-1, as well as NO. Apart from that, C3G also triggers the antioxidant effect of atorvastatin through downregulating NOX1 and increasing Nrf2 ARE signaling activity, as well as reducing NADPH protein synthesis and increasing SOD activity. So, these results support that the combination of atorvastatin and C3G has the potential to regulate the atherosclerosis process mediated by oxidative stress by inhibiting the NF-kB pathway and Nrf2 signaling activity induced by AngII [40]

The results of this study strengthen the effectiveness of purple sweet potato tuber extract in reducing LDL cholesterol levels. The results of this study also show that the ethanol extract of purple sweet potato tubers not only impacts LDL cholesterol levels but, in the long term, can also impact the thickness of the aortic IMT. Several studies also suggest that the anthocyanins in purple sweet potato tuber extract are safe to use. The combination of evidence obtained from this research and evidence from previous research provides a solid basis for continuing this research to the next stage.

International Journal of Scientific Advances

The limitations of this study were that it provided treatment with only one type of purple sweet potato tuber dose or did not compare several doses of purple sweet potato extract for the desired effect. So, the information produced is limited to the impact of purple sweet potato extract on the effects of supplementation on LDL levels and aortic IMT. More specific information should be provided regarding the most effective dose to achieve this goal.

CONCLUSION

- Giving a combination of ethanol extract of purple sweet potato tubers (Ipomoea batatas L.) 3 cc/day and atorvastatin 1.4 mg/day had a significantly lower mean LDL cholesterol level compared to giving ethanol extract of purple sweet potato tubers 3 cc/day and giving therapy. Single atorvastatin 1.4 mg/day.
- 2. The combination of ethanol extract of purple sweet potato tubers (Ipomoea batatas L.) 3 cc/day and atorvastatin 1.4 mg/day had a significantly lower mean aortic IMT compared to the administration of ethanol extract of purple sweet potato tubers 3 cc/day and single therapy. atorvastatin 1.4 mg/day.

Acknowledgments

All patients, all authors, and all support in the paper

Declarations

Funding: No funding sources Conflict of interest: None declared Ethical approval: Udayana University approved the study under the number 0456/UN14.2.2VII.14/LT/2024.

REFERENCES

[1] World Health O. Cardiovascular diseases. 2021 n.d.

http://www.who.int/mediacentre/factsheets /fs317/en/.

- [2] Riskesdas. Hasil Riset Kesehatan Dasar Tahun 2018. Kementrian Kesehatan RI 2018;53:1689–99.
- [3] Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J 2020;41:111–88. https://doi.org/10.1093/eurheartj/ehz455.
- [4] Kementerian Kesehatan RI. Panduan Hari Obesitas Sedunia. Direktorat Pencegahan Dan Pengendalian Penyakit Tidak Menular Ditjen Pencegahan Dan Pengendalian Penyakit 2023:1–26.
- [5] Wali JA, Jarzebska N, Raubenheimer D, Simpson SJ, Rodionov RN, Sullivan JFO. Cardio-Metabolic Effects of High-Fat Diets and Their Underlying Mechanisms — A Narrative Review. Nutrients 2020;12:1–18.

[6]

- [7] Liu T, Zhao D, Qi Y. Global Trends in the Epidemiology and Management of Dyslipidemia. J Clin Med 2022;11:6377.
- [8] Willeit P, Tschiderer L, Allara E, Reuber K, Seekircher L, Gao L, et al. Carotid Intima-Media Thickness Progression as Surrogate Marker for Cardiovascular Risk: Meta-Analysis of 119 Clinical Trials Involving 100 667 Patients. Circulation 2020;142:621–42. https://doi.org/10.1161/CIRCULATIONAHA. 120.046361.
- [9] Mahajan N, Mirowska KK, Mewada A, Kahn J, Afonso L, Williams KA, et al. Effect of Long-Term Exposure to Lower Low-Density Lipoprotein Cholesterol Beginning Early in Life on the Risk of Coronary Heart Disease A Mendelian Randomization Analysis. J Am Coll Cardiol 2012;60. https://doi.org/10.1016/j.jacc.2012.09.017.
- [10] Silverman MG, Ference BA, Im K, Wiviott SD, Giugliano RP, Grundy SM, et al. Association Between Lowering LDL-C and Cardiovascular Risk Reduction Among Different Therapeutic Interventions A Systematic Review and Metaanalysis. JAMA Cardiol 2016;02115:1289–97. https://doi.org/10.1001/jama.2016.13985.
- [11] Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement fromthe European Atherosclerosis Society Consensus Panel. Eur Heart J 2017;38:2459–72. https://doi.org/10.1093/eurheartj/ehx144.
- [12] Toth PP. Low-Density Lipoprotein Cholesterol Treatment Rates in High Risk Patients: More Disappointment Despite Ever More Refined Evidence-Based Guidelines. Am J Prev Cardiol 2021;6:100186. https://doi.org/10.1016/j.ajpc.2021.100186.
- [13] Faridi KF, Desai NR. Moving toward combination lipid-lowering therapy for all patients with atherosclerotic cardiovascular disease. Am J Prev Cardiol 2023;14:10–2. https://doi.org/10.1016/j.ajpc.2023.100491.
- [14] Setiawan M, Putra ST, Sargowo D. Efficacy of purple sweet potato (*Ipomoea batatas*) extract to prevent the aortic wall thickness of white male rat's atherosclerosis model. Medicinal Plants - International Journal of Phytomedicines and Related Industries 2019;11:55. https://doi.org/10.5958/0975-6892.2019.00002.9.

- [15] Jawi IM, Budiasa K. Ekstrak Air Umbi Ubijalar Ungu Menurunkan Total Kolesterol serta Meningkatkan Total Antioksidan Darah Kelinci. Jurnal Veteriner 2012:120–5.
- [16] Yasa IWPS, Jawi IM, Astawa P. The Comparative Effect of Liquid and Tablet Preparation of Purple Sweet Potato (Ipomoea batatas L) Extract to Lipid Profile, MDA, and SOD Level in Male Wistar Rats After Given High-Cholesterol Diet. Global Pharma Technology 2018;10:536–61.
- [17] Murwani S, Ali M, Muliartha K. Diet Aterogenik Pada Tikus Putih (Rattus novergicus strain Wistar) sebagai model hewan Aterosklerosis. Jurnal Kedokteran Brawijaya, 2006;XXII.
- [18] Jawi IM, Yasa IWPS, Suprapta DN, Mahendra AN. Antihypertensive effect and eNOS expressions in nacl- induced hypertensive rats treated with purple sweet potato. Universal Journal of Medicine and Dentistry 2012;1:102–7.
- [19] Setiawan M, Abdi D, Zatalini SK, Kevin Muliawan Soetanto. The Potential Effect of Sweet Potato (Ipomoea Batatas L.) Extract on Total Cholesterol and Low-Density Lipoprotein (LDL) In Hypercholesterol-Model Wistar Rat (Rattus Norvegicus). Biomolecular and Health Science Journal 2022;5:33–6. https://doi.org/10.20473/bhsj.v5i1.31689.
- [20] Balzan S, Hernandes A, Reichert CL, Donaduzzi C, Pires VA, Gasparotto A, et al. Lipid-lowering effects of standardized extracts of Ilex paraguariensis in high-fat-diet rats. Fitoterapia 2013;86:115–22. https://doi.org/10.1016/j.fitote.2013.02.008.
- [21] Gu L, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D, et al. Concentrations of Proanthocyanidins in Common Foods and Estimations of Normal Consumption. Journal of Nutrition 2004;134:613–7. https://doi.org/10.1093/jn/134.3.613.
- [22] Suprapta D, Antara M, Arya N, Sudana M, Duniaji A, Sudarma M. Kajian Aspek Pembibitan Budidaya dan pemanfaatan Umbiumbian Sebagai Sumber Pangan Alternatif. Laporan Hasil Penelitian Kerja Sama BAPEDA Bali Dengan Fakultas Pertanian UNUD 2004.
- [23] Lilly LS. Atherosclerosis. Pathophysiology of Heart Disease, Lippincott Williams & Wilkins; 2022, p. 112–35.
- [24] Mozaffarian D. Nutrition and Cardiovascular and Metabolic Diseases. In: Zipes DP, Libby P, Bonow RO, Mann DL, Tomaselli GF, Braunwald E, editors. Braunwald's heart disease: a textbook of cardiovascular medicine. 11th ed., Philadelphia, Elsevier/Saunders; 2019, p. 983– 95.

- [25] Sizar O, Khare S, Jamil RT, Talati R. Statin Medication. StatPearls Publishing, Treasure Island (FL) 2023. https://www.ncbi.nlm.nih.gov/books/NBK4 30940/.
- [26] Sutirta Yasa IWP, Jawi IM, Astawa P. The Comparative Effect of Liquid and Tablet Preparation of Purple Sweet Potato (Ipomoea batatas L) Extract to Lipid Profile, MDA, and SOD Level in Male Wistar Rats After Given High- Cholesterol Diet. Global Pharma Technology 2018;10:356–61.
- [27] Jang HH, Hwang IG, Lee YM. Effects of anthocyanin supplementation on blood lipid levels: a systematic review and meta-analysis. Front Nutr 2023;10. https://doi.org/10.3389/fnut.2023.1207751.
- [28] Mattioli R, Francioso A, Mosca L, Silva P. Anthocyanins : A Comprehensive Review of Their Chemical Properties and Health E ff ects on Cardiovascular and Neurodegenerative Diseases. Molecules 2020;25:3809.
- [29] Salehi B, Sharifi-Rad J, Cappellini F, Reiner Z, Zorzan D, Imran M, et al. The Therapeutic Potential of Anthocyanins: Current Approaches Based on Their Molecular Mechanism of Action. Front Pharmacol 2020;11:1–20. https://doi.org/10.3389/fphar.2020.01300.
- [30] Setiawan M, Putra ST, Sargowo D. Efficacy of purple sweet potato (Ipomoea batatas) extract to prevent the aortic wall thickness of white male rat's atherosclerosis model. Medicinal Plants 2019;11:55–9. https://doi.org/10.5958/0975-6892.2019.00002.9.
- [31] Ikezaki H, Furusyo N, Yokota Y, Ai M, Asztalos BF, Murata M. Small Dense Low-Density Lipoprotein Cholesterol and Carotid Intimal Medial Thickness Progression. J Atheroscler Thromb 2020:1108–22.
- [32] Morofuji Y, Nakagawa S, Ujifuku K, Fujimoto T, Otsuka K. Beyond Lipid-Lowering: Effects of Statins on Cardiovascular and Cerebrovascular Diseases and Cancer. Pharmaceuticals 2022;15:1–26.
- [33] Jawi IM, Indrayani AW, Sutirta-Yasa IWP. Aqueous extract of balinese purple sweet potato (Ipomoea batatas l.) prevents oxidative stress and decreases blood interleukin-1 in hypercholesterolemic rabbits. Bali Medical Journal 2015;4:37–40.
- [34] Nanashima N, Horie K, Maeda H, Tomisawa T, Kitajima M, Nakamura T. Blackcurrant Anthocyanins Increase the Levels of Collagen, Elastin, and Hyaluronic Acid in Human Skin Fibroblasts and Ovariectomized Rats. Nutrients 2018;10:495.

https://doi.org/10.3390/nu10040495.

International Journal of Scientific Advances

- [35] Aboonabi A, Singh I. Chemopreventive role of anthocyanins in atherosclerosis via activation of Nrf2-ARE as an indicator and modulator of redox. Biomedicine and Pharmacotherapy 2015;72:30–6. https://doi.org/10.1016/j.biopha.2015.03.00 8.
- [36] Mozos I, Flangea C, Vlad DC, Gug C, Mozos C, Stoian D, et al. Effects of anthocyanins on vascular health. Biomolecules 2021;11:1–22. https://doi.org/10.3390/biom11060811.
- $\begin{bmatrix} 37 \end{bmatrix} Luo X, Fang S, Xiao Y, Song F, Zou T, Wang M, et al. Cyanidin-3-glucoside suppresses TNF-\alpha-induced cell proliferation through the repression of Nox activator 1 in mouse vascular smooth muscle cells: Involvement of the STAT3 signaling. Mol Cell Biochem 2012;362:211–8. https://doi.org/10.1007/s11010-011-1144-3.$
- [38] Pantan R, Tocharus J, Suksamrarn A, Tocharus C. Synergistic effect of atorvastatin and Cyanidin-3-glucoside on angiotensin IIinduced inflammation in vascular smooth muscle cells. Exp Cell Res 2016;342:104–12. https://doi.org/10.1016/j.yexcr.2016.02.017.