Int J Adv Life Sci Res. Volume 4(3) 26-31
https://doi.org/10.31632/ijalsr.2021.v04i03.004

International Journal of Advancement in Life Sciences Research
Online ISSN: 2581-4877
journal homepage http://ijalsr.org

Original Article

Effect of Sambiloto (*Andrographis paniculata*) Leaves against Cholesterol Levels in Wistar Rats with Atherogenic Diet

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Abstract

Introduction : The risk of atherosclerosis increases when elevated blood cholesterol levels. Flavonoids may help inhibit the absorption of fat, which indirectly helps in lower cholesterol levels.Flavonoids are widely available on the *Andrographis paniculata* leaves (APL).This study aims to examine the effect of flavonoids in APL stewto lower the cholesterol levels. **Material and Methods:** This research was carried out using five different groups of Wistarrats. Each group consists of five Wistar rats. Group (K-) received normal diet, group (K+) received atherogenic diet and three groups of atherogenic diets rats that pre-treated with three different doses (0.6, 1.2, and 2.4g/day) of APL leaf stew. APL stew is orally gavaged for 60 days. The cholesterol blood serum was analyzed using the CHOD-PAP method. Statistical analysis using One-Way ANOVA and Post-Hoc Tukey. **Results :** The results showed a significant difference in cholesterol levels between the groups of theatherogenic diet with other treatment groups ($p = 0.000$).When compared with thenormal diet group, treatment with 1.2 g and 2.4 g of APL produce cholesterol levels not significantly different.APLstew has been shown to inhibit the elevated levels of serum cholesterol in male Wistar rats that fed with the atherogenic diet which contributed 54.8%.While fat intake increased the cholesterol level by 16.3%.Both APL stew and fat dose intake together affect the formation of cholesterol with a contribution of 69.2%. **Conclusion :** The dose that is considered as the most effective in lowering cholesterol is 2.4 g becauseit producescholesterol level closest to normal.

Keywords: *Andrographis paniculata; atherogenic diet; cholesterol*

Introduction

Prevalence of hypercholesterol is 39.6% women and 30% men in Riskesdas, Indonesia 2013. WHO data in 2015 showed that deaths worldwide caused by heart and vascular disease by 45%. Riskesdas 2018 shows a prevalence of heart disease of 1.5%. This is in line with research conducted by *Sample Registration System* Indonesia in 2014 showing that the highest cause of death in Indonesia from heart disease is 12.9% (KKRI 2019).

Consumption of high-fat, low-fiber, and antioxidants is one of the risk factors for atherosclerosis. Saturated fatty acids can increase cholesterol levels, while flavonoids can help inhibit the absorption of fats that indirectly help lower cholesterol levels (Widjajakusuma *et al.* 2019).

At the moment the group of drugs that are widely used is the group of statins associated with muscle disorders through the mechanism of inhibition of HMG-CoA enzymes that can decrease the production of mevalonate. In addition, statins can also provide side effects in the form of discomfort in the stomach in the form of bloating and abdominal pain (Hariadini, A.L., 2020). One of the natural ingredients used for cholesterol-lowering is APL. APL contains tannins and flavonoids in the form of andrographin as antioxidants. APL is known as a king of bitter reported as an anti-inflammatory, immunomodulator, atherosclerosis, and antihyperglycemic (Nugroho *et al.* 2012; Dai *et al.*2019). In this study, we determined the effects of APL stew against decreasing cholesterol levels in ratswith atherogenic diet.

Materials and Method

Dry APLwas purchased from Materia Medica, Batu, East Java, Indonesia. A marker for cholesterol serum was obtained from the Anatomy Laboratory, University of Brawijaya.

Extraction of APL

The dried APL was milled with ablender and sieved 80 mesh. The powder of APL was weighed as much as 50 grams and heated in 50 cc aquades at 100°C for 5 minutes. Next, it was refrigerated until the temperature of 40°C. It was then filtered and added aquades until the volume is 50 cc.

Animal Experiments

Male Wistar rats weighing 150-200 g were placed in the control room with a cycle of 12 hours of light, 12 hours of darkness. Animals were fed and drank throughout the day. After 1 week of acclimation, the rats were randomly divided into 5 groups (5 rats/group). Negative control (K-) of a normal diet and positive control (K+) are given an atherogenic diet and given mineral water orally every day. The other three groups were given an atherogenic diet and administered doses of APL stew (0.6, 1.2, and 2.4 g/day) for 60 days. The protocol of the animal experiment was approved by the ethical committee of University of Brawijaya, Malang, Indonesia (No. 0305/EC/KEPK-S1-GZ/10).

Cholesterol Blood Serum Analysis

Blood serum cholesterol levels in Wistar rats were measured on day 61. Rat blood serum was taken from the heart of the rat as much as 150 mL. Serum samples are then taken as much as 100 cc and mixed with 1000 cc reagent kit then homogenized. The mixture is incubated at 37°C for 5 minutes and read absorbance at a wavelength of 500 nm.

Statistical Analysis

Blood serum cholesterol analysis was performed using One-Way ANOVA at the 95% of confidence level ($\alpha = 0.05$). if the ANOVA calculation showed significant differences ($p<0.05$), then continued with the Post-Hoc Tukey test at 95% of confidence level ($\alpha = 0.05$).

Results

Effects of APL Stew on Cholesterol Levels of Wistar Rat

Serum cholesterol levels of rat blood after being given APL stew treatment for 60 days calculated using chod-PAP method.Table 1 shows the obtained cholesterol levels. Cholesterol levels in rat blood serum differ significantly ($p<0.05$). Cholesterol levels in group K+ were 183.40±15.04 higher than the P2 group (157.60±14.77), while the P4 cholesterol level (122.20±5.89) was close to the cholesterol level of group K- (108.60±7.40). Values are means of triplicate samples.

Blood cholesterol level analysis of Wistar rats using One-way ANOVA showed that there were significant differences from the five treatment groups with a value of $p=0.000$ ($\alpha=0.05$). The analysis continued by using Post-Hoc Tukey to find out the differences between groups.

Table 1. Cholesterol Blood Serum Rats Wistar (mg/dl)

Treatment	Cholesterol Blood Serum
K-	108.60 ± 7.40^{a}
K+	183.40 ± 15.04^{b}
0.6 g	157.60 ± 14.77^{c}
1.2 g	129.60 ± 8.98^{d}
2.4 g	122.20 ± 5.89^{a}

Note : Different letters demonstrate a significance ($p<0.05$) in the same rows according to Post Hock Tukey test

Effects of APL Stew on Wistar Rat Weight

The weight of Wistar rat was measured before and after treatment. The measurements were taken once a week to determine whether there is any increase in weight of Wistar rat. The weight analysis was conducted using the One-way ANOVA statistical test. The initial weight results show that there was no significant difference with the value p=0.258. Therefore, it concluded that the weight of the Wistarrat before the treatment was the same (homogeneous) in all treatment groups as seen in table 2, while the weight gain results also show no significant difference with p=0.068.

Table 2. Body Weight Rats Wistar

Weeks	Treatment				
	K-	K+	0.6 g	1.2 g	2.4 g
Early Body Weight (g)	120.4+8.41[a]	133+13.78[a]	121.6+ 6.87[a]	125+6.55[a]	125.6+ 8.38[a]
Weight Gain (g)	68.2+ 21.29[a]	103.20+7.67[b]	85.60+20.95[a]	113.7+51.09[b]	114+25.37[b]

Note : Different letters demonstrate a significance (p<0.05) in the same rows according to One-Way Anova test

Relationship between Dose of APL Leaf and Cholesterol Levels

The results of a simple linear regression analysis test with cholesterol level-dependent variables and independent variables of APL leaf grafting dose can be known that cholesterol levels in the P2, P3, and P4 treatment groups were negative and significant with the given dose of APL leaf grafts. Itrevealed that the smaller the dose of leaf stews given, the greater the cholesterol levels in the blood of the Wistarrat. This is reinforced by a probability value of 0.001. The amount of cholesterol level relationship with the dose of APL leaves is 0.740.

The magnitude of the contribution of the effect of the dose of APLleaf on cholesterol levels in the treatment group P2, P3, and P4 were proven through the results of linear regression test.Thecalculation test R square show a result of 0.548. This concluded that the dose of leafy stews of APL has a contribution of 54.8% in affecting cholesterol levels of Wistar rats.

Relationship Between Dose of APL Leaf and Fat Intake on Cholesterol Levels

The effect of fat intake and dose of APL leaf was given to cholesterol levels bymultiple linear regression tests were conducted. The results show that the fat intake of animals had a positive relationship, the greater the intake of fat, the greater the measurable cholesterol levels. The magnitude of the relationship between fat and cholesterol levels is 0.403 with a probability of 0.023. While the dose of APLleaf has a negative relationship to cholesterol levels. It was expressed that the larger the dose of leaf APLstewgiven, the smaller the measured cholesterol value. The relationship between the dose of leafy DekokAPL and cholesterol levels is 0.357.

The amount of fat intake contribution and the dose of APLleaf stew given, from the results of linear regression tests are known that the dose of leaf stews and animal fat intake try to contribute 69.2% to the formation of cholesterol serum levels.

Relationship of Energy Intake and Nutrients on Cholesterol Levels

Intake of macronutrients, in this case only fats that have a positive relationship with cholesterol levels in Wistar rat, the greater the fat consumed by Wistar rat, the greater the cholesterol levels in serum measured. The magnitude of the relationship between fat intake and cholesterol levels was 0.403 with p = 0.023 which was significant. In addition to fat, weight gain also had a positive relationship with cholesterol levels in the Wistar rat, the higher the cholesterol levels of the Wistar rat, the higher the weight gain of the Wistar rat. However, itdid not show a significant association with p = 0.355.The weight gain was influenced by fat intake which increased the cholesterol level too.

The amount of contribution of macronutrients in affecting cholesterol levels was showed by linear regression tests. The results show that adjusted value of R square analysis of the influence of macronutrient intake on cholesterol level is 0.216. This shows that carbohydrates, fats, and proteins contributed 21.6% in producing cholesterol levels in serum rat.

The contribution of carbohydrates to the formation of cholesterol levels in animal serum trials from the results of linear regression tests showed that carbohydrate intake contributed by 19.3% in affecting cholesterol levels inWistar rat.In addition to carbohydrate intake, fat intake in the diet of each treatment group also had a role in the formation of cholesterol in this study.The relationship of fat intake with cholesterol levels in ratshows that fat intake from the diet has a role of 16.3% in affecting cholesterol levels. While other macronutrients, namely protein, also contributed to measure cholesterol levels at the end of the study. Linear regression test results show that protein contributed 19.9% in determining cholesterol levels of Wistar rat.

Discussion

The formation of body cholesterol comes from two sources, namely, those derived from foods called exogenous cholesterol, and cholesterol produced by the body itself called endogenous cholesterol. If cholesterol comes from food is small, to meet the needs of other tissues and organs, then the synthesis of cholesterol in the liver and intestines will increase. Likewise, if the amount of cholesterol in the diet increases then the synthesis of cholesterol in the liver and intestines will decrease (Violi *et al.* 2002, Widyani, R., 2019).

So the importance of energy for our body must still be considered the balance because if the energy value of food was eaten is greater than the energy lost due to heat and work, or that is censored by the body, then energy will be stored and will be able to increase weight (Lakshmia *et al.* 2014).

A diet high in fat can result in the presence of LDL oxidation. Increased LDL oxidation is caused by high triglyceride levels that can convert VLDL metabolism into a large form of VLDL, this can damage HDL which will eventually complicate the cholesterol content of blood vessels (Lakshmia *et al.* 2014, Widyani, R., 2019).

Plants that are efficacious to lower the levels of cholesterol and high triglycerides including plants APLhave a mechanism of action that is flavonoids as antioxidants that inhibit the oxidation of human LDL. Flavonoids also inhibit platelet aggregation in the blood which plays a role in reducing the risk of heart disease (Violi *et al.* 2002). Flavonoids also affect cholesterol metabolism directly in hepar. Hypotheses that support the decrease in cholesterol levels and the activity of HMG-CoA reductase and sterol enzyme 0-acyltransferase-2 in cholesterol metabolism after giving flavonoids in rats (Lakshmia *et al.* 2014).

APL also contains tannins that have a mechanism of action to reduce the oxidative stress of macrophages and inhibit the formation of atherosclerosis. Tannins in the body will bind to the body's proteins and will coat the intestinal wall so that the absorption of fat is inhibited (Sudarmi *et al.* 2018). In addition, tannins protect the intestines against unsaturated fatty acids. The process of protection is carried out by tannins in the form of compaction of the mucus layer of the gastrointestinal tract thus inhibiting the absorption of food substances (including cholesterol fats) by the gastrointestinal tract (Liu *et al.* 2020). In addition, tannins are known to spur the metabolism of glucose and fat, so that the deposits of these two sources of calories in the blood can be avoided, meaning cholesterol and blood sugar drop (Bharati *et al*. 2011).

Decreased cholesterol levels are caused by the presence of antioxidant effects, improving endothelial function, and improving the balance of nitrite oxide. In addition, the decrease in cholesterol levels in the blood is due to the APL contained fiber and vitamins called can lower cholesterol and triglyceride levels with the help of fiber that binds to fatty acids free of bile acids which are then released through feces. Fiber is also fermented by microflora in the intestine producing acetic acid, propionate, and butyric acid which can inhibit cholesterol synthesis (Warditiani *et al.* 2020).

Saponins are also present in APL, the role of saponins against cholesterol reduction is that saponins can form complex bonds that are insoluble with cholesterol (from food) in the intestines so that the cholesterol can not be absorbed. In addition, saponins can combine with bile acids and exogenous cholesterol forming rattle which also cannot be absorbed by the intestines. Then saponins can also increase exogenous cholesterol by fiber, so it cannot be absorbed by the intestines (Agrawal and Pandey 2019).

The andrographolide effect in stimulating the immune system in the body, and especially phagocytic activity that stimulates the production and flow of bile, protects the hepar from toxins, against the damage effects caused by free radicals. Andrographolide also showed protective activity in the fight against chemical-induced toxicity (Polash *et al.* 2017; Soltani *et al.* 2021; Rachmani E. 2019). APL serves as an appetite enhancer, but high doses of APL can result in nausea vomiting, bad stomach and loss of appetite (Mustafa *et al.* 2010). However, from various studies conducted, in general APL does not cause serious side effects, so it is safe to consume.

Conclusion

The method used in this study is a preventive method, where APL leaf stew is given in conjunction with an atherogenic diet. The administration of leaf stews APL decreased the cholesterol levels in Wistar rats.The effective dose of APL leaf stew for lowering the total cholesterol level is 2.4 grams.

Acknowledgments

The authors wish and sincerely thank to authority of Sekolah Tinggi Ilmu Kesehatan Hang Tuah Surabaya, Indonsia for providing necessary laboratory facilities.

Conflicts of Interest

The authors declare no conflict of interest.

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