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Guava (*Psidium guajava*) leaf extract affects lipid profile changes Wistar rats on an atherogenic diet

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Abstract

The present works examines the effect of giving guava leaf extract on total cholesterol in white Wistar rats, which were given an atherogenic diet. Experimental research with a completely randomized design (CRD) was employed in this research. Guava (*Psidium guajava*) leaf extracts were administered to white rats with three levels of treatment (P0 as the control, P1 given an atherogenic diet and P2 given an atherogenic diet with guava leaf extract) with five replications. All data were analyzed using the Shapiro walk test, Kruskall-Wallis (as an alternative to the one-way ANOVA test) and the Wilcoxon test (as an alternative to the t-test 2 free samples) with a value of $\alpha = 0.01$. The results showed that giving an atherogenic diet had an effect on changes in total cholesterol. There was a change in total cholesterol after the intervention. In the P0 pre-test, the total cholesterol was 84.26±3.17 mg/dl, and this was increased to 88.03±4.36 mg/dl ($p = 0.018$) post-test. The cholesterol level in the pre-test and post-test for P1 was 84.26±3.66 mg/dl and 180.82±3.87 mg/dl ($p = 0.000$), respectively. The cholesterol content for the pre and post-test of P2 was 85.91±2.00 mg/dl and 114.26±4.94 mg/dl ($p = 0.000$), respectively. In conclusion, atherogenic diet contributes to the increasing cholesterol and guava leaf extract can reduce blood pressure in white wistar rats.

1. Introduction

Non-communicable diseases are notoriously known as the number one killer worldwide. Cardiovascular disease contributes the most to non-communicable diseases (48%) compared to other non-communicable diseases. The number of deaths from heart disease increased by more than 2 million since 2000, to nearly 9 million in 2019. Heart disease now represents 16% of total deaths from all causes. More than half of the 2 million additional deaths were in the WHO Western Pacific region (WHO, 2020). Riskesdas data for 2018 shows that 1.5% of Indonesia's population suffers from coronary heart disease. Coronary heart disease (CHD) is caused by narrowed walls of the coronary arteries due to the formation of fatty material (Rahayu *et al*., 2016). According to the latest RI Ministry of Health data (2014), the number of elderly people in Indonesia is estimated to reach 9.77% or 23.9 million people in 2010 and will increase significantly by 11.4% or 28.8 million people in 2020 (Isroin *et al*., 2019).

Indonesia is a country recognized for its largest biodiversity in the world after Brazil. Biodiversity includes ecosystems, species and genetics. By that, optimum utilization of such resources is crucial, one of which is natural medicine. The process of finding drugs from plants is a rigorous process and requires a long period of time. The process encompasses ethnopharmacological studies, chemotaxonomy, screening of bioactive compounds, the possibility of synthesizing single compounds, pre-clinical and clinical studies, to large-scale production for medical purposes. One of the medicinal plants that has just been explored for development is guava (*Psidium guajava*).

Psidium guajava is found in tropical and sub-tropical regions, including Latin America, Europe, Asia and Africa. *Psidium guajava* is a medium-sized tree belonging to the *Myrtaceae* family which has traditionally been used for the treatment of various diseases covering all parts of the plant. Guava plant leaves contain phytochemicals, including flavonoids, tannins, triterpenoids and carotenoids. Various studies

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have also proven that this plant has polyphenolic compounds, which are pharmacological as anti-inflammatory, antioxidant, antimicrobial and antiproliferative.

Hence, the present study aimed to scrutinize the effect of guava leaf extract *(P. guajava*) on changes in lipid profiles in experimental animals.

2. Materials and methods

2.1 Research design

The present work employed a completely randomized design (CRD). Guava leaf extracts were administered to white rats and this treatment comprised three levels with five replications. The purpose was to examine the effect of giving sambilito extract on blood pressure in white rats experiencing hypertension.

2.2 Time and location of research

This research was conducted from April to July 2021 at the PSPG (Center for Food and Nutrition Studies) laboratory, Gadjah Mada University, Yogyakarta. *Rattus norwegicus* wistar strain rats were selected randomly by considering the inclusion and exclusion criteria derived from seven weeks of observation and then observed the effect of sambilito extract on the rats' blood pressure. The inclusion criteria included male rats, aged 2-3 months and weighed 150-200 g, while the exclusion criteria included rats whose condition declined during the study and rats which refused to eat during the study.

2.3 Material and research tools

Normal diet rat feed ingredients, atherogenic diet rat feed ingredients and sambiloto feed extract were the material of this study. Each 100 g of feed comprised 90.9 g of comfeed PAR-S, 9.09 g wheat flour obtained from animal feed shops mixed with water. Meanwhile, ingredients for 100 g of atherogenic diet rats (for treating hypertension) were 57.26 g comfeed PAR-S, 31.8 g wheat flour, 9.54 ml cow oil, 1.9 g cholesterol, 0.12 g cholic acid and water. Fresh guava leaves were picked and processed into extracts. The leaf extracts were added into 100 g of feed. The control and treatment groups consisted of a control group (P0) with a normal feed diet (Comfeed PAR-S), and two treatment groups: P1 with an atherogenic diet and P2 with the same diet and the administration of guava leaf extract.

Research tools were used for different purposes such as for making rat feed, raising and weighing mice, and measuring blood cholesterol, triglycerides, HDL and LDL. Triple beam scales, plastic basins, wooden stirrers, and measuring cups were used in processing rat feed. A plastic tub with a size of 45 cm × 35.5 cm × 14.5 cm and wire cages of 36.5 cm × 28 cm × 15.5 cm functioned to confine the rats. All rats were weighed using a Tanita type Tokyo Japan electric scale CAP 2.25 kg. Grad 109. Cholesterol concentrations were examined using test tubes, centrifuges and spectrophotometers

Research phases included acclimatization, distribution of control and treatment groups, maintenance of experimental animals. The aclimatization was performed before treatment, and the adaptation was carried out for three days to enable the rats to adjust with the laboratory conditions. During the acclimatization period, all rats received a normal diet. The experimental animals were given an atherogenic diet for 2 weeks until they experienced hypercholesterolemia. All the rats were divided into three groups: a control group (P0) with a normal diet (Comfeed PAR-S), a treatment group (P1) with an atherogenic diet, and a treatment group (P2) with guava leaf extract-added atherogenic diet. Five rats were randomly selected from each treatment; the rats were confined separately. Maintenance of rats took four weeks, where the remaining feed was weighed every day to calculate energy intake. All selected rats were weighed every seven days. During the treatment stage, the rats were given an atherogenic diet only, while the other treatments were given an atherogenic diet plus sambiloto extract. The examination of lipid profile levels was used in the form of blood serum. It was carried out after cleavage and collection of serum in the heart of the rat.

2.4 Data analysis

A descriptive data analysis was performed to determine the frequency distribution of the characteristics of the experimental animals. The present work employed different tests to the research variables between the control and treatment groups: the Shapiro walk test, the Kruskall-Wallis test (as an alternative to the one-way ANOVA test), and the Wilcoxon test (as an alternative t-2 test for free samples) with a value of $\alpha = 0.01$. All data were examined in Microsoft Excel and SPSS 16.0.

3. Results and discussion

3.1 The results of the data normality test with the Shapiro walk test

Table 1 showed the data normality test results with the Shapiro Wilk test. It showed that the variables are normally distributed in all intervention groups. The total cholesterol, triglycerides, HDL and LDL before and after the intervention showed normal data distribution.

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Table 1. Data normality test results with the Shapiro Wilk test

Variable	Treatment Group	Means	Sig.	Interpretation
Total Pre-Cholesterol (mg/dL)	P0	84.26±3.17	0.351	Normal
	P1	84.26±3.66	0.466	Normal
	P2	85.91±2.00	0.582	Normal
Total Post-Cholesterol (mg/dL)	P0	88.03±4.36	0.686	Normal
	P1	180.82±3.87	0.457	Normal
	P2	114.26±4.94	0.909	Normal
Pre-triglycerides (mg/dL)	P0	80.14±3.40	0.884	Normal
	P1	80.73±2.61	0.685	Normal
	P3	78.98±2.03	0.656	Normal
Post-Triglycerides (mg/dL)	P0	82.99±3.93	0.829	Normal
	P1	139.26±4.31	0.721	Normal
	P2	100.00±1.67	1.000	Normal
Pre-HDL (mg/dL)	P0	88.86±2.11	0.941	Normal
	P1	82.91±1.14	0.486	Normal
	P2	84.29±1.14	0.497	Normal
Post-HDL (mg/dL)	P0	85.65±2.44	0.254	Normal
	P1	23.21±2.12	0.979	Normal
	P2	66.51±4.34	0.467	Normal
Pre-LDL (mg/dL)	P0	21.28±1.40	0.755	Normal
	P1	23.70±1.46	0.746	Normal
	P2	22.76±2.20	0.547	Normal
Post-LDL (mg/dL)	P0	24.15±2.00	0.980	Normal
	P1	74.07±2.16	0.977	Normal
	P2	32.44±2.06	0.653	Normal

3.2 Differences in lipid profiles before and after the intervention

Figure 1 displays the animal lipid profile before and after treatment. The cholesterol and triglyceride concentrations in P1 and P2 showed a significant trend. In the post-test, the cholesterol of P1 was 84.2 mg/dl and it increased to 180.2 mg/dl after the intervention. The total triglycerides before the intervention of P1 was 80.7 mg/dl and increased to 139.2 mg/dl, signifying a greater trend than that of the P2 group, in which the total cholesterol before and after the intervention was 85.9 mg/dl and 114.2 mg/dl, respectively. After the intervention, the triglyceride was increased to 100 mg/dl from 78.9 mg/dl in P2. The HDL measurement showed a decline in P1 and P2 after the intervention. A decline in HDL concentration was greater in P1 (23.2 mg/dl) compared to P2 (66.5 mg/dl). Measurement of LDL concentrations showed a significant increase in P1 after the intervention (74 mg/dl) compared to P2 (32.4 mg/dl).

The lipid profiles in experimental animals before and after the intervention are shown in Table 2. There was no difference in cholesterol concentration before the intervention (p = 0.080). After the intervention, there was a significant difference among the three groups, in which the P0 cholesterol concentration was 82.62–93.44 mg/dl, P1 was 176.01–185.63 mg/dl, and P2 was 108.13 –120.39 mg/dl (p = 0.000). In other words, triglyceride concentrations showed no significant difference among the three groups (p = 0.402). Differences were noticed after the intervention, in which the concentration of triglycerides in P0 was 78.11–87.86 mg/dl, P1 was 133 .91–144.60 mg/dl, and P2 was 97.93–102.07 mg/dl (p = 0.000).

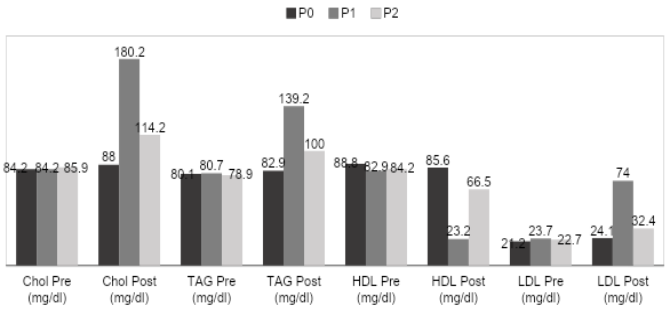

Figure 1. Animal lipid profile before and after treatment.

Table 2 shows the differences in lipid profiles of experimental animal groups before and after intervention. The HDL concentrations before and after the intervention showed that there were differences between the three groups (p = 0.000). It can be seen that the LDL concentration before the intervention does not differ significantly in three groups (p = 0.218): P0 at 21.28±1.40 mg/dl, P1 at 21.89-25.52 mg/dl, and P2 at 20.03-25.50 mg/dl. Differences were identified after the treatment (p = 0.000) as indicated by the LDL concentration of P0 at 24.15±2.00 mg/dl, P1 at 74.07±2.16 mg/dl, and P2 at 32.44±2.06 mg/dl.

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Table 2. Differences in lipid profiles of experimental animal groups before and after intervention.

Group Treatment	Average	95% CI	Sig*
Pre-Cholesterol (mg/dL)			
P0 (n = 5)	84.26±3.17	80.32 - 88.20	
P1 (n = 5)	84.26±3.66	79.72-88.80	0.08
P2 (n = 5)	85.91±2.00	83.42-88.40	
Post-Cholesterol (mg/dL)			
P0 (n = 5)	88.03±4.36	82.62–93.44	0
P1 (n = 5)	180.82±3.87	176.01–185.63	
P2 (n = 5)	114.26±4.94	108.13–120.39	
Pre-triglycerides (mg/dL)			
P0 (n = 5)	80.14±3.40	75.92-84.37	
P1 (n = 5)	80.73±2.61	77.49-83.97	0.402
P2 (n = 5)	78.98±2.03	76.46-81.49	
Post-triglycerides (mg/dL)			
P0 (n = 5)	82.99±3.93	78.11 – 87.86	0
P1 (n = 5)	139.26±4.31	133.91 – 144.60	
P2 (n = 5)	100.00±1.67	97.93 – 102.07	
Pre-HDL (mg/dl)			
P0 (n = 5)	88.86±2.11	86.24-91.48	0
P1 (n = 5)	82.91±1.14	81.49-84.32	
P2 (n = 5)	84.29±1.14	82.88-85.70	
Post-HDL (mg/dL)			
P0 (n = 5)	85.65±2.44	82.61 – 88.68	0
P1 (n = 5)	23.21±2.12	20.58 – 25.84	
P2 (n = 5)	66.51±4.34	61.12 – 71.90	
Pre-LDL (mg/dL)			
P0 (n = 5)	21.28±1.40	19.55-23.02	0.218
P1 (n = 5)	23.70±1.46	21.89-25.52	
P2 (n = 5)	22.76±2.20	20.03-25.50	
Post-LDL (mg/dL)			
P0 (n = 5)	24.15±2.00	21.48 – 26.67	0
P1 (n = 5)	74.07±2.16	71.11 – 77.04	
P2 (n = 5)	32.44±2.06	30.37 – 35.56	

*One-way ANOVA Test.

Table 3 depicts the differences in the average blood lipid profile of rats before and after the intervention. Comparative analysis of lipid profiles before and after the intervention based on the paired sample test statistic was shown in Table 3. In the control group (P0) it showed that there were significant differences in lipid profiles, including the total cholesterol, triglycerides, HDL and LDL before and after the intervention. In this sample group, the p value is below or equal to the value $\alpha = 0.01$. As seen in P0, there was an increase in total cholesterol, triglycerides and LDL after the intervention, while the HDL concentration decreased after the intervention. In the atherogenic diet group (P1), the lipid profile before and after the intervention was significantly different: there was a drastic increase in total cholesterol, triglyceride, and LDL concentrations, while HDL concentrations decreased dramatically after the atherogenic diet for four weeks. The same trend is also identified in P2: there was an increase in total cholesterol, triglyceride, LDL, and HDL concentrations after four weeks of intervention, although it is lower than P1.

Table 3. Differences in the average blood lipid profile of rats before and after the intervention.

Treatment Group	Variable	Average	Sig
P0	Total pre-cholesterol	84.26±3.17	0.019**
	Total post-cholesterol	88.03±4.36	
	Pre-triglyceride pre	80.14±3.40	0.018**
	Post-triglycerides	82.99±3.93	
	Pre-HDL	88.86±2.11	0.010**
	Post-HDL	85.65±2.44	
	Pre-LDL	21.28±1.40	0.001**
	Post-LDL	24.15±2.00	
P1	Total pre-cholesterol	84.26±3.66	0.000**
	Total post-cholesterol	180.82±3.87	
	Pre-triglyceride pre	80.73±2.61	0.000**
	Post-triglycerides	139.26±4.31	
	Pre-HDL	82.91±1.14	0.000**
	Post-HDL	23.21±2.12	
	Pre-LDL	23.70±1.46	0.000**
	Post-LDL	74.07±2.16	
P2	Total pre-cholesterol	85.91±2.00	0.000**
	Total post-cholesterol	114.26±4.94	
	Pre-triglyceride pre	78.98±2.03	0.000**
	Post-triglycerides	100.00±1.67	
	Pre-HDL	84.29±1.14	0.001**
	Post-HDL	66.51±4.34	
	Pre-LDL	22.76±2.20	0.000**
	Post-LDL	32.44±2.06	

*Paired Sample Test.

The results showed that giving guava leaf extract contributed to the changes in the lipid profile of experimental animals. Extract from guava leaves does not have short-term harmful effects as long as the dose is 5 g/kg BW. A study on ethanol extract of guava leaves on reducing total cholesterol levels in male white rats (*Rattus norvegicus*) found that administration of ethanol extract of guava leaves at a dose of 600 mg/BB for 2 weeks reduced total cholesterol levels in rats but statistically not significant because $p>0.05$ (Supriosa, 2017).

Guava contains a number of vitamins, antioxidants, polyphenols, and flavonoids that can prevent CVD. A number of studies on experimental animals have shown that all parts of guava starting from leaves, fruit, roots and twigs have medicinal potential (Sandhar *et al*., 2011; Kumar, 2012). Guava can also be used as an antimicrobial, anti-bacterial and anti-inflammatory. Guava has a hepatoprotective effect with antioxidant and anti-cancer properties (Mekoya, 2007; Vanitha *et al*., 2012). Guava also has anti-diarrhea and antimicrobial

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activity (Ezekwesili *et al*., 2010). The beneficial effects on guava may be related to the content of nutrients and non-nutritional substances such as vitamin C, vitamin A, iron, calcium, manganese, phosphorus, oxalate, and malic acid, saponins in combination with oleanolic (Elias *et al*., 2017).

The results of the phytochemical analysis of the ethanol extract of guava leaves showed 3,049.5 mg/100 g QE of flavonoid, 67,741.38 mg/L GAEAC of antioxidant capacity, 7,471.70 mg/100 g body weight of vitamin C, 12,308.56 mg/100 g TAE of tannin content, 15,884.36 mg/100 g (GAE) total phenol (polyphenol) and 102.69 mg/L inhibitor concentration (IC) 50%. Judging from the IC value of 50%, this guava leaf is a moderate (close to strong) antioxidant. Guava leaves can improve lipid profiles in diabetic rats and diabetic patients. In rats and rabbits administered with a hypercholesterolemia diet, guava leaves can suppress plasma cholesterol levels, increase HDL levels, and lowers LDL at a dose of 250 mg/kg body weight (Rahayu *et al.*, 2016).

Extract from guava leaves has no harmful short-term effects and is nontoxic to rats at a dose of 5 g/kg BW. Lethal doses are more than 5 g/kg BW. Another study examining the phenolic content of extracts found decreased cholesterol and triglyceride levels, increased HDL levels, and decreased VLDL and LDL in rats that were given a hypercholesterolemia diet.

4. Conclusion

After the treatment, both the control and intervention groups showed an increase in total cholesterol, triglycerides, LDL and a decrease in HDL concentrations. Before the intervention there was no significant difference in the average blood pressure in the three groups. Differences in the average lipid profile in the experimental animals were noticed after the intervention. Analysis of the paired sample test showed that in the control group, the atherogenic diet group, and the atherogenic diet group with guava leaf extract, there were differences in lipid profiles before and after intervention for 4 weeks in *Rattus norwegicus* wistar strain white rats.

Conflict of interest

The authors declare no conflict of interest.

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