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Hepatoprotective Effects of Methanol Extract of *Syzygium polyanthum* L. Leaves (Salam) on High Fat Diet

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ABSTRACT

Dyslipidemia (hyperlipidemia) is a strong risk factor for cardiovascular disease. *Syzygium polyanthum* (Wight) Walp. var. *polyanthum* leaves are widely consumed as a traditional Indonesia treatment. The study aimed to investigate the effect of *Syzygium polyanthum* (Wight) Walp extract in decreasing hypercholesterolemia in Wistar rats. *Syzygium polyanthum* (Wight) Walp extract (0.72, 0.9 and 1.08 g/day) was administered by gavage to the high fat diet-induced hypercholesterolemia in rats for 2 weeks. The result revealed that the extract administration reduced total cholesterol levels ($P < 0.05$), low-density lipoprotein (LDL) ($P < 0.05$), and triglyceride (TG) ($P < 0.05$) without alteration of body weight. Furthermore, the histological analysis showed that *Syzygium polyanthum* (Wight) extract decreased lipid accumulation in the liver ($p < 0.05$). It is concluded that *Syzygium polyanthum* (Wight) extract significantly lowered total cholesterol and led to lipid accumulation reduction in the liver in hypercholesterolemic Wistar Rat model.

Keywords: Hepatoprotective, Hypercholesterolemia, *Syzygium polyanthum* (Wight), Lipid accumulation.

Introduction

Epidemiological data in the 20th century underwent changes where there was a decrease in mortality and morbidity due to infectious diseases and an increase in non-communicable diseases (NCDs). One of the non-communicable diseases (NCDs) is cardiovascular disease which is currently the leading cause of mortality and morbidity worldwide.¹ Based on the epidemiological research, the risk factors for cardiovascular disease include dyslipidemia, hypertension, smoking, diabetes mellitus, stress, obesity which are modifiable risk factors and heredity, age, and gender which are non-modifiable risk factors.² The high serum cholesterol level are prevalent risk factor of cardiovascular disease.^{3,4} Cholesterol is a product of lipid metabolism comes from food synthesis and distribution through the blood vessels and is known as hypercholesterolemia.^{5,6} Hypercholesterolemia is a risk factor for atherosclerosis, which is an inflammatory disorder inside the walls of the arteries.⁷ Globally, research on natural products derived from plants has been widely carried out. A diversity of plants have biological activity and contains various active substances. Bay leaf (*Syzygium polyanthum*) is an herbal plant that has been shown to reduce cholesterol levels in the blood,^{8,9} this is because bay leaves contain secondary metabolites such as flavonoids, saponins, terpenoids, polyphenols, alkaloids, and essential oils.¹⁰

Several studies have shown that flavonoids and phenolic acids, which are classes of polyphenolic compounds have antioxidative activity, including anti-inflammatory action, inhibition of oxidative enzymes, and scavenging of free radicals.¹¹ In addition, Lee *et al.* proved that flavonoids can reduce cholesterol levels by inhibiting HMG-CoA reductase activity.¹² This study aimed to determine the anti-hypercholesterolemia activity of *S. polyanthum* leaves extracts in High-Fat-Diet-Induced hypercholesterolaemia in Wistar Rats.

Materials and Methods

Plant material collection

The leaves of *Syzygium polyanthum* (Wight.) were taken from the plantation in July 2016 and dried, which were imported through from PT. HRL International, Pasuruan East Java, Indonesia with sample number: PID-171011-10 which has been deposited in the FRIM herbarium.

Preparation of extracts of *Syzygium polyanthum* (Wight.)

The leaves of *Syzygium polyanthum* (Wight.) were collected in July 2016 from the plantation in PT. HRL International Indonesia, Pasuruan, East Java. The leaves were dried, powdered and then extracted sequentially with three solvents by the maceration method. About 150 g of *S. polyanthum* leaves powder was immersed in 350 mL of n-hexane and sonicated for about 30 min. The solution was filtered using Whatman Paper No. 1 and the residue was put in a fume hood until n-hexane was completely evaporated. The above-mentioned steps were repeated using ethyl acetate. After the ethyl acetate has completely evaporated, the residue was then immersed in methanol at three different cycles: 400 mL, 200 mL and 50 mL. The solution from each methanol cycle was also filtered using the Whatman No. 1 filter paper. The remaining plant residue was discarded while the filtered supernatant was evaporated using rotary evaporator (Labortechnik, AG CH-9230, Postfach, Flawil, Switzerland) at low pressure to produce a concentrated extract. To remove residual solvent, the

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concentrated extract was oven-dried at 50°C and stored in a freezer (-25°C) until further use.

Animals

Wistar rats of weights between 150 and 200 g were purchased from Animal laboratories (Universitas Airlangga, Surabaya). Ethical approval was granted by the animal ethics committee of Airlangga with the number 106/HRECC.FODM/VII/2017. All the experiment procedures conformed to the guidelines for animal experiments of Universitas Airlangga. The animals were acclimatized at room temperature (25–30°C) for one week under 12 hours of light/12 hours of darkness with free access to feed and drinking water.

The rat hyperlipidemia model was carried out by administering a high-fat diet orally with a beef fat emulsion composition consisting of 5 grams of beef fat, 10 grams of egg yolk and up to 100 ml of water.¹³ After administration of a high-fat diet for 2 weeks, bay leaf extract was given at doses of 0.72, 0.9 and 1.08 grams/Kg body weight orally for 2 weeks.

Blood glucose and lipid profile analyses

The blood samples of wistar rats were collected by cardiac puncture into EDTA-containing tubes. Plasma lipid levels; total cholesterol, LDL-cholesterol, and triglycerides were measured at BBLK Airlangga University and blood glucose levels was measured by blood glucose measuring device.

Histological and immunohistochemical analyses

All the animals were sacrificed on the 14th day from each animal was removed, fixed in 10% formalin solution, and processed by the paraffin technique.¹⁴ Sections of 5 µm thickness were cut and stained by hematoxylin and eosin (H&E) for histological examination.¹⁵

Statistical analysis

All results were expressed as mean ± SEM. The unpaired Student's t-test was performed to compare of parameters between two groups. Comparisons of dose-response curves were made by two-factor repeated measures ANOVA, followed by Tukey's post hoc test for comparison between groups. A value of P < 0.05 was considered significant.

Results and Discussion

Effects of high fat diet in plasma cholesterol and body weight

The high-fat diet administered for two weeks caused a significant increase in body weight compared to the normal diet group as shown in Figure 1. The results of this study showed that weight gain is closely associated with increased cholesterol levels.

These results indicated that oral high-fat diet administration has caused hypercholesterolemia. These results are in line with previous study which reported that mice consuming a high-fat diet showed increased plasma glucose levels, cholesterol and triglyceride levels.¹⁶ The saturated fat present in high-fat diet is responsible for the increase in lipid profiles.¹⁷

The effects of extract *Syzygium polyanthum* (Wight) on metabolic parameters

The plasma characteristics are given in detail in Table 1. There was a statistically significant reduction in total plasma cholesterol in the whole group with administration of bay leaf extract as compared to the high-fat diet group without administration of bay leaf extract. There was no significant difference in serum triglycerides and LDL in HFD-treated group. Previous study suggested that *S. polyanthum* (wight) walp leaves extract had anti-hypercholesterol effect,⁸ although, the underlying mechanism are not fully understood. In this study, we found that the *S. polyanthum* (wight) walp extract decreased hypercholesterolemia in *Rattus norvegicus*.

Previous study found that the *S. polyanthum* (wight) walp has many compounds in the leaves, such as flavonoids, quercetin, and vitamin B3 (Niasin) were alleged to reduced total cholesterol, LDL cholesterol, HDL, and blood triglyceride.¹⁸ However, flavonoid and tannin act as free radical scavenger and inhibit the LDL oxidation. Tannin also reduced cholesterol by inhibiting cholesterol absorption in the intestine.¹⁹ This study showed similar results that the *S. polyanthum* (wight) walp extract administration decreased the triglyceride and LDL levels, but they were statistically not significant.

The effects of extract of *Syzygium polyanthum* (Wight) on liver inflammation in HF diet fed Rats

Representative haematoxylin livers from each treatment group are shown in Figure 2.

In this study, the liver tissue histology from the control group showed normal shape with normal hepatocyte cells, no lipid deposits and no infiltration of inflammatory cells (Figure 2A). In the high-fat diet group, there were degenerative changes in hepatocytes along with lipid deposition and infiltration of inflammatory cells (Figure 2B). Previous study showed that the high-fat diet administration caused elevated serum liver enzyme levels and enhanced damage to the liver histological structure.²⁰ However, the administration of extract of *Syzygium polyanthum* (Wight) in rats showed that the higher the dose administered, the more normal hepatocyte was, less lipid deposition and infiltration of inflammatory cells (Figure 2C-D) and showed a similar pattern to the control group which demonstrated the hepatoprotective effect of *Syzygium polyanthum* extract.

Table 1: The effect of methanol extract of *Syzygium polyanthum* L. (Salam) on blood glucose, body weight, triglyceride, cholesterol and LDL-cholesterol changes in Wistar rats

	Ctrl (n = 7)	HFD (n = 7)	HFD + D1 (n = 7)	HFD + D2 (n = 7)	HFD + D3 (n = 7)
Blood glucose (mg/dL)	110 ± 6.04	231 ± 7.66†	105 ± 4.96**†	123.4 ± 3.99*†	129.9 ± 2.51*†
Body weight (g)	234.1 ± 19.6	173 ± 14.4	216.8 ± 10.7*†	214 ± 10.8*†	215.0 ± 1.18*†
Total cholesterol, md/dl	94.8 ± 5.40	169.8 ± 7.49	88.4 ± 5.02*†	51.4 ± 3.35***†	68.8 ± 5.85***†
Triglyceride, mg/dl	62.2 ± 2.76	52.6 ± 2.99	59.8 ± 2.77	50.8 ± 2.76	47.4 ± 2.58
LDL-cholesterol, mg/dl	13.8 ± 1.88	9.0 ± 1.31	10.2 ± 3.19	11.6 ± 1.76	9.4 ± 1.06

Note: A statistically significant difference in the values is indicated by different letters within one column (p<0.05). Ctrl; control, HFD; High-fat-diet, HFD+D1; high-fat-diet+ low dose (0.72g/day), HFD+D2; high-fat-diet+ mild dose (0.9 gr/day), HFD+D3 (high-fat-diet+high dose (1.08 gr/day).

*P<0.05; **P<0.01 vs control group. †P<0.05, ††P<0.01 vs HFD group. All values are mean ± SEM.

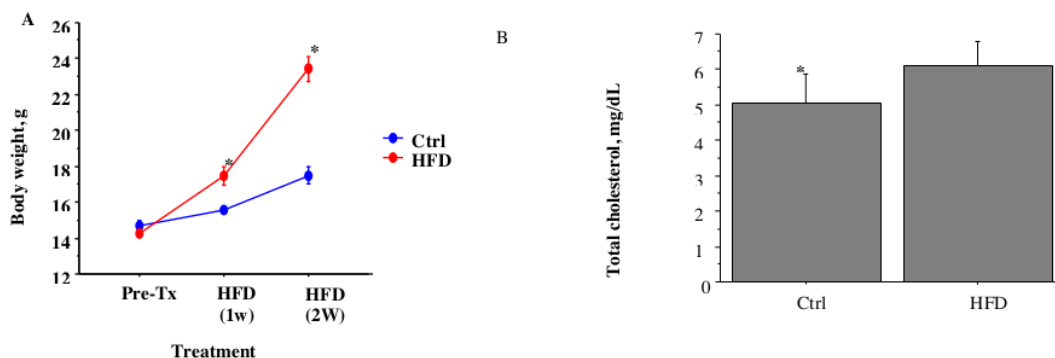


Figure 1: Effect of high-fat diet fed Wistar rats on body weight and total cholesterol in serum.

(A) Body weight increased after high-fat diet fed for two weeks (B) total cholesterol increased in the group high-fat diet compared with control group. Results are expressed as Mean \pm SEM; n=7; *p<0.05, **p<0.01

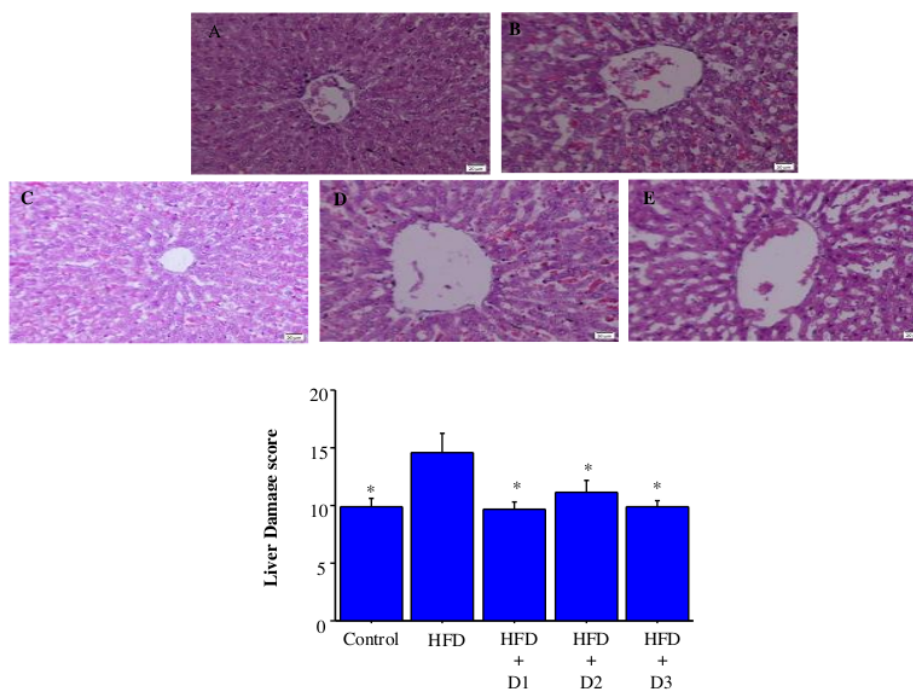


Figure 2: The effects of extract *Syzygium polyanthum* (Wight) in histological of liver

(A) Normal rats with no treatment (control group), and (B) HFD group (C) HFD + low dose (D) HFD+mild dose, (E) HFD + high dose treatment group. (HE staining, 400x magnification). (F) Representative of histological analysis showed the extract *Syzygium polyanthum* (Wight) decreases the isolated of hypercholesterolemia on dose-dependent. N=7, per group. Ctrl;control, HFD;High-fat-diet, HFD+D1; high-fat-diet+ low dose (0.72g/day), HFD+D2; high-fat-diet+ mild dose (0.9 gr/day), HFD+D3 (high-fat-diet+high dose (1.08 gr/day)). *P<0.05, **P<0.01 vs HFD group. All values are mean \pm SEM.

Steatosis and inflammation in the liver were assessed according to the degree of damage shown in Figure 2F. In the high-fat diet group, there was a significant increase in the infiltration of inflammation cells ($p<0.05$) compared to control group. However, the *Syzygium polyanthum* extract administration reduced the inflammation scores significantly ($p<0.05$). The bioactive compound in leaves reduced endogenous cholesterol synthesis in the liver. β -sitosterol can decrease the synthesis of the enzyme HMG-CoA reductase

(Hydroxymethylglutaryl CoA-reductase) in the biosynthesis of cholesterol. In addition, previous study demonstrated that the *S. polyanthum* (wight) walp extract decreased the inflammatory cells in the liver which was associated with isolated cholesterol to developed hypercholesterolemia.¹² *S. polyanthum* leaves extracts were previously shown to exhibit anti-diabetic properties on alloxan-induced,²¹ and on streptozotocin-induced diabetic rats.²² The extract of *S. polyanthum* (wight) walp also

attenuated vascular dysfunction including impaired vasomotor responses. Previous study demonstrated that the *S. polyanthum* (wight) walp leaves extract was also known for their anti-inflammatory property.²³

Conclusion

The findings from the study showed that the methanol extract of *Syzygium polyanthum* (Wight) reduces lipid accumulation in the liver and reduce the total cholesterol and triglyceride levels.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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