The Effect of Banana Pseudostem Flour and Food Bar of Edible Canna Substituted with Banana Pseudostem Flour on Lipid Profile of Hypercholesterolemia Mice

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Abstract— Cavendish Jepara 30 banana pith (EBP J30) flour and canna starch-based food bar, which was substituted with EBPJ30 Flour, were reported to contain dietary fiber and resistant starch so that it has the potential to improve the lipid profile. However, there has been no reference, research related to the effects of both on the lipid profile, and their ability to bind bile acids. The study aims to analyze the effect of EBP J30 flour diets and canna starch-based food bar, which was substituted with EBPJ30 Flour on hypercholesterolemia Sprague Dawley’s lipid profile mice and its ability to bind bile acids. Thirty male mice, aged two months, divided into six groups, namely groups that were given a standard diet including normal mice, negative controls, and positive controls, hypercholesterolemia mice fed the natural EBP J30 flour, blanched EBP J30 flour, and food bar. The 4-week diet intervention and lipid profile analysis were done once a week, and lipid profile analysis was carried out regularly every week. Diet intervention on blanched EBP J30 flour and food bar reduced total cholesterol, LDL, triglycerides, and increased serum HDL cholesterol levels in mice. The results of in vitro studies suggested that the diet of blanched EBP J30 flour and food bar could increase bile acid-binding capability. Both diets can improve the lipid profile of hypercholesterolemia mice suspected due to the bile acids' binding capacity in the blood.

Keywords— Banana pseudostem flour; lipid profile; bile acids; hypercholesterolemia.

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I. INTRODUCTION

Dyslipidemia is a condition of abnormal lipid metabolism indicated by increased cholesterol or LDL cholesterol and triglyceride levels and decreased levels of HDL cholesterol in the blood plasma [1]. Dyslipidemia should be treated because it could trigger the formation of atherosclerosis, which could increase the risk of infection factors for cardiovascular diseases and coronary heart disease, stroke, and even death [2], [3]. Conditions of the lipid profiles are often used to deal with dyslipidemia [4], [5]. Bioactive components such as dietary fiber and resistant starch can improve the plasma lipid profile due to its hypo cholesterol [6]. Handling dyslipidemia conditions are currently more dominant to use cholesterol-lowering drugs, such as simvastatin [4]. Medically, drug use was clinically safe but could result in adverse side effects [7]. Consumption of local food that contained lots of fiber and resistant starch was able to be used as an alternative to improve dyslipidemia conditions due to the availability of abundant. It did not produce adverse side effects because it was derived from secured natural materials. Plenty of dietary fiber and resistant starches could be found in plants or natural fruits. It was also could be found in a variety of processed flour that consciously enriched with those components, such as banana pseudostem flour (EBP flour)

Cavendish EBP Jepara 30 (EBP J30) flour mixed with 10-minute blanching treatment contained, total dietary fiber $43.82 \pm 0.36\%$, soluble dietary fiber $5.90 \pm 0.27\%$, insoluble dietary fiber $37.92 \pm 0.62\%$, resistant starch $13.13 \pm 0.32\%$, total phenolic $132.81 \pm 2.14$ mg/100 g, and the antioxidant activity of $18.04 \pm 0.23\%$ RSA [8]. The EBP flour was proven to increase soluble dietary fiber content by $0.83\%$, total dietary fiber $4.81\%$, resistant starch $2.89\%$, total
phenolic 43.01 mg/100g, and the antioxidant activity of 3.98% of RSA of canna starch-based food bars were substituted with EBP [9]. Bioactive components such as soluble dietary fiber and resistant starch were hypocholesterol (leading to improved human lipid profiles). The ability of dietary fiber and resistant starch in improving the lipid profile and its mechanism were affected by several factors such as the origin of fiber and processing techniques. On the other hand, dietary fiber and resistant starch's hypocholesterol properties were closely related to binding bile acids [10]. Therefore, the health benefits of EBP flour in improving the lipid profile and its mechanism were important to investigate.

However, there is no research reference related to the effect of Cavendish Jepara 30 EBP flour from Indonesia and China's starch-based food bar, which was substituted with EBP J30 Flour (a food bar) on the lipid profile of hypercholesterolemia Sprague Dawley mice. Their ability to bind bile has also not been reported. The study aims to analyze the effect of EBP J30 flour diets and food bar on hypercholesterolemia Sprague Dawley mice's lipid profile and its ability to bind bile acids.

II. MATERIAL AND METHOD

A. Material

The raw material used was natural EBP J30 flour (A1), and a 10-minute blanched EBP J30 flour/ blanched EBP flour (A2) [8], and a canna starch-based food bar, which was substituted with EBP J30 flour/food bar (A3) [9].

C. Blood Serum Lipid Profile of Mice

Blood samples were obtained from mouse's eyes (retro-orbital plexus). Blood samples were centrifuged at 2000 rpm for 12 min to obtain blood serum used for lipid profile testing using the CHOD-PAP diagnostic kit. Total cholesterol (TC), LDL cholesterol (LDL-C), triglyceride (TG), and HDL cholesterol (HDL-C) were measured at λ 550 nm. The following equation calculated the concentration (moll / L):
Asp, Abl, and Ast were absorbance samples, blanks, and standards, whereas [sp] and [st] were the sample concentrations and standards. The resulting colour was measured for its absorbance [15]. LDL cholesterol levels were obtained through calculations with the formula Rubenfire et al. [16].

\[ \text{LDL-C} = (\text{TC} - \text{TG}/5 - \text{HDL-C}) \]  \hspace{1cm} (2)

HDL cholesterol was set enzymatically by the CHOD-PAP method. LDL and VLDL were centrifuged and then separated and precipitated using magnesium chloride and phosphotungstic acid. The absorbed supernatant plus the enzyme was then measured. HDL cholesterol was calculated using the method of Eckel et al. [17].

D. Calculation of Atherogenic Index Plasmas (AIP)

Atherogenic Index Plasma was estimated using the Niroumand et al. method with the formula \( \log(\text{triglyceride} / \text{D. phosphotungstic acid}) \). The absorbed supernatant plus the enzyme was then measured. HDL cholesterol was calculated using the method of Eckel et al. [17].

E. Analysis of the Binding Capacity of Bile Acids

Bile acid-binding capability (cholic acid, deoxycholic acid) were measured using Soral et al. methods [19]. Samples of 100 mg were added to 10 ml of bile acid solution then heated in an incubator at 37ºC for 30 min. Subsequently, the sample was centrifuged at a speed of 2000g for 5 min. Samples of 50 µL were mixed with 71% sulphuric acid and 1 ml of fresh furfural solvent. After 80 minutes, the optical density was scanned with a spectrophotometer at \( \lambda 510 \) nm.

F. Statistical Analysis

The study was designed using a fully randomized design with six treatment factors (M1, M2, M3, M4, M5, and M6) with five replicates. The experimental data were analyzed by one-way ANOVA and continued with Fisher’s Least Significant Difference test at a level of \( \alpha = 0.05 \) [20]. Data analysis was produced using SPSS v20.

III. RESULTS AND DISCUSSION

The results of the assay of the average feed consumption of mice showed that treatment significantly affected the average feed consumption of mice \( (p <0.05) \) presented in Fig.1. Mice feed consumption tended to increase during the intervention period. This condition was suspected because of diet food for normal mice, and hypercholesterolemia mice were a standard food diet.

The results of the scrutiny of the average body weight of mice showed that treatment significantly affected the average body weight of mice \( (p <0.05) \) presented in Fig. 2. The highest weight gain and significantly different from other groups from beginning to end intervention hypercholesterolemia demonstrated by a group of mice without treatment. This weight gain was presumably due to the accumulation of fat-induced cholesterol that could lead to fat reserves accumulation, which occurred to the untreated ones.

A. Total Serum Cholesterol

The results of the analysis of total serum cholesterol levels of mice showed a significant consequence \( (p<0.05) \) presented in Fig.3. Dietary interventions of EBP J30 flour and food bar could lower the total serum cholesterol levels with the highest decline were shown in M4 followed by M5 flour. The decrease in M4 was not significantly dissimilar compared to the M6 as a positive control diet only was significantly dissimilar from the other four groups, while the M3 diet showed the significant lowest decrease. The decrease in total serum cholesterol levels by M4, M5, and M3 diet allegedly due to the consequence of soluble fiber and resistant starch contained on the diets [8]. Dietary fiber and resistant starch affected the lowering of total cholesterol [21]. The mechanism of decrease the total cholesterol by soluble fiber and resistant starch was possibly by adding to the excretion of bile acids and cholesterol in the faeces [22], [23].
The results of the analysis of mice serum LDL cholesterol levels showed that the treatment significantly affects LDL cholesterol levels (P <0.05) depicted in Fig. 4. Dietary interventions of EBP J30 flour and food bar could lower serum LDL cholesterol. The highest decrease was shown in M4, followed by M5 diet. The M4 diet decrease was not significantly different compared to M6 as the positive control diet only notably dissimilar from the other four groups. The significant lowest decrease was shown in the M3 diet. The increase of HDL levels shown in the M4, M5, and M3 diet was allegedly because of soluble fiber and resistant starch contained by the feed [8]. Feeding dietary fiber and resistant starch could increase HDL blood levels of diabetic mice. Changes in HDL in the body could be seen from the changes in LDL levels. In this study, increased HDL and LDL conversely decreased during the period of dietary intervention. In terms of their functions were different, HDL...
contained protein and served as transportation of cholesterol from tissues to the liver so that it could prevent the occurrence of calcification in the arteries. Instead of LDL, it could trigger atherosclerosis because LDL carried cholesterol in large amounts and could lead to calcification in the arteries [14].

Improvement of lipid profiles such as increasing HDL and decreasing total cholesterol, triglycerides, LDL on HPV treatment was suspected due to cholesterol-lowering medication with simvastatin during the intervention. Giving simvastatin at a dose of 20-80 mg could lower LDL 18-55%, 7-30% TG, and increased HDL 5-15% [30]. Reversibly, statins inhibited HMG-CoA reductase from catalyzing the transition of HMG-CoA reductase into mevalonate. Through the prohibition of these enzymes, intracellular statins reduced the cholesterol levels and accelerated LDL’s clearance from plasma [31].

The ability of banana pseudostem flour in improving the lipid profile such as increased levels of HDL and lowering total cholesterol, LDL, and triglycerides were supported by data from a group of normal feds with standard AIN (M1) and hypercholesterolemia fed with standard AIN (M2) during the period of dietary intervention. These two groups showed opposite changes in lipid profile with diet treatment of banana pseudostem flour, which decreased serum HDL and increased levels of total cholesterol, triglycerides, and LDL cholesterol that significantly contrast to other groups fed with banana pseudostem flour diet. This condition was suspected as the cholesterol was fully compacted in the blood that interfered with lipoprotein metabolism, so that affected in decreasing HDL cholesterol and increasing total cholesterol, LDL, and triglycerides.

Fig. 6 HDL cholesterol serum of mice in each treatment. Normal fed with standard AIN (M1), hypercholesterolemia fed with standard AIN (M2), hypercholesterolemia fed with natural EBP flour (M3), hypercholesterolemia fed with blanched EBP flour (M4), hypercholesterolemia fed with food bar (M5), and hypercholesterolemia fed with standard AIN and simvastatin (M6). Different letter notations above the same colored bar represent significant differences (p <0.05)

E. Atherogenic Index Plasma

The analysis of mice atherogenic index plasma showed that the treatment significantly affects atherogenic index Plasma (p<0.05) depicted in Fig.7. Dietary interventions of EBP J30 flour and food bar could lower plasma AIP. The highest decline was shown by the M4 diet, followed by the M5 diet. The decrease of AIP in M4 was not significantly dissimilar compared to M6 as the positive control diet only importantly dissimilar from the other four treatments. The significant lowest decline was shown in the M3 diet. AIP was one way to predict the risk of atherosclerosis [32]. AIP had a significant relationship with risk factors for cardiovascular disease [33].

AIP could be calculated by the formula log (TG / HDL) [34]. Based on the value of AIP in Fig.7, the M5 and M4 group in the late period of intervention had a value of AIP, 0.10 ± 0.01, and 0.16 ± 0.02, respectively. Based on atherosclerosis risk criteria, the M4 group was categorized as a low-risk group, and the M5 group was categorized as medium risk. Based on AIP, the risk of atherosclerosis could be divided into three groups; low risk (less than 0.11), medium-risk (0.11 to 0.21), and high risk (greater than 0.21) [19]. Meanwhile, the value of AIP for the M2 and M1 group was contrarily increased. This condition reinforced the suspect that EBP J30 flour and food bar could reduce the risk of atherosclerosis. The decrease in AIP value indicated the decreased levels of pro-atherogenic lipoproteins (triglycerides and LDL) and the increased anti-atherogenic (HDL) [35].

F. Bile Acid Binding Capacity

Bile acid-binding capacity performed in vitro using cholic acid and deoxycholic acid depicted in Fig.8. The results of the analysis of the binding capacity of the bile acid of mice showed that treatment significantly affected the binding capacity of bile acids (p<0.05) in Fig.8. The dietary intervention of EBP J30 flour and food bar could either bind bile acids cholic acid and deoxycholic acid. The highest binding capacity of cholic acid is shown in the blanched EBP flour diet (M4) despite the below capacity of cholestyramine compounds as a cholesterol-lowering drug that bile acid-binding mechanism that was used as a benchmark [36], [37].

Fig. 7 Atherogenic index of plasma of mice in each treatment. Normal fed with standard AIN (M1), hypercholesterolemia fed with standard AIN (M2), hypercholesterolemia fed with natural EBP flour (M3), hypercholesterolemia fed with blanched EBP flour (M4), hypercholesterolemia fed with food bar (M5), and hypercholesterolemia fed with standard AIN and simvastatin (M6). Different letter notations above the same colored bar represent significant differences (p <0.05)

Fig. 8 Bile acid binding capacity of mice in each treatment. Cholic acid and deoxycholic acid binding capacity was measured in vitro using cholic acid and deoxycholic acid depicted in Fig.8. The results of the analysis of the binding capacity of the bile acid of mice showed that treatment significantly affected the binding capacity of bile acids (p<0.05) in Fig.8. The dietary intervention of EBP J30 flour and food bar could either bind bile acids cholic acid and deoxycholic acid. The highest binding capacity of cholic acid is shown in the blanched EBP flour diet (M4) despite the below capacity of cholestyramine compounds as a cholesterol-lowering drug that bile acid-binding mechanism that was used as a benchmark [36], [37].
Meanwhile, the highest deoxycholic acid-binding capacity shown in the standard feed (M1/M2/M6) was still below the cholestyramine compound’s capacity. Cholic acid was a primary bile acid produced by our bodies from the synthesis of cholesterol. Deoxycholic acid, secondary bile acid, was synthesized from primary bile acids. Cholic acid and deoxycholic acid composition in a healthy body was 41% and 15% [38]. The power to bind bile acids from the diet of blanched EBP flour (M4), M5, and M3 was probably blanched EBP flour (M4), M5, and M3 was probably the best binding capability mechanism.

IV. CONCLUSION
Diet EBP J30 flour (M3), EBP J30 blanching flour (M4), and food bar (M5) can reduce the concentration of total cholesterol, LDL cholesterol, triglycerides and increase the concentration of HDL cholesterol in mice blood Sprague Dawley hypercholesterolemia. EBP J30 flour diet and food bar could also reduce IAP. The second feed diets could also bind the cholic acid and deoxycholic acid with the best binding ability that was demonstrated by feeding the mice with blanched EBP flour (M4). EBP J30 flour and food bar had the potential of anti-dyslipidemia, which was a bile acid-binding capability mechanism.

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