Beetroot (*Beta vulgaris L.*) reduces cholesterol and triglyceride in dyslipidemic male rats sprague–dawley model

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ABSTRACT

Background: High-fat diets habit can lead to metabolic disorders, such as dyslipidemia which a significant contributor for cardiovascular diseases. Dyslipidemia occurs as a result of metabolically interrelated abnormalities of plasma lipids and lipoproteins, including low level of high-density lipoprotein cholesterol, and increasing of low density lipoprotein cholesterol, total cholesterol, and triglyceride. In order to enhance anti-lipid treatment, nutritional therapy containing bioactive compounds are investigated extensively, including those found in beetroot which shown potential in preventing and treating metabolic disorders.

Objective: This study evaluated the effect of beetroot extract and beetroot juice on cholesterol and triglyceride levels as a dyslipidemia treatment, in comparison to simvastatin. Ethanol extract can attract flavonoids and betalain, but the extraction process can degrade fiber. Meanwhile, juice still contains quite a lot of fiber.

Materials and Methods: Male Sprague Dawley rats were divided into seven groups and fed different diets for 56 days. The groups were: normal control (K0), negative control-HFD (KN), positive control-HFD + simvastatin (KP), single-dose treatment with BE (P1) or BJ (P2), and combination treatment with BE or BJ + simvastatin (P3 and P4), each intervention was given for 28 days. After blood drawn, cholesterol total and triglyceride serum were examined and analyzed.

Results: Administration of beetroot extract and juice in single dose or combination with simvastatin gave a significant decrease in cholesterol and triglyceride levels compared to before the intervention. The average reduction levels of cholesterol in P1, P2, P3, and P4 were 54.81, 56.31, 94.19 and 69.11 respectively. Whereas the average decreasing level of triglyceride were 43.28, 30.78, 54.28 and 46.37 in P1, P2, P3 and P4 groups. Combination treatment with simvastatin gave more reduction level compared with single dose beetroot extract or beetroot juice. The most effective reduction was in the beet extract combination with simvastatin group were -94.19±4.08 mg/dL (cholesterol) and -54.28±6.93 mg/dL (triglyceride).

Conclusion: Both beetroot extract and juice, as single or combined with simvastatin, were able to decrease cholesterol and triglyceride levels, these indicated their potential for prevention and therapeutic in dyslipidemia. Further research is needed to investigate its mechanisms and establish optimal dosages for human consumption.

Keywords : Dyslipidemia; Beetroot; High Fat Diet; Cholesterol; Triglyceride.

BACKGROUND

Sedentary lifestyle and eating behaviour change have given impacts on human life such as increasing mortality risk factors such as cancer, cardiovascular, metabolic disorders and diseases. The extended consumption of high fatty acids (HFD) can have important consequences resulting in the emergence of metabolic disorders.[1] Excessive HFD consumption is a significant contributor to the development of dyslipidemia which later induces cardiovascular disease.[2,3] Dyslipidemia occurs as a result of metabolically interrelated abnormalities of plasma lipids and lipoproteins, including low level of high density lipoprotein cholesterol (HDL) and increased of low density lipoprotein cholesterol (LDL), cholesterol total, and triglyceride.[4]

The prevention or treatment of metabolic disorders caused by malnutrition, such as dyslipidemia, can be achieved through nutritional therapy that contains bioactive nutrients. In some studies, beetroot has been reported to promote health and protect against disease.[5] The medicinal plant beetroot or *Beta vulgaris L.*is found mainly in Asia, Europe, and North America.[6,7] Beetroot contains phytochemical compounds, such as betalains [8], fiber, and flavonoid (viteixin, rutin, apigenin, luteolin, quercetin, orientin).[9] Betalain as an antiradical ingredient is able to be an efficient ROS scavenger by donating hydrogen to reactive species so as to delay or prevent lipid oxidation,[10] fiber is able to bind bile acids, which are then excreted by the body so that they can reduce cholesterol levels in the blood,[11] and flavonoid function to inhibit the activity of the

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cholesterol-forming enzyme, namely HMG-CoA reductase, so that the formation of mevalonate from HMG-CoA decreases.[12] In rats fed HFD and given luteolin experiment showed that luteolin inhibited hepatic lipogenesis and lipid absorption thus restored from hepatic steatosis, and luteolin also reduced hepatic lipotoxicity by increasing expression of PPARɣ protein in adipose tissue. Luteolin also upregulated genes expression of lipolysis controlled-genes and the tricarboxylic acid cycle which act before lipid droplets formation caused adiposity reduction.[13] Quercetin correlated with a reduction in cholesterol levels as it inhibited cholesterol absorption.[14]

So far, various interesting studies of polar compounds in the form of flavonoids have used several methods and the most frequently used are the extraction methods with ethanol solvent and juice with water solvent. [15] The advantages of extraction are that it is not easy for microbes to grow, is more selective and has quite good absorption properties.[16] However, extracts that are packaged as nutraceutical products orally can cause toxic effects if consumed in excess, and there are pharmacokinetic interactions with drugs.[17] On the other hand, processed fruit products in the form of juice have the advantage of being easy in the manufacturing process with an emphasis on maintaining the organoleptic properties of the authenticity of the fruit so that they are suitable for all age groups and people who are busy with daily activities.[18] Juice preparations have the limitation that they must be drunk immediately before they undergo oxidation.[19] Ethanol extract can attract flavonoids and betalain, but the extraction process can degrade fiber. Meanwhile, juice still contains quite a lot of fiber. Therefore, this study aims to analyze the effectiveness of beetroot extract and juice on total cholesterol and triglyceride levels in dyslipidemia rat model.

As beetroot contains several bioactive compounds, therefore this study was conducted to evaluate beetroot juice and extract activity in dyslipidemic rat model, by giving HFD in rats and cholesterol total and triglyceride levels were measured after given a single dose daily of beetroot extract (BE) 100 mg/200 g body weight (BW) and beetroot juice (BJ) 3.6 ml/200 gBW and a combination BE or BJ with 0.18 mg simvastatin. The dose of beetroot extract in this study refers to research conducted by Al-Harbi (2021) which stated that administration of 100 mg/200 gBW/day was able to reduce cholesterol and triglycerides.[20] The dosage of beetroot juice refers to research conducted by Lotfi (2020) which states that giving 200 ml can reduce total cholesterol and triglycerides. Then, the ml dose for humans weighing 70 kg was converted to a dose for mice weighing 200 grams, which resulted in a dose of 3.6 ml/200 gBW/day given per mouse per day. [21]

MATERIALS AND METHODS

This study was an experimental animal experiment consisted of a pre and post-test, randomized controlled group design. All experiment activities were conducted at the Pusat Studi Pangan dan Gizi, Gadjah Mada University from end of January to end of March 2023.

Beetroot extract was obtained using maceration technique. The beetroot was peeled and sliced into thin pieces. These fresh slices were subjected to a drying process for seven days to optimize the drying of materials so that they are easily ground into powder and stop enzymatic reactions.[22] The dehydrated beetroot sample was immersed into 70% ethanol for three consecutive days. Subsequently, the mixture was filtrated, followed by evaporation using a rotary evaporator for 4.5 hours. In this research, extraction was carried out with the aim of extracting polar compounds in the form of flavonoids. 70% ethanol has properties that can dissolve polar, semipolar and nonpolar substances so that it is able to separate flavonoid compounds from the test material optimally. [23] This is proven by research by Olumese and Oboh (2016) that beetroot extract has a high flavonoid content of 96.67 mg QE/g compared to fresh beetroot which contains 63.34 mg QE/g. [24] Beetroot juice was prepared using blending technique. The beets were first peeled and sliced and weighed (in accordance with the chosen dose) to ease the subsequent smoothing process. In addition, fruit was blended without water addition to produce a thick liquid juice as a result.[25]

Male rats weighing 150-200 g were acclimated for 7 days to get used to the experimental environment. 8 weeks mature male Sprague Dawley rats were used in this experiment with average body weight 150 to 200 grams. For each group used 6 rats after calculating the sample size using the World Health Organization (WHO) method. In this study there were seven groups so the total number of samples in the experiment was 42 samples. Six rats were obtained in each group using a random sampling technique. Rats were classified into 7 different groups:
1. Group K0 (normal standard diet), given food and drink adlibitum
2. Group KN (negative control, HFD),

3. Group KP (positive control, HFD, and simvastatin 0.18 mg),
4. Group P1 (single dose treatment group, HFD, and beetroot extract 100 mg/200 gBW),
5. Group P2 (single dose treatment group, HFD and beetroot juice 3.6 ml/200 gBW),
6. Group P3 (combination treatment group, HFD, and beetroot extract 100 mg/200 gBW + simvastatin 0.18 mg),
7. Group P4 (combination treatment group, HFD, beetroot juice 3.6 ml/200 gBW + simvastatin 0.18 mg).

The composition of HFD given to rats was wheat 27.8%, cholesterol 2%, cholic acid 0.2%, and lard 10% with comfeed AD II. Dyslipidemic rats model were started by giving daily HFD in KN, KP, P1, P2, P3 and P4 groups for 28 days and continued feeding until day 56, whereas interventions were given from day 29 until day 56. The administration of beetroot extract or beetroot juice was conducted through a scheduled round at 9 am. Simvastatin was given one hour after the ingestion of beetroot extract or beetroot juice. The rat in each group was weighed once a week from day 1 until the last day of experiment.
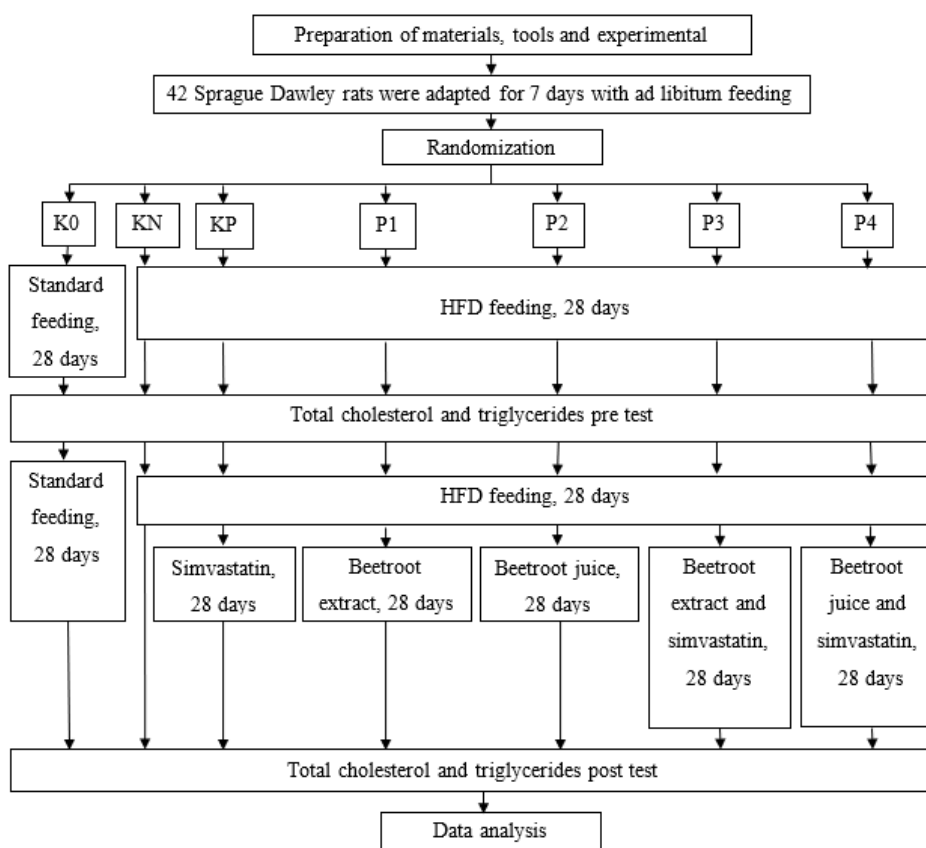

Figure 1. Animal Experimental Design

Blood drawn were done on day 28 and day 57, and rats were fasted for 10 hours and given only water. Blood were collected from orbital vein using a micro hematocrit approximately 2 ml, and transferred to a microtube and were left for coagulation process and followed by centrifugation for 10 minutes at 1620 RPM. Furthermore, serum was collected from each tube and tested for cholesterol total and triglyceride.

Cholesterol and triglyceride level was examined using a Microlab 300 spectrophotometer with the cholesterol oxidase-paminophenazone (CHOD-PAP) enzymatic photometric method. The principle of the CHOD-PAP method is hydrolyzation of cholesterol in its ester form by the cholesterol esterase enzyme into cholesterol and free fatty acids. Cholesterol will be oxidized to hydrogen peroxide with presence of the cholesterol oxidase enzyme, then hydrogen peroxide will convert 4-aminoantipyrine and phenol by catalase peroxidase enzyme into quinoneimine which is colored and color intensity will be measured photometrically. Triglyceride was evaluated with an enzymatic colorimetric method utilizing glycerol-3-phosphate-oxidase

(GPO). GPO method is used for estimating fatty oil level, fatty substances are hydrolyzed into glycerol and free unsaturated fats by the activity of lipase compounds. The free glycerol will be phosphorylated by adenosine triphosphate in the presence of glycerol kinase and later become glycerol-3-phosphate. Glycerol-3-phospate is oxidized by molecular oxygen in the presence of GPO to produce dihydroxyacetone phosphate and hydrogen peroxide. Hydrogen peroxide will react with 4-aminoantipyrine and chlorophenol with the help of the enzyme catalase peroxidase to form a colored 4-0-benzoquinone-monoamine complex and the absorbance intensity can be measured photometrically. Normal range of cholesterol total and triglyceride levels in Sprague Dawley rats at 13-22 weeks are 55-89 mg/dL and 62-92 mg/dL respectively.[26]

An analysis of the data was conducted using SPSS software. Statistical analysis of the data was performed using a paired t-test and one way ANOVA, followed by the LSD method for determining the differences between groups. The level of significance was established at a threshold of $p < 0.05$. The experiment was given ethical approval with number 44/UN27.06.11/KEP/EC/2023 from the Health Research Ethics Commission (KEPK) Faculty of Medicine Universitas Sebelas Maret.

RESULTS

After feeding HFD on rats in groups KN, KP, P1, P2, P3 and P4 for 28 days before giving intervention, indeed the rats had gain weight as compared to K0 group as shown in Figure 2.
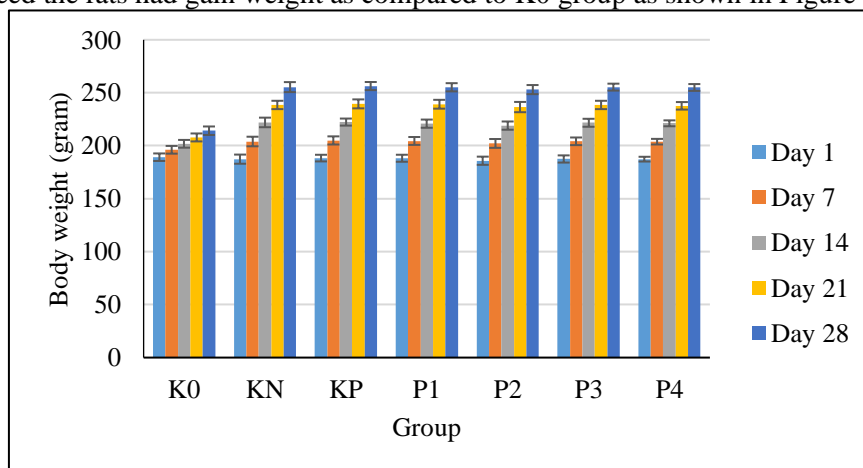

Figure 2. Rats Body Weight after 28 Days Feeding with HFD

Day 29 rats were given intervention and their body weight were evaluated weekly, from Figure 3, it was shown that 28 days intervention has made rats were slower in gaining weight in P1,P2, P3 and P4 groups than in KN group, and comparable weight with KP group.
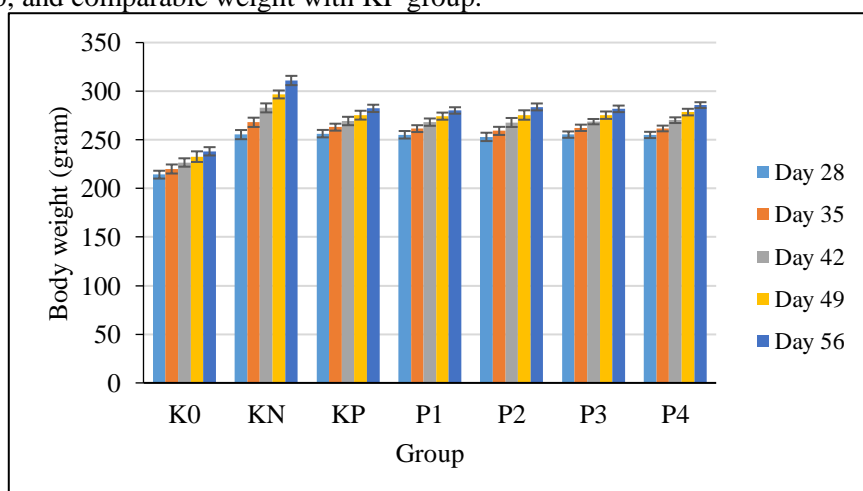

Figure 3. Rats Body Weight after 56 Days Feeding with HFD and Having 28 Days Intervention

The results showed that cholesterol level before and after intervention for 28 days had a significant difference (p <0.05). Based on Table 1, all treatment groups experienced a decrease in the average of cholesterol total except for groups K0 and KN. Treatment in groups P1, P2, P3, and P4 after dyslipidemic rats established for 28 days showed a decreasing cholesterol level in those groups when compared with its level before the intervention (Table 1). Cholesterol reduction is sorted from largest to smallest, namely in the P3 group at 50.32%, P4 at 37.45%, P1 at 29.93%, and P2 at 29.86%. The biggest decrease level was in the P3 group (50.32%), which was greater when compared to the KP group (41.54%) which were only given simvastatin. The average decrease in the P3 group was not much different when compared to the KP (simvastatin 0.18 mg/200 gBW) with a difference of -79.70 ± 3.76 mg/dL.

Table 1. Cholesterol Levels Before and After The Intervention of Beetroot Extract and Juice in Single Dose or Combination with Simvastatin

| Group | Mean (mg/dL) ± SD | | | p value |
	Pre Test	Post Test	Δ	
K0	85.11±2.01	91.18±2,13	6.07±2.95	0.004[x*]
KN	192.32±3.32	195.72±2,36	3.40±1.67	0.004[x*]
KP	191.87±5.15	112.16±2,75	-79.70±3.76	0.000[x*]
P1	183.16±3.60	128.34±2,74	-54.81±4.71	0.000[x*]
P2	188.54±5.61	132.23±6,09	-56.31±11.49	0.000[x*]
P3	187.17±3.92	92.97±2,00	-94.19±4.08	0.000[x*]
P4	184.53±1.66	115.42±7,54	-69.11±9.00	0.000[x*]
p value	0.000[y*]	0.000[y*]	0.000[z*]	

Description: K0: Normal Control (Healthy Rat); KN: Negative Control (HFD Rat); KP: Positive Control (HFD + simvastatin 0.18 mg); P1: HFD + beetroot extract (100 mg/200 gBB); P2: HFD + beetroot juice (3.6 ml/200 gBB); P3: HFD + beetroot extract (100 mg/200 gBW) + simvastatin 0.18 mg); P4: HFD + beetroot juice (3.6 ml/200 gBW) + simvastatin (0.18 mg); [x]) paired t-test (p <0.05); [y]) one way Anova test (p < 0.05); [z]) Kruskal Wallis test (p <0.05) ; [*]) There are significant differences.

The cholesterol total level after intervention showed significantly different results (p <0.05) due to variations in treatment. From the LSD Post Hoc analysis indicated that there was no statistically significant difference (p> 0.05) seen between the KP group (simvastatin 0.18 mg/200 gBW) to P4 (BJ 3.6 ml/200 gBW + simvastatin 0.18 mg), P1 (BE 100 mg/200 gBW) against P2 (BJ 3.6 ml/200 gBW), and P3 (BE 100 mg/200 gBW + simvastatin 0.18 mg) against K0 (healthy rat).

The results of the paired t-test for triglyceride level in all treatment groups in Table 2 showed significant results, there was a mean difference before and after intervention for 28 days in each group (p <0.05). The reduction in triglycerides was sorted from largest to smallest, namely in the P3 group at 40.45%, P4 at 34.12%, P1 at 31.53%, and P2 at 22.71%. The mean reduction in triglyceride levels was highest in the P3 group (BE 100 mg/200 gBW + simvastatin 0.18 mg) with a reduction difference of -54.28 ± 6.93 mg/dL. The average decrease in the P3 group was in a comparable level to the positive control (simvastatin 0.18 mg/200 gBW) with a difference of -46.68 ± 5.21 mg/dL.

Table 2. Triglyceride Levels Before and After The Intervention of Beetroot Extract and Juice in Single Dose or Combination with Simvastatin

| Group | Mean (mg/dL) ± SD | | | p value |
	Pre Test	Post Test	Δ	
K0	69.09±4.58	71.03±4.24	1.93±0.66	0.001[x*]
KN	131.87±4.65	133.70±4.70	1.83±0.55	0.000[x*]
KP	133.82±3.01	87.14±3.30	-46.68±5.21	0.000[x*]
P1	137.23±1.66	93.93±3.44	-43.28±2.89	0.000[x*]
P2	135.52±2.94	104.74±3.34	-30.78±4.96	0.000[x*]
P3	134.18±3.00	79.90±4.77	-54.28±6.93	0.000[x*]
P4	135.89±1.69	89.51±1.81	-46.37±2.12	0.000[x*]
p value	0.000[y*]	0.000[y*]	0.000[z*]	

Description: K0: Normal Control (Healthy Rat); KN: Negative Control (HFD Rat); KP: Positive Control (HFD + simvastatin 0.18 mg); P1: HFD + beetroot extract (100 mg/200 gBB); P2: HFD + beetroot juice (3.6 ml/200 gBB); P3: HFD + beetroot extract (100 mg/200 gBW) + simvastatin 0.18 mg); P4: HFD + beetroot juice (3.6 ml/200 gBW) +

simvastatin (0.18 mg); [x]) paired t-test (p <0.05); [y]) one way Anova test (p < 0.05); [z]) Kruskal Wallis test (p <0.05) ; *) There are significant differences.

Evaluation of triglyceride levels before and after the intervention in all groups was calculated using the one way Anova test. After intervention, the triglyceride levels showed significantly different (p <0.05) due to variations in treatment. From LSD Post Hoc analysis revealed that there was no significant difference (p> 0.05) in the KP group (simvastatin 0.18 mg/200 gBW) to P4 (beetroot juice 3.6 ml/200 gBW + simvastatin 0.18 mg), P1 (beetroot extract 100 mg/200 gBW) against P2 (beetroot juice 3.6 ml/200 gBW), and P3 (beetroot extract 100 mg/200 gBW + simvastatin 0.18 mg) against K0 (healthy rat).

DISCUSSION

HFD induction can increase cholesterol and triglyceride levels in all dyslipidemic groups. Pork fat can increase triglyceride higher than vegetable fat. This is probably due to the high content of palmitic acid in lard.[27] When palmitic acid is consumed through food, palmitic acid will be broken down by lipase enzymes into free fatty acids. These free fatty acids are then absorbed by intestinal cells and enter the bloodstream. In the bloodstream, palmitic acid will be transported by lipoproteins, to adipose tissue and liver. In adipose tissue, palmitic acid can be stored in the form of triglycerides as an energy reserve. Excessive palmitic acid can increase the amount of triglyceride in adipose tissue and liver.[28] The composition of cholic acid in HFD can increase the absorption of cholesterol from food sources in the intestine and suppress the action of the cytochrome p450 family 7 subfamily A member 1 (CYP7A1) enzymes therefore cholesterol was raised up in the liver.[29] Maegawa (2022) found that giving cholic acid supplements of 0.5 g/kg to rats for 4 weeks can increase cholesterol in the liver.[30] Lard contains large amounts of saturated fatty acids,[31] hence rat given HFD containing lard will have more calories and increased body weight.[32] As lard contains long-chain fatty acids such as oleic and linoleic, it makes the taste better and increases appetite.[33]

The rats treated with beetroot extract and juice had lower cholesterol and triglyceride level than the group was only given HFD feed without medication or intervention. It showed that the flavonoid, fiber, and betalain content of beetroot in extract and juice could decrease cholesterol and triglyceride level in dyslipidemic rat model. Cholesterol and triglyceride in the intervention beetroot extract combination with simvastatin were the lowest compared to the other three groups of rats. Such lower levels, presumably due to the active substance in the extract namely betalain and flavonoid. Betalain as an antiradical ingredient is capable of being an efficient ROS scavenger. Betalain's excellent antioxidant activity is due to its unique molecule as reflected in its ability to donate hydrogen to reacting species so as to delay or prevent lipid oxidation.[10] Flavonoid which function to inhibit the activity of the cholesterol-forming enzyme, namely HMG-CoA reductase, so that the formation of mevalonate from HMG-CoA decreases, are higher than in juice more effective in decreasing cholesterol and triglyceride. This is related to the impact of simvastatin which has a synergistic interaction on beetroot. This observation aligned with previous research, indicating that the administration of beetroot ethanol extract, in combination with a dosage of 0.18 mg of simvastatin, exerted an impact on cholesterol reduction in rats subjected to an atherogenic diet.[34] Apart from that, it is also possible that the juice method using a kinetic rate can cause the degradation of betalain and flavonoid compounds more quickly than the extraction method, resulting in the group of rat in the juice intervention having less than optimal betalain and flavonoid performance.[35]

It is important to consider several limitations when interpret the above mentioned results. The reduction in cholesterol and triglyceride levels observed in the dyslipidemic rat model can not be associated with the presence of flavonoids alone, but also to the presence of other active compounds that were not tested. Furthermore, further research is required to determine the molecular mechanism by which beetroot's activity in reducing dyslipidemia, and establish optimal dosages for human consumption.

CONCLUSION

Giving beetroot extract and juice as single or combined with simvastatin have effect on reducing cholesterol and triglyceride levels and these have different effectiveness, these indicated their potential for prevention and therapeutic in dyslipidemia. Administration of beetroot extract 100 mg/200 g BW with a combination of 0.18 mg simvastatin for 28 days provided the most effective effect in reducing cholesterol and triglyceride levels in dyslipidemic rat models.

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