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The effects of ethanolic extract of green algae (ulva lactuca l.) on blood cholesterol levels in male rats induced by a high fat diet

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ABSTRACT

Background: Nowadays, cardiovascular disease is increasing in number each year. It is triggered by high cholesterol diet consumed by urban people. Green algae (Ulva lactuca L.) contains phytomelatonin levels that can ease cholesterol.

Objective: To know about the effect of giving green algae ethanolic extract in male strain Wistar mice's cholesterol level.

Methods: This research involved 36 male strain Wistar mice divided to six groups. Negative control injected with 2 ml/200 gBW lard, positive control injected with 0,18 mg/200 gBW simvastatin and lard, group III, IV, V each given lard and injected with ethanolic extract of green algae at a dose of 50 mg/kgBW, 100 mg/kgBW and 200 mg/kgBW respectively, and normal control given AD 2 dietary treatment. Each group was given the same treatment peroral in 56 days and the blood was taken every 14 days through sinus orbitalis. The blood cholesterol level was analyzed by using enzymatic CHOD-PAP method. Data obtained were statistically examined using Two way ANOVA , Kolmogorov Smirnov, Levene's method and LSD with confidence level at 95%.

Results: The blood cholesterol level were not significanly different between the negative and normal control. The result of cholesterol level for 14, 28, and 42 days for all group did not increase compared to negative control, but cholesterol level in group with 200 mg/kgBW dose extract indicated significant difference with negative control for 56 day.

Conclusion: Giving ethanolic extract of green algae for 56 days decreased blood cholesterol level in male rats.

Latar Belakang: *Penyakit kardiovaskuler mengalami peningkatan setiap tahunnya, hal ini disebabkan karena kebiasaan masyarakat dalam mengkonsumsi makanan yang tinggi kandungan kolesterol. Ulva lactuca L. mengandung phytomelatonin yang dapat digunakan sebagai penurun kadar kolesterol dalam tubuh.*

Tujuan: *Untuk mengetahui efek pemberian ekstrak etanol Ulva lactuca L. terhadap kadar kolesterol pada tikus putih jantan galur Wistar.*

Metode: *Penelitian ini menggunakan 36 ekor tikus putih jantan galur Wistar yang terbagi menjadi 6 kelompok. Kontrol negatif diberi diet lemak babi 2 ml/200 gBB, kontrol positif diberi simvastatin dengan dosis 0,18 mg/200 gBB dan diet lemak babi, perlakuan I, II, III masing-masing diberi diet lemak babi serta ekstrak etanol Ulva lactuca L. dengan dosis 50 mg/kgBB, 100 mg/kgBB, dan 200 mg/kgBB, kontrol normal diberi pakan AD 2. Semua kelompok diberi perlakuan selama 56 hari secara peroral dan setiap 14 hari sekali darah hewan uji diambil melalui sinus orbitalis. Darah dianalisis kadar kolesterolnya dengan metode enzimatik CHOD-PAP. Data yang didapat diuji statistik dengan Two way ANOVA, Kolmogorov smirnov, uji levene dan LSD dengan taraf kepercayaan 95%.*

Hasil: *Data kadar kolesterol menunjukkan adanya perbedaan yang signifikan antara kontrol negatif dengan kontrol normal. Data hasil kadar kolesterol pada hari ke-14, 28 dan 42 untuk semua perlakuan belum menunjukkan perbedaan yang signifikan terhadap kontrol negatif, sedangkan pada perlakuan ekstrak dosis 200 mg/kgBB menunjukkan adanya perbedaan yang signifikan dengan kontrol negatif pada hari ke-56.*

Kesimpulan: *pemberian diet lemak babi selama 14 hari sudah dapat meningkatkan kadar kolesterol darah dan pemberian ekstrak etanol Ulva lactuca L. dosis 200 mg/kgBB selama 56 hari dapat menurunkan kadar kolesterol darah.*

INTRODUCTION

Hypercholesterolemia is a condition where the blood cholesterol concentration increased. If blood cholesterol concentration exceeds 200 mg/dL, deposits could form in the vascular endothelial cells causing obstruction or atherosclerosis.[1] One way to prevent or decrease the incidence of atherosclerosis is to use antioxidant.[2]

Green algae (Ulva lactuca L.) contain compounds like carbohydrate, protein, vitamin B, vitamin B1, vitamin C, vitamin E, and vitamin B9, amino acids, zinc, iron, fiber and pigment that contain a few lipids.[3-5] Green algae also contain antioxidant compound, which is phytomelatonin.[6] According to Kolar and Machackova[7] phytomelatonin concentration in green algae is 240µg/kg wet weight.

Phytomelatonin (N-acetyl-5-methoxytryptamine) has a lot of benefits, including: decreasing ischaemic reperfusion injury of the myocard due to its antioxidant properties,[8] decreasing oxidative stress,[9] preventing brain haemorrhage, decreasing total cholesterol and LDL,[10] as well as antihypertensive.[11]

The consumption of antioxidant b-caroten, vitamin C, vitamin E and flavonoid from food would counter-balance the effect of free radicals from oxidative stress, which would affect lipid profile.[12] Due to the prescence of phytomelatonin antioxidant activity in the green algae, a study to determine the effect of ethanolic extract of green algae (*Ulva lactuca L.*) on blood cholesterol levels in male 1,5 months old Wistar rats induced with 56 days pork fat diet is needed. The inducement of pork fat diet in this study is considered because pork fat contain more saturated fat compared with another form of fat, thus it is hoped to increase the concentration of blood cholesterol in rat models. The study was done for 56 days because previous research showed that 8 weeks inducement of high-fat diet in animal models were able to induce obesity.[13]

METHODS

Ingredients collection and Extract production

The sample used in this study was green algae (Ulva lactuca L.) taken from Pantai Drini Kabupaten Gunungkidul on 22 January 2015. Green algae extraction was done by maceration. After 70% ethanolic extract of Green algae was gained, the thicker extract was made by evaporating ethanol using rotary evaporator at 40°C.

Phytomelatonin Identification on *Ulva lactuca L.*

Identification was preceded by preliminary test to determine the presence of melatonin which is a type of alkaloid. The powder of *Ulva Lactuca L.* was measured until ± 200 mg, then it was tested with Dragendorf and Mayer reactan. Ethanolic extract of *Ulva Lactuca L.* was dissolved in HCl 2 N, it was considered positive of containing alkaloids if a yellowish brown sediment formed after being reacted with Mayer reactant and orange-brown sediment formed after being reacted with Dragendorf reactant.[14]

Second identification was done by Thin Layer chromatography (TLC). In this stage, purification of *phytomelatonin* in ethanolic extract of *Ulva lactuca L.* by measuring the ethanolic extract of *Ulva Lactuca L.* until 500 mg and added 15 ml acetylic acid 15%. The solvent was strained, then the filtrate was extracted with 50 ml ether petroleum for 3 times. The acetylic acid extract was added with NH_4OH until the pH became 10. Then the solvent was extracted with 50 ml acetylic acid twice. Ethyl acetate extract solvent

was vaporized until sediment was gained and it was re-dissolved in ethanol p.a solvent until 5,0 ml. Next, the result of purification was spotted 5 µl on silica gels plate F_{254}. Silica gel plate was then eluded with mobile phase of BAW (Butanol: Acetic Acid: Water = 12:3:5). *Phytomelatonin* was used as standard with 2 mg/ml concentration. TLC plate that had been eluded was dried and the spots were examined under UV 254.

Animal Models

This research used 36 male Wistar rats from Universitas Muhammadiyah Yogyakarta and was divided into 6 groups. Negative controls was given pork fat diets 2 ml/200 g BW, positive control was given simvastatin 0,18 mg/200 gBW and pork fat diet, intervention group I, II, III was each given pork fat diet as well as ethanolic extract of *Ulva lactuca L.* mg/kgBW, 100 mg/kgBW, and 200 mg/kgBW, normal control was given AD 2 woof. All groups were given 56 days of oral interventions and every 14 days their blood samples was taken from sinus orbitalis.

Total Cholesterol Test

Twenty four hours after intervention, the blood samples of animal models was taken from sinus orbitalis and stored in eppendorf. Blood was centrifuge for 10 minutes on 4000 rpm. Serum was taken 10µl then added with 1000 µl cholesterol reagent into the tube, and then incubated for 20 minutes in room temperature (24-30°C). Then, absorbance measurement was done in wave length 546 nm.

Data Analysis

Datas of blood cholesterol concentration measured every 14 days of every groups were statistically tested with two-way ANOVA, Kolmogorov Smirnov, levene test and LSD with Confidence Interval 95%.

RESULTS

Obtained extract was preliminary identified to determine the prescence of alkaloid compound, because *phytomelatonin* was classified as alkaloids. Identification showed that reddish

Figure 1 Identification of *phytomelatonin* in the etanholic extract of *Ulva lactuca L* (A)in Dragendrof reactant and (B) Mayer reactant

TLC was done to determine the presence of *phytomelatonin* in the extract by comparing Rf sample extract with Rf *phytomelatonin* standard. The result of TLC in stationary phase inside silica gel F_{254}, mobile phase of BAW (12:3:5) and UV 254 detector were Rf of sample extract and standars *Phytomelatonin* 0,67.

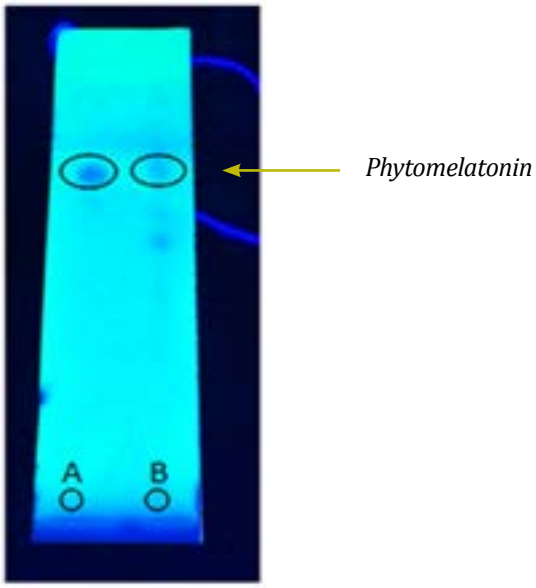

Figure 2 Results of Thin-layered chromatography (TLC) with stationery phase silica gel F_{254}, mobile phase BAW (12:3:5) and UV 254 detector,
A. Standard *phytomelatonin*, B. Ethanolic extract of *Ulva lactuca L.* Blood cholesterol concentration of male Wistar rats induced with ethanolic extract of Ulva lactuca L. and 56 days of pork fat diet, was shown in Table I.

Table 1 Results of Blood Cholesterol Concentration in 56 days

Day	Groups	Blood Cholesterol Concentration (mg/dl) Mean ± SD
14	Negative control	86,08±8,28
	Positive control	64,77±14,49*
	Intervention I	78,43±6,26
	Intervention II	78,85±8,89
	Intervention III	74,67±11,77
	Normal control	52,85±16,42*
28	Negative control	92,27±9,87
	Positive control	78,40±11,14*
	Intervention I	90,17±17,62
	Intervention II	80,57±7,00
	Intervention III	80,52±10,83
	Normal control	70,15±5,53*
42	Negative control	80,13±5,28
	Positive control	64,65±8,17*
	Intervention I	79,83±6,62
	Intervention II	69,98±8,99*
	Intervention III	66,88±7,92*
	Normal control	62,73±4,80*
56	Negative control	90.08±16.38
	Positive control	69.65±16.31*
	Intervention I	81.78±6.32
	Intervention II	76.53±7.55
	Intervention III	59.93±30.28*
	Normal control	69.5±3.77*

Note :* = p < 0,05 (statistically significant difference) compare to negative control

The results of obtained blood cholesterol concentration were analyzed statistically. Preceded by Kolmogorov-Smirnov test to determine normality of data distribution. P value of Kolmogorov-Smirnov test was 0,339 (p>0,05) which showed normal data distribution. Then, data homogenity was analyzed with Levene test. P value of Levene test was 0,191 (p>0,05) which showed homogen data variance. These results were further analyzed with parametric statistical test using two-way ANOVA with 95% confidence interval, resulting in statistically significant difference. To determine the specific difference of blood cholesterol concentration in each group, LSD (Least significant difference) test was done. The result of LSD test showed that Intervention 3 group, which was given ethanolic extract of green algae 200 mg/kgBW for 56 days, were already able to decrease blood cholesterol concentration. Statistically significant difference with negative control was obtained. In the measurement of the increased in blood cholesterol concentration, it was seen that the induction of pork fat diet for 14 days had been able to increase total blood cholesterol concentration.

DISCUSSION

This research found that pork fat diet were able to increase blood cholesterol concentration, as seen in LSD test, in which statistically significant difference was seen between negative control and normal control. This result might be due to high saturated fat contained in pork fat which would cause hyperlipidemia, including hypercholesterolemia. In chronic hyperlipidemia, LDL would accumulate, which could be oxidized due to the impact of free radicals.[15]

In positive control, which was given simvastatin, we found 22,68% reduction of blood cholesterol concentration on day 56th. This might be because simvastatin is an anti-hyperchoesterolemia Statin agents. Simvastatin lowered blood cholesterol concentration by inhibiting 3-hydroxy-3-methyl glutaryl co-enzyme A Reduktase (HMG Co-A Reduktase), which is an enzyme that catalyze HMG Co-A into mevalonic acids. It works by inhibiting cholesterol formation in the liver and increasing LDL excretion from blood circulation.[16]

The table showed no significant decrease of blood cholesterol concentration in day 56th in Intervention group I and II with dosage of 50 mg/kgBW and 100 mg/kgBW respectively. However in the dosage of 200 mg/kgBW, significantly blood cholesterol reduction as much as 33,47%, was seen on day 56th. This might be due to phytomelatonin content in green algae (Ulva lactuca L.) which act as antioxidant agents that could absorb free radicals by giving one of its electron through a reactive indol structure as well as methoxy groups and amide side

chain (N-C=O), so that it could prevent LDL modification oxidation. The decrease of blood cholesterol concentration might also be caused by the prescence of clorophil in the ethanolic extract of Ulva lactuca L., because clorophil also act as antioxidant.[17] According to Karimah[18] clorophil could decrease blood cholesterol concentration.

CONCLUSION

The administration of pork fat diet for 14 days were able to increase blood cholesterol concentration, and ethanolic extract of Ulva lactuca L. 200 mg/kg BW for 56 days were able to lower blood cholesterol concentration.

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