Black Grass Jelly (Mesona Palustris Bl) Effervescent Powder has Anti-Dyslipidemia in High Cholesterol Diet-Fed Rats and Antioxidant Activity

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ABSTRACT

Dyslipidemia that could be affected by hypercholesterol diet is a contributing factor in millions of deaths worldwide and a risk factor for ischemic heart disease and stroke. High soluble fiber diet is used as one of the non-pharmacological treatments to control hypercholesterolemia. Black Grass Jelly (Mesona palustris BL) also known as black cincau has a high soluble fiber content. This study aimed to investigate the effect of black cincau as effervescent powder form (BCEP) for anti-dyslipidemia and also the antioxidant activity. BCEP was orally administered to twenty male dyslipidemia Wistar rats induced with hypercholesterol diet for 3 weeks then plasma lipid profile was measured using enzymatic CHOD-PAP assay. The antioxidant activity also has been identified. Oral administration of 3 different doses of BCEP resulted in lipid profiles by decreasing plasma cholesterol (TC), triacylglycerol (TG), low-density lipoprotein (LDL), and also high-density lipoprotein (HDL) in dyslipidemia rats (α = 0.01). The increasing doses of BCEP revealed that it has a potential effect of decreasing plasma lipid.

Keywords : Black Grass Jelly; Mesona palustris BL; Effervescent Powder; Dyslipidemia.

INTRODUCTION

The term dyslipidemia is used to indicate the presence of one or in a combination of the increased concentration of the following abnormalities serum of TC (total cholesterol) or LDL (low-density lipid) or TG (triglycerides), or decreased concentration of HDL (Sarat Chandra et al., 2014). Dyslipidemia occurs when one of these conditions (total cholesterol ≥240 mg/dl, LDL ≥160 mg/dl, triglycerides ≥200 mg/dl, HDL <40 mg/dl) happen (Bays et al., 2013). Increasing cholesterol causes 2.6 million deaths (4.5%) and 29.7 million disability-adjusted life years (DALYS), accounting for 2% of total DALYS. As a risk factor for ischemic heart disease and stroke, elevated total cholesterol is a major cause of diseases in both developed and developing countries (WHO, 2015).

Dietary fiber is a part of the plant organs that is resistant to be digested by human enzymes and consists of hemicellulose, cellulose, lignin, oligosaccharides, pectin, gums, and waxes. Soluble fiber is one of the basic fiber categories that can delay time transit through the intestine (Dhingra, Michael, Rajput, & Patil, 2012). Today, diets high in soluble fiber represent increasing demand to control hypercholesterolemia. Black grass jelly (Mesona palustris BL), or black cincau, has a high concentration insoluble fiber. High soluble fiber has been proven to lower lipid profiles (Ramos et al., 2011). Black grass jelly effervescent powder (BCRP) was developed due to its popularity as beverages for a human.

The Indonesian Nutrition Directorate Health Department revealed that there are

6.23 grams of crude fiber per 100 grams of grass jelly gel. Therefore, if black grass jelly is consumed daily concurrently with fruits and vegetables, it helps to fulfill personal needs in daily fiber (30 g) and is useful to prevent degenerative conditions such as coronary cardiac disease. Black grass jelly contains 122 calories and 6 grams protein (per 100 grams), and it is beneficial if consumed daily due to its high fiber and low-calorie composition (Wahyono, Fitriani, & Widyaningsih, 2015). Not only does it contain fiber, the black grass jelly also contains active polyphenol. This component has been reported to prevent DNA damage in human lymphocytes when it is exposed to free radicals such as hydrogen peroxide and UV radiation. Black grass jelly extract has high antioxidant activity due to its phenol content (Tasia & Widyaningsih, 2014).

Rats have been reported to mimic human metabolism in dyslipidemia after being induced with hypercholesterol diet (Leong, Ng, & Jaarin, 2015). Using rats as animal model induced with hypercholesterol diet and treated with BCEP is an essential step before the application on a clinical trial. This research was the first study that assessed the ability of black grass jelly as BCEP for functional drinking to prevent dyslipidemia in dyslipidemia rat as an animal model.

MATERIALS AND METHODS

Twenty male Wistar rats were divided randomly into five groups (n=4). A negative control (C-) group was given a normal diet. Positive control (C+) and treatment groups were given hypercholesterol diets (HCD) with an added cocktail developed by Dhulavasant et al.: mixing of cholesterol (2 g) and cholic acid (1 g) in 20 ml of coconut oil supplemented with 1 ml of duck egg yolk (Widyaningsih & Adilaras, 2013). Treatment groups were administered BCP by gavage in low (LD-B, 126), medium (MD-B, 252), and high (HD-B, 378) (mg/day) doses every morning at 08.00 am for 3 weeks. The Animal Ethics Committee approved all experimental procedures at Brawijaya University, 181-KEP-UB.

Black Grass Jelly Effervescent Powder Preparation.

The effervescent powder was prepared using a formulation developed from previous research (Widyaningsih & Adilaras, 2013) with some modifications (Table 1). Black Grass jelly Effervescent Powder (BCRP) was prepared by the Laboratory of Food Technology, University of Brawijaya.

The formulation in Table 1 was analyzed for its physicochemical properties. These properties include antioxidant activity IC50, total phenols, water content, crude fiber content, flow time, repose angle, compressibility index, color L*, a*, b*, dissolving time, organoleptic test, and effectivity index.

Table 1. Effervescent Powder Formulation

Ingredients	Percentage (%)
Black Grass jelly Jelly Powder + Pandanus	45
Citric Acid	12
Tartaric Acid	6
Sodium Bicarbonate	12
Stevia	5
PVP	2
Total	**100**

BCRP Dose Measurement.

The given dose was converted from a human dose to rat dose. A low dose of BCRP in humans is 7 g/day/70 kg BW, equivalent to 126 mg/day/200 g BW in rats. A medium dose is double the low dose (252 mg/day/200 g BW in rats), and a high dose is 3 times the low dose (378 mg/day/200 g BW in rats). BCRP in rats was given via oral administration after dissolving in 2 ml distilled water.

Plasma Lipid Profile Measurement.

Serum was collected from sinus orbitals at baseline (week 0) and the end of the study (week 3rd). Serum was collected and centrifuged at 3000 rpm at 22°C for 25 minutes. Concentration of total cholesterol (TC) and triglycerides (TGs) in serum were analyzed using an enzymatic CHOD-PAP assay. Phosphotungstic acid and magnesium chloride were added to each sample to condense chylomicron, very low-density lipoprotein (VLDL), and LDL. HDL precipitant was analyzed with DiaSys, Germany.

Statistical Analysis.

Plasma lipids were analyzed and expressed as mean and standard errors. Statistical analysis was performed using SPSS software (version 17.0, SPSS Inc, Chicago, IL, USA). Comparisons were performed using a one-way ANOVA test, followed by Fisher Least Significant Difference (LSD), to detect differences between multiple groups. P<0.01 was considered statistically significant.

RESULTS

The Physicochemical Properties of Black Grass Jelly Effervescent Powder.

The physiochemical properties of Black Grass Jelly Effervescent Powder such as flow time, repose angle, compression index, color (L*, a*, b*), and dissolving time were measured. Flow time and repose angle were measured to know effervescent flow characteristic. Flow time and a repose angle of BCEP were 9.1 seconds and 29.5°, respectively. Compression index of BCEP was 21.33%. Color (L*, a*, b*) of BCEP in this research were 45.5, 17.9, 12.76, respectively. Dissolving time for BCEP in this research was 170 seconds. Antioxidant Activity IC50 and total phenols were 63.33 ppm and 64.92 ppm, respectively. Meanwhile, water content and crude fiber content were 3.37%, and 1.360%, respectively. The physicochemical properties of black grass jelly effervescent powder are shown in Table 2.

Table 2. Physicochemical Properties and Organoleptic Analysis Results from Black Grass Jelly Effervescent Powder

Parameter	Formula 2
Antioxidant Activity IC_{50}	63.33 ppm
Phenol Total	64.92 ppm
Water Content	3.37 %
Crude Fiber Content	1.360 %
Flow Time	9.1 seconds
Response Angle	29.5°
Compressibility Index	21.33 %
Color L*	45.5
Color a*	17.9
Color b*	12.76
Dissolving Time	170 seconds

Triglyceride Plasma.

There was a significant difference that was observed between positive control groups and the 3 treatment groups that were administered with three different doses of effervescent powder. Therefore, black grass jelly effervescent powder decreased plasma lipid profile, especially by decreasing triglyceride levels (Table 3).

Table 3. Rat Triglyceride Percentage with Black Grass Jelly Effervescent Powder Administration

Experimental Group	Triglyceride Level (mg/dl)		
	Week-0	Week-3	Mean change (%)
Positive control group	158.74	184.82	+18.09[a *]
Negative control group	120.17	146.72	+16.42[a]
Preventive dose 1	229.18	118.27	-17.89[b]
Preventive dose 2	242.89	117.25	-48.39[c]
Preventive dose 3	139.72	114.27	-51.72[c]

*Note= (+) The increasing of plasma triglyceride
Different notation shows a significant difference using LSD test (α=0,01)
*Week 0 = Week before treatment + induction (normal rats).

Total Cholesterol Plasma.

LSD test revealed that black grass jelly effervescent powder have a significant effect (α=0.01) in decreasing total cholesterol plasma percentage. Effervescent administration with preventive dose 1 (126 mg/200 g BW) was effectively decreasing plasma cholesterol levels by 5.28%; dose 2 (252 mg/200 g BW), 11.70%; dose 3 (378 mg/200 g BW), 18.42% (Table 4).

Table 4. Rat Total Cholesterol Percentage with Black Grass Jelly Effervescent Powder Administration

Experimental Group	Total Cholesterol Plasma Level (mg/dl)		
	Week-0	Week-3	Mean change (%)
Positive control group	144.33	386.67	+62.67[a] *
Negative control group	119.66	127	+6.12[b]
Preventive dose 1	149.66	141.74	-5.28[c]
Preventive dose 2	143.66	128.17	-11.70[d]
Preventive dose 3	152	124	-18.42[e]

*Note= (+) The increasing of plasma triglyceride
Different notation shows a significant difference using LSD test (α=0,01)
*Week 0= Week before treatment + induction (normal rats).

High-Density Lipoprotein Plasma.

LSD test also revealed that black grass jelly effervescent powder have a significant effect in decreasing HDL plasma percentages (α=0.01). Hypercholesterolemia diet was given to each group in conjunction with effervescent powder can change HDL cholesterol levels. Effervescent administration to rats of preventive dose 1 effectively decreased HDL levels by 22.25%; dose 2, 12.47%; dose 3, 21.30% (Table 5).

Low-Density Lipoprotein Plasma.

There was a significant difference between the negative and positive control groups. This may have arisen through the use of manual formula instead of reagent. LDL levels in the positive control group was increased by 20.5% and by 11.29% in the negative control group. There were significant differences between rats in each treatment group. This influenced the reduction in LDL levels, where dose 1 lowered LDL cholesterol by 27.35%, dose 2 by 19,16%, and dose 3 by 6,57%. The highest reduction in LDL levels was observed at dose 2 (Table 6).

Table 5. Rat High-Density Lipoprotein Percentage with Black Grass Jelly Effervescent Powder Administration

Experimental Group	High-Density Lipoprotein Level (mg/dl)		
	Week-0	Week-3	Mean change (%)
Positive control group	83.42	16.28	-80.74[e] *
Negative control group	23.57	40.85	+42.30[a]
Preventive dose 1	86.14	66.71	-22.55[d]
Preventive dose 2	79	69.14	-12.47[c]
Preventive dose 3	92.71	76.42	-21.30[b]

*Note= (+) The increasing of plasma triglyceride
Different notation shows a significant difference using LSD test (α=0,01)
*Week 0= Week before treatment + induction (normal rats).

Table 6. Rat Low-Density Lipoprotein Percentage with Black Grass Jelly Effervescent Powder Administration

Experimental Group	Low Density Lipoprotein Level (mg/dl)		
	Week-0	Week-3	Mean change (%)
Positive control group	89.83	274.04	+205[a] *
Negative control group	143.58	159.80	+11.29[b]
Preventive dose 1	76.31	55.44	-27.35[d]
Preventive dose 2	89.03	71.97	-19.16[e]
Preventive dose 3	105.47	98.53	-6.57[c]

*Note= (+) The increasing of plasma triglyceride
Different notation shows a significant difference using LSD test (α=0,01)
*Week 0= Week before treatment + induction (normal rats).

DISCUSSION

In this research, the antioxidant IC50 of black grass jelly effervescent powder was 63,33 ppm. A compound is considered as a potent antioxidant if the IC50 value is less than 50 ppm. It is considered strong if the IC50 falls between 50–100 ppm; moderate, 100-150 ppm; weak, 150–200 ppm. It is known that compounds with smaller IC50 values have a higher antioxidant activity (Molyneux, 2004). Based on the work of Widyaningsih et al. 2013, the IC50 values of ethanolic and water extracts are 49,92 ppm ± 1,88 ppm and 66,67 ± 2,54 ppm, respectively (Widyaningsih & Adilaras, 2013). Furthermore, it has been shown that black grass jelly powder water extract based on freeze-drying results in 86,892% antioxidant activity. The antioxidant activity of black grass jelly at the concentration of 50 mg/ml (98.9%) is stronger than that of 50 mg/ml of α-tocopherol (78%) (Hung & Yen, 2002). Based on this, it can be said that black grass jelly effervescent powder has high antioxidant activity.

Fiber content test of black grass jelly effervescent powder revealed the fiber content of 1,33 g/100 grams. This result is different from research conducted by the Indonesian Nutrition Directorate Health Department (2006), which showed that 100 grams of grass jelly gel contain 6,23 grams crude fiber. This difference might be the result of variations in cellulose and crude fiber in each formula due to the addition of acidic chemicals such as citric acid and tartaric acid in the processing of black grass jelly effervescent powder. Crude fiber is susceptible to acid hydrolysis, and this susceptibility could result in a difference between formulas (Qingxiang, 2002).

This research shows that administration of black grass jelly effervescent powder lower plasma TC, TGs, LDL-C, and HDL-C levels. This improvement in the lipid profile can be caused by its fiber and antioxidant content. Antioxidants are effective against lipid peroxidation, a process that leads to lipid oxidative degradation. In lipid peroxidation, free radicals release electrons from lipids in the cell membrane, leading to damage (Repetto, Semprine, & Boveris, 2012). Research focusing on effect of black grass is scarce, especially in relation to its role in dyslipidemia. The antioxidative properties of black grass jelly have been shown in other research (Adisakwattana, Thilavech, & Chusak, 2014; Chusak, Thilavech, & Adisakwattana, 2014).

Black grass jelly can improve antioxidant status due to their phenolic compounds (Chusak et al., 2014; Lih-Shiuh Lai, Su-Tze Chou, & Chao, 2001). Other studies have shown that black grass jelly prevents AGE (Advanced Glycation End Products) formation and protein oxidation, processes implicated in diabetes (Adisakwattana et al., 2014). Research focusing on black grass jelly has shown that it can decrease lipid peroxidation with malondialdehyde (MDA) concentration (Lih-Shiuh Lai et al., 2001). In this research, we examined the lipid profile instead of MDA concentrations. Protection against lipid peroxidation might be attributed to the ability of black grass jelly to donate protons. It can also be viewed as an electron donator reacting with free radicals and rendering them more stable (Lih-Shiuh Lai et al., 2001). The decrease in lipid peroxidation can decrease lipid profile. This result was observed here, where rat lipid profiles (TGs, TC, LDL-C) decreased after black grass jelly effervescent powder administration. Lipid profile were decreased might be caused by antioxidant properties in black grass jelly.

However, HDL-C level was also decreased in this research. This might be caused by natural characteristic from rats. Rat is an animal model that is resistant to LDL and lipoprotein increase, especially HDL because rat plasma is predominantly HDL-C. Rat HDL is the main transporter of plasma cholesterol (60-80% of total cholesterol). Most of cholesterol in plasma rats were transported by HDL-C, therefore the decreasing cholesterol level also decrease HDL-C level (Pisulewski, P.M. Kopec, A. Cieslik, 2002).

Black grass jelly effervescent powder can decrease cholesterol, triglyceride, and LDL-C plasma levels possibly through its soluble fiber content. Three primary biological mechanisms have been proposed to explain the cholesterol-reducing effects of dietary soluble fiber. First, prevention of bile salt re-absorption in the small intestine leading to excessive fecal bile salt excretion. Second, the decreasing of glycemic response leading to decrease in hepatic cholesterol synthesis from insulin stimulation. Third, the physiological effects of soluble fiber fermentation products, especially propionate (Gunness & Gidley, 2010). From this research, it can be proposed that soluble fiber can reduce cholesterol, triglyceride, and LDL-C by preventing the re-absorption of bile salts from the small intestine as the second mechanism. The presence of soluble fiber in the small intestine has been shown to prevent some bile salt from being re-absorbed into enteropathic circulation, resulting in excess excretion of bile salt in the feces. This causes bile acid depletion in the liver, leading to the catabolization of cholesterol in hepatocytes via the activation of cholesterol 7α-hydroxylase. Furthermore, cholesteryl esters are metabolized, and production of LDL-C surface membrane receptors is increased to enhance the uptake of LDL-C from the bloodstream, thus lowering blood cholesterol concentrations (Gunness & Gidley, 2010).

Aside from fiber content, antioxidants can also lead to a decrease in cholesterol. Mesona palustris BL possesses a polyphenolic compound as an antioxidant. Zern et al. (2003) showed that polyphenolic treatment in guinea pigs, using grape powder, can reduce VLDL cholesterol and triglyceride by 50% and 39%, respectively, compared to that in a control group (Zern, West, & Fernandez, 2003). Furthermore, Auger et al. (2002) showed that golden Syrian hamsters fed red wine phenolics had a significant decrease in plasma apo B concentration (Auger et al., 2002). These animal studies suggest that polyphenol can reduce cholesterol absorption due to the interaction between these compounds and cholesterol carriers and transporters present across the brush border membrane (Zern & Fernandez, 2005). This study suggests that black grass jelly effervescent powder induces decreasing of plasma lipid (TC, TG, LDL, and HDL in rats given a high-cholesterol diet. This research showed, modification of black cincau as an effervescent form was not change the beneficial effect of the fiber and antioxidant content for improving dyslipidemia. In conclusion, the black grass jelly effervescent powder will be a potential functional drink for improving lipid profile in dyslipidemia. However it should be pass in a clinical trial.

CONCLUSIONS

The increasing doses of BCEP revealed that it has a potential effect of decreasing plasma lipid. HDL is the primary transporter of plasma cholesterol (60-80% of total cholesterol) in rats, given that the lowering plasma lipid is showed by decreasing TC, TG, LDLD, and HDL.

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