Antihypertensive Effects of Black Cincau (*Mesona palustris BL*) Effervescent Powder and Malondialdehyde Concentration on Wistar Rats as a Hypertensive Model

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ABSTRACT

This study was investigated the effects of Black cincau (*Mesona palustris BL*) effervescent powder (BCEP) on systolic blood pressure and malondialdehyde concentration in hypertensive rats. BCEP were orally administered to hypertensive in rats for 4 weeks, and antihypertensive effects were determined. Rats were given 630 mg/kg, 1.260 mg/kg and 1.890 mg/kg BCEP on dose group 1, 2, 3 respectively. These groups were compared with a negative control group, hypertensive control group and Captopril control group. The results showed that systolic blood pressure and MDA concentrations of rats dosed with BCEP were significantly different (α=0.01) to those of control rats. Rats dose third group with of 1.260 mg/kg showed the highest percentage of lowering blood pressure. The rat dose third group had highest percentage decreased in systolic blood pressure (43.97%) and in MDA concentration (68.77%).

Keyword: Antihypertensive; Black Cincau (*Mesona palustris BL*) Effervescent Powder; Malondialdehyde.

INTRODUCTION

Throughout the world hypertension is the most frequently encountered chronic disease and affects around one billion individuals. Hypertension is associated with many chronic conditions such as insulin resistance, obesity, carbohydrate intolerance, hyperurimia, atherosclerosis and cardio-vascular diseases (Whitworth JA, 2004).

Hypertension occurs if arterial diameter decreased or blood volume increased. Hypertension caused oxidative damage in cells with reaction between free radical and poly unsaturated fatty acid (PUFA) so the result unstable compounds such as Malondialdehyde (MDA) (Armas-Padilla MC, 2007). MDA is one of the oxidative damage parameters in body.

Clinically, various antihypertensive drugs such as hypotensive diuretics, beta blocking agents, calcium antagonist, angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists and alpha-receptor blocking agents have been used to manage hypertension and alleviate symptoms. However, the efficacy of these drugs is only 40-60%, and usually, two or more antihypertensive drugs from different categories need be combined to achieve optimal results. Additionally, the side effects from these medications are an important concern (Goyal D., 2006)

The development of a safe and effective way to manage hypertension has challenged medical researchers for centuries. Nowdays, various foods have differing hypotensive. Black cincau (*Mesona palustris BL*) or black grass jelly is a traditional Indonesian food that has been used as a folk medicine and it is effective against heat-shock, hypertension and diabetes. Black cincau as herbal drink is also known in Asian countries, in China and

Taiwan similar black cincau is called hsian-tsao (*Mesona procumbens Hemsl*) (Widyaningsih T.D., 2013).

In this experiment, black cincau made in the form of effervescent powder because it is practical, good taste, and not resemble like medicine. This experiment was use Wistar rats as a hypertensive model induced with sodium chloride and Prednison. Thus, those rats treated with Captopril and Black Cincau Effervescent Powder (BCEP) in separated group to see the effect in lowering systolic blood pressure (SBP) and malondialdehyde (MDA) concentration in blood.

MATERIALS AND METHODS

Materials and Chemical.

Black cincau (*Mesona palustris BL*) effervescent powder (BCEP) were obtained from the results of previous experimental research. Reagents for analytic: 2,2-diphenyl-1-picrylhydrazyl/DPPH (Sigma), Folin Ciocal-teau, Thiobarbituric Acid Reactive Substances (TBARS) assay kit, Prednisone, NaCl, Captopril and other chemical materials.

Instrument.

The sphygmomanometer (sphygmoma-nometer S-2 Ser. N09208, Hugo Sachs Electronic, Germany) device was used to measure rat blood pressure. The glass tools were used for analysis.

Analysis Black Cincau Effervescent Powder (BCEP).

DPPH radical-scavenging activity.

Antioxidant activity was determined according to the method with slight modification (Okawa M., 2001). Sample was diluted with distilled water to get concentration of 20, 40, 60 and 80 ppm, respectively. Each sample solution (4 ml) was added to 1 ml DPPH solution (0.2 mM). The reduction of DPPH was measured at 517 nm against a blank assay for 30 minutes. The percentage of radical inhibition in medium was calculated as the different of absorbance of the blank and absorbance of the sample divided by that of DPPH control at the same time multiplied by 100. It can be calculated by this formula:

$$\%\ Inhibition = \frac{(A\ Blanko - A\ Sample)x\ 100\%}{A\ Blanko}$$

The value of sample concentration and inhibition percentage was graphically plotted for equation of linear regression (y= ax + b). The equation was used for calculating the IC50 value (*inhibitor concentration 50%*) from each sample. The IC50 value is defined as the concentration of antioxidant (sample or control) necessary to decrease the initial DPPH concentration (50%) and is expressed as mg/ml.

Total Polyphenol Content Assay.

Concentration of total polyphenol was determined by Folin Ciocalteu method with modifications (Andarwulan N., 2000). Blank solution was prepared by adding 2 ml of 96% ethanol in a 10 ml test tube. Caffeic acid 1000 ppm was used as stock solution. 50 mg tannic acid was diluted with 50 ml of 96% ethanol. The sample was weighed approximately 1 mg and diluted with 2 ml of 96% ethanol in a 10 ml test tube. The standard solution and sample were added to 5 ml deionized water, 0.5 ml of Folin Ciocalteu reagent (50% v/v) and incubated for 5 minutes. Then, it was homogenized with 1 ml of sodium carbonate solution (5% v/v), and incubated at room temperature and in the dark for 1 hour. After incubation, the solution was homogenized again. Then the total phenol content was measured with a spectrophotometer at a wavelength of 725 nm. The value of total phenolic

content is interpreted by milligram equivalents of caffeic acid per gram extract (mg/g extract).

Animals Treatment.

All animal procedures were approved by the Ethical Clearance Committee, Brawijaya University, Malang, Indonesia. Wistar male rats (180-200 g), 3-4 months old were obtained from Nutritional Laboratory PAU Gadjah Mada University, Yogyakarta, Indonesia, and given 1 week acclimation to their new environment. All rats were maintained under standard laboratory conditions at temperature of 25 ± 2 ^{o}C, 50 ± 15% relative humidity and normal photoperiod (12-hours light dark cycle). Commercial pellet diet and water were provided ad libitum for those rats. Intervention period lasted for 7 days with 3 days of 1 g/kg body weight NaCl and 1.5 mg/kg body weight Prednisone induced to promote hypertensive rat.

Dose Calculation.

Dose calculation was based on conversion of human equivalent dose to rat dose. Every rat received an equal volume of black cincau effervecent powder solution (2 ml/20 gram body weight) every day for 28 days orally by gastric gavage. Rats were weighed every 5 days. The human consumtion of black cincau effervecent powder=7 gram for rat multiply by 0.018.

- Dose 1: dose for rat=7x0.018=0.126 gram=126 mg/20 gram body weight= 630 mg/kg body weight.
- Dose 2: 2x Dose 1=1260 mg/kg body weight.
- Dose 3: 3x Dose 1=1890 mg/kg body weight.

A total of 24 Wistar rats were divided into 6 group (4 rats per group) as follows:

KN : The negative control group.
KHT : Hypertensive control group.
KO : Captropril control group.
EV125 : Treated with BCEP at a dose 1of 630 mg/kg body weight.
EV250 : Treated with BCEP at a dose 2 of 1260 mg/kg body weight.
EV375 : Treated with BCEP at a dose 3 of 1860 mg/kg body weight.

Provision of treatment by means of oral administrated.

Rats Blood Pressure Measurement.

Systolic blood pressure (SBP) were measured every week for 28 days intervention period. Systolic blood pressure measurement using tail-cuff plethysmo-graphy. Insert a rat into rat holder with the tail outside. Base of the tail paired spherical hollow device which called as pulse sensor to measure blood pressure. Counting begins when the pedal hitted. Wait until the LED lights in stop or ready indicator, and then start to read. It will show a number for systolic, heart rate and body temperature.

MDA Assay with TBARS Kit.

Blood sampling for MDA analysis were performed in the beginning after the rats in condition of hypertension and in the end of the intervention period. MDA test was using TBARS assay. The decrease in blood pressure and MDA in treatment groups are compared to KN and KHT groups. Insert 0.75 ml H_3PO_4 to polypropylene tube. Add TBA 0.25 ml and 0.05 ml blood sample. The standard sample made by replacing the blood sample with 1, 1, 3, 3-Tetra Ethoxy Propane. Add 0.45 ml aquabidest. Mix all the solutions, input into a water bath 100 °C for 1 hour. Let cool, then strain with C18 cartridges. Read the absorbance with spectrophotometer 532 nm wavelength.

Statistical Analysis.

The results are expressed as the mean ± SD for each group. Statistical differences

were evaluated using analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) Results were sidered to be statistically significant at $p<0.05$.

RESULTS

Analysis results of BCEP revealed in Table 1. The percentage scavenging activity of the DPPH by 50% has been used to measure antioxidant activity.

Table 1. Analysis Results of Black Cincau Effervescent Powder.

Parameters	Results
Antioxidant Activity IC_{50}	63.33 ppm
Totak Phenolic Compounds	64.92 ppm
Water Content	3.37 %
Rough Fiber Content	1.360 %
Flow Time	9.1 second
Idle Angle	29.5^{0}
Compressibility Index	21.33 %
Color L*	45.5
Color a*	17.9
Color b*	12.76
Dispersion Time	170 second

Rats treatments which consists three dose levels 630 mg/kg body weight, 1260 mg/kg body weight and 1890 mg/kg body weight showed statistically significant different ($p<0.01$) with rats SBP and MDA different ($p<0.01$) with rats SBP and MDA concentration.

Table 2. Effect of Black Cincau (*Mesona palustris* BL) Effervescent Powder on Systolic Blood Pressure hypertensive rats.

Group	Means Systolic Blood Pressure in mmHg/Weeks					
	0	1	2	3	4	5
KN	84 ± 4.19	85 ± 2.6	84 ± 2.75	85 ± 2.58	84 ± 1.50	85 ± 3.32
KHT	84 ± 2.99	209 ± 3.77	208 ± 3.95	209 ± 3.92	208 ± 2.06	208 ± 4.69
KO	89 ± 5.97	211 ± 2.36	161 ± 1.29	146 ± 2.58	126 ± 3.32	101 ± 1.29
EV 125	88 ± 1.41	209 ± 5.74	191 ± 3.50	156 ± 4.43	148 ± 3.87	130 ± 2.63
EV 250	88 ± 4.27	212 ± 2.65	181 ± 3.92	144 ± 2.99	137 ± 2.89	119 ± 3.87
EV 375	87 ± 2.50	209 ± 4.69	180 ± 4.55	142 ± 6.27	131 ± 3.92	108 ± 2.50

Note : KN: The Negative group control; KHT: Hypertensive group control; KO: Captopril group control; EV 125: Treated with BCEP at a dose 1 of 630 mg/kgBW; EV 250: Treated with BCEP at a dose 2 of 1260 mg/kgBW; EV 375: Treated with BCEP at a dose 3 of 1860 mg/kgBW.

The most effective in lowering SBP indicated by dose group 1260 mg/kg body weight with a decrease percentage of 43.97 in SBP and 68.77 % in MDA concentration. Table 3 shows means of rats. MDA concentration each week and Fig. 2 shows effectiveness of MDA concentration reduction.

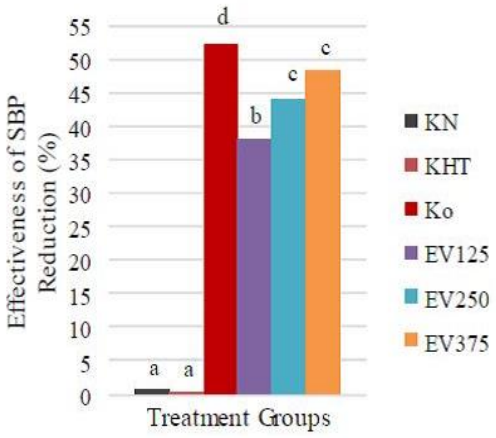

Figure 1. Percentage of Effectiveness Systolic Blood Pressure Reduction

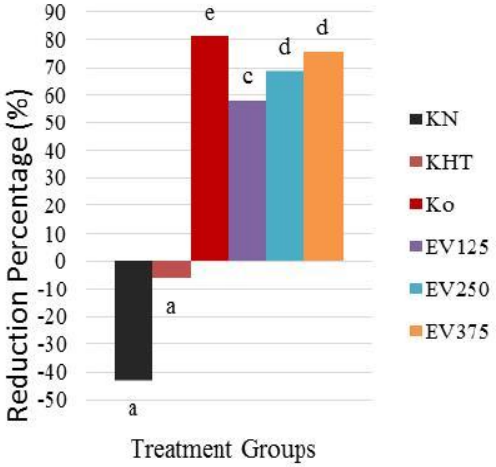

Figure 2. Means of Reduction Percentage of MDA Concentration. Data expressed as mean ± SD (bars) value (n=4) rats per group. Different subscript are mean significantly different $p<0.05$.

Table 3. Effects of Black Cincau (*Mesona palustris* BL) Effervescent Powder on MDA Concentration Before and After hypertensive Treatment.

Means of MDA Concentration (nmol/ml)		
Group	**Before Treatment**	**After Treatment**
KN	1.04 ± 0.07	1.48 ± 0.12
KHT	7.07 ± 0.26	7.49 ± 0.29
KO	7.41 ± 0.26	1.38 ± 0.11
EV 125	8.36 ± 0.33	3.50 ± 0.14
EV 250	8.18 ± 0.53	2.55 ± 0.09
EV 375	7.95 ± 0.42	1.93 ± 0.12

Note : KN: The negative group control; KHT: Hypertensive group control; KO: Captoril group control; EV 125: Treated with BCEP at a dose 1 of 630 mg/kgBW; EV 250: Treated with BCEP at a dose 2 of 1260 mg/kgBW; EV 375: Treated with BCEP at a dose 3 of 1860 mg/kg.

RESULT & DISCUSSION

Polyphenolic compounds are widely available in some plants, fruits and vegetables. They are derivatives and/or isomers of flavones, isoflavones, flavonols, catechins and phenolic acids that exhibit cardiovascular protection and improvement of the endothelial function (Han X., 2007). Treatment with polyphenols resulted in a greater decrease in blood pressure and an increased NO synthase activity in the heart and aorta than in rats with spontaneous recovery (Bernátová I., 2002). It is known that the kidney also responds sensitively to changes of blood pressure.

Previous research has found bioactive compound in black cincau, which is as a main ingredient of BCEP that can reduce blood

pressure. Caffeic acid were in aqueous extract of black cincau (*Mesona procumbens* Hemsl.) have a major role in lowering blood pressure (Yen G.C., 2009). Caffeic acid was also contained in black cincau (*Mesona palustris* BL.) as a bioactive compound (Widyaningsih T.D., 2013). Caffeic acid attenuates the proliferative reaction of vascular smooth muscle cells to Ang II stimulation in both SHRSP and WKY rats by inhibiting the generation of reactive oxygen species and then partially blocking the JAK/STAT signaling cascade and the Ras/Raf-1/ERK1/2 cascade (Gao-li P., 2005). Caffeic acid also has scavenging activity to reduce oxidative damage in cell (Nadanasabapathi S., 2013).

Addition of red gingers and pandan leaves also contribute in lowering blood pressure and MDA concentration (Oboh G., 2010 and Sukandar D., 2009). Both materials contain phenolic compounds that have scavenging activity on free radicals (Hung C.Y., 2002). Phenolic compound in red ginger can protect the body over free radicals, which is forming composition with natural metabolite from aerobic cells (Oboh G., 2010), Blood pressure reduction in BCEP treatment groups one through mechanism of diuretic. Tannin is a bioactive compound that has a role in diuretic (Yen G.C., 2009). Diuretic activity of the extract can be linked to a number of possible mechanisms. Experimental studies have demonstrated that cardiac glycosides and tannins are endowed with both diuretic and vasodilator actions (Herrera D.M., 2008). With the diuretic activity, sodium level in blood secreted so the plasma volume back to normal and blood pressure settle (Han X., 2007).

The decreased of SBP due to diuretic mechanism of bioactive compound, caffeic acid, besides it effect as scavenger against free radicals (Yen G.C., 2009). MDA concentration decreased due to phenolic compounds in BCEP which consists of main ingredients black cincau, pandan leaves, and red gingers. The phenolic compounds inhibit lipid peroxidation which causes oxidative damage resulting MDA. Because of the phenolic compound, the oxidative damage can be reduced so that MDA concentration decreased (Oboh G., 2010).

Captopril treatment group indicated the highest lowering percentage compared with BCEP treatments. It happens because the captopril had been specifically design to lowering blood pressure by inhibiting Angiotensin Converting Enzyme (ACE) in Renin-Angiotensin-Aldosteron (RAAS)[3]. System RAAS thus reducing blood pressure faster than BCEP treatment groups.

In conclusion, our study suggests that BCEP has potential blood pressure-lowering effects in hypertensive rats, which can be reduced so that MDA concentration decreased.

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