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THE EFFECT OF MAXIMUM PHYSICAL ACTIVITY AND MANGGONG BAMBOO (*Gigantochloa manggong*) LEAF EXTRACT ON CATALASE ACTIVITY IN LIVER ORGAN OF RATS (*Rattus norvegicus*)

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ABSTRACT

Gigantochloa manggong, one of endemic bamboo plant in Indonesia is suspected to have exogenous antioxidant potential. Exogenous antioxidant can help the activity of endogenous antioxidant in the body when overtraining occurs. Antioxidant can be measured by catalase enzymes activity. This study was carried out to determine the effect of maximum physical activity and leaf bamboo extract on catalase activity in liver organ of rats. This research used experimental method with completely randomized design (CRD). The test groups were the control rats (E_0R_0), leaf extract induced rats (E_1R_0), swimming activity treated rats (E_0R_1) and leaf extract induced and swimming activity treated rats (E_1R_1). Data were analyzed by the two-way ANOVA statistical test. Bamboo leaf extract non-toxic and leaf extract contained flavonoids, alkaloids,triterpenoids dan saponins. Catalase units in the control group is 1.00 unit/ml, the induced leaf extract group is 0.89 unit/ml, the maximum physical activity group is 0.78 unit/ml and the maximum physical activity treated and induced leaf extract group is 0.56 unit/ml. Based on statistical test, catalase activity has no effect ($p>0,05$) on rats. It was concluded that maximum physical activity could not reduce catalase activity. Manggong bamboo leaves extract could not increase catalase activity and there was no effect between maximum physical activity and manggong bamboo leaf extract on catalase activity in liver organ of rats.

Keywords: antioxidant, catalase, manggong bamboo, physical activity.

BACKGROUND

Physiologically in human body there is formation of free radicals as the result of metabolism which can be anticipated by endogenous antioxidant. In the condition when human body do excess activity, the free radicals production will be increased and endogenous antioxidant will sometime could not overcome this level. Many synthetic antioxidants were produced to help human with their endogenous antioxidant but unfortunately they are sometime very carcinogenic. Natural antioxidant will be the most suitable solution to acquired high activity and safely antioxidant. Bamboo has been known for centuries as the source of antioxidant. Research by Wang *et al*.(2012) has revealed that this Sasa Bamboo has antioxidant compound of flavonoid, lactone, and phenolic acid. The same compound might be found on other bamboo. *Gigantocloa manggong* is an Indonesian endemic bamboo from Meru Btiri East Java. Research on the use of this bamboo has not found yet specifically on its antioxidant activity. This research try to investigate the antioxidant activity of manggong bamboo leaves extract with phytochemistry assay and catalase activity as stress oxidative parameters on rat liver given physical swimming exercise.

MATERIALS AND METHOD

Research was done in Physiology and biochemistry laboratory, Biology department, State University of Jakarta. Method used was experimental with completely randomized design (CRD) with two factors of bamboo extract and activity as stress induction. Physical activity given was swimming based on Herwana (2005) with 3x5 minute of swimming and 15 minute resting period once a day. Research group samples were describe as follow, E1RI : extract bamboo and swimming, EOR1: extract bamboo without swimming, E_0R1: without extract with swimming, E0R0: without both extract and swimming. Replication used was 6 samples.

Phytochemical assay

Materials used were dried leaves of manggong bamboo, 70% ethanol, Mg, HCl, Amyl Alcohol, Follin-Ciocalteu, Lieberman-Burchard reagent, $CHCl_3$, chloroform, aquadest, phosphate buffer, and H_2O_2. Extraction was done according to Harborne (1996), with sample and solvent of 10:1. Extract was processed in paste before treated using evaporator vaccum. Alkaloid test was done with Wagner reagent with positive result of brown sedimentation and Dragendorff reagent with positive result of white sedimentation. Flavonoid test was done with Mg, HCl and amyl alcohol addition on extract. Positive result was shown by red, yellow or orange color. Triterpenoid test was done by Lieberman-Burchard with positive result of purple ring on the top layer of solution. Saponin was done by adding HCl with positive result of permanent and rigid foam. Phenolic assay was done by adding NaOH with positive result of red sedimentation. Tannin test was done by adding FeCl3 with positive result of dark blue or dark green.

Animal sample treatment

The research used 24 male Wistar rats (*Rattus norvegicus*) of 200-250 gram, 2-2,5 month. Rat was adapted to cage for a week before treatment. Food and drink were given ad libitum. Extract was given on 400 mg/kg BW dose according to Lin *et al.* (2012). Body weight was measured everyday. Physical activity in the form of swimming was given an hour before extract. All animal procedure has been approved under animal ethic committee University of Indonesia number 79/H2.F1/ ETIK/2014.

Liver sample preparation

All animal samples were euthanized on day 14 with ether. Necropsy was done to get liver organ for catalase test. Liver organ was added by 1 ml of PBS (Phosphate Buffer Saline). Homogenate was then centrifuge by 4000 rpm.

Catalase activity measurement

Catalase activity test was done with spectrophotometry by adding 1900 µl H_2O_2 and 100 µl of PBS with 100 µl of liver sample. Absorbance was noted in 240 nm in the first to second minute (Anatreira, 2012). Catalase activity was measured by formulation of:

$$\text{Catalase activity (U/mL)} = \frac{(\Delta S - \Delta B) \times F}{(\text{mol } H2O2) \times V}$$

S = sample absorbance (Å/minute)
B = blank absorbance (Å/minute)
Mol = molarity of H_2O_2 (mM)
V = volume of sample measured (µL)
F = titration factor

Data Analysis

Phytochemical study was analyzed qualitatively whilst catalase activity was analyzed by two way ANOVA (α =0.05).

RESULTS

Bamboo Manggong Leaves extraction

Bamboo leaves were originated from Meru Btiri National Park East Java of 10 kg/wet weight. Table 1 showed the result.

Table 1. Manggong bamboo leaves extraction

Bamboo Leaves	Weight
Wet Weight	10 kg
Drt Weight	3 kg
Simplisia powder	2,8 kg
Extract solvent	16 L
Paste weight	13,38 g

Phytochemical Assay

Phytochemical assay showed that manggong bamboo leaves contained alkaloid, flavonoid, triterpenoid and saponin (Table 2).

Animal Samples Body Weight

One indicator of stress is body weight. Rat body weight of control group showed increased level while treatment group given physical exercise and extract shown decrease weight (Table 3).

Catalase Activity

Catalase activity was measured by spectrophotometer in 240 nm. Result showed that highest catalase activity found in control group. The lowest catalase activity was showed in physical activity group. Data analysis showed that swimming and extract didn't significantly affect catalase activity (Table 4).

Table 2. Phytochemical assay of Mangong Bamboo leaves

Phytochemical test		Result
Alkaloid	Mayer Reagent	+
	Wagner Reagent	+
	Dragendorf Reagent	-
Flavonoid		+
Saponin		+
Triterpenoid		+
Phenol		-
Tannin		-

Table 3. Rat body weight within the 1st and 14th day.

Sample group	1st day BW (g)	14th day BW (g)	Δ weight (g)
E_1R_1	219,83 ± 14,47	213,5 ± 13,28	-6,33
E_1R_0	227,67 ± 13,20	229,0 ± 13,93	1,33
E_0R_1	216,63 ± 14,29	209,5 ± 16,51	-6,83
E_0R_0	213,33 ± 9,58	227 ± 14,3	13,67

Table 4. Catalase activity

Sample Group	Catalase Activity (U/ml)
E_1R_1	0.78 ± 0.38
E_1R_0	0.89 ± 0.40
E_0R_1	0.56 ± 0.28
E_0R_0	1.00 ± 0.69

DISCUSSION

Human body has mechanism to protect themself against ROS (Reactive Oxygen Species). The most important protection is the enzyme one of which catalase. Catalase ana~~y~~lysis was done to liver since it has been the center of the metabolism. Liver has peroxisome with abundant oxidative enzyme, such as catalase (Liang, 2008). Rats treated with physical activity in the form of swimming, will be easily suffer from oxidative stress which in turn will decrease their body weight. Another effect of stress is Corticotrophin-Releasing Hormone (CRH) secretion with the effect of food consumption limitation (Nagaraja, 2001). This hormone acts as inducer for cortisol in catabolic pathway.

On the other side, rat having weight gain in the control group suffer no stress at all and sufficiently feed. Under physiologic condition, free radicals, as the result of metabolism, can be overcome by endogenous antioxidant. Increase activity or oxidant will lead to higher need of the antioxidant. Under streesfull condition, catalase will be lesser since this enzyme component as a complex protein has been damaged by free radicals (Winarsi, 2007).

Based on catalase activity measurement on Table 4, the enzyme on rat treated with manggong bamboo extract showed stabile level or slightly higher than those with swimming. It means that antixiodant compound found in manggong Bamboo leaves has helped the endogenous antioxidant to combat free radical. According to Takara (2002) in Sugito (2012), phenolic compound will be able to induce antioxidant enzyme gene in order to reduce damage High flavonoid activity will be able to stabilize reactive oxygen (Simanjuntak, 2012). Borra (2006) has mentioned that flavonoid has capability in increasing gene producing enzyme and for this reason manggong bamboo leaves extract also able to stabilize catalase. Triterpenoid and saponin has capacity as metabolism regulator (Soetan, 2008).

Statistic evaluation has shown that physical activity and manggong Bamboo Leaves extract did not significantly affect catalase. This might be due to the dose use was not significant to stabilize catalase. 400mg/BW of extract dose has proven could not significantly affect catalase activity and higher dose will be needed. Catalase enzyme has structure of mostly contain protein which is easier to be damage by many factors such as temperature and acidity. Hot and extreme temperature and improper acidity will lead to decrease enzyme activity.

CONCLUSION

Maximum physical activity could not decreased catalase activity. Manggong bamboo leaves extract could not increased catalase activity and there was no effect between maximum physical activity and manggong bamboo leaf extract on catalase activity in liver organ of rats.

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