THE EFFECT OF EXTRACT ROSELLA (*HIBISCUS SABDARIFA* L) CALYX ON INCREASING CATALASE ACTIVITY AGAINST 7,12 DIMETHYLBENZ-(A)ANTRASENA (DMBA) INDUCED IN RATS

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ABSTRACT

Background : Free radical is included into a reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the body which can cause degenerative diseases such as neurodegenerative, aging, and cancer e radicals.The excessive concentration of ROS causes oxidative stress as indicated by the activity of catalytic enzyme. Roselle (Hibiscus Sabdariffa L.) has been activity as free radical inhibitors. This study aims to determine the antioxidant activity of ethanol extract of roselle calyx to see cattalase enzyme activity in the treatment of 7 days.

Objective : This experimental research was arranged as a post-test controlled group design. Twenty five Male Sprague Dawley rats age of 4 weeks divided into 5 groups: Group are treatment of 7 days, group I are baseline, group II DMBA control (Single dose 75 mg/kg BW), group III, IV and V are roselle extract treatment groups with variations dose group of 10, 50 and 100 mg/kg BW.

Methods : Roselle was extracted by maceration 60 % ethanol and followed by evaporator to get concentrated extract of 10, 50 and 100 mg/kg BW. Twenty five Male Sprague Dawley rats age of 4 weeks divided into 5 groups: Group are treatment of 7 days, group I are baseline, group II DMBA control (Single dose 75 mg/kg BW), group III, IV and V are roselle extract treatment groups with variations dose group of 10, 50 and 100 mg/kg BW. Animal was fasted for 16 hours before sampling. after that, the animal were sacrificed and organ were observed using hematocylin and eosin staining, measured cattalase activity. Data were analyzed using Anova, Least Significant Difference, Kruskal-wallis and Mann-whitney. Content of Polyfenol and Flavonoids use gallic acid and quercetin standart.

Results : The activity of cattalase was found to increase in catalase enzyme activity of roselle extract at a dose of 10, 50, 100 mg/kg BW in the treatment of 7 days in respectively (5.550 ± 0.683), (6.186 ± 0,764), (5.678 ± 0.813) and the group of DMBA (4.067 ± 0.770). The effect of roselle extract in DMBA control group shows increase in catalase activity of roselle extract dose of 50 mg / kg (6.186 ± 0.764) compared to administration of DMBA (4.067 ± 0.770) ($p < 0.05$) on day 7 of treatment. Prolonge roselle extract treatment on day 7 to show significant differences in catalase enzyme activity.

Conclusion : The rosella flowers ethanol extract has an antioxidant activity showed by the increased catalase activity.

Key Word : Hibiscus sabdarifa L, Catalase, DMBA, Antioksidan

INTRODUCTION

Free radical is a compound which isformed naturally in the body and involved in most ofthe biological processes of organisms. The human body naturally experiences a process of formation of free radical as a side-product of metabolism processes (Carutti, 1991). Free radical is included into a reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the body which can cause degenerative diseases such as neurodegenerative, aging, and cancer (Harman, 1994; Simonian and Coyle, 1996). Reactive oxygen species which is produced in vivo includes the superoxide radical (O2) and hydrogen peroxide (H2O2) is a compound that can cause lipid oxidation and DNA damage in the cells (Halliwell and Anuoma, 1991).

Defense system conducted by the enzymatic reaction is the formation of free radical termination reaction using primary antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (Blokhina et al., 2003). In addition, the defense system can also be conducted by preventing free radical reactions using secondary antioxidants found in plants such as vitamin C, vitamin E, flavonoids, isoflavones, isoflavones and anthocyanins (Sies and Stahl, 1995). Enzyme catalase is an endogenous antioxidant, as an antioxidant, this enzyme inhibits the formation of free radical, by converting it into another product that is stable, so that the antioxidant group also called chain-breaking-antioxidant (Winarsih, 2007).

The utilizations of herbs as antioxidant agentshave been conducted widely.One of the utilizations is the use of antioxidants in Rosella plant (Hibiscus sabdariffa L.). The active substances in Rosella (Hibiscus sabdariffa L.) which play the key role as the antioxidant are gossypetin, anthosianin, hibiscine glucoside (Wiyarsi, 2011). The results of a research conducted by Kelvin (2012) concludes that the Rosella calyx extract in a dose of 250 mg / kg BB lowered malondialdehyde which is the end product of lipid peroxidation (oxidative stress, lipid peroxidation is arising due to the triggering of free radical) of 28.0 % in rats fed with used cooking oil and Rosella calyx extract in a dose of 500 mg / kg BB lowered malondialdehyde 50.2%.

This research is conducted to discover the activity of Rosella calyx ethanol extract whether it experiences an increased catalase activity on the DMBA induced rats or not.

RESEARCH METHOD

Materials

The Rosella plants are obtained from KabupatenKulonProgo, Yogyakarta. Meanwhile, the 4 weeks rats SD are obtained from the Veterinary of GadjahMada University.

Extraction

1500 g rosella flowers calyxes powder is extracted with ethanol 70% of 7500 mL (1: 5) using the method of maceration with a stirring for approximately 3 hours, then it was left up to 24 hours. The macerates are collected and evaporated with a rotary vacuum evaporator at a temperature of 60^{o} C and concentrated above the water bath with a temperature of 60-70^{o} C to obtain a viscous extract. The ethanol extract of rosellaflowers calyxes is weighed and its soaked is calculated (Anonymous, 2004).

Standardization of the Extract

Qualitative Analysis with KLT silica gel 254 with a movement phase (toluene; acetone; formic acid) (6:6:1). Then, the determination of flavonoids content with a quercetin standard is conducted by the method of Chang*et al*, 2002.

The determination of total flavonoids content is conducted by spectrophotometry using aluminum chloride reagent according to the procedure of Chang. Take a 50 mg quercetin which is then diluted with 50 ml of ethanol which is diluted until the upper limit. It is the mother solution which is further diluted with ethanol in order to obtain a minimum of 7 different concentrations. Each concentration in the pipette as much as 2 mL of solution which is then added with 0.1 ml of aluminum chloride (AlCl3) 10% that has been diluted with ethanol, 0.1 ml of Na acetate, 2.8 ml of distilled water and then divortex, incubation solution mixture at room temperature for 30 minutes. Furthermore, the absorption is measured using UV-Vis spectrophotometer at 430 nm using a blank solution.

Treatment of tested animals

The tested animals were divided into 5 groups, each group consists of 5 rats, SD strain male rats in the age of 4 weeks. The rats were treated in a well-ventilated room, quite light (12 hours of light and 12 hours dark) and with a preserved humidity. The rats were fed and watered by *ad libitum*. The rats were adapted in a cage for a week before the test. The grouping of the tested animals is as follows: Group I Normal (Base line),fed by standard feeds and watered by distilled water. Group II is a DMBA induced group of 75 mg / kg bb rats through Intragastric in a single dose and corn oil solvent on day 8. Group III, IV and V is a DMBA, corn oil and solvents induced group on the eighth day. Each is induced in a dose of 10 mg/kgBB, 50 mg/kgBB and 100 mg/kgBB of rosella flowers ethanol extract for 7 days. A week after being induced by DMBA, the rats are fasted for 16 hours, then, they were sacrificed and the liver tissues of the rats were taken to measure the catalase activity.

The determination of catalase activity

The measurementwas conducted by following the procedures Iwai et al. (2002) with slight modifications. Catalase activity was measured by the magnitude of the reduction of hydrogen peroxide. In a quartz cuvette, a 0.5 ml (pH 7.0) contains 10 Nm of hydrogen peroxide. The change in absorbance was measured with a UV Vis spectrophotometer at a wavelength of 240 nm which is recorded for 15 seconds in a minute. The catalase activity was calculated using data from the slope curve of the absorbance of the sample solution (SL) and the blank solution (SLB) follows the formula:

Catalase Activity (U/mg) = (SL-SLb)/0,436 x 2.5/0.5

Data Analysis

The data analysis of catalase enzyme activity amongstthe treated groups was conducted by examining the normality and homogeneity of the data with a level of trust of 95%. The normality test conducted using the Kolmogorov-Smirnov test to determine whether the data were normally distributed or not, and then proceed with a homogeneity test which is a Levenetest to determine the homogeneity of the data. The data is said to be normally distributed and homogeneous if the significant value is more than 0.05. If the data are homogenous and normally distributed, then, the analysis will be continued by some Anova testswhich are followed by test of Least Significant Difference (LSD).

RESULTS AND ANALYSIS

Flavonoids Content Analysis

In the determination of the flavonoids content determination, the rosella flowers extract is added by $AlCl_3$ with a purpose of creating a complex form with otho hydroxyl group. So that, it gives an intensive yellow color which is then added by Na acetate with a purpose of creating a stable color complex and its absorption can be read. The results obtain a viscous extract of 615,45 gram and the immersion of EEKBR of 41,03%. Then, the results of flavonoids average with a standard curve of y = 0,003 x + 0,179 and R2=0,964 which are obtained as follows:

Tabel 1. Flavonoids Content Analysis

Replication	Absorbance	X (µg/mL)	Total of Flavonoid/100 g	Ẋ ± SD
1	0,235	14,50	0,48	0,617±0,127
2	0,264	22,02	0,73	
3	0,253	19,17	0,64	

The Analysis of Thin Layer Chromatography

The qualitative test of Thin Layer Chromatography was conducted to prove that the rosella flowers extract contains quercetin compound. The quercetin compound testwas conducted by spotting it on a silica gel of F254 using a 5µl capillary tube. The spots are eluted with movement phase of toluene: acetone: formic acid (6: 6: 1). The results showed the blue fluoresced samples and standards at UV254 nm. It can be seen that the sample had Rf which is closed to quercetinRf, 0.38 for the samplesand 0.87 for the quercetin.

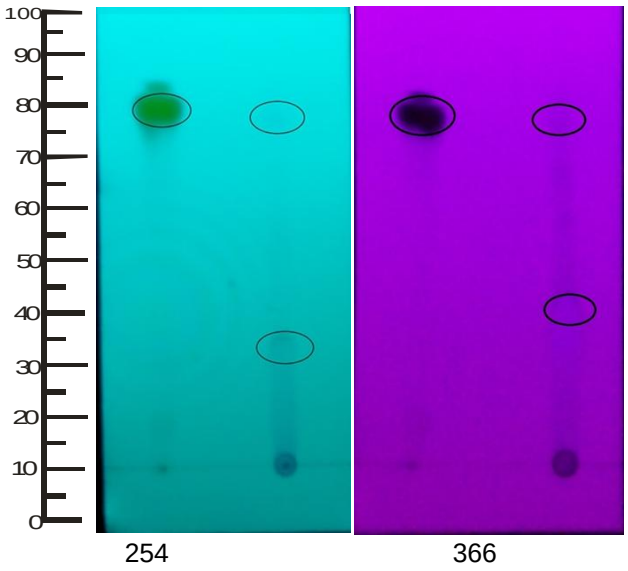

Figure 1. TLC Cromathogram of rossela extract

Rf states degree of retention of a component in the stationary phase. The greater Rf of the sample, the greater the distance of movement of these compounds on KLT. It can be seen that the samples had the same Rf with the Rf of the quercetin, so it can be said that these compounds have same characteristics, and it can be concluded that the positive samples containing quercetin. The Rf price is a characteristic parameter of the paper chromatography and thin layer chromatography. This price is a measure of the migration speed of a compound on the chromatogram and in the constant condition which is the magnitude of the characteristic and reproducible.

The Determination of Catalase EnzymActivity

This measure was conducted in the seventh day after DMBA inducing. The results can be seen on Table 1 and were presented on Figure 2.

Table 2. Activity of enzyme catalase SD rat DMBA induced on day 8 (mean±SD)

Group	Activity of enzyme catalase
Baseline	8,881 ± 0,680
DMBA	4,067 ± 0,770
Roselle extract 10 mg	5,551 ± 0,683
Roselle extract 50 mg	6,186 ± 0,764
Roselle extract 100 mg	5,677 ± 0,810

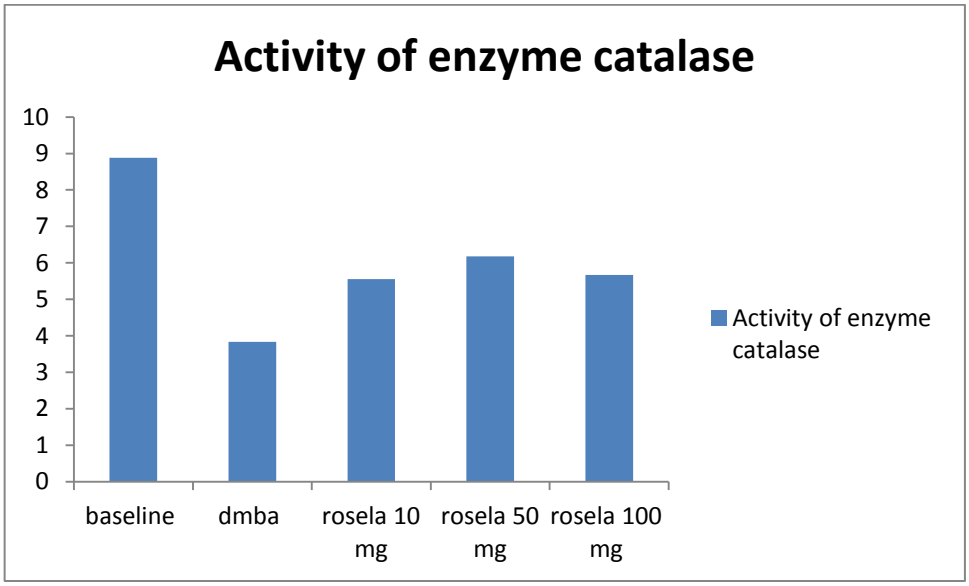

Figure 2. The graphic of catalase enzyme activity of DMBA induced SD rats on the eighth day which is different significantly between the groups given rosella extract in a dose of 10,50,100mg/kgBB compared to the DMBA groups.

The catalase enzyme activity on the seventh day, the baseline group has an increased catalase enzyme activity (8,881±0,680) compared to the DMBA one (4,067±0,770). It can be concluded that the DMBA inducing in a dose of 75 mg/KgBB, the rats can increase the formation of hydrogen peroxide which is marked by the decreased catalase enzyme

activity on the groups of rosella flowers extract in a dose of 10, 50 and 100 mg/kgBB (5,551±0,683),(6,186±0,764), (5,677±0,810) which is different significantly ($p<0,05$) with the DMBA group (4,067±0,770). It shows that the inducing of rosella extract in a dose of 10, 50, 100 mg/kgBB has a protection activity to the free radical with an increased activity of catalase enzyme. However, all of the three doses did not have any significant difference.

Overall, the specific activity of catalase enzyme is decreasing compared to the baseline group. The decrease of the catalase enzyme activity is significantly different if it is compared to the baseline group ($p<0,05$). Catalase is an enzyme which plays a role converting H2O2 to be H2O and O2. The decrease of catalase activity of the DMBA induced group is suspected to be caused by the accumulation of the ROS. On the given rosella ethanol extract treated group, group III, IV and V experiencing an increase of catalase activity compared to group II. It shows that the rosella ethanol extract has an antioxidant ability which proves to be able to prevent the further damage caused by the DMBA. When DMBA interacts with AhR, Enzyme CYP1A1 will change its substrate which is in the form of DMBA to be a reactive form of DMBA diol-epoxide.

CONCLUSION

The rosella flowers ethanol extract has an antioxidant activity showed by the increased catalase activity. The inducing of rosella extract in a dose of 10, 50, 100 mg/kgBB has an activity in increasing the catalase enzyme activity significantly on the seventh day treatment, compared to the DMBA group in a dose of 75mg/kg BB. The length of the treatment of the extract on the seventh day in a dose of 10, 50 and100 mg/kgBB did not shows any significant difference on the catalase activity. The flavonoids content on the total of rosella flowers calyx ethanol extract is0,617 µg/ml.

CONFLICTS OF INTEREST

Author declare no conflicts of interest.

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