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Gaharu Leaf Water Extract Reduce MDA and 8-OHdG Levels and Increase Activities SOD and Catalase in Wistar Rats Provided Maximum Physical Activity

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Background: Oxidative stress occurs due to an imbalance of the number of free radicals by the number of endogenous antioxidant produced by the body i.e. Superoxide Dismutase (SOD), Gluthathione Peroxidase (GPx), and Catalase. The imbalance between the number of free radicals and antioxidants can be overcome with the endogenous antioxidant intake that exogenous oxidative stress can be reduced. One of exogenous antioxidants is natural Gaharu leaf water extract. **Objective:** This research focus on the effect of Gaharu leaf water extract in reducing MDA and 8-OHdG and increase the activity of SOD and Catalase. **Methods:** This study was an experimental with post only controls group design. Experiment was divided into 5 groups of wistar rats, each consisting of 5 animals, i.e. negative control group without extract [K (-)], treatment 1 treated 50 mg/kg BW/day of the extract (T1), treatment 2 treated 100 mg/kg BW/day of the extract (T2), treatment 3 treated 200 mg/ kg BW/day of the extract (T3), and positive control group [K (+)] treated with vitamin Cat a dose 50 mg/kg BW/day. All groups treated for 10 weeks. Every day, before treatment, each group was given a maximum swimming activity for 1.5 hours for 10 weeks. ELISA was used to measure MDA, 8-OHdG, SOD, and Catalase activities. **Result:** The research results showed that treatment of extract of leaves of Gaharu with an higher dose from 50 mg/kg BW up to 200 mg/ kg BW significantly decline ($p < 0.05$) levels of MDA with the average ranging from 6.37±0.23, 5,56±0.27 and 4.32±0.27, 8-OHdG with a mean of 1.64±0.11, 1.26±0.46, and 1.09±0.17. On the other hand the treatment also increase SOD activity with less ranging from 12.15±1.04, 15.70±2.02, and 18.84±1.51, and Catalase ranging from 6,68±0.63, 8.20±1.14 and 9.29±0,79 in the blood of Wistar rats were given a maximum activity compared to the negative control group. This is probably higher phenol compounds (bioflavonoids) quantity content of the extract. These compounds synergistically affect their activity through capture and neutralize the free radicals that oxidized the lipid to less that automatically MDA production as the end result of oxidation lipid levels down, 8-OHdG down, the activity of SOD and Catalase ride. **Conclusion:** Gaharu leaf water extract reduced oxidative stress of Wistar rats through reduction of MDA and 8-OhdG mechanism. On the other hand, the extract increases SOD and Catalase activity.

Keywords: Gaharu leaf water extract, SOD, MDA, 8-OHdG and Catalase

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INTRODUCTION

Maximum physical activity can trigger an imbalance between production of free radicals and the body's antioxidant defense system known as oxidative stress[1], during maximal exercise, whole body oxygen consumption increased 20 times while the oxygen consumption of the muscle fibers expected to increase 100 folds. This can result in the body will huge lack of oxygen, known as hypoxia. This resulted in an increase in oxygen consumption increased production of free radicals which can oxidize fat in muscle tissue (fat tissue oxidation muscle), causing damage to muscle cells.[2]

Start physical training physiological and biochemical response is complex. Each movement begins with a rapid muscle

anaerobic metabolism. Strength comes from the breakdown of ATP to ADP or AMP results and takes place in the mitochondria. The energy release is accompanied by increasing flow of electrons in mitochondrial respiration circuit causes the formation of reactive oxygen (O_2^-) and H_2O_2 in an effort to ATP formation.[3]

Physical training tends to empty the ATP and ADP which would stimulate the conversion of ADP catabolism and Xanthine dehydrogenase (XDH) into oxidase. Xanthine Oxidase (XOD) will form free radicals (O2-). Excessive formation of free radicals that will cause an imbalance known as oxidative stress damage with the end result of fat, proteins and DNA.[3]

Oxidative stress also occurs due to decreased amount of oxygen and nutrients, causing micro vascular damage. This situation is referred to reperfusion injury. It can also lead to tissue damage due to excess production of free radicals from the metabolism of fats and proteins stored in the body due to lack of intake from outside the body.[2]

Oxidation reactions involving free radicals *OH can damage cell membranes and damaging the surrounding normal DNA composition which can result in a mutation. Mutations or DNA damage can lead to degenerative diseases such as cancer, heart disease, cataracts, premature aging and other.[1] Compound 8-OHdG is a marker that indicates the occurrence of DNA damage caused by free radicals excess. This is due to the oxidation of DNA constituent bases (guanosine). Oxidized guanosine will form 8-OHdG. Deoxyguanosine (dG) is one of the constituent bases of DNA and the oxidation reaction when experiencing will be 8-hydroxy-2'-deoxyguanosin (8-OHdG).

Guanosine can also undergo hydroxylation in response to normal metabolism or environmental pollution, especially heavy metals.[4] Increased levels of 8-OHdG pathology associated with the disease include depression, cancer, diabetes, and hypertension.[4-5] This is evidenced by several studies found that 8-OHdG was significantly decreased in the group of pregnant women who are given the sport compared with the group without exercise.

The imbalance between the number of free radicals and antioxidants can be overcome with the exogenous antioxidant intake or reduced the exogenous oxidative stress. One of exogenous antioxidants is natural Gaharu leaf water extract. This is evidenced overcome from in vitro analysis of the water extract of leaves of Gaharu that have a high antioxidant capacity with IC_{50} value = 3.44 ppm, which means with a concentration of 3.44 ppm has been able to inhibit 50% of reactive oxygen species (ROS) and the content of phenolic compounds / bioflavonoid = 14.980 mg GAE/100 g) that can be protected endothelial dysfunction.[6] Phenolic compounds including bioflavonoid can directly capture superoxide radicals, peroxynitrite that bioflavonoid can prevent lipid peroxidation, preventing DNA damage, stimulating the formation of enzymatic antioxidants Superoxide Dismutase (SOD) , catalase and Glutathione peroxidase (GPx) as an anti-inflammatory and as a chelating agent of metal Cu and Fe. It inhibits advanced initiation and propagation reactions of radicals.[7-8] However, the mechanism in protecting endothelial dysfunction is not known with certainty.

The aim of this study was to prove the ability of Gaharu leaf water extract in decreasing MDA and 8-OHdG and increase the activity of SOD and Catalase on Wistar rats which administered to a maximum swimming activity of 1.5 hours per day for 10 weeks.

METHODS

Materials and Instrument

Materials and instruments in this research including : thirty white male rats, Wistar descent, (weighing 200g – 250g, aged 2-4 months). Gaharu leaf extract (*Gyrinops versteegii*), ELISA Kits, SOD Bio Vision K355 - 100, Bio Vision Catalase, ELISA Kit K773-100, 8-OHdG ELISA Kit, DNA Damage Cell Bio labs STA-320, 1,1,3,3-tetraethoxypropane (Sigma), *thiobarbituric* acid, phosphate and methanol. UV-Vis double beam (Varian), analytic Digital Balance Scale, Memmert heating and dry oven, polypropylene tube, centrifuge, Buchi rotary evaporator, water bath, pyrex measuring cup, pyrex test tubes, micropipette, a set of mouse cage.

Procedure

1. Manufacture of Gaharu leaf water extract

Total of 1000 grams Gaharu leaf powder is put in 2 litres of hot water (60^{o}C) for approximately 2 hours while stirring, let it stand for 24 hours. Then, filter extraction results. Liquid extract obtained was concentrated used freeze dryer extracts obtained thick. Viscous extract, was stored in -20^{o}C for further research.

2. Treatment of Mice

A total of 30 white male rats was divided into 5 groups, each of 6 rats. All groups of rats were given a maximum activity of 1.5 hours of swimming every day for 10 weeks. The groups been awarded the maximum activity and each group was given the following treatment. Group I / negative control [K (-)] is given only distilled water, group II (P1) Gaharu leaf water extract given 50 mg/kg BW, group III (P2) given Gaharu leaf water extract 100 mg/kg BW, group IV (P3) given Gaharu leaf water extract 200 mg/kg BW and group V / positive control [K (+)] Vitamin C given 50 mg/kg BW.[9] After the treatment, blood was taken, apply in the test tube of blood containing EDTA. Blood plasma were then analyzed levels of MDA, SOD of activity, Catalase activity and levels of 8-OHdG.

3. Analysis of MDA

Measurement of MDA levels following the procedure [10] A total of 0.5 ml of blood plasma was added 2.0 ml of cold Hcl (0.25 N) containing 15% TCA, 0.38% TBA and 0.5% BHT. This mixture is heated 80°C for one hour. After boiling, the tubes were placed into an ice bath to cool the samples. The cooled samples were mixed well and applied to SEP-Pak C18 column, then centrifuged at 3500 rpm for 10 minutes. Obtained supernatant absorbance was measured spectrum photo metrically at 532 nm. Unit of measurement is nm / ml.

4. Analysis of SOD activity

A total of 0,08 ml of blood plasma was treated with a mixture consisting of 2.90 ml of Na_2CO_3 buffer containing 0.1mm EDTA (pH 10), 0:06 ml xanthine, 10 mM, bovine serum albumin 00:03 (BSA) 0.5%, 2.5 mM NBT 0:03 ml. Furthermore, the addition of xanthine oxidase (0:04 units). The resulting absorbance after 30 minutes was measured at a wave length of 560 nm. Furthermore, the regression curve of standard solutions made SOD Assay Kit Bio Vision with measurement unit U/mL.

5. Analysis of Catalase Activity

Measurements were performed by manual procedure of Bio Vision Assay Kit. Catalase activity was measured with magnitude of the reduction of hydrogen peroxide. In quartz cuvette, the blood plasma of 0.5 added to 2.0ml of K_3PO_4 buffer (pH7.0) containing 10 mM hydrogen peroxide. Changes in absorbance at 240nm recorded every 15 seconds for one minute. Furthermore, the regression curve of standard solutions made CAT Assay Kit Bio Vision. Unit of measurement is U /mg.

6. Analysis of 8-OHdG levels

Measurement of 8-OHdG levels following the procedure in product instruction 8-OHdG ELISA Kit STA-320 Cell Bio-labs. Unit of measurement is ng/mL.

RESULTS

Prior to the treatment of wistar rats (*Rattus norvegicus L.*) males adapted for 1 month. Furthermore, given the maximum activity in the form of swimming 1.5 hours per-day for 10 weeks. Having been awarded the maximum activity of mice given water extract of leaves of Gaharu with increasing doses from 50 mg/kg BW, 100 mg/kg BW and 200 mg/kg BW in the treatment group.

These results were compared with the control (-) with no feed Gaharu leaf water extract, only mice given distilled water alone, control (+) to give vitamin Cat a dose of 50 mg/ kg BW. Treatment of the extract was carried out for 10 weeks, blood in the eye of mice subsequently retrieved and examined the levels of MDA, 8-OHdG levels, enzyme activity of SOD and catalase enzyme activity.

Analysis of the results showed that MDA levels that leaf water extract of Gaharu with increasing doses ranging from 50 mg/kg BW up to 200 mg/ kg BW was able to significantly ($p < 0.05$) lower levels of MDA with the average ranging from 6.37 ± 0, 23, 5.56 ± 0.27 and 4.32 ± 0.27 compared to the negative control group without being administered the extract with a mean of 6.82 ± 0.48. This is due to the quantity of the content of this class of compounds phenol /bioflavonoid which is more and work synergistically in the capture or neutralize free radicals (*OH) are usually to peroxide fat to less that automatically MDA production as the final result of the oxidation of PUFA and oxidative stress levels down can be prevented.[11] The decrease in MDA levels mentioned above can be seen in the Table 1 and Figure 1.

Table 1. The decrease of MDA Levels

Subject Group	N	Mean of MDA Levels (nm/mL)	*F*	P
K (-)	5	6,82±0,48		
Extract 50 mg	5	6,37±0,23		
Extract 100 mg	5	5,56±0,27	70,1	0,0001
Extract 200 mg	5	4,32±0,27		
K (+)	5	3,60±0,39		

Table 2. The decrease of 8-OHdG Levels

Subject group	N	Mean of 8-OHdG Levels (ng/mL)	*F*	P
K (-)	5	1,92±0,61		
Extract 50 mg	5	1,63±0,11		
Extract 100 mg	5	1,26±0,46	4,62	0,008
Extract 200 mg	5	1,09±0,17		
K (+)	5	0,89±0,55		

Analysis of the levels of 8-OHdG, shows that the water extract of the leaves of Gaharu with increasing doses ranging from 50 mg/kg BW up to 200 mg/kg BW was able to significantly ($p < 0.05$) lower levels of 8-OHdG with the average ranging from 1,63±0.11, 1.26±0.46 and 1.09±0.17 compared to the negative control group without being administered the extract with a mean of 1.92 ± 0.61. This is due to the quantity of the content of this class of compounds phenol /bioflavonoid which is more and work synergistically in the capture or neutralize free radicals (*OH) that normally oxidize guanosine bases of the DNA becomes less automatic 8-OHdG production as an end result of oxidation levels down and DNA damage can be prevented. The decrease in 8-OHdG levels mentioned above can be seen in the table 2 and figure 2.

SOD enzyme activity analysis showed that the results of leaf water extract of Gaharu with increasing doses ranging from 50 mg/kg BW up to

200 mg/kg BW was able to significantly (p <0.05) increase in SOD enzyme activity with a mean ranging from 12.15 ± 1, 04, 15.70 ± 2.02 and 18.84 ± 1.51 compared to the negative control group without being administered the extract with a mean of 10.25 ± 1.35.

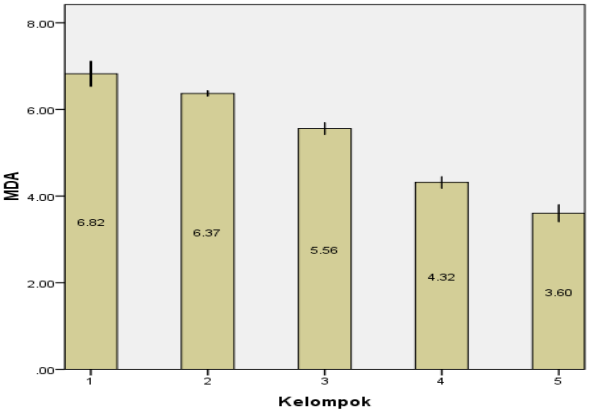

Figure 1.
The decrease of MDA Levels

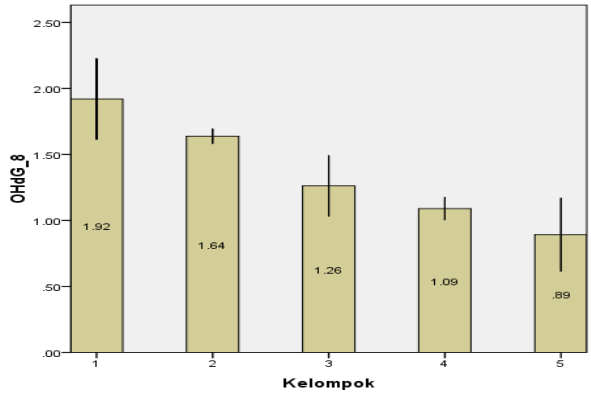

Figure 2.
The decrease of8-OHdG Levels

Table 3. The Increase of SOD Activity

Subject group	N	Mean of SOD Activity (U/mL)	*F*	P
K (-)	5	10,25		
Extract 50	5	12,15		
Extract 100	5	15,70	61,69	0,000
Extract 200	5	18,84		
K (+)	5	23,55		

This is due to the quantity of the content of this class of compounds phenol / bioflavonoid which is more and work synergistically, can directly capture or neutralize the SOD, peroxynitrite so flavonod stimulate the formation of enzymatic antioxidants SOD and endothelial dysfunction can be reduced.[6] The increase of SOD Activity mentioned above can be seen in the table 3 and figure 3.

Table 4. The Increase of Catalase Activity

Subject group	N	Mean of Catalase Activity (U/mg)	*F*	P
K (-)	5	6,10±0,76		
Extract 50	5	6,68±0,63		
Extract 100	5	8,20±1,14	46,22	0,000
Extract 200	5	9,28±0,79		
K(+)	5	12,13±0,46		

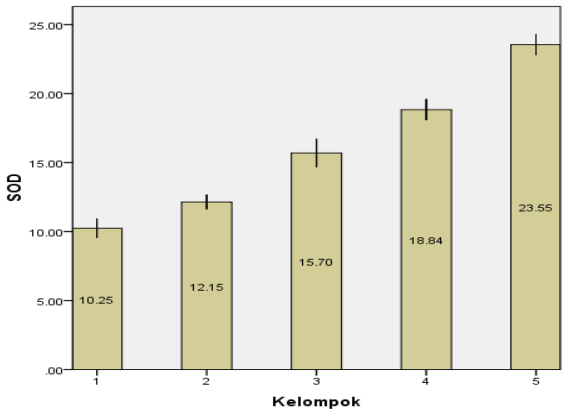

Figure 3.
The Increase of SOD Activity

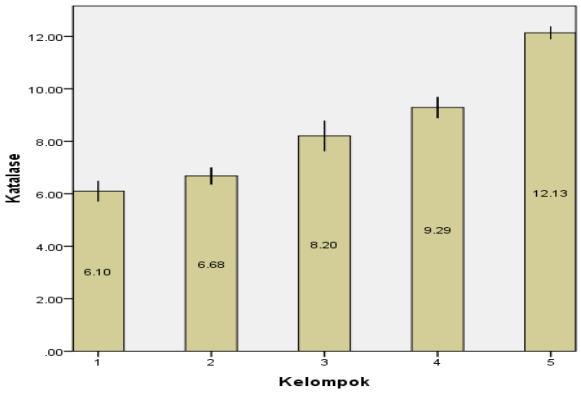

Figure 4.
The Increase of Catalase Activity

Catalase activity analysis showed that the results of Gaharu leaf water extract with increasing doses ranging from 50 mg/kg BW up to 200 mg/kg BW was able to significantly (p <0.05) increase the activity of the enzyme catalase, with the average ranging from 6.68±0,63, 8.20±1.14 and 9.29±0.79 compared to the negative control group without being administered the extract with a mean of 6.1±0.76.

This is due to the quantity of the content of this class of compounds phenol/bioflavonoid which is more and work synergistically, can directly capture or neutralize the free radical superoxide, peroxynitrites, bioflavonoid stimulate the formation of enzymatic antioxidants catalase and endothelial dysfunction can be reduced.[12-13] The decrease of

Catalase activity mentioned above can be seen in the table 4 and figure 4.

CONCLUSION

Gaharu leaf water extract with increasing doses of 50 mg/kg BW, 100 mg/kg BW and 200 mg/kg BW significantly ($p < 0.05$) can reduce oxidative stress through the mechanism of reduction in MDA and 8-OHdG and SOD enzyme activity and the increase catalase in blood of Wistar rats given maximal activity. The higher the dose given greater reduction in levels of MDA and 8-OHdG, and given the higher dose the greater the increase in enzyme activity of SOD and catalase. This is due to the content of phenolic compounds / flavonoids greater will be stronger in the capture and neutralize free radical superoxide, hydroxyl and peroxynitrite that endothelial dysfunction and oxidative stress can be prevented.

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