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Effect of *Spirulina platensis* administration on liver catalase specific activities of various ages Wistar Rats

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Abstract

Oxidative stress is one of the conditions that increases following an increase in age with a significant oxidative stress can be found in the liver. *Spirulina platensis* is a plant that has antioxidant activity that can prevent oxidative stress. Therefore, researchers will determine the effect of *Spirulina platensis* on catalase specific activities in rat liver tissue of various age groups. This study is an experimental study using rat liver tissue of 30 rats aged 12 weeks, 18 weeks, and 24 weeks that were given aquadest, and aged 12 weeks, 18 weeks, and 24 weeks that have been treated with *Spirulina platensis* administration, compared to only treated by Aquadest administration. Specific activity of the catalase of the liver will be measured using the Claiborne method. The specific activity of catalase in rat liver is the highest in rats aged 12 weeks that were given aquadest while the lowest in mice aged 24 weeks that were given Spirulina extract. A significant difference of specific activity of catalase can be seen between rats aged 24 weeks and rats aged 12 weeks and rates aged 18 weeks. Rats that were given Spirulina extract have lower specific activity of catalase than rats that were given aquadest, with a significant difference of specific activity of catalase can be seen between rats aged 18 weeks. All rats that were given *Spirulina platensis* extract have a lower specific activity of catalase than rats that were given aquadest, with a significant difference can be found in rats aged 18 weeks.

Keywords: Increasing Age; Antioxidant; Oxidative Stress; Reactive Oxygen Species

1. Introduction

Oxidative stress is an imbalance between biochemical processes related to the production of reactive oxygen species (ROS) and the production of antioxidants. Oxidative stress can be linked to the aging process.[1] Oxidative stress is thought to increase as the organism ages through the free radical theory of aging. This is because oxidative stress can induce damage in cells and body tissues.[2]

The body has the ability to protect itself from oxidative stress in the form of enzymatic and nonenzymatic antioxidants. One antioxidant that plays an important role in preventing oxidative stress is catalase contained in peroxisomes.[3] Catalase plays a role in breaking down H_2O_2 into water and oxygen.[4] In addition, catalase also plays an important role in preventing the accumulation of H_2O_2. If H_2O_2 accumulates in large quantities, the Fenton reaction can be induced and cause the production of -OH which is highly reactive and can interfere with biological systems.[5]

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Catalase deficiency can be linked to the pathogenesis of various diseases associated with increasing age such as diabetes mellitus, hypertension, and cancer.[4] Increasing catalase concentration can help inhibit a person's aging because it can reduce the release of H_2O_2 from heart mitochondria, reduce the accumulation of oxidized DNA, and reduce the susceptibility of mitochondria to H_2O_2 damage. This is consistent with increased antioxidant protection of mitochondria.[6] Decreased catalase expression in older populations can lead to impaired protection against oxidants.[7]

The liver is one of the organs that has various physiological processes. One of the roles of the liver is to perform lipid metabolism.[8] Oxidative metabolism has one of the ROS products in the form of H_2O_2 which can be produced in peroxisomes. The product will also be broken down by peroxisomes that can contain catalase.[9] Peroxisomes can be found in high amounts to counter the amount of ROS produced due to the amount of metabolism that occurs in the liver.[10]

Spirulina platensis is a blue green algae that is widely found in Indonesian waters and is easy to cultivate and harvest.[11] Spirulina is known to have various benefits such as food, environmental pollution control, and fertilizer. In addition, Spirulina has potential in the health sector such as antioxidants, antibacterial and antimicrobial, anti-inflammatory, and immune enhancers.[12] One of the Spirulina contents that has beneficial properties for health is phycocyanin. Phycocyanin is a substance that provides colouring to Spirulina which has antiplatelet, hepatoprotective, antioxidative, anti-inflammatory, and cholesterol-lowering properties.[13] Phycocyanin can help reduce the effects of induced oxidative stress significantly through its antioxidative effects. Phycocyanin also have high superoxide dismutase (SOD) activity that acts as an ROS scavenger.[14] Spirulina given in extract form has many beneficial ingredients and has the potential to become a therapeutic drug.[15]

Efforts to prevent oxidative stress due to increasing age are one of the efforts to prevent damage from occurring in the body. Therefore, this research was conducted for determine the effect of Spirulina extract, which has antioxidant properties, in helping to prevent oxidative stress in male Wistar rat model through its effect on the specific activity of catalase enzyme in liver tissue.

2. Material and methods

2.1. Samples preparation

The samples were stored liver tissue from male Wistar rats that had been treated with *Spirulina platensis* ethanol extract 200 mg/kgBB. Spirulina platensis ethanol extract was made at the Department of Chemistry, Faculty of Medicine, University of Indonesia. Groups of male Wistar rats consisted of 12-week, 18-week, and 24-week groups. The 12-week group is equivalent to the age of puberty in humans, the 18-week group is equivalent to post-pubertal age in humans, and the 24-week age is equivalent to adult age in humans. Each male Wistar rat in the test group was given 200 mg/kgBB of ethanol extract once per day for 29 days. Male Wistar rats in the control group will be given distilled water for 29 days.

2.2. Tissue homogenization

Frozen stored liver tissue samples were removed from the -20°C Deepfreezer. Each liver tissues were weighed and recorded. Then, 100 mg liver pieces were taken and put into a 1.5 ml test tube. One hundred milligrams of tissue from each liver tissue was dissolved with 500 µL of Phosphate buffered saline (PBS) pH 7.4 reagent then homogenized. The homogenization process was carried out using a homogenizer and micropestle. After the sample was homogenized, centrifugation was carried out at 3500 rpm within 10 minutes. The supernatant produced were taken and stored for analysis related to catalase activity.

2.3. Protein level measurement

Measurement of protein levels in liver tissue is measured using the Christian Warburg method. The principle of this examination is the absorption of light by proteins in the ultraviolet region with a wavelength of 280 nm. The amino acid residues tryptophan and tyrosine in the protein can absorb the light emitted by the device. A standard curve has been made using BSA as the base. The standard curve that has been made becomes a reference for calculating the protein content in the homogenate.

2.4. Catalase specific activities measurement

Catalase enzyme activities were measured using Claiborne method.[16] The samples were measured based on the decomposition of H_2O_2 free radicals by catalase in liver cells' samples then the catalase activities were measured through their Absorbance values using a spectrophotometer.

Before conducting samples examination, time catalase activities optimization were carried out. The best absorbance results were obtained between the 30 and 150 second time reactions. The absorbance results were averaged and entered into the formula to determine the catalase activity (U/mL). Specific catalase activity (U/mg protein) in liver tissue was obtained by dividing catalase activity by protein content (mg/mL).

The catalase level calculated based on formula:

$$Catalase\ activity\ (\frac{U}{ml}) = \frac{\text{A}(\Delta\text{t0}-\text{t1})\text{sample} - \text{A}(\Delta\text{t0}-\text{t1})\text{Blank}}{\text{H2O2 molarity}}\ X\ dilution$$

With:
A= Absorbance
t0= after 30 seconds; t1= after 150 seconds

Then, catalase specific activity was measured with formula:

$$Catalase\ specific\ activity\ (\frac{U}{mg}) = \frac{\text{catalase activity } (\frac{\text{U}}{\text{ml}})}{\text{protein concentration } (\frac{\text{mg}}{\text{ml}})}$$

2.5. Statistical analysis

All data obtained were processed using the SPSS program. Normality test has been conducted before conducting the analysis. The normality test used is Shapiro-Wilk because the sample used is less than 50. Based on the Shapiro-Wilk test, the significance value for all data groups is> 0.05. Therefore, it can be concluded that the data is normally distributed.

Analysis has been conducted to determine the comparison between groups of rats between ages given distilled water using the ANOVA method and the comparison between groups of Spirulina-treated rats and distilled water treatment using the independent t-test method. The data of specific activity of catalase enzyme in liver tissue of rats given distilled water at various ages are homogeneous. Therefore, ANOVA test followed by Tukey's post hoc was conducted to compare the means.

3. Result and Discussion

3.1. Liver cells' catalase specific activities of rats' with increasing age

The specific activity of catalase enzyme from Wistar rat liver tissue can be seen in Figure 1. The highest specific activity of catalase enzyme can be found in the 12-week-old distilled water treatment group, i.e. 0.028 U/mg. The lowest specific activity of catalase enzyme can be found in the Spirulina treatment group at the age of 24 weeks, i.e. 0.007 U/mg.

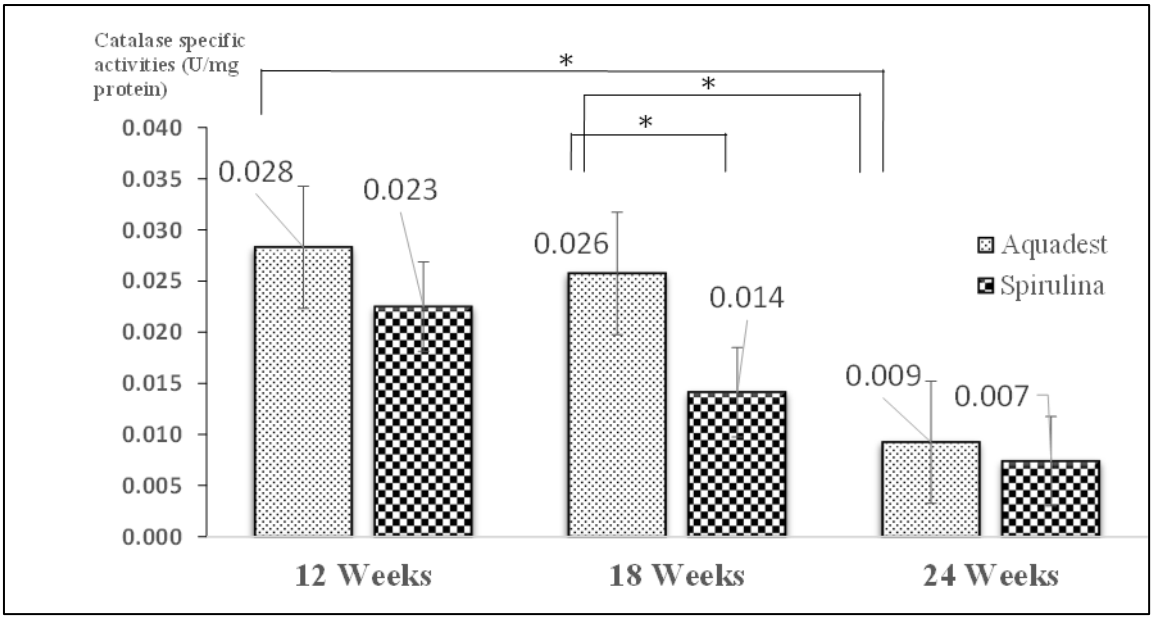

Figure 1 Catalase specific activities of 12-, 18-, and 24-weeks old age Wistar rats' liver after treated with Aquadest and *Spirulina platensis*

The catalase specific activities in rats' group without *Spirulina platensis* treatments were decreasing as the age increase. Catalase specific activities from 12-weeks old age, 18-weeks old, and 24-weeks old were 0.028 U/mg protein, 0.026 U/mg protein, and 0.009 U/mg protein respectively. The group of Spirulina-treated Wistar rats at 12 weeks, 18 weeks, and 24 weeks of age had specific enzyme activities of 0.009 U/mg, 0.006 U/mg, and 0.003 U/mg, respectively. There is significant different of catalase specific activity between 12- and 24-weeks aquadest group ($P< 0.001$) and between 18- and 24-weeks age group ($P< 0.001$). (Figure 1)

Oxidative stress is a condition caused by excessive levels of oxidants compared to antioxidants in the body. Oxidative stress has a relationship with degenerative diseases and aging because it plays a role in aging.[3] Long-term oxidative stress will cause disruption of cell structures such as proteins and DNA. The rate of lipid peroxidation will increase rapidly under oxidative stress conditions, disrupting the physiological and structural functions of a cell.[17] The process of increasing age itself can cause an increase in ROS formation due to degeneration of the mitochondrial system so that cell death increases due to oxidative stress.[18] Antioxidants are compounds that act as oxidation preventers of a substrate. The way antioxidants work is by fighting free radicals in the body.[19] Antioxidants play a role in preventing and eliminating oxidative stress by fighting the adverse effects of ROS so that they play an important role in maintaining cell function.[20] One of the antioxidants that plays a role in slowing down oxidative stress is catalase.[21] Catalase has an important role in regulating oxidant levels in the body. Catalase can break down H_2O_2 into H_2O and O_2. Deficiency or disruption of catalase can cause metabolic diseases such as diabetes and osteoporosis as well as age-related neurological diseases such as Alzheimer's disease and Parkinson's disease.[4] Decreased antioxidant activity, including catalase, can be found in various animal tissues.[22] Based on previous research, catalase enzyme activity was found to decrease with age. Vida et al. stated that older mice have a decrease in catalase activity and response which causes a buildup of H_2O_2.[23] A decrease in catalase activity with age can also be found in other animals, such as Caenorhabditis elegans.

Research by Samarghandian et al. showed that there was a significant decrease in catalase enzyme activity between animal liver tissue at the age of 20 months compared to 10 months.[24] Another study also mentioned that catalase activity in rat liver tissue decreased in association with aging.[25]

3.2. Liver cells' catalase specific activities of 12-, 18-, and 24-weeks old rats after *Spirulina platensis* administration

The liver catalase specific activities are found to be lower in *Spirulina*-treated groups compared to the only aquadest-treated in 12-, 18-, and 24-weeks old age. (Figure 1) Catalase specific activities of 12-weeks old Spirulina-treated group were 0.795 times than the Aquadest-treated group. Catalase specific activities of 18-weeks old Spirulina-treated group were 0.549 times than the Aquadest-treated group. Catalase specific activities of 24-weeks old Spirulina-treated group were 0.799 times than the Aquadest-treated group. (Table 1)

Table 1 Total decrease of specific catalase activity after Spirulina administration in all age groups

Age	12 Weeks	18 Weeks	24 Weeks
Catalase specific activity ratio (Aquadest : Spirulina platensis treatment)	1 : 0.795	1 : 0.549	1 : 0.799

Independent T-test was conducted to analyze the difference between the Spirulina-treated group and the Aquadest-treated group. There are no significant difference in catalase enzyme activity between Spirulina- and Aquadest-treated in the liver tissue of rats aged 12 weeks (p=0.156) and 24 weeks (p=0.154). Significant differences is found between the liver tissue of rats Spirulina- and Aquadest-treated in the 18-week age group (p=0.002).

Spirulina has a strong antioxidant effect because it has antioxidants, some of which are Phycocyanin and ß-carotene. These antioxidants have good effects on reducing oxidative stress and inflammation. Phycocyanin has the effect of eliminating antioxidants by reducing peroxidation and inflammation. Therefore, phycocyanin has a role in recycling free radicals. ß-carotene has a role in inhibiting singlet oxygen that can cause lipid damage due to peroxidation. [26]

Teimourpour et al. revealed that the concentration of spirulina administration can affect catalase enzyme activity. Low concentration can help increase catalase enzyme activity while high concentration can decrease catalase enzyme activity. Spirulina platensis can play a role in increasing or decreasing catalase activity which will affect the concentration of H_2O_2. The mechanism that is thought to play a role in the effect of spirulina on catalase activity is related to its effect on its structure. Spirulina will bind to the two binding sites on the catalase enzyme through hydrogen bonds and van der Waals interactions. Spirulina in low concentrations will bind to catalase in a folded state so that activity and stability will increase. However, higher concentrations of spirulina will bind to catalase in a semi-folded or unfolded state, changing the shape of the active site and inhibiting enzyme activity.[27]

4. Conclusion

The specific activity of catalase enzyme in the liver tissue of Wistar rats was lower at older ages with significant differences found in the 24-week age group compared to the 12-week and 18-week age groups. The catalase specific activity in the liver tissue of Wistar rats treated with *Spirulina platensis* are lower than treated with Aquadest both in the 12-, 18-, and 24-weeks old age groups with significant differences in the 18 week age group.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

Statement of ethical approval

The animal study protocol had obtained Ethical Clearance from Research Ethic Committee of Faculty of Medicine, Universitas Indonesia No. KET- 699/UN2.F1/ETIK/PPM.00.02/2020.

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