Broccoli Effects on HIF-1α in Heart Endothelial Cells of Rats that Induces Psychological Stress

Haqqelni Nur Rosyidah[1], Bambang Wirjatmadi[2], Bambang Purwanto[3]

[1]Researcher and Student Master of Public Health at Airlangga University, Surabaya Indonesia, 60115, [2]Professor Department of Nutrition, Faculty of Public Health, Airlangga University, Surabaya Indonesia, [3]Senior Lecturer Department of Physiology School of Medicine Airlangga University, Surabaya Indonesia, 60115

Abstract

Psychological stress possibly caused by sleep dissoder, which can cause oxidative stress. Oxidative stress can attack the heart by endothelial dysfunction. Which is expected to be reduced by administering antioxidants from steam of broccoli juiced. Broccoli known had high antioxidant activity, beside that broccoli known as one of resource of sulforaphene. Sulforaphene is an indirect antioxidant and can be an antidepressant. The purpose of this study was to determine the benefits of broccoli in reducing the number of Hif-1α expression of heart cells in male white rats wistar strain psychological stress model. This study used the Randomized Post Test Only Group Design method and statistical analysis used One Way Anova with SPSS 23. The results showed that steamed broccoli juice had a significant effect on reducing the number of Hif-1α expression in male white rat heart cells that models of psychological stress, with a sig value of 0.000 ($p < 0.05$). So steamed broccoli juice can help reduce the risk of endothelial dysfunction caused by psychological stress.

Keywords: *Psychological stress, Oxidative stress, Endothelial dysfunction, Broccoli juice, Hif 1α expression*

Introduction

Psychological stress is normal for every human being. the challenges that humans accept are getting stronger and the psychological state considers that unable to face these challenges. This psychological condition is called distress which causes a person to experience sleep disorders.(1)

The World Health Organization states that psychological disorders/anxiety and depression are common mental disorders. The organization estimates that around 4.4% of the global population has a depressive disorder and 3.6% has an anxiety disorder. The phenomenon that occurred from 2005 to 2015 was that the number of people with depression increased rapidly by 18%. More than 80% of this disorder affects citizens of low- and middle-income developing countries. According to WHO, people with depression who receive treatment are about 25 percent of the total 100 million people with depression in the world. It is estimated that by 2020 depression will become an important medical condition in the world. (2)

Indonesia is categorized as a country that is aware that mental disorders affect health. The Ministry of the Republic of Indonesia includes mental disorders in Basic Health Research (Riskesdas). The results of Riskesdas showed that the prevalence of mental emotional disorders showing symptoms of depression and anxiety was 6% of the total population of Indonesia or reached 14 million people.(3)

Broccoli is a food source that has high antioxidant activity and a food source that contains lots of sulforaphene. Based on the evaluation using the utilizing Oxygen Radical Absorbance Capacity (ORAC) or the free radical absorption ability test, the ORAC number in cooked broccoli is 1590 µmol TE per serving and in boiled broccoli is 2160 µmol TE per serving. (4) Sulforaphene is a content that high in broccoli.

Corresponding author:
Bambang Purwanto,
Department of physiology school of medicine Airlangga university. Surabaya, Jawa Timur 60115, Indonesia. E-mail : bambang-purwanto@fk.unair.ac.id

Sulforaphene increase phase II antioxidant enzyme in the human upper airway.[5] Sulforaphene has been shown to prevent angiotensin II by activation of Nrf2.[6] Other effects of sulforaphene are significantly decreased the IL-1β and endothelin contens in end. 3 cells.[7] Aside from being an antioxidant, Sulforaphane is also capable of producing effects such as antidepressant anxiolytic.[8]

Method

This research is a experimental laboratories. This study used a posttest control group study design with a completely randomized. The experimental animal in this study used a male white mouse (Rattus norvegicus) strain of Wistar who was exposed to psychological stress form of an inverted sleep pattern. The research sample was 8 tails obtained from the calculation results using Lameshow and devided in two group. Control group and treatment group exposed psychological stress form of an inverted sleep pattern. Treatment group also given steamed broccoli juiced. Experimental laboratories conducted in the laboratory of biochemistry and anatomy airlangga university in 2020. Data were analyzed descriptively and presented in mean, standard deviation, minimum and maximum values. Statistical analysis with One Way Anova. One Way Anova has two steps insist normality and homogeneity test. The dependent variable in this study is the number of Hif-1α expression. While the independent variables in this study are dose of steamed broccoli juice and treatment. Each male rats after get psychological stress exposed in treatment group given 3,6 gram of steamed broccoli. Juiced steamed broccoli is made using an 80 rpm slow juicer. The flavonoid and polyphenol content in 100 grams of broccoli extracted using a slow juicer with a speed of 80 rpm were 1,018.32 ± 57.80 mg / L and 1,226.24 ± 36.74 mg / L.[9]

Result

Characteristics of Respondents

Data were analyzed descriptively and presented in the table below:

Table 1. Descriptively of Research Subjects

Group	Mean ± SD	Minimum value	Maximum value
Control group	9,38 ± 0,82	8,40	10,40
Treatment group	6,85 ± 0,13	6,70	7,00

One Way Anova of Affect the Broccoli juiced of the number of Hif-1α expression with 3 step :

The normality test was tested using the Shapiro-Wilk test with the results presented in the table ;

Table 2. Normality test

Group	n	P value	Category
Control group	4	0,804	Normal
Treatment group	4	0,972	Normal

Homogeneity using the Levene Test, shows the data of all experimental animal groups with a value of $p = 0.332$, which means that the variance of Hif 1α expression data is homogeneous.

One Way Anova which was carried out on all experimental animal groups on the expression of Hif 1α endothelial cells showed $p = 0.000$ ($p < 0.05$), so it can be concluded that there is a difference in the expression of Hif 1α endothelial cells in the treatment group.

Discussion

Based on the data obtained, psychological stress treatment due to exposure to the reverse sleep pattern can increase the expression of Hif 1α cardiac endothelial cells in treatment groups exposed to psychological stress. The data also showed that giving broccoli juice could reduce the expression of Hif 1α cardiac endothelial cells in the treatment group exposed to psychological stress.

In this study it was also proven that the mean Hif 1α expression showed that treatment group after receiving a psychological stressor and given broccoli juice is 6.85 ± 0.13 percent. Expression of Hif 1α in the treatment group which after receiving a psychological stressor given broccoli juice was lower than the mean expression of Hif 1α in the control group which only received psychological stressors had a mean of 9.38 ± 0.82 percent.

The statistical calculation also shows a significant difference between the psychological stress control group and the psychological stress treatment group given broccoli juice with a significant value of 0.000 (p value <0.05). So it can be stated that broccoli juice is effective in reducing the expression of Hif 1α heart cells in mice treated with psychological stress.

Psychological stress that occurs continuously can result in an increase in free radicals or reactive oxygen species (ROS) in the body. ROS, especially the superoxide anion, can combine and destroy the peroxynitrate which produces NO. The mechanism of decreasing NO production is the formation of an L-arginine analogue, namely ADMA (NG-dimethyl-L-arginine) which is an endogenous competitor for NOS. This results in an impaired vascular endothelial response (endothelial dysfunction), resulting in decreased vasodilation activity. Endothelial dysfunction reduces nitric oxide (NO) in the vascular wall. Endothelium-dependent vasodilation has decreased activity due to increased blood flow. Free radical gain through the electron transport chain reaction in the mitochondria. The reaction catalyzed by NADH / NADPH oxidase results in increased superoxide production in the endothelium. So that there is an imbalance between ROS and antioxidants, causing oxidative stress.[10]

The antioxidant components contained in broccoli include flavonoids. This content causes broccoli to have potential as an antioxidant which can reduce ROS in the body. So as to prevent endothelial dysfunction caused by psychological stress. Prevention of endothelial dysfunction can reduce the impact of oxidative stress in the form of preventing tissue hypoxia in the form of reduced Hif 1α expression in heart cells. Research by Qian states that the flavonoid components in plants can provide benefits in improving vascular endothelial cell function in NO bioavability disorders due to glucotoxic conditions.[11]

Sulforaphane as an inductor of Nrf2 has the ability to react with sulfhydryl (-SH) and Nrf2 groups bound to sulfur compounds. SFN can prevent Ang II-induced cardiomyopathy through Nrf2-mediated activation of exogenous antioxidant defenses, and regulation and activation of Nrf2 by SFN through the Akt / GSK-3β / Fyn pathway.[6]

Conclusion

1. Psychological stress with reverse sleep pattern can cause endothelial dysfunction that causes hypoxia, as indicated by the increased expression of Hif 1α

2. Broccoli juice can reduce the expression of Hif 1α so that it can prevent cardiac endothelial dysfunction so that cells do not experience hypoxia and reduce the risk of damage to the heart organ

Suggestion

This study can provide information to the public that psychological stress can increase free radicals and hypoxia which have an impact on cardiac endothelial dysfunction. Other education regarding broccoli consumption can have a positive effect in overcoming the effects of psychological stress. Broccoli can lower the risk of cardiac endothelial dysfunction.

Acknowledgements : On this occasion, the authors would like to thank the ethical clearance committee of Public Health Airlangga University, Laboratories of Biochemistry and Anatomy Airlangga University

Conflict of Interest: The authors have no conflicts of interest associated with the material presented in this paper.

Source of Funding: The researcher conducted individual funding.

References

1. Elias H, Ping WS, Abdullah MC. Stress and academic achievement among undergraduate students in Universiti Putra Malaysia. In: Procedia - Social and Behavioral Sciences. 2011.
2. WHO. Depression. WHO Factsheet. World Health Organization. 2015.
3. Departemen kesehatan. Riset Kesehatan Dasar [Internet]. 2018. Available from:http://www.depkes.go.id/resources/download/infoterkini/materi_rakorpop_2018/Hasil Riskesdas 2018.pdf
4. Haytowitz D, Bhagwat S. ORAC of Selected Foods, Release 2. Nutr Data Lab Beltsv Hum Nutr Res Cent Agric Res Serv US Dep Agric [Internet].2010;1–46. Available from: http://www.ars.usda.gov/Services/docs.htm?docid=15866
5. Riedl MA, Saxon A, Diaz-Sanchez D. Oral sulforaphane increases Phase II antioxidant enzymes in the human upper airway. Clin Immunol [Internet]. 2009;130(3):244–51. Available from: http://dx.doi.org/10.1016/j.clim.2008.10.007
6. Xin Y, Bai Y, Jiang X, Zhou S, Wang Y, Wintergerst KA, et al. Sulforaphane prevents angiotensin II-induced cardiomyopathy by activation of Nrf2 via stimulating the Akt/GSK-3ß/Fyn pathway. Redox Biol [Internet]. 2018;15(November 2017):405–17. Available from: https://doi.org/10.1016/j.redox.2017.12.016
7. Wu H, Chen J, Yuan M, Xu G, Lin S. Effects of sulforaphane on TNF-α-induced proinflammatory cytokine production and reactive oxygen species generation in mouse brain endothelial cells. Int J Clin Exp Med. 2016;9(2):4929–38.
8. Wu S, Gao Q, Zhao P, Gao Y, Xi Y, Wang X, et al. Sulforaphane produces antidepressant- and anxiolytic-like effects in adult mice. Behav Brain Res. 2016;301:55–62.
9. Lee SG, Kim JH, Son MJ, Lee EJ, Park WD, Kim JB, et al. Influence of extraction method on quality and functionality of broccoli juice. Prev Nutr Food Sci. 2013;18(2):133–8.
10. Weidinger A, Kozlov A V. Biological activities of reactive oxygen and nitrogen species: Oxidative stress versus signal transduction. Vol. 5, Biomolecules. 2015.
11. Cao J, Zhang X, Wang Q, Jia L, Zhang Y, Zhao X. Influence of flavonoid extracts from celery on oxidative stress induced by dichlorvos in rats. Hum Exp Toxicol. 2012;31(6).