Broccoli Effects on Hif-1 A in Spermatozoa Cells of Ratsthat Induces Psychological Stress

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Abstract

Psychological stress which has an impact on oxidative stress and impaired endothelial function. Oxidative stress and endothelial dysfunction can lead to infertility. Broccoli with SFN as an active ingredient to prevent further damage due to endothelial dysfunction. The purpose of this study was to analyze the effect of broccoli juice (Brasica oleracea L. var italica) endothelial function of spermatozoa cells in male white rats Wistar strain psychological stress model. The design used in this study is the Randomized Post Test Only Control Group Designand statistical analysis used One Way Anova. Exposure to psychological stress by changing the watchful sleep pattern, so that the experimental animals experiencing oxidative stress were found, then administering broccoli juice to the treatment group with a single dose of 3.6 g / head. The results showed significant data changes in expression of HIF 1 alpha sperm cellswith a P value of 0,000. The conclusion of this study is that there is a significant effect between the provision of broccoli juice on the expression of HIF 1 alpha spermatozoa cells exposed to psychological stress from sleep disorders.

Keywords*: Oxidative Stress, Endothelial dysfuction, SFN, Spermatozoa Cells, HIF-1a*

Introduction

Sleep is the body's healing process that plays an important role in physical and psychological health. A person's sleep needs vary and can be classified according to age. The average sleep requirement for a person at middle age ranges from 7-9 hours per day and 7-8 hours for someone aged 65 years and over. However, with age, a person's need for sleep decreases due to various types of activities characterized by reduced sleep duration at night. The amount of sleep lost for a certain period of time from the optimal sleep time required and cause sleep cycle disruption is also called sleep deprivation (SD).(1)

Sleep deprivation can cause stress and affect the health of the body. The reduction in sleep time may be associated with oxidative stress mechanisms due to an imbalance between the formation and elimination of Reactive Oxygen Species (ROS). Someone who is too often exposed to oxidative stress can interfere with fertility, especially in men through spermatogenesis and erectile function. In Indonesia, there are 40% of couples of childbearing age and 10% of them experience infertility. Disturbance factors in men account for 40% of the causes of infertility. One way to determine the diagnostic value of male infertility is spermatozoa analysis. As much as 25% of the spermatozoa analysis results showed teratozpermia results or abnormalities in the morphology of spermatozoa.(2,3,4)

Sleep deprivation can cause stress that affects the quality of spermatozoa through the activation pathway of the Hypothalamus Pituitary Adrenal (HPA) axis and has an effect on glucocorticoid production. Excess production of glucocorticoids decreases the production of Gonadotropin Releasing Hormone (GnRH) by the hypothalamus which in turn reduces the production of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH).(5) Decrease in the amount of FSH and LH causes a decrease in testosterone secretion. Decreased testosterone levels cause nutrition for spermatogenesis to be disturbed. Increased glucocorticoids also cause oxidative stress due to increased production of Reactive Oxygen Species (ROS). The effect of decreased

testosterone and increased production of ROS plays a role in impaired spermatogenesis. [6,7]

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.[8] In broccoli, the main glucosinolate is glucoraphanin. The result of the breakdown of glucoraphanin is sulforaphane, which triggers the production of phase II enzymes, which are included in phase II enzymes, namely, glutathione S-transferase (GST), sulfotransferase, N-acetyl-transferase.[9] The active sulforaphane compound in broccoli is believed to improve Hif-1α expression. on the cell. Sulforaphane (SFN) is a natural compound with antioxidant, anti-inflammatory and neuroprotective activities. Sulforaphan is associated with normalization of stress-induced HPA axis dysfunction and an inhibitory effect on the inflammatory response to stress, which focuses on the neuroimmune mechanisms of psychological stress or depression. As a low toxicity dietary phytochemical, sulforaphane is widely consumed and has been eligible to be considered as a supplement, food, or drug, depending on its intended use.[10]

Method

This research is an experimental study, using experimental animals with a post test control group design. Samples were adult male white rats (Rattus norvegicus) aged 2-3 months and weighing 180-200 grams. Rats were divided into two groups (control group and treatment group), and each group consisted of 4 rats. The determination of the sample size is based on the Lemeshow formula. Control group and treatment group, exposed psychological stress, form of an inverted sleep pattern. The treatment group also given steamed broccoli juiced. Each male rats after get psychological stress exposed in treatment group, given 3.6 grams of steamed broccoli. Juiced steamed broccoli is made using an 80 rpm slow juicer. The flavonoids 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.[11] Data analysis used SPSS 22 for Windows. Data analysis began with the Shapiro-Wilk normality test. The data obtained were normally distributed, then continued with the Oneway ANOVA parametric test.

Result

Descriptive analysis used mean, standard deviation (SD), minimum value and maximum value of HIF-1α expression of spermatozoa cells after treatment.

Table 1: Descriptive Analysis of Research Results

Subject Group	n	Mean	SD	Minimum	Maximum
Expression of HIF-1α Spermatozoa Cells					
Control Group	4	10,08	0,67	9,50	10,70
Treatment Group	4	8,50	1,36	7,00	10,30

The expression of HIF-1α spermatozoa cells after treatment was tested for normality using the Shapiro Wilk test. If the result shows p> α (0.05) then the data is normally distributed.

Table 2: Shapiro Wilk Test of Research Results

Subject Group	n	P	Category
Expression of HIF-1α Spermatozoa Cells			
Control Group	4	0,053	Normal
Treatment Group	4	0,797	Normal

n = number of samples; p = rate of significance

Homogeneity of the HIF-1α expression of spermatozoa cells after treatment was tested using Lavene's test. The results showed that the data variant of the research results was homogeneous (p> 0.05).

Table 3 Results of Research Data Homogeneity Test Results

Subject Group	n	P	Catergory
Expression of HIF-1α Spermatozoa Cells	8	0,297	Homogenous

Anova statistical test to determine the difference between the four treatment groups. The ANOVA results showed that the four treatment groups had a significance value or p (0.000) <α (0.05), so it could be concluded that at least one treatment group was different or there was a difference in the expression of HIF-1α spermatozoa cells in the treatment group.

Discussion

ANOVA results showed a significant difference with p <0.05, indicating that there was an effect of treatment on the expression of HIF-1α spermatozoa cells in the control group, there was a difference in the effect of treatment with the treatment group that was given broccoli 3.6 g / head with a value of P 0.000. The mean of the control group was 10.08 ± 0.67, which was greater than the treatment group with a mean of 8.50 ± 1.36. These results prove that giving broccoli at a dose of 3.6 g / head can reduce the expression of HIF-1 α white spermatozoa cells Wistar strain rats.

Oxidative stress is a condition in which there is increased cellular damage caused by oxygen and oxygen-derived oxidants which are better known as ROS (Reactive Oxygen Species). This process is the result of an imbalance between the production and elimination of ROS, where there is an increase in the formation of ROS without being balanced by their elimination by antioxidants in the body. The formation of ROS is a physiological process of the body, however if there is an excessive increase it can negatively affect the body. In this case it is associated with male infertility. 40.88% of infertile male patients have sperm with high ROS levels. [12] In addition to damaging the plasma membrane, oxidative stress can also damage the integrity of DNA in the nucleus of spermatozoa. This DNA damage will eventually induce cell apoptosis which ultimately leads to a decrease in the number of spermatozoa.[13]ROS is a free radical that has high oxidative ability. Free radicals are compounds (not only oxygen derivatives) that contain one or more free electrons so they are unstable. The most important ROS compounds in the reproductive system are superoxide (O2-), hydrogen peroxide (H2O2), peroxyl (ROO-), and hydroxyl (OH-). In addition, nitrogen derivatives such as nitrogen oxides (NO-) and peroxynitrate (ONOO-) also play an important role in fertility and the reproductive system.[14] The plasma membrane and cytoplasm of spermatozoa cells contain large amounts of unsaturated fatty acids, so that ROS can easily penetrate plasma membrane.[15]The main mechanism in the process of damage to the spermatozoa membrane by ROS is the lipid peroxidation reaction or LPO (Lipid peroxidation). Hydrogen Peroxide (H2O2) is the compound that produces the largest ROS in human spermatozoa. Increased hydrogen peroxide will decrease intracellular ATP and axonemal protein phosphorylation. In addition, it can also induce lipid peroxidation processes which will cause cell death.[16]

Broccoli affects the decrease in blood pressure and pulse rate by reducing arterial contraction caused by vasocinstriction such as phenillaryine and endothelin-1. Broccoli assists eNOS activation and increases NO bioactivation which results in relaxation of blood vessels by means of alpha adrenegic blockade thereby helping to decrease cardiac contractation. Decreased heart contractions can prevent blood turbulence so that signs of hypoxia are stable. Sulforaphan is an isothiocyanate derivative as an anticancer and antioxidant. As an antioxidant, sulfraphan activates phase 2 detoxification of antioxidant enzymes through nuclear factor E2-related factor 2 (Nrf2). Sulforaphan has the ability to react with

sulfhyhydryl (-SH) groups or bind with sulfur chemical compounds. Sulfyhydryl is a group that plays a role in nitric oxide synthase in the endothelium.[17]

Conclusion

1. Psychological stress with an inverted sleep pattern can lead to endothelial dysfunction leading to hypoxia, as indicated by 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 testicular organ damage.

Suggestion

It is hoped that further research can be carried out on the effect of other antioxidants on the expression of HIF-1α in Spermatozoa Cells. And tt is hoped that further research can be carried out on the effect of exposure to psychological stress on organs other than the reproductive organs.

Conflict of Interest: The authorshavenoconflicts of interest associated with the material presented in this paper.

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