RESEARCH ARTICLE

Indonesian Journal of Anti-Aging Medicine
Volume 3, Number 1, J anuary-June 2019 : 20-23

IJAAM
Indonesian Journal of Anti Aging Medicine

Administration of broccoli extract (*Brassica oleracea var. italica*) inhibited the increase of malondialdehyde level and the decrease of aortic endothelial cells in male wistar rats (*Rattus norvegicus*) exposed by cigarette smoke

Hayatul Firi[1*], I Gusti Made Aman[2], Gde Ngurah Indraguna Pinatih[3]

ABSTRACT

Introduction: In the environment, there are many free radicals for the body, one of them is cigarette smoke which can cause oxidative stress conditions and result in damage to body cells. The broccolis extract containing flavonoids, glucosinolates, vitamins C, E and beta-carotene which have a high antioxidant protective effect.

Methods: An experimental post-test only control study was conducted using 36 males Wistar rats, aged 10-12 weeks, and 200-300 gr weight which were divided in 2 groups (control and treatment group). The broccoli extract (120 mg/ 200 grBB) was given to treatment group one hour before exposure to cigarette smoke. After 28 days of the treatment, the rats were taken for examination of MDA levels and underwent a surgery for aortic tissue for endothelial histopathology examination.

Results: The Finding indicated that the data were in normal distribution through data analysis of the Shapiro Wilk test. Lavene's test showed that both the data distribution and variety of both groups was homogenous ($p>0.05$). Through independent t-test, there were significant differences between the two groups on the value of MDA and aortic endothelium number ($p <0.05$) as the result of the comparison result of both groups.

Conclusion: The administration of broccoli extract (*Brassica oleracea var.italica*) inhibits the increased levels of blood malondialdehyde and a decreased of aortic endothelial cells in Wistar male rats exposed to cigarette smoke.

Keywords: broccoli extract, oxidative stress, MDA levels, endothelial cell counts

[1]Master Program in Biomedical Sciences Study Program Specificity of Anti-Aging Postgraduate Program Udayana University Denpasar
[2]Department of Pharmacology, Faculty of Medicine, Udayana University
[3]Department of Community Medicine/ Pre-Clinical Medical Sciences, Medical Faculty

*Corresponding Author:
Dessy Indriana Hatta;
Master Program in Biomedical Sciences Specificity of Anti-Aging;
Postgraduate Program Udayana University Denpasar;
drcanthique@gmail.com

Submitted: 17 December 2018
Accepted: 7 March 2019
Published: 1 June 2019

INTRODUCTION

The polluted environment and unhealthy lifestyle are commonplace in nowadays' industrial era. In the environment there are many pollutants as free radicals to the body, such as cigarette smoke, the fumes of motor vehicle, UV radiation, X-rays, gamma rays, the consumption of foods high in fat, alcohol, pesticides or other toxic substances that contribute to the increase of endogenous free radicals that are believed as the cause of premature aging.

In the concept of Anti-Aging Medicine, all the aging factors can be prevented, slowed or even inhibited. Aging is treated as a disease, can be prevented, treated and even returned to the original organ function, so that the life expectancy can be longer with a better quality of life[1].

There are many theories about the mechanism of aging from experts. However, the theory that most widely adopted is the free radical theory which states that the aging process begins with the initiation of free radical reactions that progressively cause damage to the biological system[2].

Cigarette smoke contains more than 7000 toxic chemical substances in form of gases and particles, including nicotine, tar, Plumbum (Pb), CO, ammonia, formalin, cadmium, acetone, methane, urea and many more. Each cigarette contains 10^{14-16} reactive oxygen species (ROS) such as superoxide (O_2'), hydrogen peroxide (H_2O_2), hydroxyl radical (OH'), peroxyl radical (ROO')[3].

The high accumulation of exogenous free radicals from cigarettes and endogenous free radicals causes oxidative stress condition to the occurrence of lipid peroxidation. Lipid peroxidation causes damage to cells. The oxidative stress due to cigarette smoke affects both coronary and peripheral circulation, which causes problems in thrombogenesis, fibrinolysis, platelet activity and inflammatory

systems, hence caused the occurrences of endothelial dysfunction, coronary atherosclerosis, plaque rupture and acute coronary syndrome[4,5]. The dysfunction of endothelial refers to the inability of endothelial cells to regulate vascular homeostasis[6]. The oxidative stress condition affects the high levels of Malondialdehyde (MDA) which is the final product of lipid peroxidation, a compound that can describe the activity of free radicals in the cell so that it is used as one of the indicators of oxidative stress that caused by free radicals. The high level of MDA is a sign of high free radicals[7].

Broccoli is a type of *Brassicaceae* (kind of cabbage), vegetable containing flavonoids, glucosinolates, vitamins C, E, and beta-carotene. The results of the examination by the Laboratory Services Unit of the Faculty of Agricultural Technology at Udayana University obtained the IC50 value of broccoli extract was 41.87 mg/ L, this indicates that broccoli has a high antioxidant ability in counteracting free radicals.

The aim of this study was to identify the administration of broccoli extract in inhibiting the increase level of blood malondialdehyde levels and decrease the aortic endothelial cells of Wistar rat exposed to cigarette smoke.

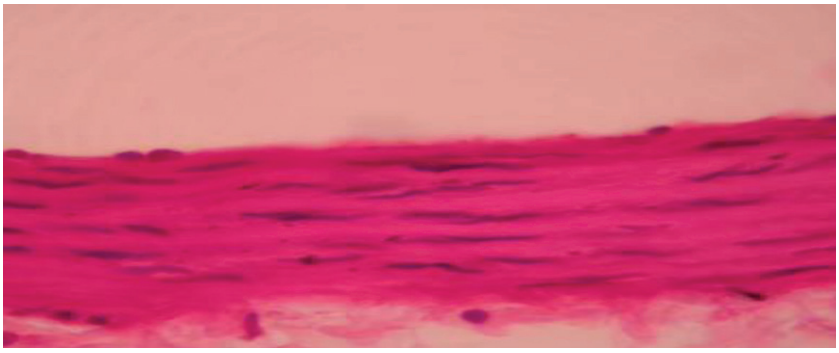

Figure 1. Histopathology of aortic endothelial of control rats (P0) showed a small number of endothelial cells along the aortic intima tunica

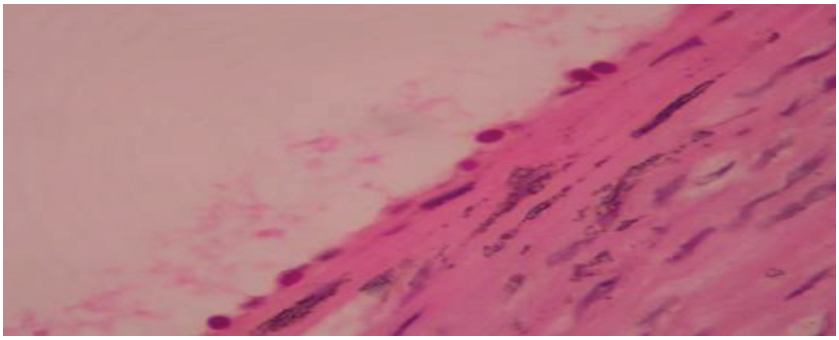

Figure 2. Histopathology of aortic endothelial of treated rats (P1) showed a number of endothelial cells along the aortic intima tunica.

METHODS

The broccoli extraction was done in the Laboratory Services Unit of the Faculty of Agricultural Technology at Udayana University. 36 male Wistar rats (*Rattus norvegicus*) aged 10-12 weeks weighing 200-300 grams were used in this study. The acclimatization of rats was carried out for seven days at the research site for adjustment to the environment. After the 7th day, the subjects were divided into two groups, each group consists of 18 rats. For the first group (P0), 2 ccs of placebo were given one hour before exposure to cigarette smoke. As for the second group (P1), 120 mg/ 200 grBB of broccoli extract was given one hour before exposure to cigarette smoke. Then, both groups of rats were exposed to cigarette smoke in a special cage, one cigarette per rats for 3 hours. After 28 days of treatment, the blood of all rats was taken for MDA level examination and then the aortic tissue surgery was performed for endothelial histopathology examination. Then, the data were analyzed and presented using descriptive analysis, data normality, data homogeneity, and comparability tests.

RESULTS

After 28 days of treatment, the examination of MDA levels and surgery on both groups were done, aortic tissue was taken and histopathological examination of aortic tissue was performed to observe endothelial cells, using HE (Hematoxylin-Eosin) staining (**Figures 5.1** and **5.2**). The calculation of aortic endothelial cells was undergone using an electron microscope of Olympus CX21 with 400 times magnification.

From **Table 1**, the mean MDA value of the control group (P0) was 7.87 ± 3.47 and the mean of the treatment group (P1) was 4 ± 3.28. The independent sample t-test analysis showed that the value of t = 3.43 and the value of p = 0.002. This indicated that the two groups had a significantly different MDA value after the treatment ($p < 0.05$).

From **Table 2**, the mean of endothelial cells in the control group (P0) was 37.28 ± 10.26 and in the treatment group (P1) was 73.17 ± 6.87. The Independent Sample T-test analysis showed that the value of t = -12.3321 and p-value = 0.000. This indicated that the two groups had a significantly different mean of endothelial cells count after receiving treatment ($p < 0.05$).

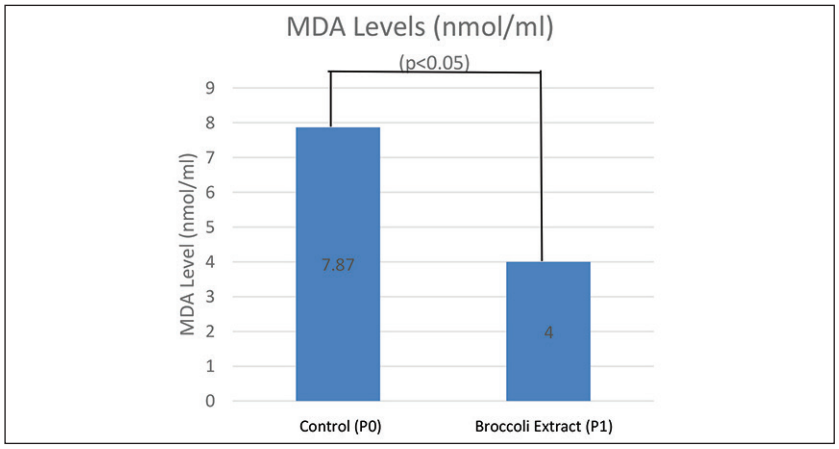

Figure 3. Difference of malondialdehyde (MDA) mean value from both group after the treatment

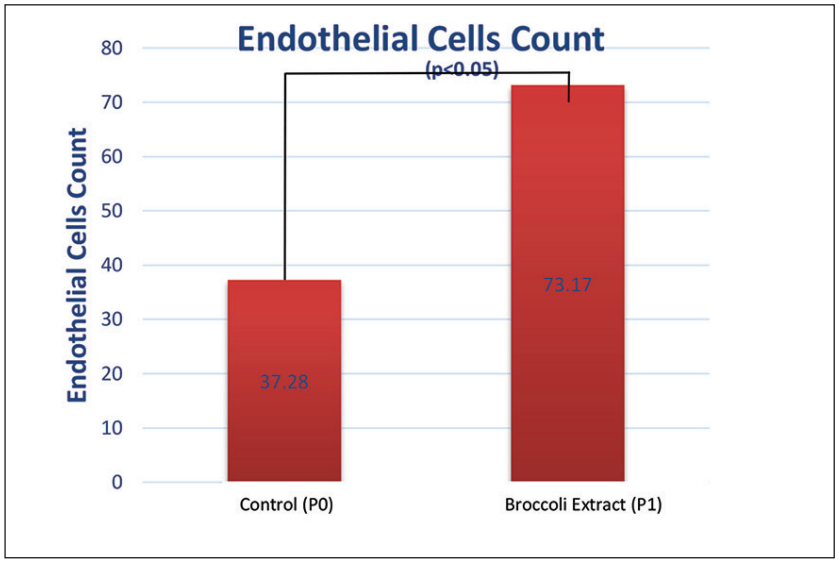

Figure 4. Difference of mean aortic endothelial count from both group after the treatment

Tabel 1. The difference of the mean of MDA value from both groups after the treatment

Variable	N	Mean	SB	*T*	P
MDA Value	Group P0	7.87	3.47	3.432	0.002
	Group P1	4.00	3.28		

Table 2. The difference of the mean of aortic endothelial count to both of group after the treatment

Variable	Groups	Mean	SB	*T*	P
Endothelial Count	Group P0	37.28	10.26	-12.321	0.000
	Group P1	73.17	6.87		

DISCUSSIONS

The integrity of endothelial function could be disrupted by various factors such as hyperglycemia, hypercholesterolemia, toxic substances including free radicals, drugs and immunologic processes[7]. Cigarettes contain more than 7,000 chemicals. Each cigarette contains 10^{14-16} reactive oxygen species (ROS) such as superoxide (O_2'), hydrogen peroxide (H_2O_2), hydroxyl radical (OH'), peroxyl radical (ROO')[2]. A high number of free radicals and all chemical components contained in cigarette smoke could create oxidative stress condition and lipid peroxidation. Lipid peroxidation in the cell membrane produces the final product of malondialdehyde. The high level of lipid peroxidation is caused by the high number of free radicals/ ROS which affects the high levels of Malondialdehyde (MDA). Malondialdehyde is toxic which can react with proteins and DNA, hence caused cell damage[8].

Free radicals caused the chain of reactions of lipid peroxidation in cell membranes, altered mitochondrial function, decreased the enzyme of NADPH-oxidase and activated inflammatory cells and caused cell death. Moreover, endothelial disturbances were also affected by disturbances in the bioavailability of Nitric Oxide (NO), and cigarette smoke contributes to the interference of NO^6 activity.

The main mechanism of cellular defense against oxidative stress was through the signal path of Nrf2-ARE which could be activated by the intervention of nutrients (phytochemicals, phenolic acids)[4,9]. Broccoli contains the main flavonoids in the form of quercetin and kaempferol which had the ability to donate hydrogen atoms from its hydroxyl group, binding (chelation) metal ions as well as the ability to stimulate the activation of phase II detoxification enzymes. This ability was also shown by other components of broccoli, namely glucoraphanin. The activation of this phase-2 detoxification enzyme caused an increase in cellular glutathione (GST, GPX) levels, which had the ability to remove hydrogen peroxide to protect the cells against oxidative stress and toxicity[10,11]. Cell protection against oxidative stress was also done by other antioxidant abilities which were also found in broccoli extracts, namely vitamin C, E, and beta-carotene. Broccoli had an IC50 value of 41.87 mg/ L which means it had the high antioxidant ability.

In this study, the antioxidant component of broccoli extract could reduce the number of endothelial cells released due to cell damage by free radicals

through a mechanism of decreasing oxidative stress conditions in which characterized by the decreased levels of MDA in the blood. This condition also indirectly reduced the disturbances that threaten the bioavailability of NO. Whereas, as generally known that the bioavailability of NO is very important in maintaining endothelial homeostasis.

CONCLUSIONS

Based on the results of this study it can be concluded that the broccoli extract (*Brassica oleracea var. italica*) has been proved to inhibit the increase of blood malondialdehyde levels and decrease the number of aortic endothelial cells in rats that exposure to cigarette smoke.

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