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Antioxidant Efficacy of Flavonoid in Broccoli (Brassica oleracea var. italica) to Decrease Alveolar Macrophages in Lung Tissue Due to Cigarette Smoke Exposure

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Brassica oleracea; Alveolar Macrophages; Lung tissue; Free radicals

ABSTRACT

Cigarette smoke has shown various kinds of free radical content. The increasing number of free radicals from exposure to cigarette smoke that enters the respiratory tract may result in lung tissue damage. The reason is because excessive free radicals can trigger oxidative stress due to an imbalance in the number of antioxidants in the body and trigger an inflammatory response. Alveolar macrophages are one of the defence systems in lung tissue which will increase when the number of free radicals is increasing. Giving antioxidants from outside the body can help reduce the impact of free radicals. This study aims to determine the effect of broccoli extract on the reduction of alveolar macrophages. Study is experimental using Randomized Controlled Trial design. Research process was carried out for 21 days by dividing the experimental animals into 6 groups. Each group was given a treatment cigarette smoke 2 times per day for 2 minutes. The extract of broccoli in different doses (0.5ml;0.75ml;1 ml;1.25 ml). The assessment of the number of alveolar macrophages with Hematoxylin Eosin (HE) staining, the measurements were carried out by calculating the average of 10 visual fields in each preparation. The results showed that the administration of broccoli extract resulted in a decrease in the number of alveolar macrophages (ANOVA, p=0.000). In addition, the increased administration of broccoli extract would further reduce the number of alveolar macrophages ($P<0.05$). It can be concluded that the intake of broccoli extract can reduce the number of alveolar macrophages due to exposure to cigarette smoke.

1. INTRODUCTION

Tobacco cigarettes contributes to the increase in the number of non-communicable diseases in the world that may cause morbidity and mortality [1]. The increased death threat is estimated to exceed that of the people with HIV, tuberculosis, and malaria. By 2030, deaths caused by smoking are estimated to reach 8 million people per year or almost 10% of the total deaths [2]. In developing countries, the risk of death caused by increasing cases of non-communicable diseases due to smoking is greater than in developed countries [3]. The adult age group is the highest group in the use of tobacco cigarettes. The decrease in the use of cigarettes

has been carried out through various health promotion efforts. From 2005 to 2015, there was a significant decrease in the prevalence of adolescent smokers, but there has been no decline since 2015 until now [4]. The availability of tobacco cigarettes, affordable prices, traditions, lifestyles, promotion of the cigarette industry as well as the influence of friends and external parties are among the factors that someone wants to start trying to smoke cigarettes [5], [6].

The increasing risk of death from smoking has been proven for a long time. Deaths caused by the disease from smoking are estimated to have reduced about 10 years of life compared to non-smokers. This effect is not only found in active smokers, but also found in smokers who have quitted smoking [7]. Most of the diseases caused by smoking are related to the respiratory tract such as Chronic Obstructive Pulmonary Disease (COPD), asthma, to lung cancer. In addition, the negative health effects of smoking are cardiovascular disease, blood vessel disorders, osteoporosis and stroke [8], [9]. There are known carcinogenic substances content and other harmful chemicals in cigarettes such as nicotine, tar, and benzopyrene. Moreover, cigarette smoke also contains carbon monoxide (CO) which can affect the bond of oxygen (O_2) and haemoglobin (Hb) [10]. Those various kinds of harmful content will be inhaled into the respiratory tract and will be considered as a form of free radicals in the body [11]. The free radicals that enter the respiratory tract will respond to the body's first defence system, namely the alveolar macrophages. These cells are the body's first defence system that has the functions to kill pathogens and pollutants that enter the respiratory tract [12]. Alveolar macrophages will perform phagocytosis and stimulate the secretion of pro-inflammatory cytokines. This process can take place continuously and is directly proportional to the number of free radicals from the cigarette smoke that enters the respiratory tract. Lung tissue damage can be caused by the reaction to an increase in the number of alveolar macrophages and the response of inflammatory [13].

Free radicals have an important role for the body; in small to moderate concentration, they are needed to synthesize cellular structures and the body's immune system. Excessive increase in free radicals is usually obtained from outside the body, and it can cause damage to death of a tissue. Actually, a human body has the ability to prevent and reduce the impact of tissue damage due to free radicals by neutralizing it with antioxidants [14]. However, if the ratio between free radicals and antioxidants is not appropriate, then it is necessary to provide intakes from outside the body. Various types of vegetables and fruits are believed to be the main sources of antioxidants [15], [16]. Both can play a role in helping to increase antioxidant activity in the body (enzymatic antioxidants) or directly neutralize free radicals that enter the body (non-enzymatic antioxidants) [17]. One type of vegetable that contains plenty antioxidants is broccoli (Brassica oleracea var. italica). This vegetable is known to contain high levels of flavonoids, so it is believed to act as anti-inflammatory, anti-cancer, and antioxidant [18]. Giving broccoli to reduce free radicals caused by exposure to cigarette smoke has never been done. Therefore, this study aims to determine the changes in the number of alveolar macrophages to the administration of broccoli extract (Brassica oleracea var. italica) due to the exposure to cigarette smoke.

2. METHODS

This research was experimental with post-test control group design. The research sample used was male Wistar rats (Rattus novergicus) for 21 days. This study divided the experimental animals into 6 groups, namely a negative control group, a positive control group, and four treatment groups. The maintenance and care of experimental animals was carried out using the 3R principle (Replacement, Reduction, and Refinement). The positive control group and the treatment group will be exposed to clove cigarette smoke because these cigarettes are the most often consumed in Indonesia. The nicotine and tar content in the cigarettes is 2.4 mg and 38 mg, respectively. Exposure to the cigarette smoke is given for 2 cigarettes per day for 21 days.

The production of broccoli extract (Brassica oleracea L. var. Italica) through the maceration process uses 96% ethanol as the solvent, and it was continued with a vacuum rotary evaporator to obtain the preparation. After the extract was available, the flavonoid content (18 mg/ml) was measured. The intake of broccoli extract in experimental animals was carried out using a conversion table between Laurence and Bacharach organisms through a comparison of the adequacy rate of flavonoids per day (8 mg/day), in order to obtain a dose of 0.5 ml; 0.75 ml; 1 ml; 1.25 ml.

In this study, the experimental animals were divided into 6 groups. The first group was the negative control group, where experimental animals were only given daily intake. The second group was the positive control group, where experimental animals were only exposed to cigarette smoke. The four treatment groups were those that were exposed to cigarette smoke and given different doses of Broccoli extract in the amount of 0.5 ml; 0.75 ml; 1 ml; 1.25 ml respectively. After the intervention given for 21 days, the experimental animals were terminated for lung organ harvesting. The histopathological preparations of lung tissue were made by histotechniques process and Hematoxylin Eosin (HE) staining. The parameter in this study was the average number of alveolar macrophages in 10 fields of view per lung tissue preparation.

3. RESULTS

Table 1. shows the average number of alveolar macrophages in 10 visual fields. The highest mean value was obtained in group II of 5.40 ± 0.65, while the lowest value was obtained in group I of 2.32 ± 0.23. These results will then be tested for normality between groups.

Table 1: Average Number of Alveolar Macrophages Between Groups

	Groups	Mean ± SD
I	Negative control group	2,32 ± 0,23
II	Positive control group (exposure to cigarette smoke)	5,40 ± 0,65
III	Treatment group I (exposure to cigarette smoke and administration of 0.5 ml of extract)	5,04 ± 0,54
IV	Treatment group II (exposure to cigarette smoke and administration of 0.75 ml of extract)	4,32 ± 0,54
V	Treatment group III (exposure to cigarette smoke and administration of 1 ml extract)	3,56 ± 0,17
VI	Treatment group IV (exposure to cigarette smoke and administration of 1.25 ml of extract)	3,36 ± 0,26

Based on table 2. It shows that the results of the normality test have a p value greater than 0.05 so it can be concluded that all groups have data that are normally distributed. Meanwhile, the homogeneity test (p = 0.009) obtained a p value <0.05 so that the data can be said to be inhomogeneous. Because the data has a normal distribution and is not homogeneous, the calculation of the difference between groups will be carried out using a T-test.

Table 2: ANOVA test results in each group

Groups	Normality test	Homogeneity test
I	0,814	0,009
II	0,111	
III	0,201	
IV	0,117	
V	0,314	
VI	0,421	

The results of the differences between one group and another using the T-test in each group. Based on table 3. shows that the negative control group has a significant difference ($p < 0.05$) against all groups. In the positive control group, an insignificant difference was found in the comparison of the first treatment group ($p = 0.206$). In the first and second treatment groups there were significant differences ($p<0.05$) against all groups. The third treatment group also had an insignificant difference with the fourth treatment group ($p = 0.477$).

Table 3. T-test test results in each group

Groups	I	II	III	IV	V	VI
I	-	-	-	-	-	-
II	0,000	-	-	-	-	-
III	0,000	0,206	-	-	-	-
IV	0,000	0,001	0,016	-	-	-
V	0,000	0,000	0,000	0,011	-	-
VI	0,001	0,000	0,000	0,002	0,477	-

The figure below shows the comparison of the negative control group (figure 1.) with the treatment group (figure 2.) on the number of alveolar macrophages (red arrow).

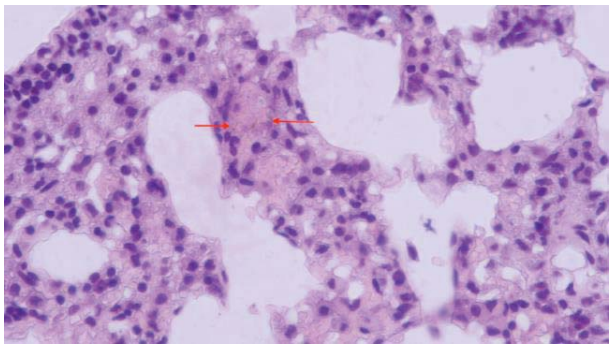

Figure 1: Negative Control Group

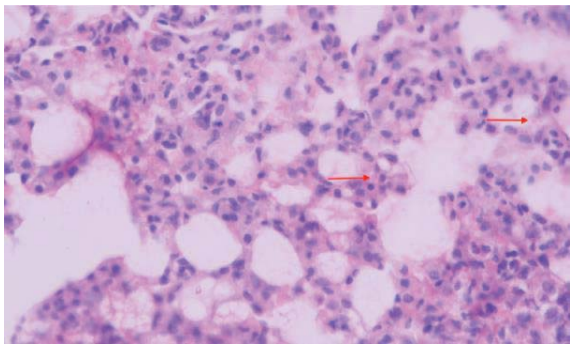

Figure 2: Treatment Group

4. DISCUSSION

The increase in free radicals in the body is caused by cigarette smoke that enters the respiratory tract. These free radicals will stimulate non-specific antibodies in the lung tissue, namely alveolar macrophages. Increased exposure to cigarette smoke is directly proportional to the increase in the number of alveolar macrophages in the lung tissue. The positive control group that received exposure to cigarette smoke showed an increase in the number of alveolar macrophages compared to the other groups [19]. Macrophage cells can be found in almost all body tissues and are the main bacterial killing mechanism through the process of phagocytosis. In the lungs, macrophage cells are often referred to as alveolar macrophages and have a fairly large number compared to other organs. The role of alveolar macrophages in innate immunity represents more than 90% of immunity in the airways. In addition, alveolar macrophages also produce inflammatory mediators such as Reactive Oxygen Species (ROS) under physiological conditions, express pro-inflammatory cytokines such as interleukins (IL-1, IL-6, IL-8), Tumor Necrosis Factor Alpha (TNF-α) and Interferon gamma (IFNγ) and the release of anti-inflammatory mediators [20], [21].

The administration of antioxidants from broccoli extract showed a decrease in the number of alveolar macrophages. Broccoli contains a variety of antioxidants; one of the main antioxidants is flavonoids [22]. Flavonoid antioxidants have various benefits and considerable pharmacological activities, and their potential as antioxidants have been widely found in various studies as one of the micronutrients capable of neutralizing

free radicals [23]. Its ability to reduce free radicals is carried out by suppressing the synthesis of Reactive Oxygen Species, inhibiting the enzymes produced and increasing antioxidant activity [24]. Flavonoids protect cells and tissues from damage caused by oxidative stress caused by normal oxygen metabolism processes or induction from outside the body [25].

Flavonoids protect lipid membranes against various oxidative damage from free radicals. Lipid peroxidation is one of the causes resulting from an imbalance in the number of radicals from cigarette smoke with antioxidants in the body [26], [27]. One of the free radicals as the main cause is superoxide radicals obtained from burning cigarette smoke. Superoxide radicals can be neutralized by the superoxide dismutase enzyme and be turned into hydrogen peroxide, which will then be converted again by glutathione peroxidase into water and oxygen. However, metal ions can trigger an increase in free radicals by reducing hydrogen peroxide to a highly reactive hydroxyl radical. Flavonoid antioxidants can also bind to these metal ions to inhibit the formation of free radicals [28], [29].

Increasing the administration of antioxidants can further reduce free radicals caused by exposure to cigarette smoke [30]. However, the administration of antioxidants with a certain dose will have an insignificant impact on reducing free radicals. This is because free radicals will still be formed physiologically from cell metabolism.

5. CONCLUSION

The intake of broccoli extract (Brassica oleracea L. var. Italica) can affect the number of alveolar macrophages in lung tissue due to exposure to cigarette smoke. Increasing the dose of broccoli (Brassica oleracea L. var. Italica) extract intake will boost the decrease in alveolar macrophages.

6. ACKNOWLEDGMENTS

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7. CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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Journal ID : **TMJ-08-01-2022-10980** Total View : **353**

Title : Adenoid Cystic Carcinoma of the Head and Neck Survival Analysis and Oncologic Outcome Single Institute Experience

Abstract :

To presents our experience, in diagnosis and management of ACC of the head and neck. This is a retrospective review of 57 patients with ACC managed during the period from January 2011 to January 2016. Data about the characteristics and management of the disease were recorded. All patients were followed up to detect the development of local recurrence and distant metastasis and their management. The mean age was 45.5±15.1, with a female-to-male ratio of 1.5:1. The minor salivary glands were affected in 61.4% of cases. Four patients (7%) were metastatic at presentation. The main presenting symptom was swelling, followed by pain. Surgical resection was performed in 48 patients (84.2%) followed by adjuvant radiotherapy in 36 of them. Four patients received radical radiotherapy. Treatment failed in 3 patients. Recurrences were recorded in 21 out of the 50 cured patients; 9 had locoregional recurrence, 9 had distant metastases, and 3 had both. The overall survival (OS) and disease-free survival (DFS) at three years were 79% and 57.1%, respectively. Surgical resection improved OS ($p< 0.001$). Advanced T-stage, lymph node invasion, solid tumors, close or positive margins worsened OS. Adjuvant radiotherapy was associated with better DFS ($p = 0.003$), while solid tumors were associated with worse DFS. Despite aggressive management with radical surgery and adjuvant radiotherapy, recurrence affects 42% of the patients within three years. Patients with unrespectable tumors have a poor prognosis. Adjuvant radiotherapy improves DFS but not OS.

Full article

Journal ID : **TMJ-08-01-2022-10979** Total View : **469**

Title : Antioxidant Efficacy of Flavonoid in Broccoli (Brassica oleracea var. italica) to Decrease Alveolar Macrophages in Lung Tissue Due to Cigarette Smoke Exposure

Abstract :

Cigarette smoke has shown various kinds of free radical content. The increasing number of free radicals from exposure to cigarette smoke that enters the respiratory tract may result in lung tissue damage. The reason is because excessive free radicals can trigger oxidative stress due to an imbalance in the number of antioxidants in the body and trigger an inflammatory response. Alveolar macrophages are one of the defence systems in lung tissue which will increase when the number of free radicals is increasing. Giving antioxidants from outside the body can help reduce the impact of free radicals. This study aims to determine the effect of broccoli extract on the reduction of alveolar macrophages. Study is experimental using Randomized Controlled Trial design. Research process was carried out for 21 days by dividing the experimental animals into 6 groups. Each group was given a treatment cigarette smoke 2 times per day for 2 minutes. The extract of broccoli in different doses (0.5ml;0.75ml;1 ml;1.25 ml). The assessment of the number of alveolar macrophages with Hematoxylin Eosin (HE) staining, the measurements were carried out by calculating the average of 10 visual fields in each preparation. The results showed that the administration of broccoli extract resulted in a decrease in the number of alveolar macrophages (ANOVA, $p=0.000$). In addition, the increased administration of broccoli extract would further reduce the number of alveolar macrophages ($P<0.05$). It can be concluded that the intake of broccoli extract can reduce the number of alveolar macrophages due to exposure to cigarette smoke.

Full article

Journal ID : **TMJ-08-01-2022-10978** Total View : **391**

Title : Cathelicidin in Mesenchymal Stem Cell Conditioned Medium Inhibit Pro-Inflammatory Cytokine and Osteoclastogenesis: A Bioinformatic Approach

Abstract :
Mesenchymal stem cell conditioned mediun (MSC-CM) possessed abundant beneficial active compounds such as Cathelicidin or LL-37. Cathelicidin is antimicrobial peptide that may inhibit the microbial pathogen, pro-inflammatory cytokine and osteoclastogenesis. Cathelicidin may potential for drug development to treat osteolysis due to excessive pro-inflammatory cytokines induced by endotoxin. The aim of this study is to investigate MSCs-CM active compound namely Cathelicidin (LL-37) effect binding to tumor necrosis factor alpha (TNF-α), Interleukin 1 beta (IL-1β), Receptor activator of nuclear factor κB (RANK), and tumor necrosis factor receptor 1 (TNFR1), interleukin- 1 receptor complex type 1 (IL-1RI), receptor activator complex of nuclear factor kappa-B ligand and osteoprotegerin (RANKL / OPG) by means of bioinformatics approach, in silico study. Sample preparation of target protein from RCSB PDB database. Then, the sample went through molecular docking method (rigid-body docking). Last, the sample visualize as 3D structure using PyMol software. The molecular docking simulation results showed that the binding condition with the cathelicidin TNFR_Cathelicidin complex at Rank 1 has the lowest binding energy with a global score of -83.94 kcal/mol, the IL1βR_ Cathelicidin complex at Rank 1 has the lowest binding energy with a global score of -8.43 kcal/mol. The RANK / TNFRSF11A_Cathelicidin complex in Rank 1 has the lowest binding energy with a global score of -21.67 kcal/mol. Ligand-receptor binding of Cathelicidin demonstrated by molecular docking inhibits various pro-inflammatory cytokines that may inhibit osteoclastogenesis in silico.

Full article

Journal ID : **TMJ-08-01-2022-10977** Total View : **474**

Title : Salivary Biomarker Potential for Early Detection of Oral Squamous Cell Carcinoma by Surface Acoustic Wave Technology: A Narrative Review

Abstract :
Oral squamous cell carcinoma (OSCC) is the most frequent oral cancer that occurs with a prevalence of about 90%. OSCC ranked as the sixth most common oral cancer in the world with morbidity and mortality rates of more than 50%. Southeast Asia has the highest prevalence compared to other countries, which is 6.4/100.000, 20-40% of new OSCC cases are detected after experiencing metastasis to the lymph nodes that cause further complications. Therefore, OSCC early detection is needed for better patient management. Surface Acoustic Wave (SAW) is the latest technology that can detect OSCC using salivary biomarkers. Analysis of the potential of saliva-containing biomarkers for early detection of OSCC using Surface Acoustic Wave technology. Salivary biomarkers such as IL-1β, IL-8, and Galectin-3-binding protein (LGALS3BP) increased significantly even in the early stages of OSCC. SAW technology provides accurate label-free detection of various analytes, from molecular to cellular levels through the inverse piezoelectric effect of interactions between specific biomarkers as good as gold standards ELISA. SAW electromechanical between the piezoelectric crystal and the single input electric crystal that combines sends a surface wave to the SAW substrate then binds to OSCC biomarkers and changes SAW frequency. SAW velocity is sensitive to changes in mass loading, causing shifts in latitude and design phases that allow high sensitivity detection. The concentration of IL-1β, IL-8, and LGALS3BP can be defined by this frequency shift measurement, then OSCC can be detected earlier. Salivary biomarkers potentially utilized for early detection of OSCC disease using Surface Acoustic Wave technology.

Full article

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Title : Role of learning organization paradigm informing and developing the intellectual capital in Jordanian hospitals

Abstract :
The purpose of this study is to examine the role of learning organization (LO) paradigm in forming and developing the intellectual capital (IC) in Jordanian hospitals. As well as, to assess differences between governmental and private Jordanian hospitals regarding practices the learning organization, and intellectual capital dimensions. Scientific methodology been followed based on deductive approach, as well as descriptive and analytical methods are used. Hypothesis was set to validate if: There is a significance influence for LO paradigm in forming and developing the IC in hospitals under study. where the field of application was, Jordanian hospitals running under healthcare sector, and data collection methods represented with secondary data, which obtained through the records of organizations under study, as well as the primary data through participants' perceptions toward the research terms by using the

questionnaire as an instrument for research. The study was limited to a sample of four private hospitals and four government hospitals, which could be considere as the largest hospitals working in the healthcare sector in Jordan, with n = 351 and 355, for private

and public hospitals respectively, and total sample 706. The outcomes of the study indicate that there is a significant impact for practicing the overall dimensions of LO in Jordanian hospitals on forming and developing each of human, structural, and customer capital. As well as, found that there is a significant impact for practicing the overall dimensions of LO in Jordanian hospitals on forming and developing the overall IC. The study identifies the LO and IC dimensions in the jordanian healthcare sector, which might be regarded as a developing country and its important for such countries to consider. The study came with developing questionnaire as an instrument to evaluate LO and IC. Application of the tool facilitates other divisions in such sector to analyze the status.

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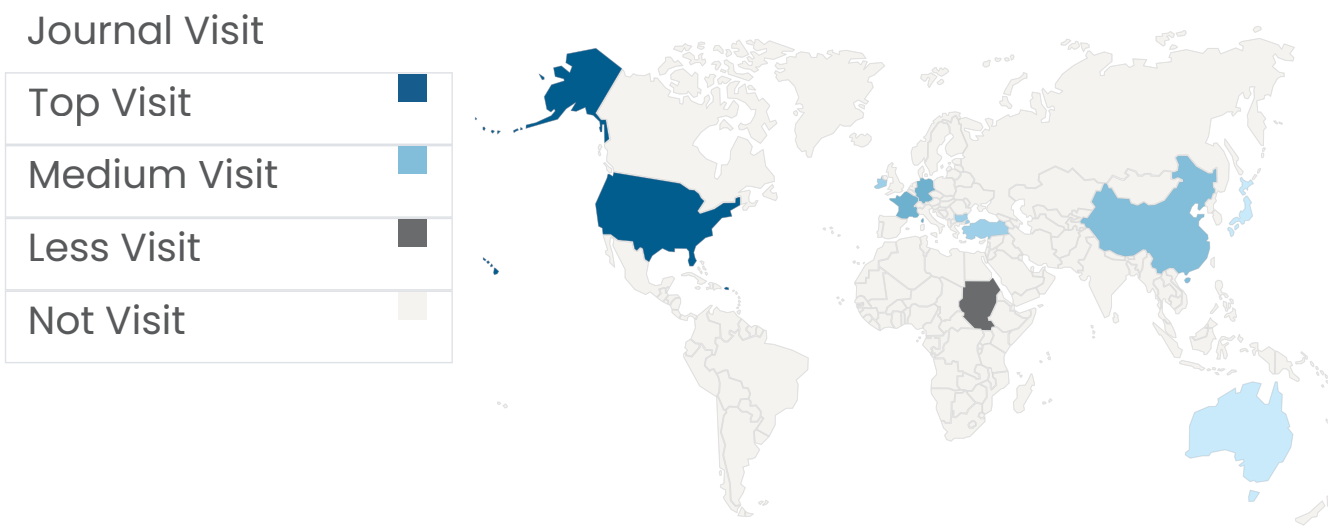

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Antioxidant Efficacy of Flavonoid in Broccoli (Brassica oleracea var. italica) to Decrease Alveolar Macrophages in Lung Tissue Due to Cigarette Smoke Exposure

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Keywords:
Brassica oleracea; Alveolar Macrophages; Lung tissue; Free radicals

ABSTRACT

Cigarette smoke has shown various kinds of free radical content. The increasing number of free radicals from exposure to cigarette smoke that enters the respiratory tract may result in lung tissue damage. The reason is because excessive free radicals can trigger oxidative stress due to an imbalance in the number of antioxidants in the body and trigger an inflammatory response. Alveolar macrophages are one of the defence systems in lung tissue which will increase when the number of free radicals is increasing. Giving antioxidants from outside the body can help reduce the impact of free radicals. This study aims to determine the effect of broccoli extract on the reduction of alveolar macrophages. Study is experimental using Randomized Controlled Trial design. Research process was carried out for 21 days by dividing the experimental animals into 6 groups. Each group was given a treatment cigarette smoke 2 times per day for 2 minutes. The extract of broccoli in different doses (0.5ml;0.75ml;1 ml;1.25 ml). The assessment of the number of alveolar macrophages with Hematoxylin Eosin (HE) staining, the measurements were carried out by calculating the average of 10 visual fields in each preparation. The results showed that the administration of broccoli extract resulted in a decrease in the number of alveolar macrophages (ANOVA, p=0.000). In addition, the increased administration of broccoli extract would further reduce the number of alveolar macrophages ($P<0.05$). It can be concluded that the intake of broccoli extract can reduce the number of alveolar macrophages due to exposure to cigarette smoke.

1. INTRODUCTION

Tobacco cigarettes contributes to the increase in the number of non-communicable diseases in the world that may cause morbidity and mortality [1]. The increased death threat is estimated to exceed that of the people with HIV, tuberculosis, and malaria. By 2030, deaths caused by smoking are estimated to reach 8 million people per year or almost 10% of the total deaths [2]. In developing countries, the risk of death caused by increasing cases of non-communicable diseases due to smoking is greater than in developed countries [3]. The adult age group is the highest group in the use of tobacco cigarettes. The decrease in the use of cigarettes

has been carried out through various health promotion efforts. From 2005 to 2015, there was a significant decrease in the prevalence of adolescent smokers, but there has been no decline since 2015 until now [4]. The availability of tobacco cigarettes, affordable prices, traditions, lifestyles, promotion of the cigarette industry as well as the influence of friends and external parties are among the factors that someone wants to start trying to smoke cigarettes [5], [6].

The increasing risk of death from smoking has been proven for a long time. Deaths caused by the disease from smoking are estimated to have reduced about 10 years of life compared to non-smokers. This effect is not only found in active smokers, but also found in smokers who have quitted smoking [7]. Most of the diseases caused by smoking are related to the respiratory tract such as Chronic Obstructive Pulmonary Disease (COPD), asthma, to lung cancer. In addition, the negative health effects of smoking are cardiovascular disease, blood vessel disorders, osteoporosis and stroke [8], [9]. There are known carcinogenic substances content and other harmful chemicals in cigarettes such as nicotine, tar, and benzopyrene. Moreover, cigarette smoke also contains carbon monoxide (CO) which can affect the bond of oxygen (O_2) and haemoglobin (Hb) [10]. Those various kinds of harmful content will be inhaled into the respiratory tract and will be considered as a form of free radicals in the body [11]. The free radicals that enter the respiratory tract will respond to the body's first defence system, namely the alveolar macrophages. These cells are the body's first defence system that has the functions to kill pathogens and pollutants that enter the respiratory tract [12]. Alveolar macrophages will perform phagocytosis and stimulate the secretion of pro-inflammatory cytokines. This process can take place continuously and is directly proportional to the number of free radicals from the cigarette smoke that enters the respiratory tract. Lung tissue damage can be caused by the reaction to an increase in the number of alveolar macrophages and the response of inflammatory [13].

Free radicals have an important role for the body; in small to moderate concentration, they are needed to synthesize cellular structures and the body's immune system. Excessive increase in free radicals is usually obtained from outside the body, and it can cause damage to death of a tissue. Actually, a human body has the ability to prevent and reduce the impact of tissue damage due to free radicals by neutralizing it with antioxidants [14]. However, if the ratio between free radicals and antioxidants is not appropriate, then it is necessary to provide intakes from outside the body. Various types of vegetables and fruits are believed to be the main sources of antioxidants [15], [16]. Both can play a role in helping to increase antioxidant activity in the body (enzymatic antioxidants) or directly neutralize free radicals that enter the body (non-enzymatic antioxidants) [17]. One type of vegetable that contains plenty antioxidants is broccoli (Brassica oleracea var. italica). This vegetable is known to contain high levels of flavonoids, so it is believed to act as anti-inflammatory, anti-cancer, and antioxidant [18]. Giving broccoli to reduce free radicals caused by exposure to cigarette smoke has never been done. Therefore, this study aims to determine the changes in the number of alveolar macrophages to the administration of broccoli extract (Brassica oleracea var. italica) due to the exposure to cigarette smoke.

2. METHODS

This research was experimental with post-test control group design. The research sample used was male Wistar rats (Rattus novergicus) for 21 days. This study divided the experimental animals into 6 groups, namely a negative control group, a positive control group, and four treatment groups. The maintenance and care of experimental animals was carried out using the 3R principle (Replacement, Reduction, and Refinement). The positive control group and the treatment group will be exposed to clove cigarette smoke because these cigarettes are the most often consumed in Indonesia. The nicotine and tar content in the cigarettes is 2.4 mg and 38 mg, respectively. Exposure to the cigarette smoke is given for 2 cigarettes per day for 21 days.

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The production of broccoli extract (Brassica oleracea L. var. Italica) through the maceration process uses 96% ethanol as the solvent, and it was continued with a vacuum rotary evaporator to obtain the preparation. After the extract was available, the flavonoid content (18 mg/ml) was measured. The intake of broccoli extract in experimental animals was carried out using a conversion table between Laurence and Bacharach organisms through a comparison of the adequacy rate of flavonoids per day (8 mg/day), in order to obtain a dose of 0.5 ml; 0.75 ml; 1 ml; 1.25 ml.

In this study, the experimental animals were divided into 6 groups. The first group was the negative control group, where experimental animals were only given daily intake. The second group was the positive control group, where experimental animals were only exposed to cigarette smoke. The four treatment groups were those that were exposed to cigarette smoke and given different doses of Broccoli extract in the amount of 0.5 ml; 0.75 ml; 1 ml; 1.25 ml respectively. After the intervention given for 21 days, the experimental animals were terminated for lung organ harvesting. The histopathological preparations of lung tissue were made by histotechniques process and Hematoxylin Eosin (HE) staining. The parameter in this study was the average number of alveolar macrophages in 10 fields of view per lung tissue preparation.

3. RESULTS

Table 1. shows the average number of alveolar macrophages in 10 visual fields. The highest mean value was obtained in group II of 5.40 ± 0.65, while the lowest value was obtained in group I of 2.32 ± 0.23. These results will then be tested for normality between groups.

Table 1: Average Number of Alveolar Macrophages Between Groups

	Groups	Mean ± SD
I	Negative control group	2,32 ± 0,23
II	Positive control group (exposure to cigarette smoke)	5,40 ± 0,65
III	Treatment group I (exposure to cigarette smoke and administration of 0.5 ml of extract)	5,04 ± 0,54
IV	Treatment group II (exposure to cigarette smoke and administration of 0.75 ml of extract)	4,32 ± 0,54
V	Treatment group III (exposure to cigarette smoke and administration of 1 ml extract)	3,56 ± 0,17
VI	Treatment group IV (exposure to cigarette smoke and administration of 1.25 ml of extract)	3,36 ± 0,26

Based on table 2. It shows that the results of the normality test have a p value greater than 0.05 so it can be concluded that all groups have data that are normally distributed. Meanwhile, the homogeneity test (p = 0.009) obtained a p value <0.05 so that the data can be said to be inhomogeneous. Because the data has a normal distribution and is not homogeneous, the calculation of the difference between groups will be carried out using a T-test.

Table 2: ANOVA test results in each group

Groups	Normality test	Homogeneity test
I	0,814	0,009
II	0,111	
III	0,201	
IV	0,117	
V	0,314	
VI	0,421	

The results of differences between one group and another using the T-test in each group. Based on table 3. shows that the negative control group has a significant difference ($p < 0.05$) against all groups. In the positive control group, an insignificant difference was found in the comparison of the first treatment group ($p = 0.206$). In the first and second treatment groups there were significant differences ($p<0.05$) against all groups. The third treatment group also had an insignificant difference with the fourth treatment group ($p = 0.477$).

Table 3. T-test test results in each group

Groups	I	II	III	IV	V	VI
I	-	-	-	-	-	-
II	0,000	-	-	-	-	-
III	0,000	0,206	-	-	-	-
IV	0,000	0,001	0,016	-	-	-
V	0,000	0,000	0,000	0,011	-	-
VI	0,001	0,000	0,000	0,002	0,477	-

The figure below shows the comparison of the negative control group (figure 1.) with the treatment group (figure 2.) on the number of alveolar macrophages (red arrow).

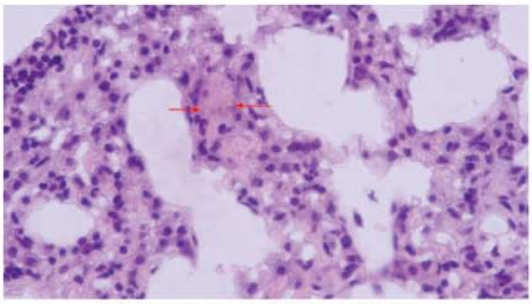

Figure 1: Negative Control Group

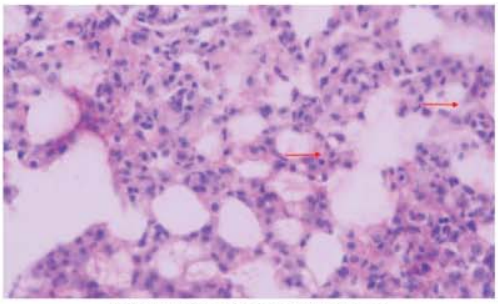

Figure 2: Treatment Group

4. DISCUSSION

The increase in free radicals in the body is caused by cigarette smoke that enters the respiratory tract. These free radicals will stimulate non-specific antibodies in the lung tissue, namely alveolar macrophages. Increased exposure to cigarette smoke is directly proportional to the increase in the number of alveolar macrophages in the lung tissue. The positive control group that received exposure to cigarette smoke showed an increase in the number of alveolar macrophages compared to the other groups [19]. Macrophage cells can be found in almost all body tissues and are the main bacterial killing mechanism through the process of phagocytosis. In the lungs, macrophage cells are often referred to as alveolar macrophages and have a fairly large number compared to other organs. The role of alveolar macrophages in innate immunity represents more than 90% of immunity in the airways. In addition, alveolar macrophages also produce inflammatory mediators such as Reactive Oxygen Species (ROS) under physiological conditions, express pro-inflammatory cytokines such as interleukins (IL-1, IL-6, IL-8), Tumor Necrosis Factor Alpha (TNF-α) and Interferon gamma (IFNγ) and the release of anti-inflammatory mediators [20], [21].

The administration of antioxidants from broccoli extract showed a decrease in the number of alveolar macrophages. Broccoli contains a variety of antioxidants; one of the main antioxidants is flavonoids [22]. Flavonoid antioxidants have various benefits and considerable pharmacological activities, and their potential as antioxidants have been widely found in various studies as one of the micronutrients capable of neutralizing

free radicals [23]. Its ability to reduce free radicals is carried out by suppressing the synthesis of Reactive Oxygen Species, inhibiting the enzymes produced and increasing antioxidant activity [24]. Flavonoids protect cells and tissues from damage caused by oxidative stress caused by normal oxygen metabolism processes or induction from outside the body [25].

Flavonoids protect lipid membranes against various oxidative damage from free radicals. Lipid peroxidation is one of the causes resulting from an imbalance in the number of radicals from cigarette smoke with antioxidants in the body [26], [27]. One of the free radicals as the main cause is superoxide radicals obtained from burning cigarette smoke. Superoxide radicals can be neutralized by the superoxide dismutase enzyme and be turned into hydrogen peroxide, which will then be converted again by glutathione peroxidase into water and oxygen. However, metal ions can trigger an increase in free radicals by reducing hydrogen peroxide to a highly reactive hydroxyl radical. Flavonoid antioxidants can also bind to these metal ions to inhibit the formation of free radicals [28], [29].

Increasing the administration of antioxidants can further reduce free radicals caused by exposure to cigarette smoke [30]. However, the administration of antioxidants with a certain dose will have an insignificant impact on reducing free radicals. This is because free radicals will still be formed physiologically from cell metabolism.

5. CONCLUSION

The intake of broccoli extract (Brassica oleracea L. var. Italica) can affect the number of alveolar macrophages in lung tissue due to exposure to cigarette smoke. Increasing the dose of broccoli (Brassica oleracea L. var. Italica) extract intake will boost the decrease in alveolar macrophages.

ACKNOWLEDGMENTS

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7. CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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Corresponding Author: 1*

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has been carried out through various health promotion efforts. From 2005 to 2015, there was a significant decrease in the prevalence of adolescent smokers, but there has been no decline since 2015 until now [4]. The availability of tobacco cigarettes, affordable prices, traditions, lifestyles, promotion of the cigarette industry as well as the influence of friends and external parties are among the factors that someone wants to start trying to smoke cigarettes [5], [6].

The increasing risk of death from smoking has been proven for a long time. Deaths caused by the disease from smoking are estimated to have reduced about 10 years of life compared to non-smokers. This effect is not only found in active smokers, but also found in smokers who have quitted smoking [7]. Most of the diseases caused by smoking are related to the respiratory tract such as Chronic Obstructive Pulmonary Disease (COPD), asthma, to lung cancer. In addition, the negative health effects of smoking are cardiovascular disease, blood vessel disorders, osteoporosis and stroke [8], [9]. There are known carcinogenic substances content and other harmful chemicals in cigarettes such as nicotine, tar, and benzopyrene. Moreover, cigarette smoke also contains carbon monoxide (CO) which can affect the bond of oxygen (O_2) and haemoglobin (Hb) [10]. Those various kinds of harmful content will be inhaled into the respiratory tract and will be considered as a form of free radicals in the body [11]. The free radicals that enter the respiratory tract will respond to the body's first defence system, namely the alveolar macrophages. These cells are the body's first defence system that has the functions to kill pathogens and pollutants that enter the respiratory tract [12]. Alveolar macrophages will perform phagocytosis and stimulate the secretion of pro-inflammatory cytokines. This process can take place continuously and is directly proportional to the number of free radicals from the cigarette smoke that enters the respiratory tract. Lung tissue damage can be caused by the reaction to an increase in the number of alveolar macrophages and the response of inflammatory [13].

Free radicals have an important role for the body; in small to moderate concentration, they are needed to synthesize cellular structures and the body's immune system. Excessive increase in free radicals is usually obtained from outside the body, and it can cause damage to death of a tissue. Actually, a human body has the ability to prevent and reduce the impact of tissue damage due to free radicals by neutralizing it with antioxidants [14]. However, if the ratio between free radicals and antioxidants is not appropriate, then it is necessary to provide intakes from outside the body. Various types of vegetables and fruits are believed to be the main sources of antioxidants [15], [16]. Both can play a role in helping to increase antioxidant activity in the body (enzymatic antioxidants) or directly neutralize free radicals that enter the body (non-enzymatic antioxidants) [17]. One type of vegetable that contains plenty antioxidants is broccoli (Brassica oleracea var. italica). This vegetable is known to contain high levels of flavonoids, so it is believed to act as anti-inflammatory, anti-cancer, and antioxidant [18]. Giving broccoli to reduce free radicals caused by exposure to cigarette smoke has never been done. Therefore, this study aims to determine the changes in the number of alveolar macrophages to the administration of broccoli extract (Brassica oleracea var. italica) due to the exposure to cigarette smoke.

2. METHODS

This research was experimental with post-test control group design. The research sample used was male Wistar rats (Rattus novergicus) for 21 days. This study divided the experimental animals into 6 groups, namely a negative control group, a positive control group, and four treatment groups. The maintenance and care of experimental animals was carried out using the 3R principle (Replacement, Reduction, and Refinement). The positive control group and the treatment group will be exposed to clove cigarette smoke because these cigarettes are the most often consumed in Indonesia. The nicotine and tar content in the cigarettes is 2.4 mg and 38 mg, respectively. Exposure to the cigarette smoke is given for 2 cigarettes per day for 21 days.

The production of broccoli extract (Brassica oleracea L. var. Italica) through the maceration process uses 96% ethanol as the solvent, and it was continued with a vacuum rotary evaporator to obtain the preparation. After the extract was available, the flavonoid content (18 mg/ml) was measured. The intake of broccoli extract in experimental animals was carried out using a conversion table between Laurence and Bacharach organisms through a comparison of the adequacy rate of flavonoids per day (8 mg/day), in order to obtain a dose of 0.5 ml; 0.75 ml; 1 ml; 1.25 ml.

In this study, the experimental animals were divided into 6 groups. The first group was the negative control group, where experimental animals were only given daily intake. The second group was the positive control group, where experimental animals were only exposed to cigarette smoke. The four treatment groups were those that were exposed to cigarette smoke and given different doses of Broccoli extract in the amount of 0.5 ml; 0.75 ml; 1 ml; 1.25 ml respectively. After the intervention given for 21 days, the experimental animals were terminated for lung organ harvesting. The histopathological preparations of lung tissue were made by histotechniques process and Hematoxylin Eosin (HE) staining. The parameter in this study was the average number of alveolar macrophages in 10 fields of view per lung tissue preparation.

3. RESULTS

Table 1. shows the average number of alveolar macrophages in 10 visual fields. The highest mean value was obtained in group II of 5.40 ± 0.65, while the lowest value was obtained in group I of 2.32 ± 0.23. These results will then be tested for normality between groups.

Table 1: Average Number of Alveolar Macrophages Between Groups

	Groups	Mean ± SD
I	Negative control group	2,32 ± 0,23
II	Positive control group (exposure to cigarette smoke)	5,40 ± 0,65
III	Treatment group I (exposure to cigarette smoke and administration of 0.5 ml of extract)	5,04 ± 0,54
IV	Treatment group II (exposure to cigarette smoke and administration of 0.75 ml of extract)	4,32 ± 0,54
V	Treatment group III (exposure to cigarette smoke and administration of 1 ml extract)	3,56 ± 0,17
VI	Treatment group IV (exposure to cigarette smoke and administration of 1.25 ml of extract)	3,36 ± 0,26

Based on table 2. It shows that the results of the normality test have a p value greater than 0.05 so it can be concluded that all groups have data that are normally distributed. Meanwhile, the homogeneity test (p = 0.009) obtained a p value <0.05 so that the data can be said to be inhomogeneous. Because the data has a normal distribution and is not homogeneous, the calculation of the difference between groups will be carried out using a T-test.

Table 2: ANOVA test results in each group

Groups	Normality test	Homogeneity test
I	0,814	0,009
II	0,111	
III	0,201	
IV	0,117	
V	0,314	
VI	0,421	

The results of the differences between one group and another using the T-test in each group. Based on table 3. shows that the negative control group has a significant difference ($p < 0.05$) against all groups. In the positive control group, an insignificant difference was found in the comparison of the first treatment group ($p = 0.206$). In the first and second treatment groups there were significant differences ($p<0.05$) against all groups. The third treatment group also had an insignificant difference with the fourth treatment group ($p = 0.477$).

Table 3. T-test test results in each group

Groups	I	II	III	IV	V	VI
I	-	-	-	-	-	-
II	0,000	-	-	-	-	-
III	0,000	0,206	-	-	-	-
IV	0,000	0,001	0,016	-	-	-
V	0,000	0,000	0,000	0,011	-	-
VI	0,001	0,000	0,000	0,002	0,477	-

The figure below shows the comparison of the negative control group (figure 1.) with the treatment group (figure 2.) on the number of alveolar macrophages (red arrow).

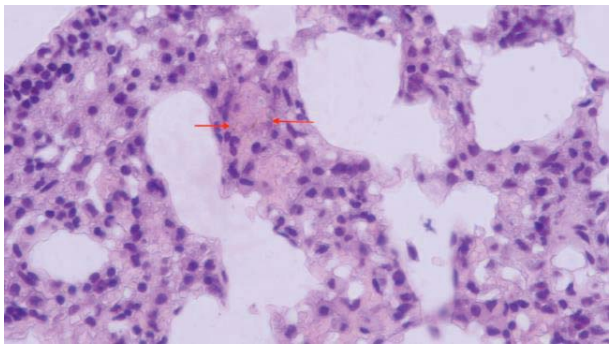

Figure 1: Negative Control Group

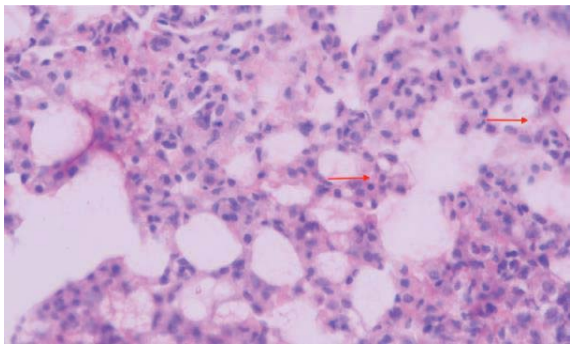

Figure 2: Treatment Group

4. DISCUSSION

The increase in free radicals in the body is caused by cigarette smoke that enters the respiratory tract. These free radicals will stimulate non-specific antibodies in the lung tissue, namely alveolar macrophages. Increased exposure to cigarette smoke is directly proportional to the increase in the number of alveolar macrophages in the lung tissue. The positive control group that received exposure to cigarette smoke showed an increase in the number of alveolar macrophages compared to the other groups [19]. Macrophage cells can be found in almost all body tissues and are the main bacterial killing mechanism through the process of phagocytosis. In the lungs, macrophage cells are often referred to as alveolar macrophages and have a fairly large number compared to other organs. The role of alveolar macrophages in innate immunity represents more than 90% of immunity in the airways. In addition, alveolar macrophages also produce inflammatory mediators such as Reactive Oxygen Species (ROS) under physiological conditions, express pro-inflammatory cytokines such as interleukins (IL-1, IL-6, IL-8), Tumor Necrosis Factor Alpha (TNF-α) and Interferon gamma (IFNγ) and the release of anti-inflammatory mediators [20], [21].

The administration of antioxidants from broccoli extract showed a decrease in the number of alveolar macrophages. Broccoli contains a variety of antioxidants; one of the main antioxidants is flavonoids [22]. Flavonoid antioxidants have various benefits and considerable pharmacological activities, and their potential as antioxidants have been widely found in various studies as one of the micronutrients capable of neutralizing

free radicals [23]. Its ability to reduce free radicals is carried out by suppressing the synthesis of Reactive Oxygen Species, inhibiting the enzymes produced and increasing antioxidant activity [24]. Flavonoids protect cells and tissues from damage caused by oxidative stress caused by normal oxygen metabolism processes or induction from outside the body [25].

Flavonoids protect lipid membranes against various oxidative damage from free radicals. Lipid peroxidation is one of the causes resulting from an imbalance in the number of radicals from cigarette smoke with antioxidants in the body [26], [27]. One of the free radicals as the main cause is superoxide radicals obtained from burning cigarette smoke. Superoxide radicals can be neutralized by the superoxide dismutase enzyme and be turned into hydrogen peroxide, which will then be converted again by glutathione peroxidase into water and oxygen. However, metal ions can trigger an increase in free radicals by reducing hydrogen peroxide to a highly reactive hydroxyl radical. Flavonoid antioxidants can also bind to these metal ions to inhibit the formation of free radicals [28], [29].

Increasing the administration of antioxidants can further reduce free radicals caused by exposure to cigarette smoke [30]. However, the administration of antioxidants with a certain dose will have an insignificant impact on reducing free radicals. This is because free radicals will still be formed physiologically from cell metabolism.

5. CONCLUSION

The intake of broccoli extract (Brassica oleracea L. var. Italica) can affect the number of alveolar macrophages in lung tissue due to exposure to cigarette smoke. Increasing the dose of broccoli (Brassica oleracea L. var. Italica) extract intake will boost the decrease in alveolar macrophages.

6. ACKNOWLEDGMENTS

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7. CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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