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The Effect of Cigarette Smoke through Biofilters with Natural Plant Materials on Mice MDA Level

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Abstract

Background: Cigarette smoke causes various health problems. Therefore, it is necessary to overcome the problem. One solution to these problems is biofilters' use to capture free radicals from cigarette smoke. This study aims to discover the effect of cigarette smoke exposure through a biofilter made from dates, olives, and pomegranates on mice's MDA levels.

Methods: The experimental study was performed to compare the result of five different treatments. Exposure of 150 ml of cigarette smoke was given every day using a suction tool (15 times each mouse) for 28 days. It was performed with five controls treatment: negative, positive, date biofilter, pomegranate biofilter, and olive biofilter. Furthermore, the data were analyzed using the one-way ANOVA followed by the Duncan test to discover differences in mice's MDA levels from each treatment.

Results: The measurement of MDA levels is using the TBA (Thiobarbitaric Acid) test. The sample is obtained from 1.8 grams of the mices' liver in each treatment.The results showed an effect of using date, pomegranate, and olive biofilters on mice's MDA levels. It shows that the best MDA levels were found in date biofilter treatment with a value of 224 ng/ml with a p-value of 0.023.

Conclusion: The results showed that the MDA levels with the Dates and Olives biofilter treatment were better than the negative control. Therefore, these treatment makes the harmful content of cigarette smoke can be minimized.

Keywords: Cigarette Smoke, Biofilters, Mice MDA Level

Conflicts of Interest: None declared
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Introduction

Breathing is the process of inhaling the air into and out of the lungs to bring in oxygen (1). Therefore, the required air content must be clean and healthy. It must contain the compounds needed by the body, including oxygen and water vapor. Meanwhile, air containing free radicals and heavy lead will have harmful effects on the body, including cigarette smoke (2, 3). Several studies have shown that one of the dangers of inhaling cigarette smoke is the accumulation of free radicals in the body, causing abnormalities in sperm (4-6).

Cigarette smoke potentially causing cancer will become harmless with the additional scavenger filter on it. The

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↑What is "already known" in this topic:
Plant-based biofilters have already been proposed as an effective technology to filter contaminated or harmful air and smoke. Through biofilters, the air can be filtered first and will produce better and healthier air output for the body's needs.

→What this article adds:
This research proposed a new plant-based material for biofilters which are dates, olives, and pomegranates. These materials will examine cigarette smoke exposure and its effect on the mice's MDA level. The result of the study is expected can be used as a comparison for related study to gain new knowledge about the effective plant-based material to reduce the cigarette smoke exposure.

scavenger's active role is to transform cigarette smoke containing harmful materials and free radicals into harmless health (7). The organic and metal components in cigarette smoke are not isolated but in the set of polymer. These polymers are formed based on chemical bonds and the component's biradical phenomena. Those sets of polymers are combined based on the magnetic field strength, accumulate, and distribute electrons to the polymer's surface.

The Biofilter is a component of a closed recirculation system that causes the neutralization of toxic substances (8, 9). There are seven types of free radicals on cigarettes without Biofilter that could be detected by Electron Spin Resonance (ESR) Leybold Heracus, which are Hidroperoxida CO_2, C, Peroxy, O_2-, CuOx, $CuGeO_3$. On the other side, Biofilter consists of natural ingredients that contain antioxidants as free radical scavengers.

Many studies on special filters made from natural ingredients have been shown to capture free radicals in cigarette smoke. One of the uses of a composite membrane of date palm seeds can absorb free radicals in cigarette smoke with a 0.7 gr date palm powder ratio with 0.3 ml PEG (10). This study also states that the mass composition of date seeds affects the Biofilter density, which also affects the effectiveness of free radical absorption.

Another study shows that the filler size in Biofilter also affects the absorption of free radicals (11). The filler with a 200 mesh sieve is better to absorb free radicals than the filler with a 120 and 80 mesh sieve. The small size of the filler will contain more antioxidants in the Biofilter, so it is better at absorbing alleged free radicals from cigarette smoke. Moreover, pomegranate leaf powder can absorb free radicals from cigarette smoke with a composition ratio of 0.9 g with PEG 0.3 ml as a matrix (12). PEG as a matrix has a higher density value than egg white.

Based on the description, it can be concluded that natural ingredients with specific compositions are proven to scavenge free radicals. However, no studies examined the effect of cigarette smoke with a biofilter on mice MDA levels and spermatozoa quality. Furthermore, this research will discover smoke biofilter composites with dates, olives, and pomegranates.

Methods

This experimental research aims to determine the effect of cigarette smoke exposure through a biofilter made from dates, olives, and pomegranates on MDA levels of mice (Mus musculus). Moreover, Figure 1 presents the research

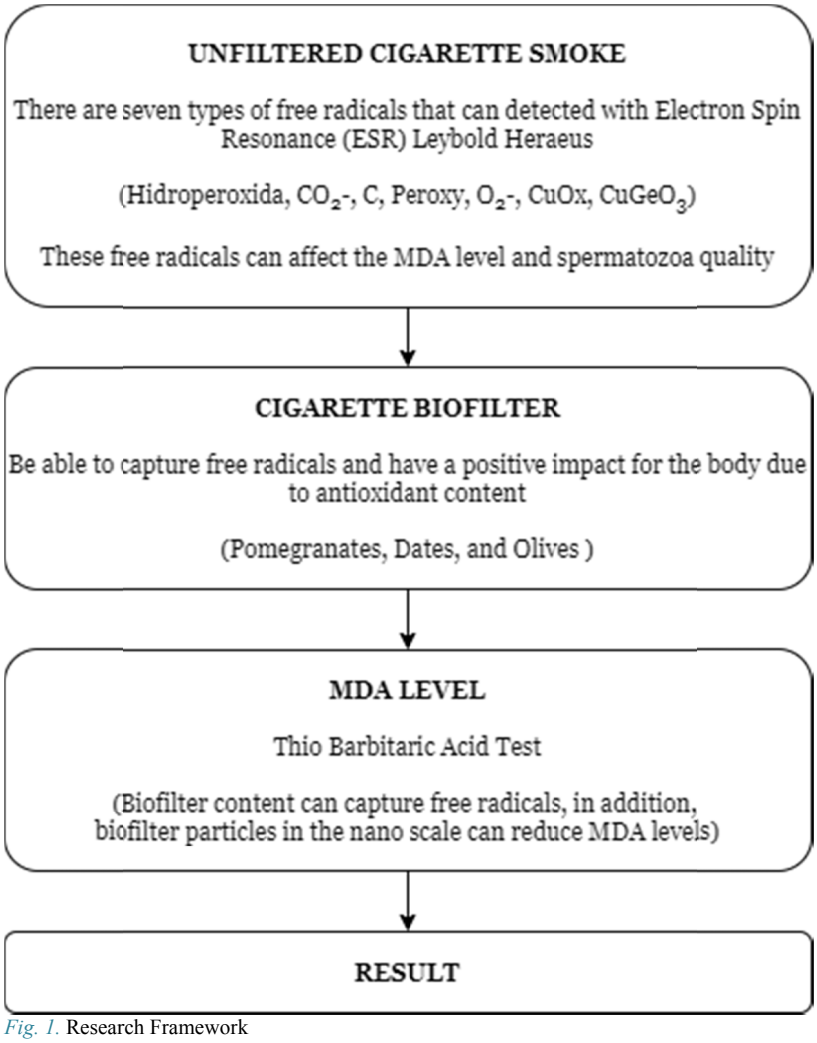

Fig. 1. Research Framework

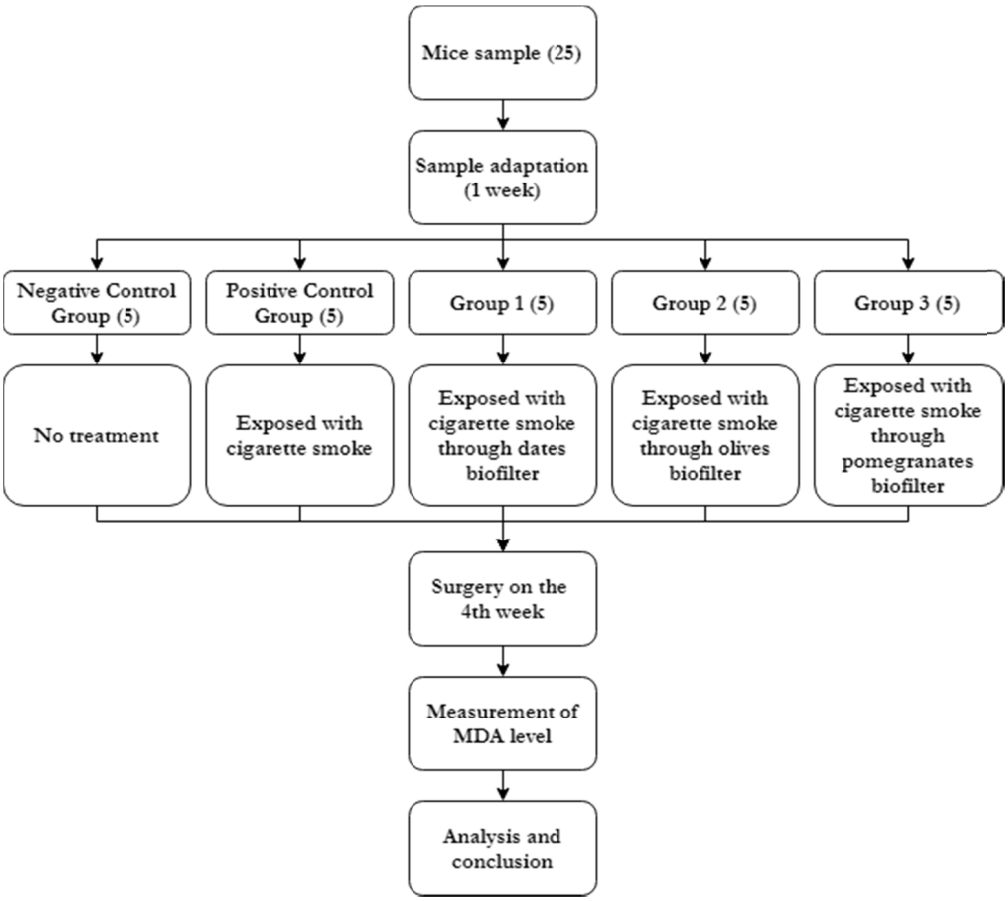

Fig. 2. Research Flow

framework. Also, the research flow is illustrated in Figure 2.

Figure 1 shows that the cigarette smoke contains seven free radicals after being seen using ESR. These free radicals have a negative impact on the human body. Therefore, a plant-based biofilter containing antioxidants is needed to capture free radicals from cigarette smoke. Previous research presents that plant-based biofilters can capture free radicals from cigarette smoke. So, it can minimize the content of free radicals of cigarette smoke.

In this research, cigarette smoke is then exposed to mice to see the MDA levels. A high concentration of MDA indicates an oxidation process in the cell membrane. High antioxidant status is usually followed by a decrease in MDA levels. The high and low levels of MDA are very dependent on the antioxidant status in the human body.

Moreover, Figure 2 presents the research flow. Preparation was firstly done by organizing the experimental animal breeding sites, which include cages, husks, places to eat, and food. Furthermore, the mice were acclimated for seven days. Then apply a biofilter made from dates, pomegranates and olives to cigarettes by attaching the biofilter to one end of the cigarette.

Next, the non-biofiltered and biofiltered cigarettes are burned and sucked using an injection or suction tool periodically for up to 15 puffs, and the smoke exposure is given to the experimental animals. At the time of exposure, the mice were transferred to a closed cube-shaped glass with limited air ventilation. This step was performed routinely for four weeks, with a dose of one exposure day, which was 15 puffs for each treatment. After that, the mice fasted before the surgery day. Furthermore, the mice were dislocated in the neck. Then surgery was performed by taking its liver. And, the liver samples were then analyzed to take the data.

Population and Research Sample

The sample object in this study were male mice (BALB/c) about 3-4 weeks old with a weight of 18-20 grams. Twenty mice were divided into five groups which are control group (-), control group (+), treatment group (1) exposed with cigarette smoke through dates biofilter, treatment group (2) exposed with cigarette smoke through olives Biofilter, and treatment group (3) exposed with cigarette smoke through pomegranates Biofilter. Each group will consist of four mice with random selection. Cigarette smoke exposure was carried out for four weeks, with 15 suction per day for 15 minutes at room temperature (20-25°C).

Treatment

The Biofilter is attached to the back end of the unfiltered cigarette, and then it will burn to extract smoke using a syringe. Upon exposure, mice were transferred to

closed glass tubes with limited air ventilation. The exposure is carried out routinely for four weeks, with a dose per day of exposure 15 times the suction or the equivalent of one cigarette. Furthermore, surgery was performed to extract the lung organs and carried out observations to collect data.

Measurement of MDA Levels

MDA kit with a concentration of 0 to 8 µg / mL was taken 100 µL each and put into a different test tube. Then added 550 µL of distilled water. Each tube containing 650 µL of standard solutions then added with 100 µL of 100% TCA, 250 µL of HC1 IN, and 100 µL of 1% Na-Thiobarbiturate. After that, it is homogenized with a vortex, and the tube is covered with plastic and given a hole. Next, it will incubate in a water heater with a temperature of 100°C for 30 minutes. After that, it is cooled to room temperature. Furthermore, the absorbance of MDA with a 4 µg / mL concentration was measured in the wavelength range of 500-600 to determine the maximum wavelength of MDA. Then, make an MDA standard curve measured at its maximum waveform (538 nm).

Furthermore, the measurement of MDA levels is using the TBA (Thiobarbitaric Acid) test. 1.8 grams of the liver is cut into small pieces and then crushed in a cold mortar, placed on the ice cubes, and added 1 mL of 0.9% NaCl. Then the homogenate was transferred into a microtube then centrifuged at a speed of 8000 rpm for 20 minutes, and the supernatant was taken. 100 µL of hepatic supernatant was added to 550 µL of distilled water, then added 100 µL of TCA, 250 µL of HC1 1 N, and 100 µL of Na-Thiobar biturate. The solution was homogenized with vortex on each reagent addition, then centrifuged at 500 rpm for 10 minutes. After that, the supernatant is taken and transferred to a new test tube. Furthermore, the solution was incubated in a heater at 100°C for 30 minutes and left at room temperature. Next, the sample absorbance was measured at the maximum wavelength for the TBA test (538.1 nm) and plotted on a determined standard curve to calculate the sample concentration.

This MDA test using Thiobarbituric Acid (TBA) method (13, 14). There are several principles of this method: the effect of acid and heat accelerates the decompression of fat peroxide to form MDA. Also, MDA is reacted with TBA to form a color, and the color change is measured by a specific wavelength (nm) spectrophotometer. MDA, which is a secondary product of lipid peroxidation, will react with TBA in an acidic atmosphere (pH 2-3), and a temperature of 97-100°c gives a pink color. Furthermore, the MDA examination works with a spectrophotometer is begins with the determination of the maximum wavelength (λ) (nm). Then, making standard absorbance curves. The final step is to measure MDA levels in the sample in units of µg/100 gr mass.

After the experiment was done, then the data were collected to perform statistical analysis. Mice's MDA level from each treatment were calculated. Then, the data were tested using one-way ANOVA with a 95% confidence level. Furthermore, the analysis was continued using Duncan's test to determine the impact level of each sample. The results of statistical tests were significant if p-value < 0.05.

Results

Figure 3 shows cigarette smoke exposure in several treatments on mice (Mus musculus) MDA levels. Based on the result, it is known that the lowest MDA level results were in the treatment using a date biofilter (224 ng/ml), and the highest was in the K(+) treatment (281 ng/ml). Furthermore, the statistical analysis using One Way ANOVA was also performed to measure the cigarette smoke exposure through dates, olives, and pomegranates biofilters on mice MDA level. Table 1 presents the result of the One Way ANOVA.

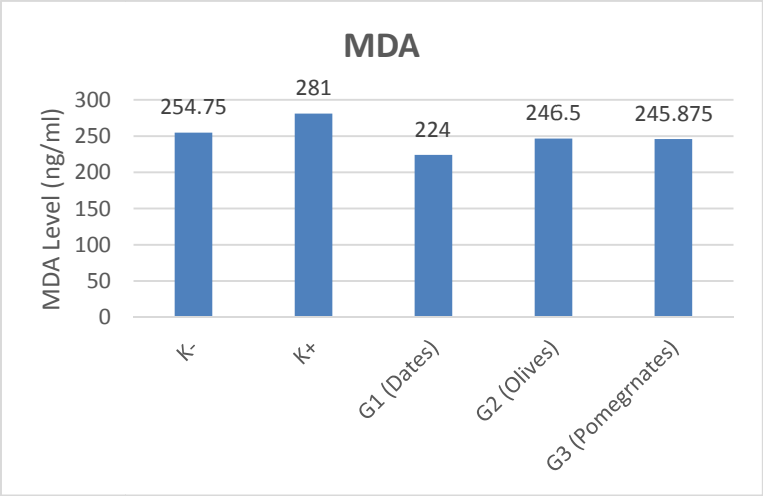

Fig. 3. Graph of treatment result on mice MDA level

Table 1. Result of One Way ANOVA

Source of variation	Sum of Squares	Df	Mean Square	F	p value
Between Groups	6751.700	4	1687.925	3.889	0.023
Within Groups	6510.938	15	434.062		
Total	13262.638	19			

Table 2. Result of Duncan test

Treatment	N	Subset of alpha=0.05	
		1	2
Dates	4	224.0000	
Pomegranates	4	245.8750	
Olives	4	246.5000	
K(-)	4	254.7500	254.7500
K(+)	4		281.0000
p value		0.072	0.023

Figure 3 shows the effect of cigarette smoke exposure through a biofilter of dates (Phoenix dactylifera I.), olives (Olea europaea), and pomegranate (Punica granatum linn) on the MDA levels of mice (Mus musculus). It shows that the MDA values of mice in each treatment are K(-) (254.75 ng/ml), K(+) (281 ng/ml), dates (224 ng/ml), pomegranate (245.875 ng/ml), olives (246.5 ng/ml). The results showed that the lowest MDA levels were in the treatment using a date biofilter (224 ng/ml) and the highest MDA levels were in the K(+) treatment, which was 281 ng/ml. Therefore, it indicates that there is a decrease in MDA levels in the treatment with biofilters.

The significant values were obtained in the One Way ANOVA statistical analysis of different tests on the effect of cigarette smoke exposure through dates, olives, and pomegranates biofilters on mice MDA level is 0<0.05. H0 is rejected if the significant value less than 0.05 means that there is an effect, while H0 is accepted if the significant value is more than 0.05 means there is no effect.

Table 1 shows that the significant value is 0.023, indicating a significant difference between cigarette smoke exposure through dates, olives, and pomegranates biofilter on mice MDA levels. After that, to determine each treatment's difference value, it was continu continued with the Duncan test at 0.05. The result of the Duncan test is presented in Table 2.

Discussion

Table 2 showed that mice exposed to cigarette smoke through dates, pomegranates, and olives biofilters were not significantly different from K(-) treatment that is not exposed to the cigarette smoke. In this experiment, the lowest value was obtained in the treatment with the dates Biofilter. It is because dates seeds contain amino acid phenolic compounds that can scavenge free radicals. The phenolics in date seeds capture the peroxy radicals (ROO-) in cigarette smoke. Free radicals attract phenolic hydrogen oxide. Also, the resulting phenoxy radicals are stabilized by resonance and react with peroxy radicals. Therefore the peroxy radicals are damaged and unable to oxidize.

Moreover, the olive and pomegranate biofilter is also not significantly different from the date of Biofilter and K(-) treatment. This is because the particles' content from the olives and pomegranates biofilter, which are apigenin, luteolin, polyphenols, and flavonoids, enrich the body's antioxidants. It is helpful as an antidote to peroxy radicals that cause the breaking of fat bonds in cell membranes that produce lipid peroxidation in the form of MDA. It means that the olive and pomegranate biofilter treatment has relatively low levels of MDA.

MDA production in K(-) treatment is on a low level because free radicals in the body did not increase due to treatment. Therefore, this causes lipid peroxidation in the MDA because there is no oxidative stress on the cells. Meanwhile, in K(+) treatment, there was oxidative stress due to the accumulation of free radicals that cause a lipid peroxidation process, leading to higher MDA levels.

Moreover, lipid peroxidation is the earliest known and most studied cell or tissue damage mechanism due to free radical attack (15, 16). Most lipid peroxidation occurs in cell membranes, especially unsaturated fatty acids, which are essential cell membranes' components (17, 18) . The level of lipid peroxidation is identified by measuring the end product, Malondialdehyde (MDA). It is an oxidation product of unsaturated fatty acids, which is toxic to cells (19). Measurement of MDA levels is an indirect measurement of free radical activity as an indicator of oxidative stress (20). This measurement was performed with the Thiobarbituric Acid Reactive Substances (TBARStest) test (13).

Moreover, based on the result, the biofilter from plant material contains antioxidants and has the ability to capture free radicals of cigarette smoke. It helps to minimize the harmful content of cigarette smoke, so the quality of cigarette smoke has better quality.

Conclusion

Based on the research result, there was a significant effect of cigarette smoke exposure through dates, olives, and pomegranates biofilters on the mice MDA levels. It is known that the antioxidants contained in the dates, olives, and pomegranates biofilters are effective at capturing free radicals in cigarettes. Therefore, it can reduce MDA levels due to these free radicals. Furthermore, it is necessary to study the cigarette smoke effect through dates, olives, and pomegranates biofilter on another organ. Hence, it can provide information related to the most effective biofilter in minimizing the harmful content of cigarette smoke.

Acknowledgments

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Ethical approval

This study and all of the experimental procedures involving animals were conducted in accordance with the animal care guidelines of Universitas Islam Negeri Maulana Malik Ibrahim Malang, Indonesia.

Conflict of Interests

The authors declare that they have no competing interests.

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