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Original Research Article

Muntingia calabura Modulates Alveolar Matrix Metalloproteinase-9 (MMP-9) and Decreases The Alveolar Diameter in *Sprague-Dawley* Rats Exposed to Cigarette Smoke

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ABSTRACT

Exposure to cigarette smoke is a major risk factor for chronic obstructive pulmonary disease (COPD), characterized by oxidative stress and inflammation. The fruit of *Muntingia calabura* demonstrates significant antioxidant and anti-inflammatory properties. This study investigates the effects of *Muntingia calabura* fruit extract on matrix metalloproteinase-9 (MMP-9) expression and changes in alveolar diameter in *Sprague-Dawley* rats subjected to cigarette smoke exposure. Eighteen male *Sprague-Dawley* rats were randomly assigned to three groups: control (K), treatment 1 (P1) receiving 100 mg/kg body weight/day of *M. calabura* extract, and treatment 2 (P2) receiving 200 mg/kg body weight/day of the extract. All groups were exposed to cigarette smoke from burning seven cigarettes daily for four weeks. MMP-9 expression was assessed via immunohistochemistry, and alveolar diameter was measured using Optilab software. Both treatment groups exhibited a significant increase in MMP-9 expression compared to the control group ($p < 0.0001$). Alveolar diameter was significantly reduced in the P2 group compared to P1 and K ($p < 0.0001$). A negative correlation was observed between MMP-9 expression and alveolar diameter ($r = -0.384$, $p < 0.0001$). Administration of *Muntingia calabura* fruit extract enhances MMP-9 expression and decreases alveolar diameter in rats exposed to cigarette smoke, suggesting its potential protective role against smoke-induced pulmonary damage.

Keywords: Cigarette smoke, *Muntingia calabura*, matrix metalloproteinase-9, alveolar diameter.

Introduction

Chronic obstructive pulmonary disease (COPD) is a serious health issue that affects people all over the world. Its prevalence and mortality rate continue to rise on a yearly basis, and it is characterized by progressive and irreversible respiratory barriers due to the inflammatory process of the lung.[1] At the moment, COPD is the fourth leading cause of death in the world, but it is expected to move up to the third spot by the year 2020.[2] Emphysematous lung damage is a key component of COPD, primarily caused by cigarette smoke.[3,4]

According to the data provided by the WHO, Indonesia has an estimated 61.4 million active smokers, making it the country with the third-highest number of active smokers, after China and India. The percentage of Indonesians who smoked cigarettes increased from 34.2% in 2007 to 36.3% in 2013.[1] Cigarette smoking results in the production of more than 4000 oxidants that are generated from combustion, and hundreds of these oxidants are additives. Each inhalation of cigarette smoke contains 1017 free radicals in the tar phase and 1015 in the gas phase. [5]

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Smoking harms not only the smoker but also those exposed to secondhand smoke. Passive smokers are exposed to five times as much carbon monoxide as active smokers, and they inhale four times as much tar and nicotine. This makes secondhand smoke more hazardous than mainstream smoke.[6]

Cigarette smoke (CS) is the primary source of highly reactive form of oxygen known as ROS. Increased ROS levels from cigarette smoke initiate the inflammatory response in the lungs by triggering the transcription factor NF-κB, which upregulates pro-inflammatory cytokine expression, thereby contributing to COPD development. [7]

Oxidative stress is the result of various biochemical pathways in which endogenous antioxidants are unable to resist oxidants, especially Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), leading to inflammatory alterations in COPD.[5] The underlying mechanisms of COPD are complex, involving recurrent inflammation and oxidative stress (an imbalance between oxidants and antioxidants), primarily driven by inflammatory cells such as neutrophils and macrophages. Emphysema can be caused by both exogenous and endogenous oxidant derivatives, which are known to inactivate antiproteases like 1-antitrypsin. This accelerates the elastin degradation in the lung parenchyma and induces apoptosis in the endothelial and epithelial cells, both of which are necessary steps in the development of emphysema.[1]

Emphysema, also known as dilatation of the alveoli wall, occurs when the alveoli lose their integrity (alveolar attachment), which is followed by the death of the parenchymal tissue. This process is initiated by the oxidation of proteins that increase the activity of proteolytic enzymes and inactivate anti proteolysis enzymes (1-antitrypsin and TIMP), resulting to enzyme imbalance. The widening of the alveolar wall is one of the objective indicators that can be assessed microscopically by measuring the diameter of the alveoli. [8] The loss of elasticity in the

alveoli results from protein oxidation, which also causes damage to the elastin fibers within interstitial tissues. [8,9]

The results showed that from 10 emphysema patients taking alveolar macrophages from Bronchoalveolar Lavage (BAL) fluid obtained an increase in MMP-9 expression compared to the control group, indicating increased proteolytic enzyme activity in emphysema. The proteases produced by alveolar macrophages. In addition to being able to reduce the amount of dissolved elastin, gelatinase is also capable of degrading type IV collagen, which is a significant structural component of the basement membrane. Gelatinase B-derived macrophages, as opposed to gelatinase A or MME, have been shown in recent research to be the primary source of increased elastolytic capacity in the emphysematous lung. [10]

Negative effects that are caused by radical molecules that cause cell damage can be prevented by the presence of antioxidants, which can originate endogenously or exogenously. The body produces antioxidant molecules in order to counteract the effects of SOD (superoxide dismutase). Exogenous antioxidants can be obtained from foods rich in chain-breaking antioxidants such as vitamin C, vitamin E, beta-carotene, and flavonoids. Meanwhile, endogenous antioxidants are produced naturally by the body to counteract oxidative stress. [11]

Butylated hydroxyanisole, butylated hydroxytoluene (BHT), and tertiary butylhydro quinone (TBHQ) are examples of synthetic antioxidants that are commercially available. However, it is not recommended that these antioxidants be taken for an extended period of time because recent research indicates that they may have hepatotoxic and mutagenic effects. Because the use of synthetic antioxidants as antioxidant agents is restricted, it is vital to produce an effective natural antioxidant that has substantial consequences for human health. This is because of the fact that synthetic antioxidants have limited usage as antioxidant agents. [12]

Medicinal plants and their bioactive constituents play a vital role in the prevention of oxidative stress mediated disease. [13] Kersen fruit, also known as *Muntingia calabura*, is a fruit commonly founding Indonesia. Although it is rich in antioxidants, its medicinal use remains limited. The findings of a citric fruit polyphenol extract suggested that the *M. calabura* plant possessed antioxidant properties. They include vitamin C at 33.6 mg AAE per gram of extract, vitamin E at 14.7 mg TE per gram of extract, total phenol compound at 121.1 mg GAE per gram of extract, flavonoids at 173.2 mg RE per gram of extract, and anthocyanin at 82.4 mg CGE per gram of extract.[12] The fatal dose of ethanol extract of *M. calabura* is 1000 mg/kg BW. [2] The best results were obtained while testing the antioxidant activity of *M. calabura* using a variety of different types of solvent.[11,14] Administration of methanol extract *M. calabura* fruit dose 200 mg/kg BW / day in hepatotoxic induced hepatotoxic rats with acetaminophen was able to significantly increase enzymatic antioxidant levels in liver tissue.[15]

Muntingia calabura is rich in flavonoids and phenolic compounds, exhibiting significant antioxidant and anti-inflammatory properties. However, the specific effects of *M. calabura* fruit extract on MMP-9 expression and alveolar structural changes in the context of cigarette smoke exposure remain unexplored. This study aims to evaluate the impact of *M. calabura* fruit extract on MMP-9 expression and alveolar diameter in *Sprague-Dawley* rats subjected to cigarette smoke exposure.

Materials and Methods

Ethical approval

This study was conducted in a controlled laboratory environment using an experimental design with a post-test only control group design. This study obtained ethical approval from the Health Research Ethics Commission (KEPK) of the Faculty of Medicine, Diponegoro University and Dr. Kariadi Hospital Semarang No. 784/EC/FK/RSDK/2016. The research was carried out at the Anatomy Pathology Laboratory of the Faculty of Medicine UGM-RS Sardjito Yogyakarta, the Laboratory of Food and Nutrition Unit of Gadjah Mada University (UGM), and the Anatomical Pathology Laboratory of the Faculty of Medicine UNDIP Semarang.

Animals

In this particular experiment, *Sprague-Dawley* male white rats served as the subjects of the investigations. The inclusion criteria included an age range of two to three months, a weight range of one hundred to two hundred grams, and good health. An active but immobile rat meets the criteria for exclusion; there is a discernible anatomical defect; and a deceased rat meets the criteria for drop out. The number of samples per treatment group must be at least five rats each group, and because there are three treatment groups, this requires a total of 15 rats. The sample size was established based on the formula developed by the WHO in 1993. To account for potential losses, the number of rats in each group was increased by one.

Muntingia calabura extraction

Kersen fruit (*Muntingia calabura*) is obtained from kersen trees in in the village of Berjo Kulon, Sidoluhur, Godean, Sleman Regency, Yogyakarta, Indonesia with GPS coordinates (Lat -7.758078^{o} Long 110.280978^{o}). The fruit is selected when it is ripe because it has a low alkaloid content. Fresh *M. calabura* was blended, then extracted with methanol 96% at a ratio of 1:3 (fruit juice to solvent) using an shaker rotator (Daihan SHO 2D Wonju, Korea) at room temperature. The mixture was then centrifuged, and the supernatant was collected. In order to obtain the macerate, a rotary evaporator (G3 Heidolph Germany) operating at a temperature of 40 degrees Celsius was utilized. After that, the macerate was subjected to nitrogen evaporation in order to obtain concentrated *M. calabura* extract with a concentration of around 99%. The manufactured kersen fruit extract dose was 100 mg/kg BW, and the extract was dissolved in 0.5% Na CMC at 2 ml/200 g BW.

Procedures for Research

Each of the three groups consisted of six male *Sprague-Dawley* rats, and the sample size was 18 male *Sprague-Dawley* rats. These rats were randomly separated into three groups. The first group served as a control and received a placebo (Na CMC 0.5%), while also being exposed to two times the normal amount of cigarette smoke daily. One treatment group (P1) received Muntingia calabura extract via stomach sonde at 100 mg/kg BW, diluted in 0.5% NaCl (2 ml/200 g BW). This group was also subjected to cigarette smoke on a daily basis at a rate of seven cigarettes. The third group served as a treatment of two (P2) and was given *M. calabura* extract orally at a dose of 200 mg/kg BW dissolved in Na CMC 0.5% 2ml / 200gr BB. Also, this group was subjected to cigarette smoke on a daily basis.

Muntingia calabura extract was administered thirty minutes before exposure to cigarette smoke. The time spent being exposed to cigarette smoke and the extract of *M.calabura* was for a total of four weeks. After a course of treatment lasting for four weeks, the mice were terminated and had their right lung tissue removed. The Anatomical Pathology Laboratory FK UGM then processed the tissue, stained it with HE, and performed immunohistochemistry for MMP-9 according to histopathology examination standards.

Matrix metalloproteinase-9 (MMP-9) expression examination

The immunohistochemical imaging approach with MMP-9 polyclonal antibody was used to investigate the level of MMP-9 expression (Bioss Antibodies bs-0397R, LTD, USA). In the Anatomical Pathology laboratory of FK UNDIP, immunohistochemical readings of MMP-9 were performed. The Anatomical Pathologist was responsible for providing an interpretation of MMP-9 expression. The intensity of the staining, in conjunction with the percentage of positively stained cells, was analyzed to determine the level of MMP-9 expression. Positive cells were characterized as those that have brown granules in the cytoplasm. The evaluation was performed semiquantitatively using the Allred score.[16] It was carried out manually at 400 times magnification, in 10 field-of-view zones, and by adding the proportion score and the intensity score, each of which had a range of 0-8.

Alveoli Diameter Assessment

The lung tissue that had been HE stained was then used to perform microscopic digital pictures, which were displayed on each preparation. Finally, the Optilab program was used to measure the diameter of the alveoli (Soft Imaging System, Münster, Germany). Alveolar diameter analysis was automatically arranged in both vertical and horizontal planes. The diameter of the alveolus was measured on the side that was the largest, and this procedure was conducted on one hundred alveolar cells for each preparation. The magnification that is being utilized is 10 times 10.

Data analysis

Differences in MMP-9 expression and the diameter of lung alveoli were statistically analyzed using (SPSS; version 21) statistical software, The Kruskal-Wallis test and the Post Hoc Mann-Whitney U test. Statistical significance was set at $p < 0.05$.

Results and Discussion

The comparison expression score on the matrix metalloproteinase-9 (MMP-9)

It was discovered that out of the three groups, in the control group (K), two samples passed away on the sixth day, and two samples died on the thirteenth day; therefore, the number of live samples up to the termination time was two. The remaining two samples had their alveolar diameters measured, as well as their MMP-9 expressions. These measurements were carried out using a different field of view. One of the samples from the first treatment group (P1) did not survive until the 13th day; hence, only the other five samples were included for the study. Throughout their time in treatment group 2 (P2), all six samples survived until the end of the study.

Samples that died on day 6 were not subjected to autopsy or organ harvesting, while samples that died on the 13th day were not carried out on autopsies but the lung tissues taken and HE-stainted were performed. The results of HE-stained preparation of the three samples that died on the 13th day by the Anatomical Pathologist indicated lymphoid tissue hyperplasia, hemorrhage and swollen lung tissue leading to chronic inflammation as shown in figure 1.

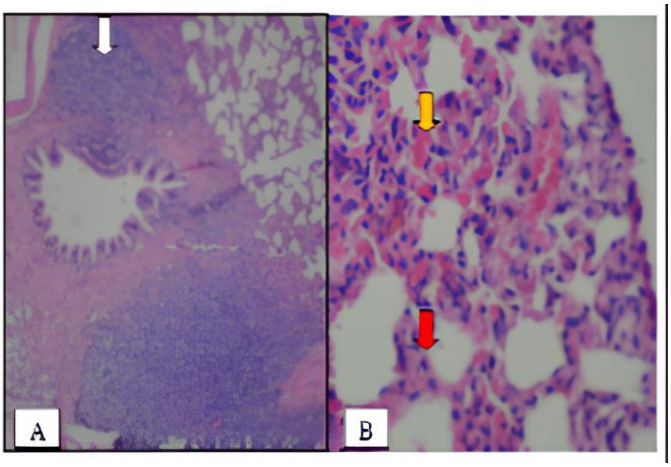

Figure 1: Anatomical and pathological examination of the lung tissue of a deceased rat. Lymphoid gland hyperplasia (image A, white arrow) (enlarged 40 times), bleeding (image B, yellow arrow), and the lung appears bloated (Figure B, red arrow) are all symptoms of the condition (magnified 400x)

The findings of the readings and calculations that were made on the Metalloproteinase Matrix (MMP-9) by an Anatomical Pathologist. The number of proportion scores of positive cell count and intensity score for each preparation were counted using the Allred technique, and the findings are presented in Table 1. There were a total of five fields of view for each preparation.

Table 1: *Matrix Metalloproteinase* (MMP-9) Expression Score in each group (N=75)

Group	*Mean*	*Median*	*Std. Deviation*	*Minimum*	*Maximum*	*P*
Control	4.2	4	1.154701	3	6	
Treatment 1	5.64	6	1.287116	3	7	ρ<0.0001*
Treatment 2	6.44	7	1.416569	4	8	

** One-way ANOVA test, P<0.05 (significant)*

The control group had the lowest mean score for MMP-9 expression (4.2), whereas the group P2 had the highest score (6.44). Following the completion of the statistical analysis using the One Way ANOVA Test, each of the three groups obtained a value of less than p<0.0001. Following an analysis using a Post Hoc Multiple Comparison Test, the following are the findings on the differences between each group:

Table 2: Differences in *Matrix Metalloproteinase* (MMP-9) Expression Score, between Treatment Group and Control Group (N=75)

	Control	Treatment 1	Treatment 2
Control	-	ρ<0.0001*	ρ<0.0001*
Treatment 1	ρ<0.0001*	-	ρ = 0.374
Treatment 2	ρ<0.0001*	ρ = 0.374	-

******Multiple Comparison Test, P*<0.05 (significant)

Table 2 demonstrates that there was a difference in the Matrix Metalloproteinase (MMP-9) expression score that was significant (p<0.0001) between the control group and treatment 1 (group kersen fruit extract 100 mg / kg BW / day) and between the control group and treatment 2 (group kersen fruit extract 100 mg / kg BW / day). However, there was no significant difference between treatment group 1 and treatment 2 (p= 0.374). This demonstrates that there is no significant difference in MMP-9 expression scores between kersen extract doses of 100 mg/kg BW/day and 200 mg/kg BW/day.

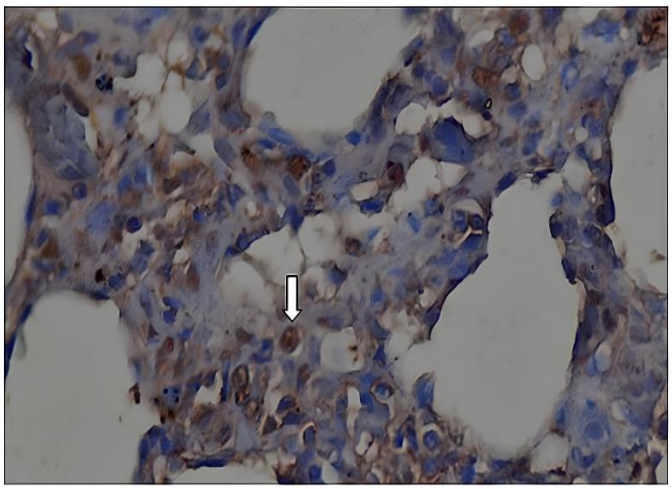

Figure 2: MMP-9 Expression. Positive cells are characterized by the presence of brown granules in the cytoplasm (white arrow). (magnified 1000x).

The comparison of the size of alveoli diameter

The results of the reading and calculation of the diameter of the alveoli were conducted by the researchers and anatomical pathologists, who had previously done a similar perception with the expert Anatomical Pathology. The diameter of the alveoli was measured using an optilab

instrument that had a scale measurement unit, and the results were recorded in microns.
A total of five pulmonary histologic preparations were used, with five rats in each group. The largest width of 100 alveoli was measured in each preparation. The results are presented in Table 3.

Table 3: Descriptive Data of Alveoli Diameter (μ) in each group (N=1.500)

Group	***Mean***	***Median***	***Std. Deviation***	***Minimum***	***Maximum***
Control	84.7	77.4	35.9	35.9	214.7
Treatment 1	65.5	62.9	18.1	18.1	170.9
Treatment 2	56.9	53.3	26.3	26.3	245.7

The maximum diameter of the alveoli in the K1 group was the most extensive (84.7 μ) whereas in the P2 group was the smallest (56.9 μ). The result of normality test of data to the research variable with One-Sample Kolmogorov-Smirnov Test P <0.05, indicating that the data of study variables were not normally distributed. Therefore, parametric statistical analysis could not be performed.

Table 4: Differences in Alveolar Diameter (μ) between Treatment Group and Control Group (N=1.500) using *Kruskal-Wallis Test* and *Mann-Whitney U Test*

	Mann-Whitney U Test			*Kruskal-Wallis Test*
	Control	Treatment 1	Treatment 2	
Control	-	ρ<0.0001*	ρ<0.0001*	
Treatment 1	ρ<0.0001*	-	ρ<0.0001*	ρ<0.0001*
Treatment 2	ρ<0.0001*	ρ<0.0001*	-	

* *P*<0.05 (significant)

The outcome of statistical analysis with Kruskal-Wallis Test obtained ρ <0.0001 value in all three groups suggesting that there is a statistical difference. To determine differences between groups, a further analysis of Post Hoc Mann-Whitney U Test with the result showed that there was a significant difference of alveoli diameter (ρ <0.0001) between control group with treatment 1, control group with treatment 2, and treatment group 1 with Treatment 2 with the results as in table 4. According to the findings of this investigation, a dose of 200 mg/kg BW/day of the extract of *Muntingia calabura* exhibited a reduction in the size of the width of the alveoli that was smaller than the dose of 100 mg / kg BW / day.

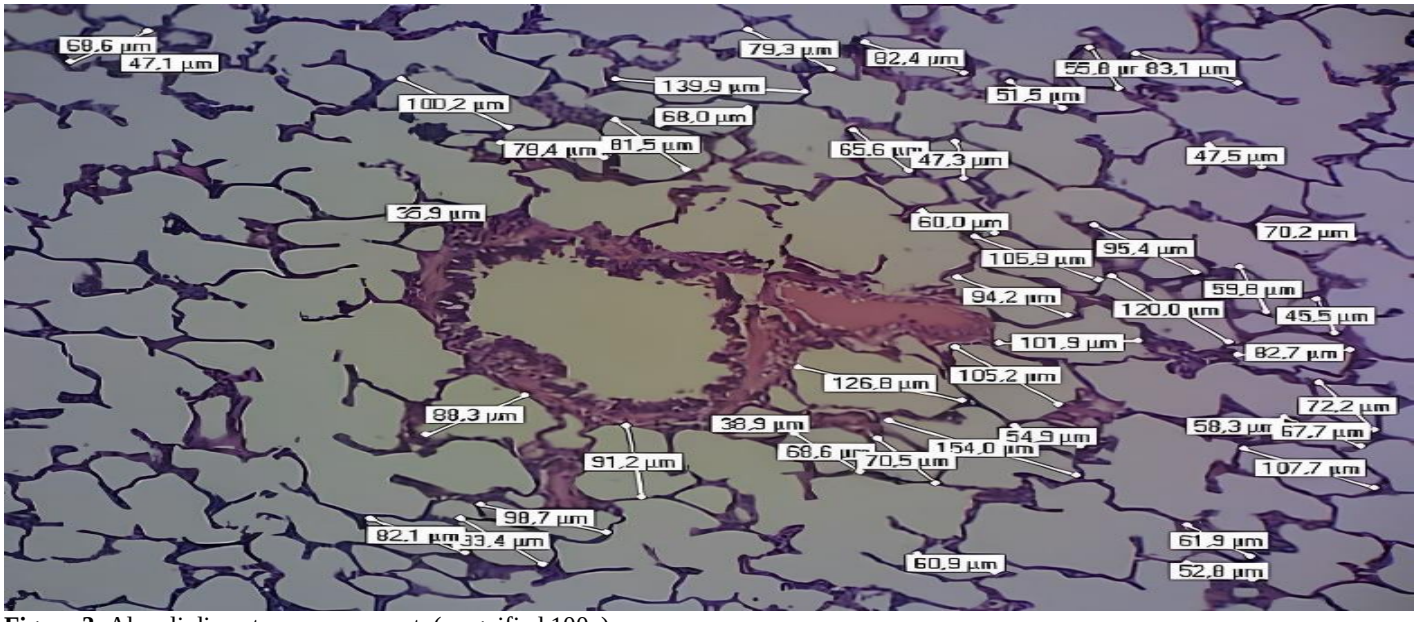

Figure 3: Alveoli diameter measurement. (magnified 100x).

The relationship between the score of matrix expression metalloproteinase (MMP-9) and the diameter of alveoli
The result of normality test of data on research variable with One-Sample Kolmogorov-Smirnov Test for the variable of alveoli diameter and expression of MMP-9 is P > 0.05, or not significantly different. This indicates that the research variables are normally distributed, allowing for a nonparametric correlation analysis using Spearman's rho test.
Nonparametric correlation statistical analysis with the Spearman's rho Test was performed in order to determine the relationship between MMP-9 expression and alveoli diameter. The results of this analysis are presented in table 5.

Table 5: Correlation Statistic Analysis Result of MMP-9 Expression and Alveoli Diameter using *Spearman's rho Test*

Correlation	*r*	*Asymp.Sig*
MMP-9 Expression - Alveoli Diameter	-0.384	ρ<0.0001*

* *Spearman's rho Test, P*<0.05 (significant)

There is a correlation between the MMP-9 expression and the diameter of the alveoli ($p < 0.0001$), and the correlation coefficient (r) is (-0.384). This indicates that there is a significant negative correlation direction, which indicates that the smaller the diameter of the alveoli, the higher the score of MMP-9 expression.

The effect of kersen fruit extract (muntingia calabura) on matrix expression metalloproteinase (MMP-9) and alveoli diameter

There is a correlation between the MMP-9 expression and the diameter of the alveoli (0.0001), and the correlation coefficient (r) is (-0.384). This indicates that there is a significant negative correlation direction, and it also indicates that the increased score of expression MMP-9 will result in a smaller diameter of the alveoli.

The results of this study demonstrated that the treatment group had a higher average rating of MMP-9 expression when compared to the control group. The group with the lowest mean score for MMP-9 expression was group K (4.2), followed by group P1 (5.64), and the group with the highest mean score was group P2 (6.44). When compared to the control group, the treatment group's results showed a smaller average width of the alveoli, while the control group's results showed no change. The broadest width of the alveoli was found to be the largest in the K1 group (84.7), followed by the P1 group (65.6), and then by the P2 group, which had the smallest diameter at 56.9.

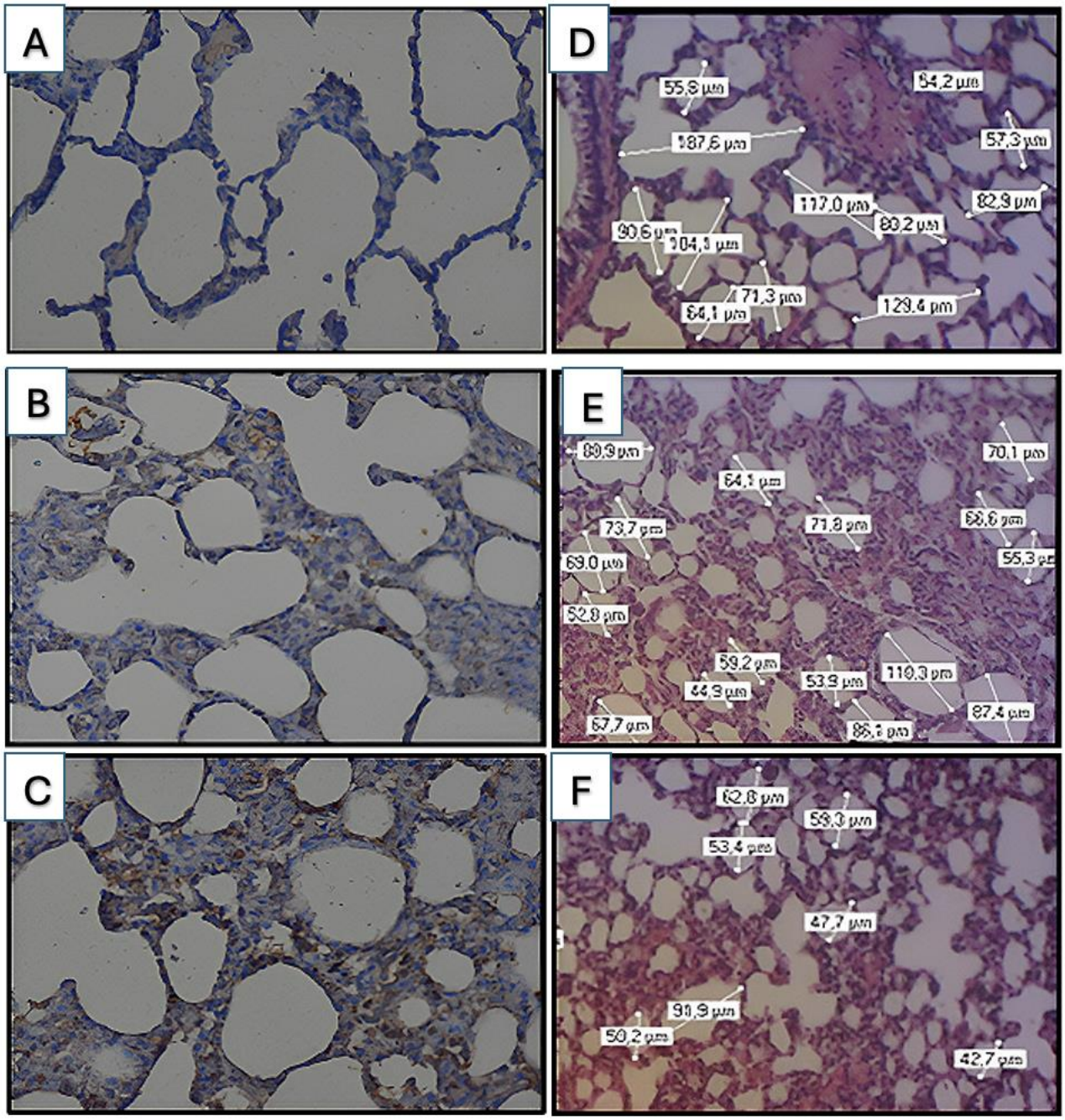

Figure 4: The comparison of MMP-9 expression and the size of the inter-group alveolar diameter were control group (Figure A), treatment group 1 (figure B) and treatment group 2 (figure C). All three show cytoplasm stained brown (magnified 400x). Alveoli diameter size pictures, in microns (µ) in the three groups of controls (Figure D), treatment group 1 (figure E) and group 2 (F). (100X)

In this particular study, the treatment group showed increased in MMP-9 expression, while their alveolar width decreased more than in the control group. Even though there was a significant difference in the size of the alveoli diameter between the control group and the treatment group, the molecular examination that looked at MMP-9 expression revealed that there was an increase in MMP-9 expression in the treatment group compared to the control group. This was the case even though there was a significant difference in the size of the alveoli diameter between the control group and the treatment group.

As revealed by the HE staining, there was an increase in the level of MMP-9 expression in the treatment group in comparison to the control group. This was caused by the presence of a greater number of inflammatory cells in the lung tissue of the treatment group in comparison to the control group. The alveoli appear to contain a large number of inflammatory cells like lymphocytes and neutrophils. An activated neutrophil will express MMP-9, which will result in a greater expression of MMP-9. In the control group, there has been an increase alveolar diameter, and the cells present between the alveoli septum, including the inflammatory cell, have decreased in number, leading to a corresponding decrease in the amount of MMP-9 that has been expressed.

Only inhibition of the process of alveolar diameter widening was observed after administration of *Muntingia calabura* extract at doses ranging from 100 to 200 mg/kg BW / day for a period of four weeks. Yet, the protease-antiprotease imbalance that contributes to the pathophysiology of emphysema is still an ongoing. This is demonstrated by the fact that MMP-9 expression in treatment groups 1 and 2 was dramatically enhanced and significantly different from that of the control group. The findings of this study are consistent with those of earlier research that linked elevated levels of BAL MMP-9 in healthy smokers (who did not exhibit signs and symptoms of emphysema on physical examination and support) to an early picture of emphysema in smokers who were more likely to develop the condition. [17]

Latest findings from scientific studies demonstrate that the pulmonary parenchyma can recover from the harm caused by exposure to secondhand smoke. Unfortunately, when it transforms to a situation in which the parenchyma is unable to make advances.[18,19] In the meantime, the exposure to clove cigarette smoke, which contains high amounts of nicotine, significantly decreases alpha-1 antitrypsin levels., which over time causes alveolar emphysema and leads to increased elastase expression. [20]

In this study, the extract of *Muntingia calabura* at doses of 100 and 200 mg/kg BW / day was unable to produce a preventative effect by reducing the inflammatory process. This was due to an imbalance between protease and antiprotease. Because the addition of the *M. calabura* simultaneously was unable to combat the oxidative stress that was causing inflammatory processes due to the continued use of cigarettes, it is imperative that either an increase in the dosage or an extension of the amount of time that the extract was administered be taken into consideration. However, the potential of *M. calabura* extract as a therapeutic agent could be considered, where at first the exposure of cigarette smoke given, then after that, the *M. calabura* extract was given as treatment.

The role of muntingia calabura extract (MCE) on the pathogenesis of emphysema

Chronic pathology of the pulmonary parenchyma (emphysema, small-airway diseases, COPD), with various cellular and molecular changes, can occur as a result of 3 (three) theories: 1) protease-antiprotease imbalance, 2) oxidant-antioxidant imbalance, and 3) Inflammatory response. [20–22] The current mechanistic explanation for COPD is that it is due to unchecked neutrophil elastase (NE) activity in the lung resulting from a functional deficiency in alpha-1 antitrypsin (AAT). NE is readily inhibited by AAT and when introduced into the lung causes emphysema in animal models. However, other serine proteases, matrix metalloproteinases, cysteinyl cathepsins, and bacterial proteases have shown similar findings.[23] The inflammation-induced overproduction of reactive oxygen species (ROS) means that endogenous antioxidants may not be sufficient to prevent oxidative damage, resulting in an oxidative imbalance in the lung.[24]

Antioxidant components such as phenol acids, polyphenols, and flavonoids can feed on free radicals and block oxidative processes. Kersen fruit contains antioxidants such as vitamin E, phenols, flavonoids, and anthocyanins. [12] Administration of cutaneous methanol extract of dosages of 200 mg/kg BW / day in hepatotoxic-induced rats enhanced enzymatic antioxidant levels in hepatic tissue significantly, among others, SOD, CAT and Glutathione peroxidase (GPx). The study also revealed a significant drop in vitamin E and C levels following MCE administration. This study indicates that hepatoprotective effects arise due to the ability of MCE to limit the bioactivation of toxins and are strong antioxidants in trapping free radicals and suppressing lipid peroxidation.[15]

The decrease in levels of ascorbic acid may be attributed to increased usage as an antioxidant defense against increasing reactive oxygen species or reduced glutathione given that glutathione is necessary for the recycling of ascorbic acid. Vitamin C works as a scavenger of free radicals and can become protective in stages of initiation and development of carcinogenesis. Vitamin E is renowned as an effective antioxidant; Converting superoxide radicals, peroxy lipid radicals into less reactive forms. Vitamin-E is one of the most essential free radical scavengers that can used as a chain-break antioxidant in the membrane. Vitamin E lowers the lipid hydroperoxides formed during the peroxidation process and protects the cell structure against cell damage.[15]

The anti-inflammatory action of MCE showed in investigations of inflammation induced *Wistar* albino rats with carrageenan. The thickness of edema (mm) has greatly decreased and is comparable to those treated with normal treatment with indomethacin. MCE, whose main active components are flavonoids, have been proven to have anti-inflammatory, antiallergic, anti-viral, and anti-carcinogenic effects. One mechanism that can be used to explain the link between anti-inflammatory and antioxidant activity is the response generated by ROS. Nitric oxide (NO) is released as a result of reactive oxygen species (ROS), which are a form of inflammatory stimulation. This study implies that limiting ROS will lead to a decrease in NO generation, which in turn will contribute to anti-inflammatory, anticancer, and antioxidant activity.[22]

The anti-inflammatory properties of flavonoids have been mentioned in various studies in the literature, but are often not connected with respiratory system disorders. There are both cellular and molecular mechanisms that are involved in the pathogenesis of COPD. These mechanisms include oxidative stress, chronic inflammation, protease imbalance, and antiproteases, all of which are contributing factors in the development of emphysema. Prior studies have established the effects of flavonoids, such as quercetin, on COPD and in reducing the formation of pulmonary emphysema in experimental animals. Quercetin displays anti-inflammatory actions through inhibition of protein tyrosine and serine/threonine kinases. [25] These findings suggest that quercetin could serve as a potential treatment for reducing IgE and histamine levels in individuals with asthma.[26] These flavonoids improve lung elasticity and prevent the progressive loss of elastic recoil and the subsequent development of emphysema; however, they do not stimulate the regeneration of damaged alveoli. [25] The role of MCE in the pathogenesis of emphysema due to exposure to cigarette smoke is more closely related to the oxidant-antioxidant theory than to the protease-antiprotease imbalance and inflammatory response theories.

These findings support previous studies indicating that oxidative stress and inflammation play critical roles in lung damage from cigarette smoke exposure. [27] Flavonoid-rich extracts have been shown to reduce oxidative stress and improve lung histopathology in experimental models. [28] Despite these findings, *M. calabura* extract did not fully prevent MMP-9 upregulation, suggesting that longer treatment durations or higher doses may be required for optimal therapeutic effects. Further mechanistic studies are needed to elucidate the pathways through which *M. calabura* exerts its protective effects.

Conclusion

Muntingia calabura extract increases MMP-9 expression while reducing alveolar diameter in cigarette smoke-exposed rats. These findings suggest a potential protective role in reducing cigarette smoke-

induced lung damage. Future studies should explore extended treatment durations and molecular mechanisms underlying these effects.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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