Abstract

Background: Air pollution due to motor vehicle emissions causes several serious health problems including respiratory problems. Fumes exposure activated alveolar macrophages and epithelial cells release mediators such as LTB₄, TNF-α, and IL-8. TNF-α are known induced acute inflammation and tissue damage. Oats are plant with high dietary fiber content and nutritional value. Oats are contain of antioxidant compounds source such as tocopherols and avenanthramides. Therefore, this research aims to determine the effect of oatmeal on TNF-α of white rats exposed to motor vehicle fumes. Methods: This research used true experiment design with Randomized Post-Test Only Control Group design using 16 white male rats divided into three groups: control group only was given standard fed (K1), second group was given standard fed and exposed with motor vehic le fumes (K2), third group was given standard fed mixed with 10 gr of oatmeal and exposed with motor vehicle fumes (K3) for ten days. After ten days of the treatment, blood of white rats from all groups will be taken intracardial then measured TNF-α levels. Results: Statistical test analyzed using SPSS Software 23. Oatmeal has an effect to decrease TNF-α levels (P<0.001). Conclusion: This study conclude that oatmeal can help reduce TNF-α levels when exposed to motor vehicle fumes.

Keywords: antioxidant, ELISA, inflammation, oatmeal

INTRODUCTION

Air pollution in urban areas is a very important problem and requires special attention (Kurniawati et al., 2017). The high level of air pollution caused by the increasing economic development of society in recent years, which in turn led to an increase in the amount of transportation, especially motor vehicles (Sari et al., 2015). The number of motor vehicle users in Indonesia increases by 6 million units every year. Exhaust or emissions from transportation contribute for 70% of air pollution that occurs in urban areas, especially by motor vehicles (Yusrianti, 2015). Thus, motorcycles are the biggest polluters, compared to the pollution produced by diesel cars, buses, and even trucks (Rahmasari et al., 2023). Motor vehicles can emit various types of emissions including high concentration of ultrafine particulates (UFP), carbon monoxide (CO), oxides of nitrogen (NOx), unburned hydrocarbons (HC), sulfur dioxide (SO₂), lead (Pb), carbon dioxide (CO₂), particulate matter (PM 2.5), polycyclic aromatic hydrocarbon (PAHs), benzene and formaldehyde (Meo et al., 2019). Globally, the several types of emissions emitted by motor vehicles, carbon monoxide (CO) is one of the most common types of emission emitted by motor vehicles with a percentage of 75% (Sari & Sofwan, 2021). Air pollution due to motor vehicle emissions causes several serious health effects including

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Effect of Oatmeal on TNF-α of White Rats Exposed to Motor Vehicles Fumes

Sukma Sahadewa¹, Fara Disa Durry²*, Zulfan Febriawan², I Putu Agus Aris Wijaya Kusuma¹

ARTICLE INFO

AUTHOR’S AFFILIATIONS
Faculty of Medicine, Universitas Wijaya Kusuma, Indonesia¹
Faculty of Medicine, Universitas Pembangunan “Veteran” Jawa Timur, Indonesia²

CORRESPONDING AUTHOR
Fara Disa Durry
Universitas Pembangunan “Veteran” Jawa Timur
E-mail: faradisa.fk@upnjatim.ac.id

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respiratory health problems (asthma, lung irritation, bronchitis, pneumonia), cancer and even early death (Jiang et al., 2016).

Fumes exposure results in direct activation of macrophages and epithelial cells. Activated alveolar macrophages and epithelial cells release mediators such as IL-8 TNF-α and LTB₄ that can induce an influx of neutrophils into the lungs. IL-8 and LTB₄ are known as neutrophil chemotactic factors that activate and recruit neutrophils to the airways (Rodrigues et al., 2021). Macrophages also release MCP-1 which functions as a chemotactic agent to attract monocytes from the circulatory system. TNF-α induces activated the Transcription Factor Nuclear Factor kB (NF-kB) which the IL-8 gene in epithelial cells and macrophages also activated. Macrophages and neutrophils that have been activated will release the MMP-9 enzyme which can damage connective tissue in the lung parenchyma (Markin et al., 2023). So TNF-α is a proinflammatory cytokine which has a main responsibility to driving the inflammatory response directly through induced the expression of inflammatory genes and also indirectly by inducing cell death (van Loo & Bertrand, 2023). Fumes exposure causes increased expression of TNF-α which is the cause of acute inflammation due to fumes exposure and results in connective tissue damage (Lugg et al., 2022).

Oats are type of cereals that consumed in smaller quantities in the world than wheat and rice (Rasane et al., 2015). Oats are known for their high levels of dietary fiber and highly nutrient values (Patra et al., 2023). Oats contain several essential amino acid such as methionine, threonine, isoleucine, tryptophan, valine, histidine, phenylalanine, tyrosine, cysteine, leucine, it's necessary for human body used. Additionally, oats are a natural source of antioxidants such as tocopherols, alk(en)ylresorcinols, and phenolic acids and their derivatives, avenanthramides and avenalumic acids (Mushtaq et al., 2014). All these phenolic compounds have the benefit to improve health due to their antioxidant activity and/or effect of membrane modulating (Rahman et al., 2022). Phenolic compounds in oat have strong antioxidants potentially through scavenging ROS (Reactive Oxygen Species), nitrogen species and chelating transition minerals (Alemayehu et al., 2023). In addition, avenanthramides in oats can inhibit the activation of transcription factors that regulate infection and inflammation (Eudes et al., 2013). So oats can be a supplement that serves to reduce inflammation (Koenig et al., 2014).

In previous study conducted by Pavadhgu et al. (2019) that hypercholesterolemic adults who consumed oat porridge (containing 70 grams of oats) can reduce TNF-α levels up to 19.5% and supported by research to the molecular level conducted by Zhang et al., (2020) that avenanthramides compounds extracted from oats (contain 300 mg of AVA) can decrease the expression of TNF-α gene in high fat diet-induced mice. Based on the background and previous study, we found that motor vehicle fumes can increase pro-inflammatory cytokine levels, especially TNF-α and the levels can be reduced through oat consumption treatment. Research on the effect of oat consumption on TNF-α levels in patients or experimental animals exposed to motor vehicle fumes has never been done. Therefore, this research was conducted to investigate the effect of oatmeal on TNF-α levels in white rat (Rattus norvegicus) exposed to motor vehicle fumes.

MATERIALS AND METHODS
Research Design
This research used true experiment with a randomized control-group only post test. The experimental animals used in this study were Wistar strain rats, male rats, aged 2-3 months old, average body weight about 150 to 200 grams, health condition i.e. clear eyes, agile movement, and shiny fur. The samples used were 18 rats taken randomly and then divided into 3 groups. The 3 groups are the control group with standard feed without treatment (K1), the group with standard feed and treated with motor vehicle fumes exposure (K2), the group with standard feed also oatmeal and exposure to motor vehicle fumes (K3).
Experimental Procedures
The first procedure performed in this study was sample selection. The sample selection was based on the criteria such as male white rats, aged 2-3 months, average body weight about 150-200 grams, and in good health (clear eyes, agile movement and shiny fur). Samples that suitable with requirements are then used as research samples. Then, the selected samples divided into 3 groups randomly. Each group consisted of 6 white rats so the total sample used was 18 rats. After that, the white rats were adapted for 7 days by given standard feed. Adaptation was conducted for 7 days to prevent stress and to observed the initial condition of the rats. After adaptation for 7 days then continued with treatment for 10 days. Before given the treatment, white rats were weighed using a torsion balance in the morning. The treatment for the control group (K1) was only given standard feed, for groups 2 and 3 both were given the treatment of exposure to motor vehicle fumes by opening the cage then the cage was filled with smoke from motor vehicles through the pipe from the motorbike exhaust and exposed for 3 minutes, this was conducted once a day every morning for 10 days at 08.00 WIB. Then specifically for group 3, the standard feed was mixed with 10 gr of oatmeal and boiled water until it thickened into porridge. Oatmeal was given once a day in the afternoon for 10 days with 140 grams of feed given. After 10 days of treatment, white rats will be taken intracardial blood. Then the blood was centrifuged to take serum and continued with the measurement of TNF-α levels using ELISA test.

Measurement of TNF-α Levels
Measurement of TNF-α was conducted by ELISA methods using KOMABIOTEK ELISA kit. Absorbance values was measured with a wavelength of 492 nm.

Data Analysis
The data results were obtained then analysed to statistical analysis using SPSS 23.0 software. First, the data were analysed through the *Shapiro-Wilk* normality test which is needed to compare the distribution of TNF-α data. If the data is normally distributed, it will proceed to the variance homogeneity test (*Levene test*) which aims to determine whether the data group has a homogeneous data variance or not. Furthermore, *ANOVA* test was conducted on TNF-α level data to determine the effect of oatmeal on TNF-α level.

Ethical Statement
This study was approved by ethics committee of Wijaya Kusuma University (certificate number 84/SLE/FK/UWKS/2019).

RESULTS
TNF-α Levels Examination Results
The *ELISA* test results of TNF-α inflammatory marker levels in each treatment group are presented in Table 1 and Figure 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>91.71 ± 6.36</td>
</tr>
<tr>
<td>K2</td>
<td>151.06 ± 2.87</td>
</tr>
<tr>
<td>K3</td>
<td>116.22 ± 0.06</td>
</tr>
</tbody>
</table>

K1: control group; K2: fed and exposed to motor vehicles fumes; K3: fed with oatmeal and exposed to motor vehicles fumes
Based on Table 1 and Figure 1, it is known that the average of TNF-α levels in the control group or fed only (K1) was 91.71 U/mL, then the average of TNF-α levels in the group of fed and exposed to motor vehicle fumes (K2) has increased TNF-α levels by 151.06 U/mL, while the average of TNF-α levels in the group of fed with oatmeal and exposed to motor vehicle fumes (K3) has decreased TNF-α levels of 116.22 U/mL. So the best treatment to reduce TNF-α levels is by adding oatmeal to the feed (K3).

**TNF-α Levels Normality Test Results**
Normality test is necessary to compare the distribution of TNF-α measurement data. Normality test results are shown in Table 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Shapiro-Wilk Statistic</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>0.814</td>
<td>0.215</td>
</tr>
<tr>
<td>K2</td>
<td>0.845</td>
<td>0.532</td>
</tr>
<tr>
<td>K3</td>
<td>0.635</td>
<td>0.492</td>
</tr>
</tbody>
</table>

According to the normality test that distribution of data of TNF-α levels normally distributed with each significance values of 0.215; 0.532; 0.492 ($P_{value}$<0.05).

**TNF-α Levels Homogeneity Test Results**
If the data from the measurement of TNF-α levels is normally distributed, it is followed by a variance homogeneity test (Levene Test) which to determine whether the data groups (K1, K2, and K3) have homogeneous data variances or not. The homogeneity test results are shown in Table 3.

<table>
<thead>
<tr>
<th>Data</th>
<th>Levene Statistic</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>4.096</td>
<td>0.44</td>
</tr>
</tbody>
</table>

As shown in Table 3, it was found that the variance of TNF-α level data in all of group was homogeneous with a significance value of 0.44 ($P_{value}$>0.05).

**TNF-α Levels ANOVA Test Results**
ANOVA results showed that treatment of oatmeal had an effect on TNF-α levels in the blood of white rats exposed to motor vehicle fumes. The ANOVA test results are shown in table 4.
According to the ANOVA test that the treatment of oatmeal had an effect to the TNF-α levels in the blood of white rats exposed to motor vehicle fumes with significance values of 0.000 ($P_{\text{value}}<0.05$).

**DISCUSSION**

Our results showed that the TNF-α levels of white rats exposed to vehicle exhaust decreased after given oatmeal. This means that giving oatmeal has an effect on TNF-α levels in the blood of white rats exposed to vehicle fumes. Oats (*Avena sativa* L.) are the best grains which has positive impact on human health. Inflammation is the complex of biological responses of immune system that can be induced by several factors including pathogens (Chen et al., 2018).

Inflammation is characterised by the releasing of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α and their levels will increase continuously (Moriya, 2019). Pro-inflammatory cytokines have the potential to initiate neutrophil infiltration, stimulate the generation of more inflammatory cytokines, leading to an inflammatory reaction that may cause tissues destruction (Li et al., 2014). Moreover, inflammatory cytokines have the capacity to activate oxidative pathways, causing the generation of reactive oxygen species (ROS) and thereby inducing oxidative stress in tissues (Mittal et al., 2023). Hence, inhibiting the production of inflammatory cytokines plays a role in diminishing inflammation and limiting ROS generation, which decrease tissue destruction (Bordon et al., 2013). An essential transcription factor in regulating inflammatory responses is nuclear factor-kappa B (NF-κB). NF-κB serves as a mediator for the expression of proinflammatory cytokines and various adhesion molecules (Hossen et al., 2017). So natural plant source are needed to reduce inflammation, especially reduce TNF-α levels. Oats are known to contain the main compounds that show antioxidant activity, vitamin E (tokol), phytic acid, phenolic acids and avenanthramides (Avns) (Wang & Eskiw, 2019). AVA, or avenanthramides, are soluble phenolic compounds characterized by their low molecular weight. They consist of a small fraction of anionic, nitrogen-containing, covalently linked hydroxycinnamic acid compounds in their structures. Notably, their structural resemblance to the pharmaceutical antioxidant Tranilast has been identified (Zhang et al., 2020). Generally, avenanthramides exhibit the ability to inhibit various key regulators in the inflammatory response, including the suppression of tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), and IL-6 (Zou et al., 2023). Avenanthramides may reduce TNF-α by inhibiting the activity of the proinflammatory transcription factor NFκB. Phenolic compounds have the capability to reduce iκB phosphorylation. The regulation of NFκB activation in the cytosol involves iκB phosphorylation and subsequent proteolysis. Degradation of iκB results in the nuclear translocation of NFκB, allowing it to bind to the transcription site of the targeted gene. NFκB role in regulating TNF-α expression, the suppression of NFκB can lead to a reduction in TNF-α expression (Hoesel & Schmid, 2013). Additionally, avenanthramides demonstrated the ability to decrease TNFα-induced gene expression of IL-6, IL-1b, and IL-8. Potential mechanisms underlying their anti-inflammatory effects involve direct actions, such as the inhibition of ERK, JNK, and NFκB activation, as well as indirect effects through the activation of PPARγ (Korbecki et al., 2019). Avenanthramides are also known to reduce the accumulation of macrophage cell numbers (Donado-Pestana et al., 2015).

The limitations of this research are that the treatment period for giving oatmeal was too short and the concentration of oatmeal used in the research was only 1 type of concentration. Future research requires information regarding how oatmeal works to reduce TNF-α levels when exposed to motor vehicle fumes.

**CONCLUSION**

Oatmeal has an effect on reduce TNF-α levels in the blood of white rats, which the result shown that oatmeal is able to reduce TNF-α levels up to 116 U/ml in white rats exposed to motor vehicle fumes.
CONFLICT OF INTEREST
The authors declared that there is no conflict of interest.

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REFERENCES


