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The Effectiveness of Basil Leaves Extract *(Ocimum basilicum L.)* on Histopathological Features of The Lung *Rattus Norvegicus* Exposure to Mosquito Coil Smoke

Efektivitas Ekstrak Daun Kemangi *(Ocimum basilicum L.)* Terhadap Gambaran Histopatologi Paru-Paru Tikus Yang Terpapar Asap Obat Nyamuk Bakar

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ABSTRACT

Background: Mosquito coil smoke is one of the exogenous free radicals. Exposure to mosquito coil smoke is associated with lung inflammation, oxidative stress and lipid peroxidation. Compounds that can inhibit the oxidation reaction by binding free radicals are known as antioxidants. **Purpose:** This study aims to determine the effectiveness of basil leaf extract (Ocimum basilicum L.) as an antioxidant on damage to the histological structure of the lung of white rats (Rattus norvegicus) exposed to mosquito coil smoke. **Method:** Rats were divided into five groups; group without treatment as negative control C(-) was given 0.5% CMC Na, group as positive control C(+) was given 0.5% CMC Na before mosquito coil smoke exposure, and three groups that treated with 150 T(1), 300 T(2), and 600mg/kgBW T(3) of basil leaves extract before mosquito coil smoke exposure. Exposure to mosquito coil smoke was given for 8 hours a day for 52 days. All rats were terminated after 52 days of treatment; the histopathology of the lungs was observed under the microscope at 400× magnification within five visual different fields. The analysis was done by observing the severity. Statistical analysis in this study used Kruskal-Wallis and Mann-Whitney test. **Results:** The result showed that the control group and treatment group had significantly different lung damage ($p<0.05$). **Conclusion:** All doses of basil leaf extract reduce lung damage in infiltration of inflammation of cells, thickening of alveolar wall, and enlarge alveolar in rats. The effective dose of basil leaf extract is 600mg/kgBW in reducing the lung damage.

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ABSTRAK

Latar Belakang: Paparan asap obat nyamuk bakar merupakan salah satu sumber radikal bebas eksogen. Obat anti nyamuk bakar dikaitkan dengan peradangan paru-paru, stres oksidatif, dan peroksidasi lipid. Senyawa yang dapat menghambat reaksi oksidasi dengan cara mengikat radikal bebas dikenal dengan antioksidan. **Tujuan:** Penelitian ini bertujuan untuk mengetahui efektivitas ekstrak daun kemangi (Ocimum basilicum L.) sebagai antioksidan terhadap kerusakan struktur histologis paru tikus putih (Rattus norvegicus) yang dipapar asap obat anti nyamuk bakar. **Metode:** Tikus dibagi menjadi lima kelompok, kelompok tanpa perlakuan sebagai kontrol negatif K(-) diberi CMC Na 0.5%, kelompok kontrol positif K(+) diberi CMC Na 0.5% sebelum paparan asap obat nyamuk bakar, dan tiga kelompok. yang diberi perlakuan ekstrak daun kemangi 150 P(1), 300 P(2), dan 600mg/kgBB P(3) sebelum paparan asap obat nyamuk bakar. Paparan asap obat nyamuk bakar diberikan selama 8 jam sehari dalam 52 hari. Setelah perlakuan selesai, histopatologi paru diamati di bawah mikroskop dengan pembesaran 400x dalam lima bidang visual yang berbeda. Semua tikus diterminasi setelah 52 hari. Analisis dilakukan dengan observasi tingkat keparahan. Analisis statistik dalam penelitian ini menggunakan uji Krusskal-Wallis dan Mann-Whitney. **Hasil:** Hasil penelitian menunjukkan bahwa kelompok kontrol dan kelompok perlakuan memiliki kerusakan paru yang berbeda nyata ($p<0.05$). **Kesimpulan:** Semua dosis ekstrak daun kemangi mengurangi kerusakan paru dengan parameter yang diamati infiltrasi sel peradangan, penebalan dinding alveolar, dan memperbesar alveolar pada tikus. Dosis efektif ekstrak daun kemangi adalah 600mg/kgBB dalam mengurangi kerusakan paru.

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Kata kunci: Asap Obat Nyamuk Bakar; Histopatologi; Paru-Paru; Tikus

INTRODUCTION

Mosquito coils are a popular insecticide for mosquito control, widely chosen by the public due to their low cost, availability, and ease of use (Rianti, *et al.,* 2017). However, mosquito coils contain active ingredients that generate free radicals. One such compound commonly found in mosquito coils is D-allethrin, which can be rapidly absorbed by the lungs. Prolonged and repeated inhalation of D-allethrin can lead to cell damage in lung tissue (Qureshi, *et al.,* 2024).

The impact caused by exposure to mosquito repellent smoke depends on the age of the body to which it is exposed, the length of exposure, and the ingredients contained in mosquito repellent burns. In individuals with a history of asthma and allergies, it will cause more dangerous effects (Dahniar, 2011). Heryanto's (2019) research showed that 66.7% of respondents who had the habit of using mosquito repellent were at risk of suffering from acute respiratory infections; this result was greater than the risk of acute respiratory infections in respondents who did not use mosquito repellent, which was 27.2% of respondents. Cellular damage caused by exposure to insecticides is linked to the generation of free radicals in the body (Metcalf, *et al.,* 2016). Free radicals consist of unpaired or missing electrons, and when they encounter non-radical molecules, they can steal electrons from nearby cell molecules (Werdhasari, 2014). An excessive accumulation of free radicals can lead to oxidative stress, resulting from an imbalance between oxidants and antioxidants in the body.

The cellular response to oxidative stress from exposure to mosquito coil smoke is influenced by the balance between oxidants and antioxidants in the body. This response can be cytoprotective through antioxidants or may trigger an inflammatory reaction, leading to the release of cytokines and cytotoxicity, which causes apoptosis in epithelial and endothelial cells. This process can result in permanent dilation of the air spaces, known as emphysema (Wei, *et al.,* 2024).

Among efforts that can be made to neutralize free radicals due to exposure to mosquito coil smoke that has entered the body, one of which is to use exogenous antioxidant compounds. Antioxidants act as electron-giving compounds or reductant of free radical molecules (Rianti, 2017). The exogenous antioxidants can be obtained from plants, one of which is basil *(Ocimum basilicum L.)*. Basil leaves contain several active compounds such as flavonoids, tannins, alkaloids, and saponins which have strong antioxidant activity (Erviana, *et al.,* 2016). The mechanism of antioxidant compounds in basil leaves works by breaking the chain reaction of free radicals (Lestari, 2018). In Mahidin's (2018) research, the administration of basil leaf extract was found to improve the number of spermatogenic cells in white rats damaged by monosodium glutamate (MSG). The results of Tazuyyun's (2020) research showed that the administration of basil leaf extract can increase superoxyde dismutase (SOD) levels in the lungs of white rats. The results of these studies reveal the potential for antioxidants contained in basil leaves. Related to the background that has been described, the researcher wanted to find out the effectiveness of administering basil leaf extract *(Ocimum basilicum L.)* on the histopathological picture of the lungs of white rats *(Rattus norvegicus)* who were exposed to smoke from mosquito burn repellents.

MATERIAL and METHOD

Material

This research was a laboratory experiment with Post Test Only Control Group Design. The object in this study are 25 healthy male white rats *(Rattus norvegicus)* Wistar strain aged of 2-3 months with an average weight of 200 grams, kept in the same location and provided with the same food. The equipment used in this study include animal, balance, water container, 1 ml syringe, feeding tube, gloves, a water bath surgical instruments, and microscope. The tools used for exposure to smoke from mosquito repellent include cages measuring 50 cm × 50 × cm × 50 cm equipped with two vents on the side of the cage with a diameter of 9 cm and a wire bulkhead inside. Materials used in this study were white rat, mosquito coil repellent, basil leaves extract, 0,5% CMC Na, rat feed, Hematoxylin Eosin (HE) stain, buffer formalin, 70, 80, 90, and 96% alcohol, xylol, and paraffin.

Basil Leaves Extract Preparation

The procedure of making basil leaf extract *(Ocimum basilicum L.)* refers to Kumalasari (2020). The extraction of basil leaves was carried out by using the method of maceration. Basil leaves powder (500 grams) were soaked in 96% ethanol (2000 mL) for 3 days. Filtration was done by using filter paper to take the macerate. The macerate result was evaporated using rotary evaporator at a temperature of 45°C with 50 rpm to obtain a viscous extract of basil leaves (Lazuardi, 2019).

Research Implementation

Treatment started after the acclimatized period for seven days. Then the animal models were weighed and randomly divided into five groups (5 rats/group). C (-) : rats were given CMC Na 0.5% solution. C (+) : rats received CMC Na 0.5% and were exposed with mosquito coil smoke for 8 hours a day for 52 days. T (1) : rats received 150 mg/kg BW basil leaves extract, and were exposed with mosquito coil smoke for 8 hours a day for 52 days. T (2) : rats received 300 mg/kg BW basil leaves extract and were exposed with mosquito coil smoke for 8 hours a day for 52 days. T (3) : rats received 600 mg/kg BW basil leaves extract and were exposed with mosquito coil smoke for 8 hours a day for 52 days.

Histopathological Observation

All rats were terminated after 52 days of treatment. The histopathological of the lungs were observed under the microscope at 400× magnification within five visual different fields. Then the observation was done by determining the score of the range of damage to the lung. Based on the research of Hansel and Barnes (2004), the parameters that were taken to determine the score of the level of lung damage (Tabel 1) included the percentage of infiltration of inflammatory cells, interalveolar septum thickness, and alveolar lumen dilation (Table 2).

Table 1. Score of the Level of Lung Damage. Hansel and Barnes (2004)

Criteria	Score	Description
Infiltration of Inflammatory Cell	0	There Is No Histopathological Change In Visual Field.
	1	Infiltration of Inflammatory Cell In < ⅓ Visual Field.
	2	Infiltration of Inflammatory Cell In ⅓ Until ⅔ Visual Field.
	3	Infiltration of Inflammatory Cell In > ⅔ Visual Field.
Interalveolar Septum Thickness and Lumen Alveolar Dilation	0	There Is No Histopathological Change In Visual Field.
	1	Interalveolar Septum Thickness and Lumen Alveolar Dilation In < ⅓ Visual Field.
	2	Interalveolar Septum Thickness and Lumen Alveolar Dilation In ⅓ - ⅔ Visual Field.
	3	Interalveolar Septum Thickness and Lumen Alveolar Dilation In >⅔ Visual Field.

Table 2. The Range of Lung Damage Score. Hansel and Barnes (2004)

Description	Damage Range
There is no damage	Normal
> 0 < 30 % lung damage	Mild
> 30 % - 60 % lung damage	Moderate
> 60 % lung damage	Severe

The score values from the three parameter were then calculated using the formula:

$$\% \text{ Damage} = (\mathbf{X}/9) \times 100\%$$

Description: **X** = the average of the three scoring parameters; 9 = maximum score (3×3 parameters = 9) (Purnama, *et al.,* 2020).

Data Analysis

Data for each group were analysed statistically using Shapiro-Wilk test. If the data were distributed normally, the statistical analysis continued with the parametric analysis of variance (ANOVA) test followed by the Post Hoc (Tukey) test to compare the differences of each groups. If the data were not normally distributed, the statistical analysis continued with non-parametric test using Kruskal-Wallis test followed by Mann Whitney test to compare the treatment effect of each group.

RESULTS

The normality test results indicated that the data did not follow a normal distribution. As a result, the data were analyzed using a non-parametric method, specifically the Kruskal-Wallis test. The Kruskal-Wallis test results m showed there were significant difference of the treatment groups ($p<0.05$). After that, the Mann -Whitney test was used to see the differences between each group. The data result can be seen in Table 3.In this study, the histopathological analysis of the lung tissue from the negative control group C(-), which received no treatment, revealed the least amount of damage compared to the positive control group C(+), and the treatment groups T(1), T(2), and T(3). Any damage observed in the negative control group C(-) could be attributed to uncontrollable external factors, such as the psychological state of the rats, which may have been influenced by their environment. The most severe lung damage was observed in the positive control group C(+), which was exposed to mosquito coil smoke. Figure 1 shows the comparison of the histopathological features of the lungs of each group.

Tabel 3. The Results Data of Lung Damage Test

Group	Lung Damage (Mean ± SD)	Damage Range
C(-)	2.32 ± 0.335	Mild
C(+)	7.40 ± 0.707	Severe
T(1)	5.88 ± 0.,335	Severe
T(2)	4.36 ± 0.740	Moderate

DISCUSSION

Increased inflammatory cell infiltration is caused by free radical molecules contained in the smoke of mosquito coils which trigger an immune response with the infiltration of leukocytes, especially neutrophils in the blood vessels of the lungs (Tohomi, *et al.,* 2014). The accumulation of inflammatory cells in the interalveolar septum leads to thickening of its structure. The dilation of the alveolar lumen is indicative of emphysema. Emphysema is primarily a pathological condition affecting the air spaces distal to the terminal bronchioles, resulting in damage to the alveoli. This damage can occur because mosquito coil smoke stimulates epithelial cells to produce cytokines, which in turn promote the release and activation of neutrophils and macrophages. Additionally, mosquito coil smoke can inactivate antiproteases, creating an imbalance between proteases and antiproteases. This imbalance leads to lung degradation, causing the dilation of the alveolar lumen.

The findings of this study revealed a gradual reduction in lung damage levels in the T(1), T(2), and T(3) groups, which were administered basil leaf extract and exposed to mosquito coil smoke. Basil leaves have strong antioxidant activity, where this antioxidant activity is produced by phenolic and non-phenolic compounds contained in basil leaves (Erviana, *et al.,* 2016). Basil leaves extract that were given to the groups T(1), T(2), and T(3) were exogenous antioxidant supplementations. The body has a defense system comprised of endogenous antioxidants, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH). These antioxidants play a key role in preventing and neutralizing oxidative damage caused by free radicals (Vinoth, *et al.,* 2016). However, these endogenous antioxidants alone are insufficient to neutralize the buildup of free radicals in the body without the support of exogenous reducing compounds obtained from food sources (Tazuyyun, 2020). Exogenous antioxidants can neutralize free radicals by inhibiting the formation of reactive oxygen compounds directly by cutting out chain oxidation reactions or by capturing free radical molecules. The addition of exogenous antioxidants is also needed to help the function of endogenous antioxidants in preventing oxidative stress, where oxidative stress occurs due to an imbalance of oxidants with antioxidants (Fitria, *et al.,* 2013). Antioxidants can transfer electrons to free radicals to stabilize their chemical bonds (Hendra, *et al.,* 2011). However, antioxidant compounds in low levels cause the damage prevention process to be not optimal.. Basil leaves extract in low concentrations has a low antioxidant content as well. This means free radical damage can still occur due to the lack of antioxidants in neutralizing free radical molecules from exposure to mosquito coil smoke.

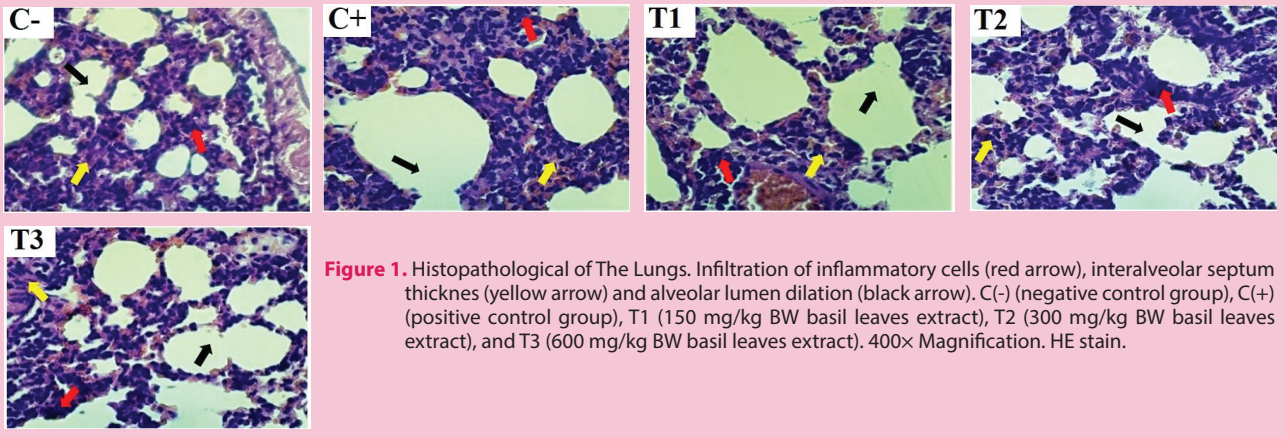

Figure 1. Histopathological of The Lungs. Infiltration of inflammatory cells (red arrow), interalveolar septum thicknes (yellow arrow) and alveolar lumen dilation (black arrow). C(-) (negative control group), C(+) (positive control group), T1 (150 mg/kg BW basil leaves extract), T2 (300 mg/kg BW basil leaves extract), and T3 (600 mg/kg BW basil leaves extract). 400× Magnification. HE stain.

CONCLUSION

Based on the findings of this study, it can be concluded that basil leaf extract *(Ocimum basilicum L.)* has the potential to prevent histopathological damage in the lungs of white rats *(Rattus norvegicus)* exposed to mosquito coil smoke. The effective dose of basil leaf extract in this study was found to be 600 mg/kg BW

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CONFLICT of INTEREST

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ETHICAL APPROVAL

This experiment was performed on the basis of approval by the laboratory animals use the research ethics committee of Faculty of Veterinary Medicine [2.KEH.139.10.2022], Universitas Airlangga, Indonesia

AUTHORS' CONTRIBUTIONS

PK: Writing – Original Draft, Data Collection, Data Processing; Analysis; ISH: Manuscript Preparation, Writing – Review & Editing, Analysis; MAMH: Writing – Review & Editing, Analysis

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