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The influence of carbofuran insecticide exposure on the number of type II Lung Cells in Mice (*Mus musculus*)

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Abstract

The aim of this research was to demonstrate lung histopathological damage in mice (*Mus musculus*) caused by carbofuran exposure. Twenty female mice (*Mus musculus*) aged 10 weeks with a body weight of 25-30 g were used in the study. The mice were divided into four groups: P0, P1, P2, and P3. All samples received 0.5 ml/day of physiological NaCl, and P1, P2, and P3 were additionally exposed to carbofuran at doses of 0.0208, 0.0417, and 0.0833 mg/kg body weight of mice/day, respectively. This treatment was carried out for 10 days. Data analysis was performed using ANOVA followed by the BNJ test. The results showed an increase in necrotic type II cells with increasing doses of carbofuran.

Keywords: Carbofuran; Lung; Necrosis; Type II Cell; Pesticide Stress

1. Introduction

The use of large and uncontrolled doses of insecticides has several negative impacts, including an increase in resistance among plant pest organisms, disruption of human and animal health, and contamination of agricultural products, water, soil, and air due to insecticide residues [1]. More than 29% of fruits, vegetables, rice, and wheat contain residues of at least 2 types of insecticides [2]. Insecticide residues have also been found in food products, including soybeans, approaching the maximum residue limits (MRLs), especially for organophosphates, carbamates, and organochlorines [3].

Carbofuran was a carbamate insecticide commonly used in agriculture and forestry [5]. The use of carbofuran can result in residues in crops such as mustard greens, water spinach, and long beans [6]. Insecticide residues, including carbofuran, in food can pose a threat to non-target organisms [7]. Clinical symptoms of carbofuran poisoning include vomiting, dizziness, muscle weakness, diarrhea, shortness of breath, convulsions, and blurred vision [8]. Bonner et al. reported that farmers exposed to carbofuran had a 95% higher risk of lung cancer [9].

Oral administration of carbofuran can increase ROS levels and trigger oxidative stress [10]. The formation of free radicals reacts with cellular components such as carbohydrates, amino acids, Deoxyribonucleic acid (DNA), and phospholipids, leading to accelerated necrosis [11]. Furthermore, Lobo et al. mentioned that free radicals can cause damage to various parts of cells [12].

Type II cells are one of the cell types found in the interalveolar septum. The main function of type II cells is the synthesis and secretion of surfactant. Surfactant is a substance that reduces surface tension and serves as an anti-collapse agent, facilitating alveolar expansion. The onset of shortness of breath is caused by a decrease in the amount of surfactant due to the death of type II cells [13].

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Increased oxidative stress triggers damage to alveolar epithelial cells, including type II cells, leading to cell death through apoptosis or necrosis [14].

Information regarding the effects of carbofuran on lung tissue is limited. Therefore, research is needed to explain the mechanisms by which carbofuran causes lung damage. This is crucial for prevention and mitigation efforts against the effects of carbofuran exposure. Understanding its effects on the lungs can lead to prevention measures to reduce carbofuran poisoning, whether through oral, inhalation, or dermal exposure.

2. Material and methods

2.1. Materials

The materials used in this study were carbofuran (Furadan 3GR, MDL MFCD00041819), complete chicken feed CP 593 (PT Charoen Pokhpand Indonesia), tap water, ether/chloroform, distilled water, 70% alcohol, physiological NaCl, formalin 10%, cotton, alcohol (70%, 80%, 96%), xylene, paraffin, Hematoxylin Eosin (HE) stain. The instruments used in this study included mouse cages, disposable syringes, surgical tools (forceps, scalpel, scissors, sonde), masks, gloves, pipettes, Erlenmeyer flasks, reaction tubes, tube racks, petri dishes, blotting paper, mouse feed and drinking containers, cover glass, and object glass.

2.2. Methods

2.2.1. Dose Determination

This research used the LD_{50} approach (the dose that can kill 50% of the test animals) for carbofuran between 1-2.5 mg/kg in rats [15]. Furadan used in this study contained 3% carbofuran. Based on preliminary research, an LD_{50} value of 0.5 mg/kg body weight (BW) was obtained in mice. The doses administered were doses that did not cause death in the test animals but caused organ damage. Thus, fractions of the LD_{50} were obtained, namely 1/24 LD_{50} (0.0208 mg/kg BW), 1/12 LD_{50} (0.0417 mg/kg BW), and 1/6 LD_{50} (0.0833 mg/kg BW) [16].

2.2.2. Experimental Animal Preparation

Mice (*Mus musculus*) were randomly selected and divided into four treatment groups (P0, P1, P2, P3), each with five replicates. The mice were then kept in cages and provided with food and water ad libitum.

2.2.3. Treatment

Mice (*Mus musculus*) were randomly selected and divided into four treatment groups (P0, P1, P2, P3). Carbofuran exposure to mice was administered orally at a dose of 0.5 ml. Carbofuran was administered for 10 days, as this period represents the peak of lung DNA changes as a manifestation of ROS due to carbofuran insecticide exposure. Euthanasia was performed on day 12 using chloroform, followed by mouse dissection to collect the lungs. The collected lungs were then placed in containers filled with 10% formalin. Subsequently, histopathological preparations were made using Hematoxylin Eosin (HE) staining.

2.2.4. Histopathological Examination

The histopathological preparations of mouse lungs were examined under a microscope at a magnification of 400x. The examination aimed to observe changes in the histopathological appearance of mouse lungs and to count the number of necrotic type II cells. Observations were made in 5 different fields of view for each histopathological preparation, followed by an assessment of the level of histopathological damage to mouse lung tissue by counting the number of necrotic type II cells.

2.2.5. Observed Variables

The observed variables involved counting the total number of type II cells within the interalveolar septum, followed by the calculation of the number of necrotic type II cells in each field of view in each preparation. Each preparation was examined in five different fields of view. The calculation of the percentage of lung damage was determined as follows:

2.2.6. Data Analysis

The experimental design of this study was a Completely Randomized Design (CRD). Data analysis was conducted using Analysis Of Variance (ANOVA) with the Statistical Programs For Social Scientific (SPSS) software. If significant

differences were found in the analysis ($p<0.05$), the Bonferroni Honestly Significant Difference (BNJ) test was performed.

3. Results and Discussion

Necrosis observations were carried out microscopically using HE-stained histopathological preparations from the lungs of Balb/C mice (*Mus musculus*). The assessment was conducted on the interalveolar septum and observed in five different fields of view using an Olympus® CX-41 microscope with a magnification of 1000 times. The data obtained were qualitative. The qualitative data resulted from observations and assessments of each carbofuran treatment. The analysis results showed significant differences ($p<0.05$), which were further examined with the BNJ test.

Table 1 Mean of Type II Cells Experiencing Necrosis after Carbofuran Exposure

Group	**Necrosis Score (Mean ±SD)**
P0 (Control)	0.00a ± 0.00
P1-Treatment 1 (1/24 LD50)	4.28b ± 0.67
P2-Treatment 2 (1/12 LD50)	7.09c ± 0.26
P3-Treatment 3 (1/6 LD50)	8.24d ± 0.38

Note: Different superscripts in the same column indicate significant differences ($P<0.05$). Control (P0), P1= Carbofuran 1/24 LD_{50} (0.0208 mg/kg BW), P2= Carbofuran 1/12 LD_{50} (0.0417 mg/kg BW), and P3= Carbofuran 1/6 LD_{50} (0.0833 mg/kg BW).

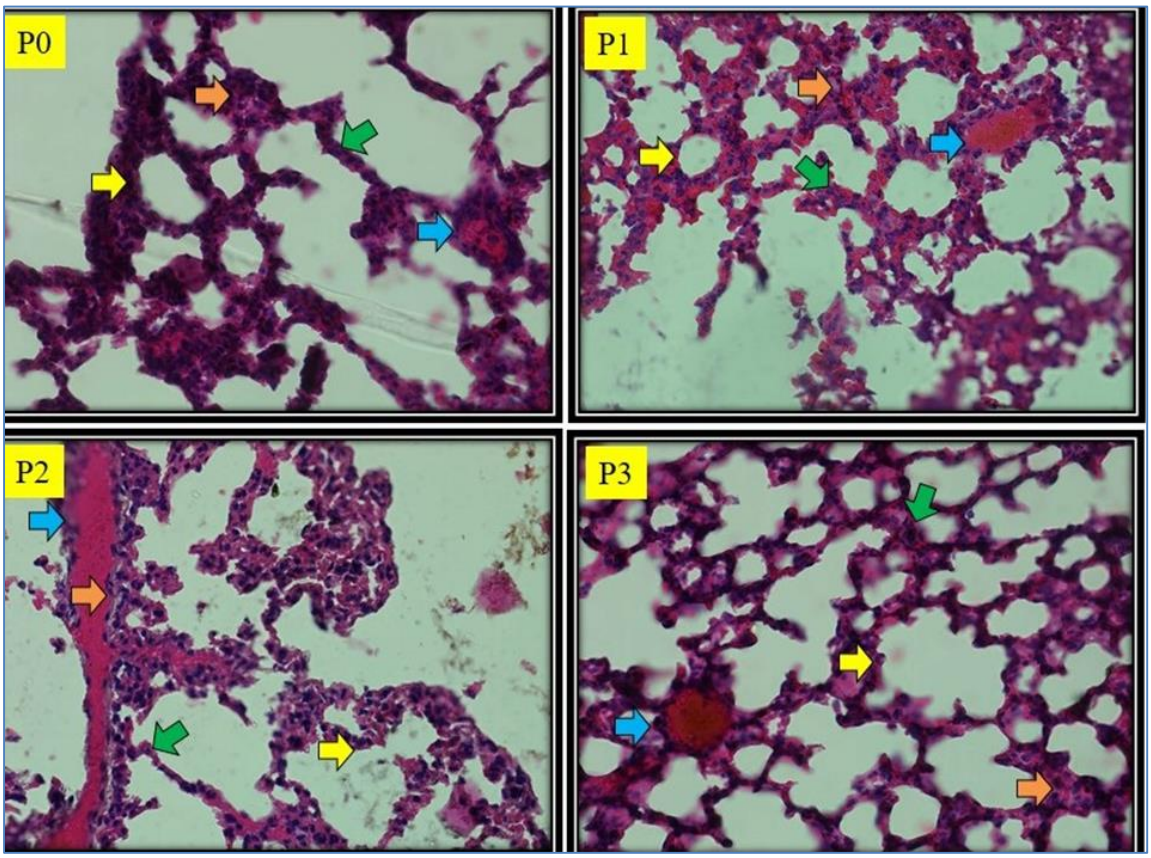

Figure 1 Histopathological image of lung tissue, Control (P0), P1= Carbofuran 1/24 LD_{50} (0.0208 mg/kg BW), P2= Carbofuran 1/12 LD_{50} (0.0417 mg/kg BW), and P3= Carbofuran 1/6 LD_{50} (0.0833 mg/kg BW). (Yellow Arrow) Alveolar Saccus, (Green Arrow) Interalveolar Septum, (Blue Arrow) Congestion, (Orange Arrow) Inflammatory Infiltration (HE Staining; 400x Magnification; Olympus CX-41 Microscope)

The research revealed significant differences among the carbofuran exposure treatments (P0, P1, P2, P3), with an F-value of 409.43. This indicates an increase in the number of necrotic type II cells with increasing carbofuran dosage (Table 1).

Based on the ANOVA results, the Bonferroni Honestly Significant Difference (BNJ) test was conducted to identify significant differences compared to the control group. The BNJ analysis revealed significant differences between each treatment group (P1 with P2, P1 with P3, P2 with P3) compared to the control (P0). Additionally, there were significant differences among the treatment groups (P1, P2, P3) themselves.

The results showed significant differences among the carbofuran exposure treatments (P0, P1, P2, P3). In P1, the average necrosis was 4.28 ($p<0.05$), which was significantly lower than in P2 and P3. This suggests that P1 contained a carbofuran dose that could still be tolerated by antioxidant enzymes in the lungs. Antioxidant enzyme capacity plays a crucial role in the defense mechanism of type II cells against oxidant induction. Antioxidant enzymes such as catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx) can prevent cell damage from oxidative stress caused by free radicals. Low levels of oxidant induction can be neutralized by antioxidant enzymes [17].

In P2, the average necrosis was 7.09 ($p<0.05$), which was higher than in P1 but lower than in P3. This indicates that the dose in P2 caused a partial decrease in antioxidant enzymes. The partial decrease in antioxidant enzymes such as catalase, SOD, and GPx still allowed them to prevent more severe type II cell damage. Free radicals can only cause cell damage when antioxidant functions are no longer able to neutralize the effects of excessive free radical production [18].

In P3, the average necrosis was 8.24 ($p<0.05$), the highest among all groups. This suggests that the P3 dose was no longer tolerable by antioxidant enzymes in the lungs. Excessive ROS production led to an imbalance between oxidants and antioxidants, resulting in a significant decrease in antioxidant enzymes. This decline in antioxidant enzymes impaired the defense and protection of cells against oxidant induction, leading to more severe damage to type II lung cells [18].

Type II cells play a crucial role in surfactant synthesis and secretion, which reduces alveolar surface tension. An increase in necrosis of type II cells leads to decreased surfactant synthesis and secretion. This reduction in surfactant synthesis and secretion results in alveolar collapse and reduced lung expansion, causing respiratory difficulties [19].

The occurrence of type II cell necrosis is caused by carbofuran exposure, leading to increased ROS levels. Increased ROS levels result in increased lipid peroxidation, DNA damage, and decreased glutathione (GSH) levels. These three factors contribute to cell dysfunction, damage, and ultimately cell death, which can occur through necrosis [20].

According to Pizzino et al, excessive ROS production can be highly dangerous to the body and can trigger the formation of highly reactive hydroxyl radicals (OH-) [17]. Hydroxyl radicals are one of the most dangerous free radicals for the body and can damage DNA, proteins, and unsaturated fatty acids (polyunsaturated fatty acids/PUFA), which are essential components of cell membrane phospholipids. DNA damage leads to temporary inhibition of protein synthesis and growth-related genes associated with the induction of antioxidant enzymes and proteins involved in cell repair [18].

The results of the study by Aoshiba and Nagai indicated that intracellular ROS increases with a decrease in GSH levels [14]. Decreased GSH levels result in inhibition of proliferation and lysis of type II cells, increasing the permeability of lung epithelial cells. The primary target of lung toxicity is alveolar epithelium, including type II cells. Damage to type II cells can manifest as cell enlargement, disruption of mitochondria, and endoplasmic reticulum.

In the histopathological images of P0, P1, P2, and P3, inflammatory infiltration was observed (Figure 1). According to Chen et al., inflammation is a defensive response to injury [21]. The result of inflammation is the neutralization and disposal of attacking agents, the destruction of necrotic tissue, and tissue repair.

The presence of inflammatory infiltration in various treatments may be due to oral carbofuran exposure. Oral administration of carbofuran leads to increased ROS levels. Excessive ROS production leads to oxidative stress [17]. Oxidative stress triggers a local immune response, resulting in decreased lung function. Additionally, oxidative stress induces an increase in neutrophils and macrophages in lung tissue [22]. Inflammatory infiltration was also observed in P0. The presence of inflammatory infiltration can be attributed to various factors, including illness in the mice, environmental stress, and bacteria [23].

In the histopathological images of P0, P1, P2, and P3, congestion was observed (Figure 1). Congestion involves the accumulation of blood within blood vessels, accompanied by the dilation of these blood vessels. Congestion can be systemic, resulting from tissue damage due to hypoxia [24]. Intracellular hypoxia occurs due to decreased lung membrane permeability, leading to congestion [25].

4. Conclusion

Based on the research analysis, it can be concluded that exposure to the carbofuran insecticide can cause lung tissue damage, including necrosis of type II lung cells. The increase in type II cell necrosis is associated with increasing carbofuran dosage.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The study was approved by the Faculty of Veterinary Medicine Animal Ethics Committee of Universitas Airlangga. All variables were considered in accordance with the Ethics Committee related to the animal handling to ensure no discomfort or pain was caused to the animals during sampling(certificate registration number: 2012/112-KE).

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