International Journal of GEOMATE, March, 2019 Vol.16, Issue 55, pp. 113 - 118
ISSN: 2186-2982 (P), 2186-2990 (O), Japan, DOI: https://doi.org/10.21660/2019.55.8260
Special Issue on Science, Engineering & Environment

THE EFFECTS OF TRANSFLUTHRIN AS THE ACTIVE SUBSTANCE OF ONE PUSH AEROSOL REPELLENT ON ORGANS DAMAGE OF MICE (MUS MUSCULUS) (CASE STUDY OF LUNG, LIVER, BLOODS, AND KIDNEY)

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*Corresponding Author, Received: 01 Sept. 2018, Revised: 10 Oct. 2018, Accepted: 12 Dec. 2018

ABSTRACT: One push aerosol repellent used by the public contains an active substance called transfluthrin. Transfluthrin is mostly pyrethroid synthetic which has functions as neurotoxin substance. It may act as free radicals in the body due to the compounds which have one free electron that makes it reactive. The purpose of this study was to investigate the effects of transfluthrin on mice organs. In this study, the experimental animals (Wistar mice) were put in an exposure chamber and exposed by one push aerosol repellent contains around 20-25% of transfluthrin one time a day for 15 days. The damage of mice organs was identified by the microscopic picture (400x of magnification). The results showed that the expose of repellent contains about 20-25% of transfluthrin on mice increase the damage of lung, liver, blood, and kidney cells from 13% to 24%, 12% to 27%, 9% to 69% and 22% to 55%, respectively.

Keywords: Transfluthrin; Lung cells; Liver cells; Blood cells; Kidney cells

NOMENCLATURE: ATN (Acute Tubular Necrosis); ATP (Adenosine Triphosphate); DNA (Deoxyribonucleic Acid); DSA (Alveolar Septal Destruction); ROS (Reactive Oxygen Species)

1. INTRODUCTION

Air as one of the natural resources which are the main need for a living thing and non-renewable. Air pollution has negative effects on human health. Substances that are often used in daily life and potentially as air pollutants (pollutants) are cigarette smoke [1], air freshener aerosol, and insecticide aerosol [2]. Increased concentrations of air pollutants are related to various diseases such as lung cancers, Parkinson, and many others [3–5].

Pollutants, both gaseous and particulates are very harmful to the health of the human body. Pollutants can pass through the body through respiration and oral processes. The presence of pollutants in the body will be able to disrupt the function of organs, including the kidney as the main organ in the excretory system [6,7], the lungs as respiratory organs [8], the liver that plays an important role in the process of detoxification, and blood as the most fluid in the body [9,10].

The degree of organ damage caused by particulate matters of pollutants is strongly influenced by particle size [11,12]. The size of the particulate matter varies greatly. Based on its size, the particles can be classified into three kinds, i.e., coarse particles, fine particles, and ultrafine particles. Ultrafine-sized particles give a greater effect than larger particles. The results showed that the fraction of ultrafine particles was the most damaging particulate matter [13].

One of the substances that are often used in daily life and potentially as a pollutant is one push aerosol mosquito repellent. Indonesia is one country that has a tropical climate, where the tropical climate is very suitable for the breeding process of insects, especially mosquitoes. Mosquitoes themselves can cause various diseases. The effort to overcome the increasing number of diseases caused by mosquitoes is to use some chemicals insecticide - containing mosquito repellent.

For each type of a mosquito repellent contains different ingredients depending on the brand and their types. One of the most popular mosquito repellents in the market is the one-push aerosol which has a variety of content one of which is transfluthrin chemicals. Transfluthrin is one of the pyrethroid insecticides that produces a rapid reaction with low persistence. Pyrethroid insecticide has a very specific effect on insect nerve cells, so it needs a little amount to have the required effect [14].

One push aerosol mosquito repellent is mostly used in a closed room. The scent of mosquito repellent is an indication that the harmful compound of mosquito repellent is still spreading in the room as well as an indication of the residue in the room. This aerosol can disrupt the

respiratory tract. If the particles enter the lung, it will flow in the blood so it will give certain effects such as inflammation of the sensitive organs [3,15]. The transfluthrin active ingredients contained in one push aerosol can decrease the erythrocytes.

The pulmonary lung is the organ that first contacts directly with particles. Particles that enter the lungs will be carried by the blood and circulated throughout the body. The blood then goes to the kidney for filtering. The filtered residues will be detoxified by the liver. So that the potential organs affected by the active ingredients of the mosquito repellent are lung, blood, kidney, and liver.

Based on the description of the dangers of mosquito repellent and the potential impact on the organs, it is necessary to conduct research to investigate the impact of mosquito repellent on organs. However, there are limited studies on the impacts of the indoor aerosol one push mosquito repellent, and no available data on the effect of transfluthrin on organ damage. Hence, this study was aimed to investigate the damage level of the kidney, liver, lung and blood organ in mice exposed by the indoor aerosol one push mosquito repellent. This study will contribute to a better understanding of the pollutant impact on the anatomy, structure, and work function of the organ.

2. MATERIALS AND METHODS

2.1 Experimental Animals and Treatments

We used 2-3-month-old male mice (Wistar) as the experimental animals that were divided into three groups: a control group, a treatment group A and a treatment group B. The control group was unexposed mice. The treatment group was sprayed with the different mosquito repellent. The group A and B were exposed by the transfluthrin content of 21.3% and 25% respectively. The exposure dose was given for five spray pushes. After the mice were sprayed, they were placed in the exposure chamber with the dimension of 20 cm x 20 cm x 30 cm for 20 minutes (Fig.1). Then the chamber was opened to the mice breathed free air. After spraying for 15 days, the mice were dissected to take the organ: lungs, blood, kidneys, and livers.

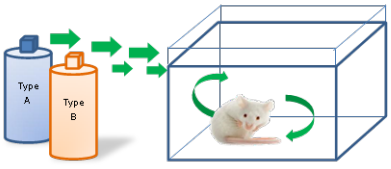

Fig.1 Chamber of exposure to mice

2.2 Preparation and Histological Examination

2.2.1 Kidneys, livers, and lungs

The mice from the control group and the treatment groups were sacrificed by cervical dislocation. The kidneys, livers, and lungs were cleaned using NaCl (0.9%). They were fixed in buffered formalin (10%) for a week and dehydrated in upgraded ethanol series. After being processed to the paraffinization, they were cut using a microtome and were colored using HE (hematoxylin and eosin) [6].

2.2.2 Blood

The blood smears were placed onto the object glasses. They were fixed with 70% methanol solution and then covered with the cover glasses. When they came dried (±5 minutes), they were stained using a Giemsa and buffer pro-Giemsa solution [10].

2.2.3 Histological examination

The cells deformation was observed using a microscope (400x of magnification). The damage level (*DL*) of the observed cells (*OC*) was calculated using Eq. (1) [6]:

$$DL = [\sum deformed\ cells / \sum OC] \times 100\% \quad (1)$$

3. RESULTS

3.1 Lung Damage

The microscopic picture of the lung treatment group mice is shown in Fig.2.

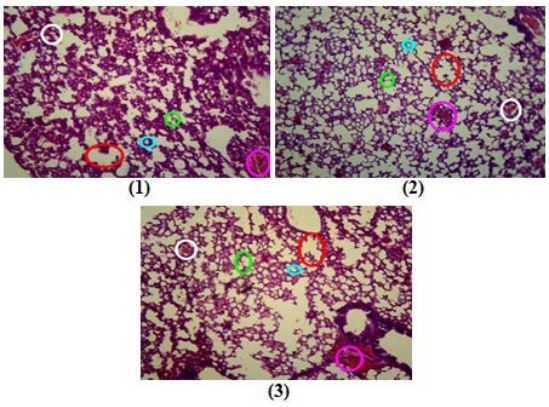

Fig.2 Microscopic pictures of lungs of mice (1) control (without treatment), (2) type-A and (3) Type-B. The red, green, and grey circles show the emphysema, DSA, and edema, respectively. The pink circles indicate the bleeding

Lung emphysema is a disease whose primary symptom is an airway obstruction because the air sacs in the lungs are excessively bulging and

suffered extensive damage. Edema is characterized by the presence of fluid-filled alveoli and an increased laxity of the alveolar septum. Alveolar septal destructions (DSAs) are characterized by the thinning of the alveolar septum, atrophy, and in some parts total damage of the alveolar septum that spherical formed and may also be accompanied by enlargement of the ducts and alveolar sacs. For normal alveolar cells characterized by an almost spherical (poly) cell shape with a size of 4-5µm [8]. The lung damage is calculated by counting the normal cells and the damaged cells in five random fields to determine the cell deformation [16]. The percentage of the cell deformation is presented in Fig.3.

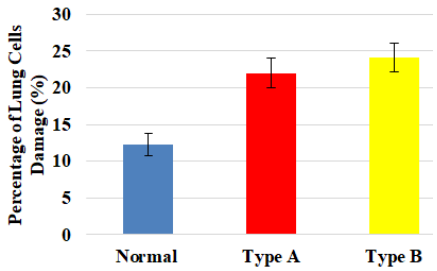

Fig.3 Damage percentages of the mice lungs

3.2 Red Blood Cells Damage

The microscopic picture and percentage of blood cells damage of red blood cells can be seen in Fig.4.

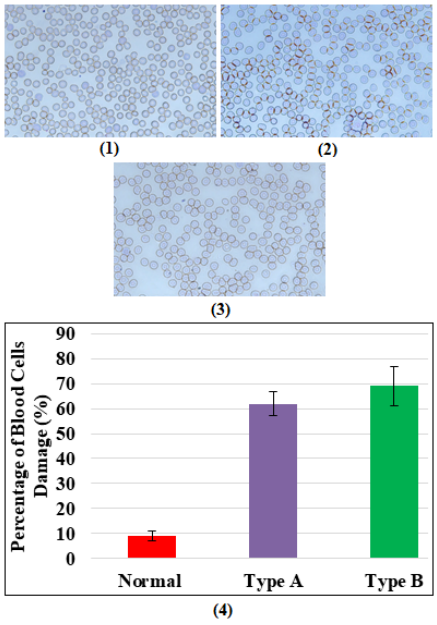

Fig.4 Microscopic pictures of red blood cells of mice (1) control (without treatment), (2) type-A and (3) Type-B. The percentage of red blood cells damage (4)

The physical changes were determined by the comparison between the size and shape of normal cells and the abnormal cells [10]. The abnormal blood cells are classified into nine types of cellular damage, i.e., cell target, teardrop, crystal, roll cell, polychromasia, sickle cell, stomatocyte, and basophilic [17,18]. Cell targets are the blood cells that have erythrocytes that look like shoots or Mexican hats. Teardrop is a pear-shaped blood cell or like water droplets. Krista is a blood cell that has a straight or crooked or slightly square shape. Roll cell is a blood cell that holds each other. Sickle cell is damage that has erythrocytes shaped crescents, stiff, and chronic hemolytic anemia. Stomatocyte is a type of blood cell that has erythrocytes in the form of a central pallor such as the mouth. Basophilic is a blood cell damage that has a slim/ rounded granule. Keratocyte is a blood cell damage that has a barrier shape.

3.3 Kidney Damage

The observation of the kidney cell damage was demonstrated by the microscopic images of the glomerular cells and tubules (Fig.5) [6]. The results of the glomerular observation (green arrows) and kidney tubular mice showed that the pollutant exposure could cause the damage of the kidney structure that is known as the glomerulus and tubular damage. Glomerular damage is characterized by the widening of Bowman space [19,20]. Tubular damage is characterized by narrowing of the tubular lumen (orange circles). The increasing of the glomerular and tubular damage will cause necrosis or cell death called Acute Tubular Necrosis (ATN). ATN is caused by lack of oxygen (ischemic ATN) and the influence of toxic substances in cells (nephrotoxic ATN). Microscopically, there is a pigment of hemoglobin pigment scattered in the tubules. The percentage of the kidney cells damage is presented in Fig.6.

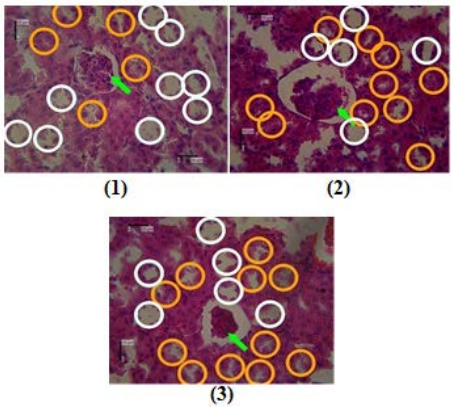

Fig.5 Microscopic pictures of kidneys of mice (1) control (without treatment), (2) type-A and (3) Type-B. White circles show normal tubules

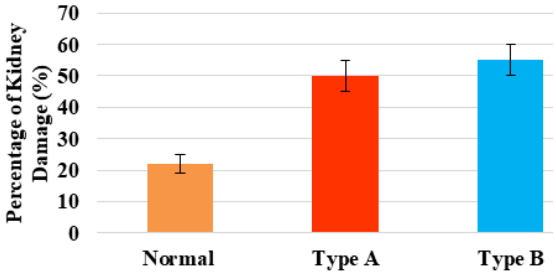

Fig.6 Damage percentages of the mice kidneys

3.4 Liver Damage

The changes in the mice liver include the central vein, hepatocyte cell form, and sinusoid blood vessel (Fig.7) [9]. The centralist vein shape that is originally a spherical shape and unbroken. After receiving treatment, the vein began to break. Similarly, hepatocyte cells also had to change the shape. Meanwhile, the sinusoidal blood vessels became wider. The observation of liver cells showed that spraying of pollutant material in mice had adverse effects on the liver organ of mice where the administration of these pollutants affected the shape and structure of mice liver cells. Dosage type B-type mosquito spray for high intensity can cause blood cells to get worse. At a spray dosage of 5 times obtained damage of liver cells reached 27.49%.

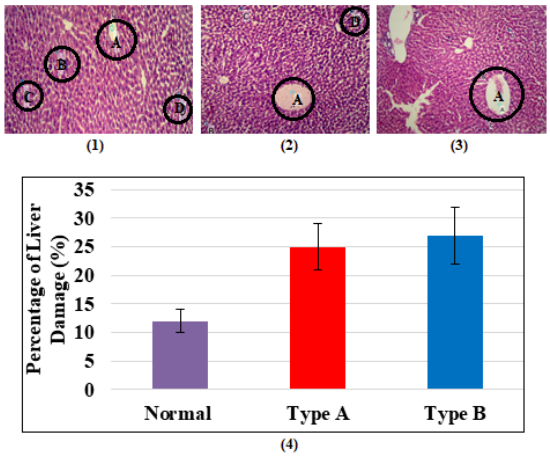

Fig.7 Microscopic pictures of livers of mice (1) control (without treatment), (2) type-A and (3) Type-B. Captions: A = Vein Centralist, B = Normal Cell, C = Binucleus, D = Parenchymal Degeneration. The percentage of liver cells damage

4. DISCUSSION

Insecticide one push aerosol is considered the most powerful in killing or eradicating mosquitoes. There are five ways insecticide work on the insect body that affects the nervous system, inhibits energy production, affects the endocrine system, inhibits cuticle production and inhibits water balance. Transfluthrin contained in both types of mosquito repellents is a pyrethroid class II obtained from the synthesis of pyrethrin. Based on their toxicity and structure, pyrethroid is classified as type I and type II. Type I is a pyrethroid group that has no α-cyano group and type II has an α-cyano group.

The interaction between blood and transfluthrin occurs when the blood passes through the lungs. Blood contains hemoglobin which acts as an oxygen binder. With the transfluthrin entering the lungs, the hemoglobin will tend to bind transfluthrin rather than oxygen gas to be circulated throughout the body. The blood containing transfluthrin can affect other substances needed by the body such as nutrients, hormones, fats, sugars, and proteins. Pyrethroid can increase activation and prevent deactivation. The impact of the event resulted in the ion channel open longer. At the cellular level, pyrethroid interferes with nerve activity, membrane depolarization, and synaptic disorders [21]. The membrane of a protein affected by pyrethroid causes the change of the action potential of the cells.

Enzymes that hydrolyze ATP have been found to be sensitive to pyrethroid reactions. ATPase neural membrane is a target for pyrethroid in invertebrate insects, whereas in rats it is synaptosome. Pyrethroid joins the lipid bilayer layer to interfere with phospholipid conditions and cause instability in the membrane. The mechanism of neurotoxicity begins with an interruption in the activity of Ion Na +, K + and ATP ions and membrane instability.

Several types of pyrethroid are known to produce poisoning symptoms similar to poisoning due to cyanide and aldehyde. Pyrethroid is thought to be capable of generating free radicals and causing stress on cells. Pyrethroid manifold cypermethrin and fenvalarete have been known to produce oxidative stress and changes in antioxidant enzymes [22]. Oxidative stress is a condition where there is a disturbance of balance between the productions of free radicals with antioxidants that cause tissue damage. Oxidative stress can be caused by a reduction in natural antioxidant levels in the body and increased free radical production [12]. Oxidative stress occurs due to the presence of reactive oxygen species (ROS) in cells more than the natural oxidant compounds in cells. Free radicals are substances, molecules, compounds that have highly cellular reactive properties. If radicals are not inactivated, their reactive characteristic can damage all types of cellular macromolecules such as carbohydrates, proteins, lipids, and DNA. Free radicals generally

stand on their own, with very fast reactions with other atoms filling in the vacancy of an unpaired orbital. Transfluthrin is a free radical of the C atoms in the structure of benzene.

The kidneys function to produce urine from the body's metabolic processes. The kidney is the main path of toxic substances (toxins). The release of the substance is carried out by three processes: filtration by the glomerulus, reabsorption by tubules, and secretion by tubules. The kidneys also concentrate toxically on the filtrate and carry the toxic via tubular cells. Glomerular damage will lead to disruption of the filtration process in the glomerulus so that the ability to filter blood is reduced, the proteins and blood cells will accumulate in the tubules.

Tubules serve as a place of reabsorption and secretion in the process of formation of urine. Damage to the kidney tubules is characterized by the presence of tubular cells that swell due to fluid shift from outside the cell into the cell. This shift occurs because the toxic substances contained in ultrafine particles from pollutants cause changes in the surface charge of the tubule epithelial cells, the active transport of ions and organic acids, and the ability to concentrate from the kidneys that eventually lead to damaged tubules, impaired urinary flow, increased via tubular pressure. This cell swelling causes the tubular lumen to become narrowed until closure. If the tubule cell is damaged, it will interfere with the system of urine formation in the kidney, then the substance that is not needed by the body cannot be removed from the body, so it will be toxic that can damage the kidney organ [23,24].

5. CONCLUSION

The results of the research have shown that there is damage to organ cells in mice due to the pollutant exposure. The transfluthrin active substances contained in one push aerosol affect the mice organ with the different damage degrees. The blood is the worst damage with the percentage of 69%. Meanwhile, the lung is the least damage of 24%. The damage depends on the transfluthrin content. A more dose of transfluthrin caused more damage to organs.

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