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THE IMPACT OF EXPOSURE TO AEROSOL MOSQUITOES REPELLENT RELATED TO FREE RADICALS ON THE ORGANS OF MALE MICE

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ABSTRACT: From year to year, human activities using chemical substances lead to pollute ambient air and affect human health. This pollutant can pass through the respiratory system and disrupts the organ function, including lung, liver, blood, and kidney. It can be identified by the deformation found in the structure and function change of the related cells in these organs and the existence of free radical. On the other hand, each substance has a different composition and health effect. The possibility of the free radical type that is formed is also different and related to the substance. According to this background, it a need to determine the presence of free radicals in the mice's organs exposed to one push aerosol mosquito repellents as the substance. This study aimed to investigate the free radicals on the mice's organs due to the exposure to aerosol mosquito repellent and its relation with the organ damage of cell deformation. This study used male mice as the experimental animals that were divided into control and treatment groups. The study included two types of repellents, Type-A (25% of aerosol concentration) and Type-B (21.3% of aerosol concentration). Free radicals were detected using an electron spin resonance device. The results showed that the cell deformation due to the exposure of one push aerosol was different for each organ. The deformation levels were about 36% to 74% that depended on the aerosol concentration. The exposed aerosol generated anion superoxide (O2-) and singlet oxygen radicals (1O2) as the free radicals found in the observed organs.

Keywords: Aerosol, Free radical, Organ damage, Pollutant, Mice

1. INTRODUCTION

Indonesia is a big 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. That is why it needs some efforts to overcome the increasing number of diseases caused by mosquitoes.

Eradication of mosquitoes tends to be carried out using free-selling insecticides. The effort to eradicate mosquitoes by using insecticides is only a temporary thing and has no preventive effects for a longer period. Insecticides that are widely used by the community can have a negative impact on the environment and humans due to the dangerous chemical compounds contained in the insecticide.

One push aerosol mosquito repellent is one of the substances that are often used in Indonesia, especially in daily life due to its practice use compared to another insecticide. However, the usage of this mosquito repellent is growing from time to time. As a consequent, it also contributes to a pollutant in the ambient air. It contains transfluthrin, as an insecticide ingredient.

In the human body, transfluthrin tends to be reactive because it has unpaired electrons [1]. These electrons come from oxygen atoms in transfluthrin in cyclic rings, as a result of their reaction to the mono oxidase acid (H+) enzyme [2]. These unpaired electrons will look for pairs from other parts of the cell [3]. Transfluthrin which enters the body may trigger the formation of free radicals.

Free radicals are atoms, elements, compounds, or molecules that have one or more unpaired electrons in their outer orbitals [4]. These unpaired electrons are very reactive and tend to look for pairs by attacking and binding electrons to stable molecules around them [5–7]. The mechanism or process of free radical formation has several stages. The stages of free radical formation are initiation (initial formation of free radicals), propagation (propagation or new radical formation), and termination (destruction or conversion into stable and unreactive free radicals) [3]. The impact of free radicals on cells is the disruption of cell function or treating abnormal cell function [8]. Disorders of cell function consist of short-age cells characterized by wrinkled skin, premature aging, and degenerative diseases. The body's defense system is reduced so that the body becomes weak and easily injured [9]. Abnormal reactions from cells can grow new cells that trigger tumor cell growth and cancer [10,11].

According to this background, it is urgently required to identify free radicals that is generated in the body related to the use of one push aerosol mosquito repellent. Thus, the purpose of this study was to identify the types of free radicals in mice's organs that were exposed to one push aerosol mosquito repellent. This study also investigated the influence of different transfluthrin concentrations on the free radical existence, so that an alternative step can be sought to deal with further organ damage.

2. MATERIALS AND METHODS

2.1 Experimental Animals

This study used healthy male mice with the age of 2-3 months old (with 23-30 grams of BW (body weight)). The mice were treated humanely and feed with food and water *ad libitum* and kept in controlled animal cages (temperature: 24 - 27^{o}C, relative humidity: 75 – 78%) with 12 h dark/ light cycle [12]. All of them (n = 21) were acclimatized in an experimental chamber (20 x 20 x 30 cm^3) for three consecutive days before being divided randomly into two treatment groups (Group-A and Group-B) and one control group (Group-C). The mice in each treatment group received similar treatment related to the transfluthrin concentration (n = 7 per group, Table 1). All procedures were in accordance with the Animal Care and Use Committee of Brawijaya University, Malang, East Java, Indonesia (Ethical Clearance Number: 541-KEP-UB).

2.2 Transfluthrin Content

This study used two different one push aerosol mosquito repellents. They were Type-A and Type-B mosquito repellents with different transfluthrin concentrations. Type-A and Type-B mosquito repellent had 25% and 21.3% of transfluthrin concentrations, respectively.

Table 1 Scheme of the experimental animals used in the study (n = 21)

Groups	Number of Sprays	Mice	Transfluthrin Type
Group-A	5 sprays	7	Type-A (25% of transfluthrin)
Group-B	5 sprays	7	Type-B (21.3% transfluthrin)
Group-C	-	7	-

2.3 Animal Treatments

According to Table 1, Group A was a group of mice exposed to one push aerosol Type-A mosquito repellent as much as five sprays. Group B was a group of mice which were exposed to one push Type-B with five times sprays. These treatments were done in a similar time and controlled room temperature. These treatments were conducted for twenty minutes per day within 30 consecutive days.

2.4 Organs Preparation

After exposure procedures, all mice were sacrificed by cervical dislocation. The kidneys, livers, and lungs (organ samples) were cleaned using NaCl (0.9%) and were fixed in buffered formalin (10%) for a week before being dehydrated in upgraded ethanol series. After that, they were processed to the paraffinization and cutting, then colored using hematoxylin and eosin. For the blood samples, the blood smears on the object glass were fixed with methanol solution (70%) and dried. After that, these samples were colored using Giemsa and buffer pro-Giemsa solution. All resulted samples were observed under a microscope (400x of magnification) to investigate the level of cell physical damages (blind counting method) [12–14].

2.5 Free Radicals Investigation

The presence of free radicals in the samples was detected using ESR (Electron Spin Resonance, Fig.1). The sample was placed in a magnetic field and given interference with electromagnetic waves in radio frequency *f*. The magnitude of the magnetic field given depending on the amount of electric current I flowing in the Helmholtz coil and could be calculated using Eq. (1) below.

$$B = \mu_0 \, . \, (\tfrac{4}{5})^{\frac{3}{2}} \, . \, \tfrac{n}{r} \, . \, I \tag{1}$$

where,

μ_0 : 1.2566 x 10^{-6} (T.m/A)
B : external magnetic field (T)
I : current on the Helmholtz coil (A)

The number of turns of Helmholtz coil was interpreted as n (n = 320 coils), with the radius (r) of 6.8 cm. The type of the detected free radicals is determined using Lande factor (g factor) calculation, as expressed in Eq. (2).

$$g = \frac{h \, . \, f}{\mu_B \, . \, B} \quad (2)$$

where,

μ_B : Bohr magneton (9.273 x 10^{-24} J/T)

h : Planck constant (6.625 x 10^{-34} Js)

f : resonance frequency (Hz)

Table 2 Values of g factor of singlet oxygen and superoxide free radicals

Free Radical Name	g Factor Value
$^1O_2$	1.501
O_2^-	1.501 - 1.750
Fe^{3+}	1.77
MnO_2	1.8367
FeS	1.86
Hydroperoxide	1.9896
CO_2^-	1.996
Cu	1.997
SO_4^-	1.9976
Hydroxyl	2.00047
CO_2	2.0007
Alkoxyl	2.00160 - 2.00197
Helium	2.002
Methanol	2.00205
Alkyl	2.00206
Hydrogen	2.00232
Methil	2.00255 – 2.00286
DPPH	2.0036
SO_3^-	2.0037
Ethyl	2.0044
C	2.00505 – 2.00548
Peroxy	2.0155 – 2.0265
CuO_x	2.098
$CuGeO_3$	2.154
$YBa_2Cu_3O_7$	2.24
Cu-HA	2.289
Hg	4.0 – 4.5

According to Eq.(2), f was determined from the existence of RF wave resonance. This wave resonance was identified by forming a V-shaped curve on the oscilloscope. This curve was a combination of two RF waves with a phase difference of 135º (Lissajous curve).

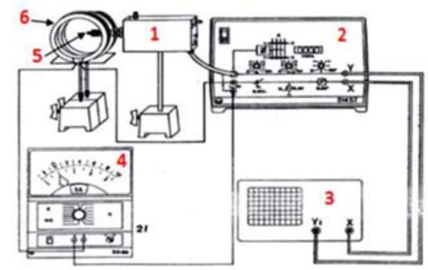

Fig. 1 The set up of Leybold-Heracus ESR devices, where: 1). radio frequency control unit; 2). ESR control unit; 3) oscilloscope; 4). multimeter; 5). sample place; and 6). Helmholtz coil.

2.6 Statistical Analysis

The resulted values were shown as mean ± SEM (standard of means). All statistical analysis was performed using Microsoft Excel 2016.

3. RESULTS AND DISCUSSION

3.1 ESR Calibration

For a better result, a DPPH calibrator (DPPH radical is a stable free radical) was used to calibrate ESR [15]. The resulted g factor was compared to the literature. Table 3 below shows the result of the calibration using DPPH. According to the calibration, the resulted calibration factor was 0.9964. The V-shaped curve (RF wave resonance) and a Lissajous curve are shown in Fig.2-Fig.3.

Table 3 Result of ESR calibration using DPPH

f (MHz)	I (A)	g Factor (literature)	g Factor (calibration)
23.7	0.199	2.0036	2.0109

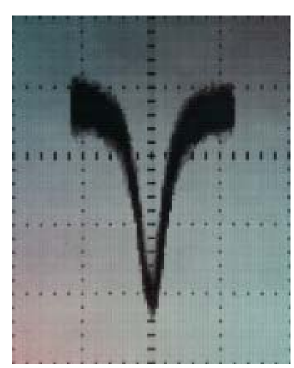

Fig. 2 DPPH resonance curve.

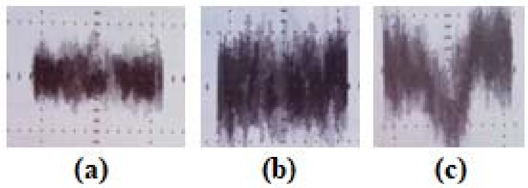

Fig. 3 The Lissajous curves for (a) research tube; (b) control group; and (c) treatment group.

3.2 Formed Free Radicals

The exposure to one push aerosol mosquito repellents (Type-A and Type-B) had a direct impact in mice's organs. According to ESR analysis, the exposure treatments generated some free radicals with similar *g* factor value (Table 4). The presences of O_2^- (superoxide) and $^1O_2$ (singlet oxygen) in mice's organs indicate the form of ROS (reactive oxygen species) [9,16,17]. ROS in the mice's organs can be used to predict the presence of oxidative stress in cells, showing the inability of cells in overcoming the excess amount of free radicals.

Based on the results, the level of organ damage varies. The most damage organ is alveolar cells (74-78%), where Type-B with lesser transfluthrin concentration has 74% of cell physical damage. Liver cells (hepatocytes) has 36% and 40% of cell physical damages, respectively for Type-A and Type-B, having the least impact of transfluthrin exposure. According to these results (see Table 4), we can see that the cell physical damages and free radical generation is directly correlated with the different concentration of the exposed transfluthrin. As an active substance, transfluthrin belongs to the pyrethroid group. According to the previous study, pyrethroids have low toxicity that is safe to use in humans. Occasionally, the pyrethroid group can be categorized to toxin when the level of deposited pyrethroid exceeds the normal dose. As an impact, if toxin enters the body continuously, it may accumulate and may cause damage to the organ due to the free radicals.

Table 4 The detected free radicals in mice's organ exposed to one push aerosol mosquito repellents

Organs	Type-A			Type-B		
	g Factor	Free Radicals	Cell Physical Damage (%)	*g* Factor	Free Radicals	Cell Physical Damage (%)
Liver	1.560	O_2^-	40±3	1.500	$^1O_2$	36±3
	1.526	O_2^-		1.490	$^1O_2$	
	1.518	O_2^-		1.517	O_2^-	
	1.492	$^1O_2$		1.593	O_2^-	
Kidney	1.560	O_2^-	47±3	1.560	O_2^-	49±3
	1.568	O_2^-		1.576	O_2^-	
	1.542	O_2^-		1.584	O_2^-	
	1.466	$^1O_2$		1.474	$^1O_2$	
	1.483	$^1O_2$		1.483	$^1O_2$	
Blood	1.509	O_2^-	71±3	1.509	O_2^-	72±4
	1.545	O_2^-		1.526	O_2^-	
	1.577	O_2^-		1.560	O_2^-	
	1.628	O_2^-		1.577	O_2^-	
Lung	1.550	O_2^-	74±4	1.507	O_2^-	72±4
	1.557	O_2^-		1.517	O_2^-	
	1.535	O_2^-		1.508	O_2^-	
	1.576	O_2^-		1.543	O_2^-	

In the human body, free radical is generated during normal cellular metabolism [15]. Besides, the human body has its antioxidant defense mechanisms to equilibrate the free radicals formations [9,17]. However, any disruption in this mechanism may disturb the equilibrium in the body resulting in oxidative stress, including cell deformation or cell physical damage [18].

The mechanism of cell damage due to transfluthrin begins when transfluthrin attaches to the cell membrane. When transfluthrin is inhaled by the respiratory organ, it may follow the bloodstream, passes through the lungs.

Besides, the function of normal blood related to the hemoglobin as an oxygen binder may be disrupted. Hemoglobin may tend to bind transfluthrin rather than oxygen to be circulated throughout the body and becomes a toxin in the body. This process then triggers other disruptions, including the bad impacts of nutrients, fats, hormones, and proteins. Moreover, pyrethroid can manifold cypermethrin and fenvalerate, resulting in oxidative stress and changes in antioxidant enzymes [19]. They have a high potential to interfere with nerve activity, membrane depolarization, and synaptic disorders [20]. They then may trigger the formation of free radical. This free radical formation (singlet oxygen) process occurs during the process of oxidative metabolism where there is an electron leak that can form O_2^- [3,21]. The entry of transfluthrin as toxins into cells may trigger mitochondria to produce more O_2^- (Fig.4).

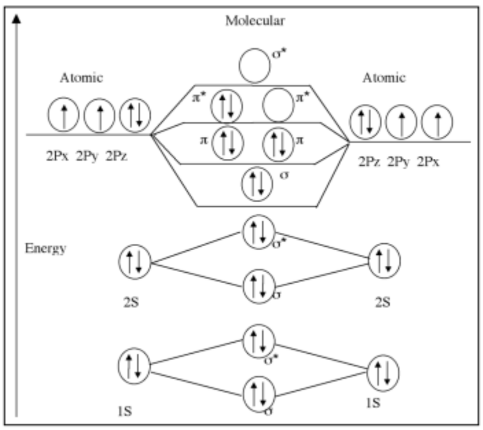

Fig. 4 Singlet oxygen electron configuration [22].

The main formation of ROS (reactive oxygen species) such as superoxide occurs in the mitochondria through the interaction of O_2 with Coenzyme Q (KoQ) [23]. Some electrons moving from NADH and other compounds to O_2 may pass when KoQH interacts with O_2 and form superoxide radicals [24]. Moreover, mitochondria also contain high SOD (superoxide dismutase) and glutathione [25]. Dismutase is determined as the main defense against oxidative stress that occurs in the body, in which it is triggered by chemical enzyme induction or conditions that increase superoxide formation [25].

4. CONCLUSION

Exposure to transfluthrin contained in one push aerosol mosquito repellents on mice has an influence on the existence of the free radical on the mice's organs. They are detected as superoxide anions (*g* factor 1.501 - 1.750, O_2^-) and singlet oxygen (*g* factor 1.501, $^1O_2$), in which generating 36% to 74% of cell deformation levels (depending on the transfluthrin concentration and mice's organs). The forming process of free radicals likely takes apart during the oxidative metabolic process where there is an electron leak that can form O_2^-. The entry of transfluthrin into cells can trigger mitochondria to produce more $^1O_2$.

5. ACKNOWLEDGMENTS

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