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Sublethal Toxicity of Organophosphate Pesticides and its Effect on Hematology Parameter, Histopatology Hematopoietic Organ of Silver Rasbora (*Rasbora argyrotaenia)*

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Abstract. Pesticides are pollutants that are found in rice fields and rivers. Pesticides that are often used by farmers in Indonesia in eradicating insects are organophosphate insecticides, where they can eradicate insects that are very toxic to fish due to strong neurotoxic substances that inhibit AchE (Acetylcholinesterase) activity. The research aims to examine the effect of organophosphate pesticides on hematology and histopathology of hematopoietic organs in silver rasbora fish. The research method used is an experimental method with a completely randomized design. The parameters investigated were hematology and histopathology hematopoietic organ (liver and kidney). The results of this study showed a hematological change in silver rasbora fish where there was a decrease in total erythrocytes (0,59±0,004) and hemoglobin (2,5±0,1) while total leukocytes increased (245,35±15,78). Also, there are differential changes in leukocytes, namely an increased in the number of monocytes (5±1) and neutrophils (24±3), but lymphocytes have decreased in number (72±1). The Histopathology of the fish liver also can found in this research, those damages that are found are erythrocyte infiltration, necrosis picnosis, and karyolysis. Histopathology of fish kidney also can found cloudy sweling, necrosis karyolysis and tubular necrosis.
Keyword: pesticide, hemathology, histopathology, silver rasbora

INTRODUCTION

The use of pesticides in agriculture only functions as much as 90%, the rest has polluted the surrounding environment, especially fish (Shoaib, Siddiqui, & Ali, 2012). Organophosphate is an active neurotoxin, it doesn't even require any conversion to inhibit the enzyme acetylcholinesterase (Setyawati, Wiratmini, & Wiryatno, 2011). Organophosphate is able to inhibit the action of the enzyme acetylcholinesterase (AChE) which causes the disruption of acetylcholine in delivering impulse stimulation from pre-synapse to post-synapse (neurotransmitter) so that the work of the muscles becomes disrupted. Muscle work that is not directed to cause

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symptoms of poisoning that affect the entire body (Richendrfer & Creton, 2015). Fish that are exposed to pesticides will experience hematological changes (Awicka, Uchta, Rzezinska, & Akota, 2015). Hematopoetic tissue damage, namely liver (Begum & Mithra, 2015) and kidney (Deka & Mahanta, 2012).

Haematological studies are important criteria for diagnosis and indicators of the severity of a disease (Clauss, Dove, & Arnold, 2008). Loss of the function of the enzyme acetylcholinesterase results in a prolonged vasoconstriction resulting in blood flow and inhibiting the supply of nutrients into the tissues (Christensen, Harper, Luukinen, Buhl, & Stone, 2009).

One of the fish affected by organophosphate pesticides is the silver rasbora fish, which is a freshwater fish and lives in the wild such as rivers or rice fields. The existence of silver rasbora is increasingly difficult to find, this is due to the widespread capture and pollution of water in rivers that affect the fish population. Based on the description above, it is necessary to research to determine the effect of sub lethal toxicity of organophosphate pesticides on hematology, histopathology of liver and kidneys of silver rasbora.

MATERIAL AND METHOD

Animal and experiment condition

The research was approved by the Animal Care and Use Committee in Veterinary Faculty University Airlangga Surabaya, Indonesia (No. 2. KE. 148. 07. 2019). The experiment hematology and histopathology parameter use male and female fish silver rasbora (5-7 cm, weight ± 0,35 gr). The fish was divided into 5 treatments with 4 replications and kept in a 20-liter volume container. The maintenance container was previously sterilized by soaking $KMnO_4$ at a dose of 2.5 ppm during 24 hours and aerated. Maintenance water is soaked with chlorine for 24 hours and aerated.

Pesticide organophosphate was used profesnofos curacron 500 in the experiment. before getting sublethal doses, a lethal test is first performed with LC 0 - 96 Hour. The dose used in the preliminary test uses multiple logarithmic 0, 0,01, 0,1, and 1 ppm. The physical and chemical parameters remained at the same level throughout the lethal test is Dissolved Oxygen 6-7 ppm, Temperatur 25 - 28 ^{0}C and pH 7-8,5. Than LC 0 - 96 value determined through probit analysis with IBM SPSS Statistics 20 software, with value is 0.04 ppm, so a dose is obtained is 0,001 ppm, 0,005 ppm, 0,01

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ppm dan 0,05 ppm. Fish was fed at 9 - day intervals with a commercial fish feed.

Hematological Parameter

Sampling of fish blood was carried out on day 9. The blood was taken from the part of the caudalis artery as much as 0.4 ml by a sterile syringe measuring 1 ml which had previously been given EDTA. Some blood taken from some treatment than is was put into a microtube to calculate the number of blood profiles. The sample blood are used to measure total erythrocytes, hemoglobin levels and total leukocytes. Erythrocytes were measured by a procedure from Blaxhall & Daisley, (1973).

Blood samples were taken using a pipette containing red ears 6 to scale 1, then Hayem's solution was added to scale 101, then stirring was carried out by shaking the same pipette for 3-5 minutes until the blood and Hayem's solution were evenly mixed. The first drop is removed and the next drop is dropped on a hemocytometer, then covered with a glass cover and observed with a 400x magnification microscope. Calculation of total erythrocytes was performed on five small boxes in a hemocytometer with the following equation:

$$\textit{Total Erythrocytes} = \sum \left(\frac{\textit{Erythrocytes } x\, 1}{\textit{Box volume}} x \text{ Diluting Factor} \right)$$

Hemoglobin was measured by the Sahli method using a sahlinometer (Wedemeyer & Yasutake, 1977). Blood samples are sucked using a Sahli pipette up to a scale of 20 mm^3 or 0.2 ml, then put into a Hb meter tube filled with 0.1 N HCl to a scale of 10 (red), then stirring and allowed to stand for 3-5 minutes.

Furthermore, the distilled water is put into the Hb-meter tube until the color changes like the color of the standard solution on the Hb-meter. The scale is read by looking at the surface of the liquid and is matched with the Sahli tube scale seen on the g% (yellow) path scale which means the amount of Hb per 100 ml of blood.

Leukocyte calculation based on (Blaxhall & Daisley, 1973). The blood samples are taken using a pipette containing white beads up to a scale of 0.5, then add a Turk's solution to a scale of 11, then stirring by shaking a pipette for 3-5 minutes until the blood and Turk's solution are mixed evenly. The first drop is removed and the next drop is dropped on a hemocytometer, then covered with a glass cover and observed under a microscope at 400x magnification. The calculation of total leukocytes is performed on four large boxes in a hemocytometer using the following equation:

$$Total\ leukocytes = \sum \left(\frac{Leukocytes\ x\ 1}{Box\ volume} x\ \text{Diluting Factor} \right)$$

Differential leukocyte observation method use based on (Blaxhall & Daisley, 1973) by observing blood smear preparations stained with Giemsa coloring under a microscope. Observation and calculation of each cell type (monocytes, lymphocytes, and neutrophils) are carried out until the number of all cell types reaches 100, and the results are expressed%.

Histopathological Parameter

Silver rasbora which has been exposed to pesticides is taken from hematopoietic organs is a liver and kidney at the end of the research. Each repetition was taken by one fish to be dissected and the liver and kidney were taken. Sections of 5μm were prepared and stained with hematoxylin and eosin stains as described by Luna (1968) and Bernet *et al*. (1999).

Statistical Analysis

Hematologic data that have been obtained are then tabulated and analyzed using IBM SPSS 20. Variance analysis (ANOVA) with a duncan test at 95% confidence interval is used to determine the effect of treatment on changes in hematological characteristics. If it has a real effect, then it will be followed by Duncan's test. Histopathology data are presented using descriptive analysis.

RESULT

Hemathology

Hematological conditions of silver rasbora fish that have been exposed to organophosphate pesticides based on total of Leukocytes, total Erythrocytes, Hemaglobin, and differential Leukocytes presented in the following table.

Table 1. Average Eritrocyte and Haemoglobin during 9 day

Parameter	Consentration (ppm)				
	0	0,001	0,005	0,01	0,05
Erythrocytes (x 10^6 sel.mm^{-3})	$1,67^{d}$±0,03	$1,35^{c}$±0,008	$1,20^{bc}$±0,12	$1,05^{b}$±0,03	$0,59^{a}$±0,004
Hemaglobin (gdL^{-3})	6^{d}±0,2	$4,6^{c}$±0,2	$3,9^{bc}$±0	$3,6^{b}$±0,2	$2,5^{a}$±0,1

The observations showed that organophosphate pesticides had an influence, in the form of a decrease in total erythrocytes, hemaglobin, hematocrit, along with increased concentration. Observation of erythrocytes showed that there was an influence ($P < 0.05$) on total erythrocytes, where 0,05 ppm treatment showed the lowest number of erythrocytes. Hemaglobin values indicate that there is an influence ($P < 0.05$) on the treatment given, where 0,005 ppm treatment has a lower amount of Hb.

Table 2. Average leukosit and differential leukosit during 9 day

Parameter	Consentration (ppm)				
	0	0,001	0,005	0,01	0,05
Leukocyte (x 10^3 sel.mm^{-3})	$157,02^{c}$±3,2	$177,34^{bc}$±4,8	$197,94^{b}$±15,12	$209,72^{ab}$±1,27	$245,35^{a}$±15,78
Monosit (%)	5^{a}±1	$4,5^{a}$±1,5	$4,5^{a}$±1,5	3^{a}±1	4^{a}±1
Limfosit (%)	71^{a}±3	72^{a}±3	$75,5^{a}$±0,5	73^{a}±3	76^{a}±3
Neutrofil	24^{a}±1	$23,5^{a}$±3,5	20^{a}±1	24^{a}±3	20^{a}±1

Leukocyte observations show an increase in total leukocytes, while differential leukocytes in monocytes and neutrophils have decreased and lymphocytes have increased percentages. Observations showed that there was an effect (P <0.05) due to organophosphate pesticides on the number of leukocytes, 0,05 ppm treatment was the most influential concentration, while 0,001 ppm treatment was the least influential treatment. The administration of organophosphate pesticides did not influence (P> 0.05) on the percentage of differential leukocytes.

Histopathology of liver

Liver organ in 0,01 ppm treatment showed damage in the form of infiltration eritrocyte, nekrosis picnosis and karyohexis.

Histopathology of kidney

The kidney organ in 0,01 ppm treatment showed cloudy sweling, nekrosis karyolysis, haemoragi and tubular necrosis.

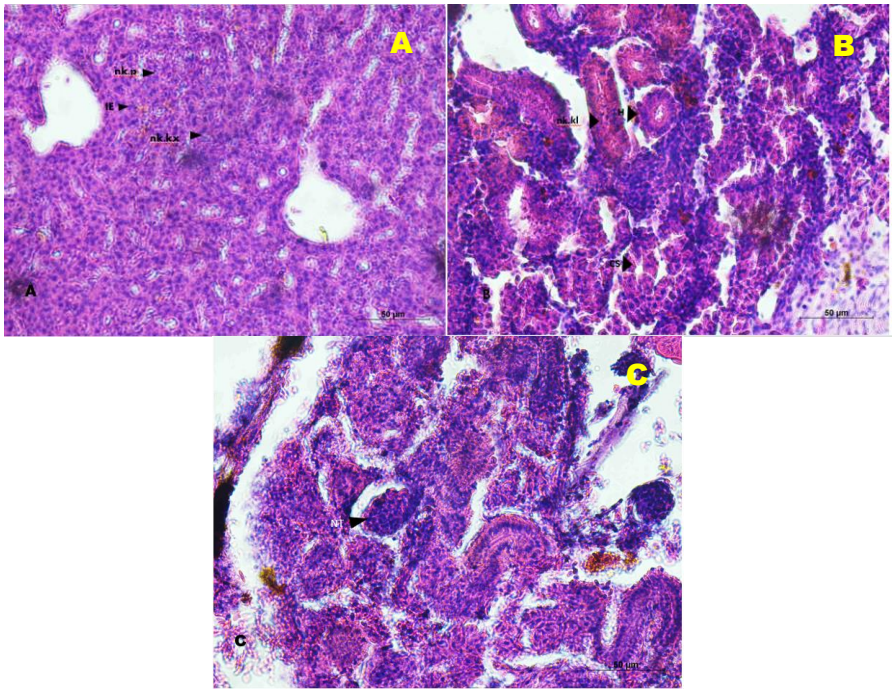

Figure 1. (A) liver of Silver rasbora, IE; infiltration eritrocyte, nk.p; piknotis, nk.k; karyohexis. B**,** Kidney of Silver rasbora, CS; cloudy sweling, nk.kl; nekrosis karyolisis, H; haemoragi. C. NT; necrosis tubular

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DISSCUSION

This study showed that there was a decreased in total eryothrocytes and hemoglobin, while total leukocytes increased. Addition, there are differential changes in leukocytes, namely a decrease in the number of monocytes and neutrophils, but lymphocytes have increased in number. These hematological changes will affect fish exposed to a substance that causes fish to experience stress (Mattsson et al., 2001).

Total decreased erythrocytes and hemoglobin indicate that the fish is anemic. The reduction in hematological value can be caused by erythropoiesis, hemosynthesis and osmoregulation dysfunction or due to an increase in the rate of erythrocyte damage in hematopoietic organs (Mattsson et al., 2001; Seth & Saxena, 2003). Anemia can occur due to an element found in organophosphate pesticides, namely enzim acetyl cholinesterase. Acetylcholinesterase enzyme can inhibit or suppress anemia (Barbieri & Ferreira, 2011). Therefore, that when the acetylcholinesterase enzyme is disrupted, it will allow anemia to occur in fish. This anemia is caused by oxidative injury induced by reactive oxygen groups through oxidation of hemoglobin or other cellular components (Žďárová Karasová, 2017).

The decrease in erythrocytes is directly proportional to the decrease in hemoglobin, along with the increasing concentration of treatment. The cause of erythrocytes is suspected in most vertebrates, including fish, the erythropoetic activity is regulated by erythropoietic which is produced by the kidneys. If the kidney is damaged, it can cause a decrease in erythropoietin. Thus reducing erythrocyte production and hemoglobin synthesis even under conditions of hypoxia (Kumar, Nagpure, & Trivedi, 2011). Decreased hemoglobin levels occur in each treatment, the decrease indicates an influence due to exposure to organophosphates. This decrease is predicted from the rapid oxidation of hemoglobin to methemoglobin or O_2 radical release caused by toxic stress from organophosphate.

It is increasingly recognized that xenobiotics capable of undergoing the redox cycle can have toxic effects through the generation of oxygen free radicals (Ramesh & Saravanan, 2008). The results of the observation showed that Leukocytes increased in each addition of higher concentrations at the end of the observation. The increase in leukocytes indicates the occurrence of damage due to the infection of

body tissues, severe physical stress, and leukocytosis.

Increased leukocytes caused by organophosphates cause leukocytosis, which is considered a tissue response under chemical stress, can also be correlated with increased antibodies that help in the survival and recovery of fish exposed to pesticides (Awicka et al., 2015; Kumar et al., 2011). The decrease in monocytes and neutrophils may be associated with the nature of the immunological challenge to which the fish was exposed at a particular period of time and in the various sub-lethal pesticides (Adewumi, Ogunwole, Akingunsola, & Falope, 2018).

The tendency to increase the number of lymphocyte shows that fish experience lymphocytosis, which is an immune response mechanism through antibody production. This increase can occur due to activation of the immune system from the presence of contaminants, which can be an adaptive response from organisms that produce a more effective immune defense (Barreto-Medeiros et al., 2005).

The function of the liver organ most associated with processes of detoxification and biotransformation, and due to its function, position, and blood supply, so the most affected by contaminants in the water (Younis, Abdel-Warith, Al-Asgah, Ebaid, & Mubarak, 2013). In this result, liver tissues showed infiltration erythrocyte, necrosis picnosis and karyolysis in the 0,01 ppm group. This result are in same with data research by (Velmurugan, Selvanayagam, Cengiz, & Unlu, 2009), reported that the liver showed cloudy swelling of hepatocytes, congestion, degeneration of vacuolar, karyolysis, karyohexis, dilation of sinusoids and nuclear hypertrophy.

Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors. Thus, based in our result of research kidney organs we have recorded cloudy swealing, haemoragi, necrosis karyolisis and tubular necrosis. That means if the process of exposure of the profesnofos will continue can be the whole shape change will be noticed. This will finally prevent the normal anatomy and function of the kidney. (M.A. Akhter, 2013) states that the kidney is one of the first organs to be affected by contaminants in the water because, the kidney is a vital organ of the body and proper kidney function is to maintain the homeostasis.

CONCLUSIONS

The results of this study showed a hematological change in silver rasbora fish

where there was a decrease in total erythrocytes and hemoglobin while total leukocytes increased. Also, there are differential changes in leukocytes, namely an increased in the number of monocytes and neutrophils, but lymphocytes have decreased in number. The Histopathology of the fish liver also can found in this research, those damages that are found are erythrocyte infiltration, necrosis picnosis, and karyolysis. Histopathology of fish kidney also can found cloudy sweling, necrosis karyolysis and tubular necrosis.

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