Organophosphate exposure time impact on acetylcholinesterase activity

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Abstract. This study aimed to determine exposure time needed by organophosphate pesticides (OPs) to decrease acetylcholinesterase (AChE) activity in wistar rats. Twenty-five male were divided into 5 groups. The control group without OPs exposure (K0), the group exposed to OPs for 1 week (K1), and groups (K2), (K3) and (K4) exposed to OPs for 2, 3, and 4 weeks respectively. The blood sample was taken every week and the AChE activity of blood sample was measured using a spectrophotometer. The results showed that AChE activity decreased with the longer OPs exposure time. Exposed treatment for 3 weeks (K3) 162.80 ± 4.56 U/L showed a significant difference (p <0.05) on control (K0) 499.20 ± 6.99 U/L. The results showed that the AChE activity of (K3) significantly decreased 162.80 ± 4.56 U/L compared to control (K0) 499.20 ± 6.99 U/L. In conclusion Exposure OPs pesticide 3 weeks can reduce AChE activity.

1. Introduction

Farmers in Indonesia still use a lot of synthetic pesticides, especially from the organophosphate group. Farmers use pesticides without regard to the presence or absence of pests (cover blankets). Widespread use of pesticides is a threat, not only for the environment but also for humans [1]. OPs containing organophosphate compounds are toxic to vertebrate organisms [2]. Chlorpyrifos poisoning (organophosphate insecticide) occurs in the central nervous system and peripheral nerves due to the inhibition of acetylcholinesterase (AChE) enzyme activity. Pesticides are considered as a technology product that is easy to implement, effective, and available at the farm level. Every year 1-5 million cases of poisoning occur in agricultural workers, and 80% of this occurs in developing countries with a mortality rate of 5.5% or around 220,000 people [3].

Insecticides OPs and carbamates are inhibitors of the AChE enzyme, by phosphorylation of this enzyme irreversibly, while carbamate reversibly regenerates this enzyme (Ellenhorn, 1997 in [4]).

The inhibition of AChE by OPs is caused by the occurrence of covalent bonds between OPs and enzymes so that their activity is inhibited [5]. Irreversible inhibition of OPs in both muscarinic and nicotinic can affect central nervous system (CNS) [6]. This causes acetylcholine (ACh) to not be hydrolyzed to choline and acetic acid and will accumulate in the nervous system so that lymphocyte mobility and cytotoxicity increases. The resistance of the enzyme causes Ach to accumulate at the synapse located between the nerves and muscles. Accumulation of acetylcholine at the neuromuscular junction causes persistent depolarization of skeletal muscle, resulting in weakness and fasciculation. One parameter of organophosphate pesticide poisoning is a decrease in cholinesterase enzyme activity. Organophosphor is a toxic compound which is a major part of a pesticide [7].
Esters of organophosphate or carbamate are used as pesticides, insecticides, medicines for health problems such as glaucoma, parasitic infections, and alzheimer disease. The chemical structure of OPs in some pesticides can be seen in Figure 1.

Figure 1. Chemical structure of OPs [8]

R1 and R2 are methyl, ethyl, or isopropyl, and X is a leaving group, usually in the form of nucleophilic substitution by oxygen from serine on the active side of the target protein. Variation of X depends on the type of OPs [9].

OPs can inhibit the function of various carboxylic hydrolase esters such as acetylcholinesterase, plasma and other hepatic carboxylesterase, paraoxonase and esterase in the body [10]. The lipophilic portion of the OPs pesticide will interact with the cell membrane and disrupt the dual-layer phospholipid structure.

2. Method
This current study was conducted at the Technical Service Unit of the Analytic Laboratory of Udayana University involving five groups of wistar rats; each group consisted of 5 rats fed with the standardized concentrate CP 51. The control group (KO) was not exposed to OPs, group 1 (K1) was exposed to OPs for 1 week, group 2 (K2) was exposed to OPs for two weeks, group 3 (K3) was exposed to OPs for three weeks and group 4 (K4) was exposed to OPs for 4 weeks. The sample of the blood of the rats was taken in the beginning, 1, 2, week 3, and week 4. Each rat was exposed to OPs pesticide at 2.5 ppm through the mouth; each day it was exposed to 1 mL using the hull zonde. The sample of the blood was taken at the end of each exposure. The activity of AChE was determined using a kit, and its absorbance was read using the spectrophotometer.

3. Result and Discussion
The result of the study can be seen in the Table 1.

Table 1. AChE activities at the each treatment

| Treatment Code | AChE activities |
|----------------|-----------------|
| K0.1           | 424             |
| K0.2           | 608             |
| K0.3           | 520             |
| K0.4           | 464             |
| K0.5           | 480             |
| K1.1           | 452             |
| K1.2           | 492             |
| K1.3           | 504             |
| K1.4           | 398             |
| K1.5           | 456             |
| K2.1           | 422             |
| K2.2           | 468             |
The average of the activity of the AChE of each group, which was different from one another, can be seen in Figure 2. The average activity of the AChE for K0 was (499.20±6.99), the average activity of the AChE for K1 was (460.4±4.15), the average activity of the AChE for K2 was (434.80±4.83), the average activity of the AChE for K3 was (162.80±4.56) and the average activity of the AChE for K4 was (107.60±3.03). The activity of the AChE decreased sharply from K2 to K3; however, the activity of AchE went down slightly from K3 to K4.

![Figure 2. The average activity of the AChE for one group of treatment was different from that for another](image)

The OPs pesticide became toxic to the back boned-organism as the function of the cholinesterase enzyme was hampered, leading to the neurotoxic effect. The exposure to OPs from week 0 to week 2 did not cause the activity of the AChE to decrease significantly; however, in week 3 it showed a significant drop, resulting from different factors such as dosage, chemical structure, and how OPs entered organism. It could enter organism through respiration, digestion, and skin. The current study shows that the exposure of OPs through digestion probably took a longer time for its toxic effect to take place than the expose of OPs through respiration.

The toxicity of the chemical structure of OPs with a sulfur cluster (P=S) was different from that of the chemical structure of OPs with a phosphate cluster (P=O). OPs hampered AChE by irreversibly phosphorylating the enzyme, causing the covalent bond among OPs to take place in which the active side of the enzyme formed a phosphorylated cholinesterase bond, causing the cholinesterase enzyme not to be able to hydrolyze acetylcholine into choline and acetate acid. The effect of neurotoxicity of OPs could take place slowly, resulting from the esters of the organophosphore classified as axonopathy; it took place for several weeks and the effect of the cumulative dosage took place after several weeks of exposure.
4. Conclusion

The conclusion which can be drawn from the current study is that it took three weeks for the exposure of the OPs pesticide to decrease the activity of AChE.

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