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Original Article

Subchronic Oral Exposure to Chlorpyrifos Decreases Butyrylcholinesterase in Wistar Rat

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Abstract

Background: Indonesia as an agricultural country has more than 33 million workers in the agriculture. Pesticides have an important role in agriculture because they function as powerful plant pest exterminators. However, its use is considered to cause various toxic effects, one of which is on the nervous system. This study aimed to evaluate the impact of pesticide exposure on cognitive impairment by assessing the butyrylcholinesterase biomarker in the blood of rats as experimental animals.

Methods: This was a true experimental study conducted during September–November 2022 at the Pharmacology and Biochemistry Laboratory, Faculty of Medicine, University of Jember with a post-test only approach using Wistar rats which were divided into 1 control group and 4 treatment groups treated with chlorpyrifos 5 mg/kg orally. Serum butyrylcholinesterase enzyme levels were measured using the kinetic photometric method and then analyzed with a regression test in IBM SPSS® Statistics software.

Results: The average value of butyrylcholinesterase was 920.23±145.27 (Control group); 904.48±91.6 (7 days after chlorpyrifos administration); 889.81±95.2 (14 days after chlorpyrifos administration); 457.84±69.5 (28 days after chlorpyrifos administration); 575.82±194.25 (56 days after chlorpyrifos administration). The results of the One-Way ANOVA test showed significant differences between groups as indicated by a significance test of 0.024 ($p < 0.05$).

Conclusions: There is a relationship between the duration of exposure to chlorpyrifos and butyrylcholinesterase levels in Wistar rats. This fact shows that the longer an organism is exposed to low doses of chlorpyrifos pesticides, the higher the level of neurological system toxicity that occurs in that organism.

Keywords: Butyrylcholinesterase, chlorpyrifos, toxicology

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Introduction

Pesticides are materials used to reduce plant pests.[1] Farmers use pesticides with the aim of increasing agricultural products.[2] Data on the use of pesticides in Indonesia in 1998 was 11,587.2 tons and this figure increased in 2000, which was 17,977.2 tons.[3] Pesticides have an important role in increasing the productivity of agricultural products, but if these pesticides are not used wisely, pesticides can cause health problems.[4] Pesticides not only affect the health of farmers and workers in the pesticide industry but will also have a health effect on the wider community due to the presence of pesticide residues.[5] Pesticide exposure to farmers is mostly through inhalation and direct skin contact. If farmers do not use personal protective equipment (PPE), it occurs in areas that are not covered such as the face and hands.[6] Exposure can also occur indirectly through pesticide residues

found in agricultural products. Exposure to even small amounts of pesticide residues, in the long term, will cause the accumulation of pesticide metabolites that are lipophilic and settled in individual fat tissue.[7]

Chlorpyrifos is a type of organophosphate insecticide that works by inhibiting the cholinesterase enzyme.[8] Chlorpyrifos is the insecticide most often used by farmers in Indonesia, and in the world, chlorpyrifos residues are found in water, soil, food, fruit, and vegetables.[9] Several studies in humans and experimental animals have shown an association between exposure to organophosphate pesticides on intestinal microbiota disturbances, obesity, and increased LPS levels.[10]

Acetylcholinesterase (AChE) activity in blood can be used as a biomarker as a proxy for cholinergic AChE found in the nervous system.[11] Once in the body, pesticides will bind to AChE which results in AChE being inactive causing accumulation of acetylcholine and binding to muscarinic receptors in the central and peripheral nervous systems.[12] Although AChE is an enzyme associated with acute exposure symptoms, butyrylcholinesterase (BchE) has been more commonly used as a biological marker to test for low levels of exposure to organophosphates.[13] BChE is an enzyme that displays complex kinetic behavior and can be used as a parameter to detect impaired liver function, especially synthesis function due to exposure to organophosphates and carbamates.[14] BChE is the more commonly used biomarker although this form of butyrylcholinesterase is not known to be directly involved in neuronal cholinergic processes. Studies have found that tests for BChE tend to produce measurements that have a greater correlation than AChE measurements, which means that BChE measurements have greater reproducibility.[15] The duration of exposure and the dose of organophosphates will certainly influence the effects of toxicity that will occur, but there is no research related to this yet. Research on the effect of long exposure to chlorpyrifos and the dose-response relationship is important for the early detection of chlorpyrifos toxicity on health. Therefore, this study aimed to evaluate the effect of long exposure to the pesticide chlorpyrifos on butyrylcholinesterase (BchE) levels in Wistar rats.

Methods

This research was a true experimental study (completely randomized design) consisting of 5 groups, namely: the normal control group given aquadest until the 56th day (Group K0), the chlorpyrifos group for 7 days (P1), the chlorpyrifos group for 14 days. (P2), the chlorpyrifos group for 28 days (P3), and the chlorpyrifos group for 56 days. The pesticide used is chlorpyrifos with the trademark PESTANAL® analytical standard from Sigma Aldrich. The inclusion criteria for rats used were male Wistar rats (Rattus novergicus), 46–84 days old, body weight 120–170 grams, physically active experimental animals, and had never been used as samples in previous studies. These animals were obtained from the Wistar Farm rat farm (city of Malang). Mice were housed and kept under regulated temperature, light, and adequate ventilation. Mice were given food and drink distilled water ad libitum. The rat cage was cleaned every 2–3 days. The study was conducted during September–November 2022 at the Pharmacology and Biochemistry Laboratory, Faculty of Medicine, University of Jember.

Animals in the treatment group were given the pesticide chlorpyrifos 5 mg/kg orally to rats for 7, 14, 28, and 56 days, meanwhile the control group was given 1 mg/kg aquades for 56 days. On the day of termination, the rats were fasted for 12 hours first. Then, the rats were terminated using pentobarbital 200 mg/kgBW, and 5 cc of blood was taken intracardially.[16]

The rat blood was put into an EDTA tube and then centrifuged at 3000 rpm for 10 minutes to obtain serum. The serum was added to reagent butyrylcholinesterase activity kit and then incubated for 40 minutes. Measurement of serum butyrylcholinesterase enzyme levels used kinetic photometric method. The tool used in this research was the semi-auto chemistry analyzer model biolyzer 100. The measurement results were expressed in units of U/L. The normal value of butyrylcholinesterase in men was 5,400 U/L–13,200 U/L.[17]

The results of butyrylcholinesterase levels were statistically analyzed with IBM SPSS® Statistics software. The type of statistical test used to determine the effect of long exposure chlorpyrifos on butyrylcholinesterase (BChE) levels of Wistar rat was the regression test.

This research was conducted under ethical clearance from the Ethics Commission of the Faculty of Medicine, University of Jember number 1.632/H25.1.11/KE/2022. Approved procedures include an oral probe and termination with pentobarbital.

Table 1 Butyrylcholinesterase Levels of Wistar Rats (Mean±SD)

	Group				
	7 Days Treatment (n=6)	14 Days Treatment (n=6)	28 Days Treatment (n=6)	56 Days Treatment (n=5)	Control (n=5)
Random Cholinesterase Levels, mean (min-max)	904.48±91.6	889.81±95.2	457.84± 69.5	575.82±194.25	920.23± 45.27

Table 2 Post Hoc Test Results for Butyrylcholinesterase Levels

Group	K	P1	P2	P3	P4
K		0.379	0.336	0.02*	0.67
P1	0.379		0.935	0.18*	0.319
P2	0.337	0.935		0.22*	0.359
P3	0.002*	0.018*	0.022*		0.142
P4	0.067	0.319	0.359	0.142	

Note: (*) there is a significant difference ($p<0.05$)

Results

After all groups of rats had received complete treatment, the rats were terminated, and their butyrylcholinesterase levels were assessed. The mean values of butyrylcholinesterase in each group were 920.23 (control group); 904.48 (7 days after chlorpyrifos administration); 889.81 (14 days after chlorpyrifos administration); 457.84 (28 days after chlorpyrifos administration); 575.82 (56 days after chlorpyrifos administration). Results showed that the administration of chlorpyrifos pesticide orally caused a decrease in butyrylcholinesterase levels in all treatment groups (P1–P4). Therefore, the chlorpyrifos treatment group had lower butyrylcholinesterase levels than the control group. The average values and ranges of all groups are shown in Table 1.

The results of the One-Way Anova test found significant differences between groups as indicated by the significance of the difference test of 0.024 ($p<0.05$). The results of the post hoc test also showed a significant difference in the butyrylcholinesterase levels of rats in each group. Group P3 showed a significant difference when compared with group K, group P1, and group P2, but there was no significant difference when compared with group P4 (Table 2).

The administration of chlorpyrifos at a dose of 5 mg/kg could reduce butyrylcholinesterase levels with values varying between 253.31–1,519.32 (Groups P1–P4). The range of normal butyrylcholinesterase values was 5,400 U/L–13,200 U/L. The butyrylcholinesterase levels decreased considerably from the normal values. The results of statistical tests using the regression method yielded a formula regarding the relationship between length of exposure and butyrylcholinesterase levels. This relationship was expressed in an equation "Y = 0.2694x2 – 19.6050x + 950.4434" (Figure 1). In this test, it was found that the peak decrease in butyrylcholinesterase levels was on day 36. Butyrylcholinesterase levels were also shown to have decreased since the first day of treatment.

Discussion

Pesticides are compounds that are widely used to eradicate plant pests in agriculture. Unwise use of pesticides can cause parasympathetic poisoning because it can work as a butyrylcholinesterase inhibitor. Chlorpyrifos is one type of organophosphate pesticide that can inhibit the butyrylcholinesterase enzyme irreversibly. This of course can cause individuals exposed to these pesticides to experience symptoms of parasympathetic and nicotinic toxicity such as weakness, hypertension, fasciculations, diaphoresis, bronchospasm, emesis, and so on.[18,19]

Cholinesterase is an enzyme that has an

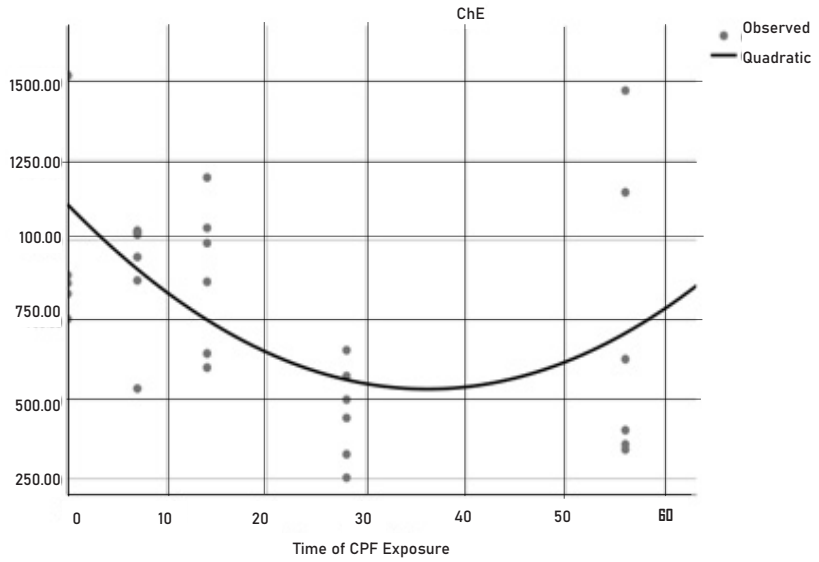

Figure 1 Correlation Between Long Exposure of Chlorpyrifos and Butyrylcholinesterase Levels

important role in the nervous system.[20] This enzyme works by hydrolyzing acetylcholine into choline and acetic acid so that acetylcholine is prevented from giving its effect continuously. Acetylcholine itself has an important role for nicotinic and parasympathetic nerves, but in excessive amounts, acetylcholine can cause symptoms of neurological toxicity. Therefore, cholinesterase works to routinely degrade acetylcholine. This study used BChE because of its greater reproducibility. This biomarker is more commonly used for cholinesterase although it not directly work in cholinergic processes.

The control group had higher butyrylcholinesterase levels than the treatment group although they were still classified as low levels. This shows that chlorpyrifos can reduce butyrylcholinesterase levels in rats. This study also showed that butyrylcholinesterase levels continued to fall on day 7, day 14, and day 28. Then, butyrylcholinesterase levels rose again on day 56. The increase in BChE levels on day 56 could be because of down regulation of cholinergic receptors and activation of anti-inflammatory pathways. Inflammation is closely related to Regulatory T cells (Treg). Tregs play an important role in maintaining host immunity when exposed to toxins or pathogens due to their ability to suppress excessive immune responses that might harm the host. Chronic exposure to chlorpyrifos promotes low-grade inflammation.[21] Statistical tests also concluded that butyrylcholinesterase levels began to fall on the first day of treatment and reached their peak on the 36th day.

This study is in line with another study which analyzed the effect of time-dependent administration of the pesticide fenitrothion on the biomarker of neurological toxicity, cholinesterase.[22] The study found that oral administration of fenitrothion for 7, 14, 21, 28, and 42 days can cause a decrease in butyrylcholinesterase compared to the control group (6963.54–9696.3 versus 16380 u/L). In addition, the study also showed that oral administration of fenitrothion (10 mg/kg) can cause toxic effects on various other organs such as the liver, kidney, and brain. Administration of fenitrothion was shown to cause an increase in alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotrasnferase (ASP), and serum bilirubin levels and a decrease in liver albumin. The pesticide can also induce a state of hyperglycemia and hyperlipidemia while simultaneously increasing serum creatinine, blood urea nitrogen (BUN,) and decreasing brain dopamine levels. Finally, fenitrothion also caused oxidative stress conditions in all organs. This study may have used a different type of pesticide, but the results are quite representative of the basis of research on other types of organophosphates such as chlorpyrifos, especially regarding cholinesterase levels.

Limitation of the study is that there is no chronic exposure group so that in cases of

more than 90 days it can only be estimated using forecasting methods. This would be different if the researcher had a 90-day group to provide more definitive information about differences in cholinesterase levels.

To conclude, oral exposure to chlorpyrifos has been shown to decrease butyrylcholinesterase levels in vivo in rats. There is a relationship between the long duration of chlorpyrifos exposure and butyrylcholinesterase levels in Wistar rats. This fact shows that the longer a living being is exposed to low doses of chlorpyrifos pesticides, the higher the level of neurological system toxicity experienced by the organism. In this study, the level of pesticide toxicity evaluated was neurological system toxicity through the assessment of butyrylcholinesterase levels. In the group with longer exposure levels, butyrylcholinesterase levels became lower because it was inhibited by chlorpyrifos. This condition can lead to a variety of life-threatening clinical symptoms related to autonomic nerves.

This study is important because there is still no study that evaluates the effect of long exposure to chlorpyrifos on butyrylcholinesterase levels. In fact, this study shows that chlorpyrifos exposure even in low doses can cause neurological toxicity to living things. This study can be a good starting point for future studies. It is possible that chlorpyrifos can also cause toxicity in other organ systems such as cardiorespiratory, genitourinary, digestive, and so on. This fact can also be a consideration for policymakers to pay more attention to the use of chlorpyrifos on the health of farmers and agro-industrial communities.

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