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COMPARATION BETWEEN TRADEMARK AND ANALYTICAL STANDARD CHLORPYRIFOS ADMINISTRATION ON BUTYRYLCHOLINESTERASE AND GLUCOSE LEVELS IN RATS

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ABSTRACT: Indonesia is one of the largest agricultural countries in the world. In 2022, approximately 88.89% of the workforce was employed in the agricultural sector. One of the most important agricultural practices is the use of pesticides, which are essential for pest control. Organophosphates are widely used pesticides, and chlorpyrifos is one of the most commonly applied organophosphate insecticides. However, chlorpyrifos exposure may cause adverse health effects, including poisoning, organ damage, mortality, and insulin resistance. Butyrylcholinesterase (BChE) activity and serum glucose levels are important biomarkers for evaluating chlorpyrifos toxicity, as exposure to chlorpyrifos can decrease BChE activity and increase serum glucose levels. Chlorpyrifos is available in two forms, analytical standard chlorpyrifos (Pestanal) and trademark formulations such as Dursban 200 EC. However, studies comparing the effects of trademark and analytical standard chlorpyrifos on BChE and serum glucose levels are still limited. This study aimed to compare BChE activity and serum glucose levels between control and treatment groups, as well as between Pestanal and Dursban treatments. Rats were orally administered Pestanal or Dursban at a dose of 5 mg/kg body weight for 28 days. Serum BChE activity and glucose levels were measured and analyzed using an independent t-test with SPSS software. The results showed that serum BChE activity differed significantly compared to the control group and between the Pestanal and Dursban groups. Serum glucose levels were significantly higher in the treatment groups compared to the control group. However, no significant difference was observed between the Pestanal and Dursban groups.

INTRODUCTION: The Indonesia is one of the largest agricultural country in the world. There are 8 million hectare agricultural land in 2015 in Indonesia and 88,89% workers work on agricultural sector ^{1,2}.

Large amount of agricultural activity cause pesticide in Indonesia are widely used. There are 1,6 tons of pesticide used between 2011 and 2020 ³.

Pesticides are beneficial to eliminate the pests in agricultural land. Beside that beneficial function, pesticides can cause poisoning and make residue ⁴. Organophosphate as one of the pesticide group is widely used around the world. 50% of all pesticide in the world used organophosphate ⁵. Chlorpyrifos as one of the organophosphate pesticide is being used as insecticide in agricultural land ⁶.

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There are 133 tons of chlorpyrifos used between 2020-2022 around the world (European Chemicals Agency, 2022). One of the trademark of chlorpyrifos used is Dursban⁷.

Exposure to chlorpyrifos for the long period can cause negative impact because of its residue. Chlorpyrifos can cause disturbance such as neurotoxicity and alteration in enzymatic activity. In acute period such as nausea, vomiting, dizziness, seizure, loss off consciousness. In chronic period can also cause alteration of liver, testis, kidney functions, and even death⁸. Enzymatic activity is also altered. One of them is the activity of cholinesterase⁹. Chlorpyrifos can inhibits activity of cholinesterase. The inhibition of cholinesterase can cause sign and symptoms of neurotoxicity and disturbance of enzymatic activity¹⁰. Butyrylcholinesterase (BChE) as one of the cholinesterases can be used to identify pesticides poisoning for asymptomatic, sub clinical, and also low dose exposure. This enzyme can also be used to determine of liver function¹¹. Low dose exposure of chlorpyrifos can also alter metabolism and cause hyperglycemia¹².

Previous study showed that chlorpyrifos exposure inhibit activity. Given dose 2,84 and 4,26 mg/KgBW of rats for 28 days significantly decrease butyrylcholinesterase serum level compare to control¹³. Other study also showed that 10 mg/KgBW also decrease level of butyrylcholinesterase serum level on rats orally for 28 days¹⁴. Study about glucose serum level for sub chronic time period showed that 16 weeks exposure of chlorpyrifos 4,75 orally to rats can increase glucose level¹⁵. In 90 days exposure of chlorpyrifos 3,26 and 8,15 mg/KgBW orally could increase glucose serum levels in rats¹⁶.

Pestanal as analytical standard chlorpyrifos is commonly use in laboratory because of its 99% purity of chlorpyrifos¹⁷. Dursban as one of the trademark of chlorpyrifos in the data showed that it has 12,6% of chlorpyrifos and 87,4% inert solvent of aromatic and aliphatic hydrocarbon¹⁸. Carbon tetrachloride (CCl₄) as one of the aliphatic hydrocarbon can cause fibrosis, vacuolization, and necrosis of hepatocyte that givel orally 0,5 mL/KgBW for 8 weeks¹⁹. CCl₄ can also lead to lipid peroxidation and elevate glucose level 3 mL

for 4 weeks²⁰. Ethanol 4 mg/KgBW orally for 12 weeks as one of the aromatic hydrocarbon also increased Kupffer and stellate cells in rats hepatocytes²¹. Many study variations about the effect of chlorpyrifos for rats was already implemented. Until now none of those study compare the differences between analytical standard and trademark chlorpyrifos.

MATERIALS AND METHODS:

Chemicals: Analytical standard chlorpyrifos (99% purity)/Pestanal was bought from sigma-aldrich company in Indonesia. Dursban 200 EC was also bought from DOW agro Indonesia. All other chemicals were generous gift from Medical Faculty of Jember University.

Animal Treatments: Adult male wistar (2-3 months) rats were obtain from malang, Indonesia. The rats were housed at Medical Faculty experimental animal house. Acclimatization firstly conducted to rats for 7 days. The temperature controlled in 25° C, given turbo 30 mg/ day as food, and tap water *ad libitum* for the drink. Previously the study was approved by Medical Faculty Ethical Committee of Jember University. The body weight of the rats maintained at 150-200 mg. Rats divided into 4 groups that assigned with simple random sampling. Each group contains of 5 rats. The first Group (K1) was given tween 20 and normal saline by oral gavage for 28 days. The second group (K2) was given distilled water by oral gavage for 28 days. The third group (P1) was given Pestanal 5 mg/KgBW by oral gavage for 28 days. The last group (P2) was given Dursban 5 mg/KgBW by oral gavage for 28 days. The Pestanal was dissolved used 5% tween 20 and normal saline to. Dursban was dissolved using distilled water. Upon sacrifice by given 200 mL/KgBW of pentobarbital to the rats, blood were soon collected from intracardiac. The blood sample waited for 1 hour and then turn them into serum and ready to to be analized for glucose and BChE serum level.

Butyrylcholinesterase Analysis: Butyrylcholinesterase was analyse with kinetic photometry method. Measurement conducted with reagen kit from DiaSys. Reagent 1 contain pyrophosphate with 7,6 PH 95 mmol/L and 2,5 mmol/L hexacyanoferrate kalium. Reagent 2 contain 75

mmol/L butyrylcholine. Kinetic photometry method were based on cholinesterase reaction that based on hydrolysis of butyrylcholine resulting thiocholine and butyrate. Thiocholine after that decreasing yellow Kalium hexacyanoferrate (II) to colorless.

Glucose Analysis: Butyrylcholinesterase was analyse with kinetic photometry method. Measurement conducted with reagen kit from DiaSys. Reagent 1 contain pyrophosphate with 7,6 PH 95 mmol/L and 2,5 mmol/L hexacyanoferrate kalium. Reagent 2 contain 75 mmol/L butyrylcholine. Kinetic photometry method were based on cholinesterase reaction that based on hydrolysis of butyrylcholine resulting thiocholine and butyrate. Thiocholine after that decreasing yellow Kalium hexacyanoferrate (II) to colorless.

Statistical Analysis: The statistical analysis were use independent t-test with help of SPSS software. At first, the K1 and P1 also K2 and P2. The significant results for the groups continue to compare P1 and P2.

RESULTS AND DISCUSSION: Based on mean of the butyrylcholinesterase (BChE) level the highest level is in the K1 group (746.43 ± 170.53). P1 group has lower BChE level than K1 (498.74 ± 125.00) and the lowest in the P2 group (322.00 ± 26.17) compare to the K2 group (498.74 ± 125.00). The BChE level is shown on the **Table 1**.

TABLE 1: SERUM BChE LEVELS

Group	BChE Serum Levels (U/L) (Mean \pm SD)
K1	746.43 ± 170.53
K2	498.74 ± 125.00
P1	468.33 ± 134.34
P2	322.00 ± 26.17

Independent t-test Sig. 2 tailed for the BChE serum level for the all the group compare show significant result ($P < 0.05$). K1 and P1 t-test result is (0.031), K2 and P2 (0.002), and P1 and P2 (0.004). The independent t-test results are shown in **Table 2**, and the differences in serum BChE levels are presented in **Fig. 1**.

TABLE 2: INDEPENDENT T-TEST FOR BChE SERUM LEVEL

Group	Sig. 2 tailed	Mean difference
K1 and P1	0.031	247.69
K2 and P2	0.002	129.03
P1 and P2	0.004	219.71

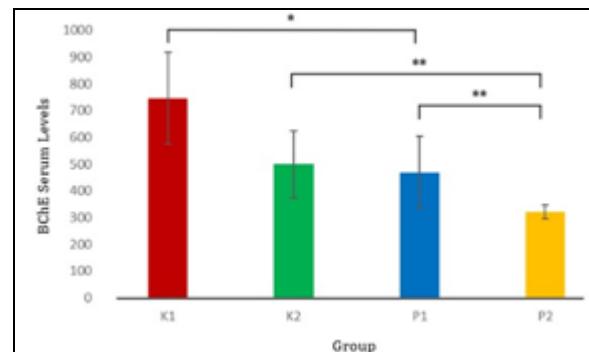


FIG. 1: DIFFERENCES IN SERUM BChE LEVELS AMONG CONTROL AND TREATMENT GROUPS. Values are expressed as mean \pm SD. $p < 0.05$ (*); $p < 0.01$ (**).

BChE serum levels show significant different compare to control and between Pestanal and Dursban group. Oral administration of Chlorpyrifos for 28 days could reduce BChE level that is corresponding to previous study. 5 mg/KgBW for 28 days could reduce BChE serum level compare to control ^{22, 23}. Other study that use trademark chlorpyrifos for 28 days also show same result compare to control. 2.84 and 4.26 mg/KgBW chlorpyrifos given by oral gavage for 28 days could reduce BChE serum level ^{13, 24}. This condition occurred because chlorpyrifos that metabolized by live transform into chlorpyrifos-oxon and inhibits cholinesterase and binding to it irreversibly ²⁵. Sub-chronic and chronic exposure could also change liver function because of inflammation caused by chlorpyrifos-oxon and created vacuolization, congestion, and fibrosis to hepatocytes. Liver as the place to synthesize BChE if there are change of structure could lead to reducing of BChE synthesis lower than normal ^{26, 27, 28}.

The significant reduction in BChE activity observed in chlorpyrifos-treated groups indicates enzymatic inhibition and possible hepatic involvement following sub-chronic exposure. As BChE is synthesized in the liver, its decreased activity may reflect hepatocellular dysfunction, which plays an important role in metabolic regulation. Hepatic impairment and cholinergic disruption induced by organophosphate exposure have been reported to interfere with insulin signaling, glucose utilization, and gluconeogenesis ¹². Therefore, alterations in BChE activity may be associated with subsequent changes in glucose homeostasis, providing a biological basis for evaluating serum glucose levels as a metabolic

outcome of chlorpyrifos exposure ¹⁵. Glucose serum level shown that K2 is lower than P2 (75.53 ± 15.64) and (110.58 ± 18.83). Meanwhile K1 is also lower than P1 (72.80 ± 7.40) and (96.97 ± 2.95). The glucose level shown on **Table 3**.

TABLE 3: SERUM GLUCOSE LEVELS

Group	Glucose Serum Levels (mg/dL) (Mean \pm SD)
K1	72.80 ± 7.40
K2	75.53 ± 15.64
P1	96.97 ± 2.95
P2	110.58 ± 18.83

Independent t-test Sig. 2 tailed for the glucose serum level show significant level compare to control, but not P1 and P2 group. K1 and P1 t-test result is (0.001), K2 and P2 (0.029). There is no significant different from Mann-Whitney analysis for P1 and P2 result is (0.386). The independent t-test results are shown in **Table 4**, and the differences in serum glucose levels are presented in **Fig. 2**.

TABLE 4: INDEPENDENT T-TEST FOR GLUCOSE SERUM LEVEL

Group	Sig. 2 tailed	Mean difference
K1 and P1	0.001	-24.17
K2 and P2	0.029	-35.04

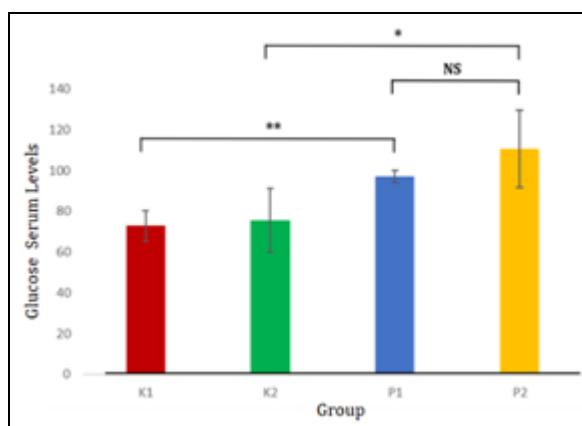


FIG. 2: DIFFERENCES IN SERUM GLUCOSE LEVELS AMONG CONTROL AND TREATMENT GROUPS. Values are expressed as mean \pm SD. $p < 0.05$ (*); $p < 0.01$ (**); Not significant (NS).

Glucose serum also shown that compare to control the groups have significant differences. Organophosphate in previous study could elevate glucose serum level by oral gavage administration for 28 days. Diazinon $\frac{1}{4}$ LD₅₀ administration for 28 days made insulin resistance and elevated glucose serum level ²⁹. Other study gave malathion for 28 days by oral gavage 200 mg/KgBW also elevated glucose serum level ³⁰.

Study of chlorpyrifos administration 5 mg/KgBW for 28 days by oral gavage also made elevation of glucose serum level ³¹. Sub-chronic and chronic exposure of chlorpyrifos could induce β pancreatic cell death. Hyperglycemia occurred when there are β pancreatic cell death ³². Elevated glucose level could also caused by liver gluconeogenesis and glycogenolysis from other tissues ³³. There were no significant different between pestanal and dursban group according to glucose serum level. It was indicate that between trademark and analytical standard had no different refer to glucose serum level for 28 days administration of chlorpyrifos.

CONCLUSION: Oral administration of Dursban and Pestanal for 28 days have significant differences compare to control group for BChE and glucose serum level. Between Dursban and Pestanal group there is significant different of BChE serum level. There is no significant different glucose serum level elevation in Dursban and pestanal group.

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CONFLICT OF INTEREST: There are no conflict of interest in this study.

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