Research Article

Effect of Low-Dose Chlorpyrifos on Hepatocyte Necrosis and Serum Albumin Levels in Wistar Rats

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Abstract

Chlorpyrifos is a broad-spectrum organophosphate pesticide that also causes various health problems. The active metabolite of chlorpyrifos can increase free radicals in the body, especially in the liver and kidneys. The continuous increase in free radicals will cause an imbalance between free radicals and oxidants, triggering oxidative stress. Oxidative stress stimulates mitochondrial dysfunction, resulting in changes in the hepatocyte shape and serum albumin levels. This study aimed to determine the effect of chlorpyrifos on the hepatic histopathology scores and serum albumin levels of Wistar rats. Experimental research was conducted at the Faculty of Medicine, University of Jember in May-December 2024. A total of 12 male Wistar rats were divided into two groups. The control group was given normal saline (+5% Tween 20), and the chlorpyrifos group was given chlorpyrifos + normal saline (+5% Tween 20) orally for 7 days. On the 8th day, all animals were euthanized with ketamine-xylazine, hepatic organs, and blood were taken intracardiac. Preparations were made using HE staining, and serum albumin levels were checked using a photometer. The results showed that hepatic histopathology scores assessed by the Manja Roenigk method and serum albumin levels had significant differences between the groups metabolized. Seven days of chlorpyrifos exposure can increase hepatocyte necrosis and decrease serum albumin levels.
Keywords: *insecticide, histopathology, hepar, albumin, serum.*

Pengaruh Klorpirifos Dosis Rendah Terhadap Nekrosis Hepatosit dan Kadar Albumin Serum Tikus

Abstrak

Klorpirifos merupakan pestisida golongan organofosfat dengan spektrum luas dan dapat menyebabkan berbagai masalah kesehatan. Metabolit aktif klorpirifos dapat menyebabkan peningkatan radikal bebas dalam tubuh, terutama dalam hepar dan ginjal. Peningkatan radikal bebas secara terus menerus akan menyebabkan ketidakseimbangan antara radikal bebas dan oksidan sehingga memicu stres oksidatif. Stres oksidatif akan merangsang disfungsi mitokondria sehingga terjadi perubahan bentuk hepatosit dan perubahan kadar albumin serum. Tujuan penelitian ini yaitu mengetahui pengaruh klorpirifos terhadap skor histopatologi hepar dan kadar albumin serum tikus Wistar. Penelitian eksperimental dilakukan di Fakultas Kedokteran Universitas Jember pada bulan Mei-Desember 2024. Sebanyak 12 tikus Wistar jantan dibagi dalam dua kelompok. Kelompok kontrol diberikan normal saline (+5% Tween 20) dan kelompok klorpirifos diberi klorpirifos+normal saline (+5% Tween 20) secara oral selama tujuh hari. Pada hari ke-8 seluruh hewan coba dieutanasia dengan ketamine-xylazine kemudian diambil organ hepar dan darah secara intrakardiak. Dilakukan pembuatan preparat dengan pewarnaan HE dan pengecekan kadar albumin serum dengan alat fotometer. Hasil penelitian menunjukkan bahwa skor histopatologi hepar yang dinilai dengan metode Manja Roenigk dan kadar albumin serum memiliki perbedaan yang signifikan antar kelompok. Paparan klorpirifos selama tujuh hari dapat meningkatkan nekrosis hepatosit dan menurunkan kadar albumin serum.
Kata kunci: *insektisida, histopatologi, hepar, albumin, serum.*

Introduction

Organophosphate pesticides are the most frequently used globally, with chlorpyrifos accounting for 40% of the total.[1] This is due to the broad-spectrum activity, high efficacy, and low toxicity of chlorpyrifos. However, continued chlorpyrifos use causes various health issues in living organisms and ecosystems.[2] Pesticide use in Indonesia continues to be underreported. However, according to the Food and Agriculture Organization in 2022, Indonesia ranked third in pesticide use, with organophosphates being the most commonly used.[3] According to research conducted in Badung Denpasar, 40%-60% of farmers utilized chlorpyrifos insecticide.[4] Workers in the agricultural centers of the Special Region of Yogyakarta also use chlorpyrifos-based pesticides, particularly for shallots.[5] In Indonesia, chlorpyrifos manages household pests such as mosquitoes, cockroaches, flies and termites. Therefore, it is widely used and readily available.[6]

Several studies have found that chlorpyrifos poisoning primarily affects the liver.[7] The liver is the body's detoxifying organ. When the liver's detoxifying capacity is insufficient to deal with excessive internal or external toxins, histopathological alterations ensue.[2,8] Chlorpyrifos is metabolized in the liver by esterase and cytochrome P450 enzymes, which can form more toxic chemicals, including *chlorpyrifos-oxon* and *3,5,6-trichloro-2-pyridinol.*[8,9] The active metabolites of chlorpyrifos can increase free radicals such as reactive oxygen species (ROS), which cause oxidative stress and liver cell damage.[8,10] The body's ability to neutralize entering chemicals is a health concern that should be addressed.[2] Pesticide residues that penetrate directly or are linked to agricultural goods at small levels are thought to have no adverse consequences; thus, they are frequently overlooked.[11]

Several investigations have found histological alterations in the liver after exposure to chlorpyrifos for 7–28 days.[10] Exposure of tilapia to sublethal doses over a 7-day period resulted in notable histopathological changes in the liver, supported by significant alterations in hepatic biomarkers. However, Hu et al[12] discovered that a rise in hepatic SGOT/SGPT biomarkers occurred on the third day after chlorpyrifos exposure, with biomarker levels returning to normal on days four to ten.[12] As a result, histological analysis of hepatic cells is required to identify specific alterations in hepatic microscopic structures, as changes in hepatic morphological structures are linked to decreased hepatic function.[7,10] An outcome of hepatic cell injury is a decrease in protein production, specifically albumin, resulting in hypoalbuminemia.[10,13] Albumin is extremely useful for transporting hydrophobic molecules such as lipid derivative hormones (cortisol, thyroid, estrogen, and testosterone), medicines, and other water-insoluble ions.[14] In the last decade, research on the effects of low-dose chlorpyrifos on liver histopathology scores and blood albumin levels has been limited. Thus, this study aims to assess the effect of low-dose chlorpyrifos on the liver histopathology scores and serum albumin levels of Wistar rats.

Methods

Male Wistar rats were used in this in vivo experimental study from May to December 2024 at the Faculty of Medicine's Experimental Animal House at the University of Jember. The Health Research Ethics Committee of FK UNEJ has approved the research protocol under letter 5240/UN25.1.10.2/KE/2024.

The degree of freedom formula was used to calculate the number of samples required to obtain a minimum sample size of 6 Wistar rats. A total of 12 rats were randomly divided into two groups, with the inclusion criteria for this study being male Wistar rats aged 8-10 weeks, weighing 110-220 grams, with active physical activity and a decent appetite. The dropout criteria included rats that died within the time span of treatment until before termination and sick rats characterized by weak rats, unwilling to eat, and

diarrhea. The rats were collected from Malang Regency in East Java. The rats were fed, watered ad libitum, and housed in cages of two rats each.

Animal Treatment

The rats were divided into two groups. The control group was only given normal saline (+5% Tween 20). The chlorpyrifos group was orally given chlorpyrifos 5 mg/kg bw + normal saline (+5% Tween 20) for 7 days. On the 8th day, termination and necropsy will be performed. Rats will be euthanized with ketamine-xylazine at a dose of 40-5 mg/kg bw[15].

Sampling and Data Analysis

The hepatic organs were washed first with distilled water and then put into an organ pot containing 10% BNF liquid. The hepatic organs in this study were not macroscopically observed, which became a limitation of this study. Preparation making in this study used the median lobe of the liver. Preparations were made using the hematoxylin-eosin (HE) staining technique. Assessment of the hepatic histopathology score using the Manja Roenigk method. Observations were made using a Leica DM500 microscope with an additional OptiLab AmScope magnification of 400x at five fields of view. Histopathology was examined by two blinded observers and confirmed by an expert. The assessment by observers 1 and 2 will be averaged for each animal by rounding up after obtaining a Cronbach's alpha significance value >0.7. Assessment was performed by observing 100 hepatocytes for each sample.

Each hepatocyte was given a score of 1 to 4, reflecting the type of damage: score 1 for normal cells characterized by hepatocytes having a polygonal shape and homogeneous red cytoplasm. Enlarged hepatocytes with cloudy and granular cytoplasm characterize score 2 for cells with parenchymal degeneration. Score 3 for cells with hydropic degeneration is characterized by hepatocytes that appear enlarged due to fluid accumulation in the cytoplasm, so that the cytoplasm appears pale and has vacuoles. Score 4 for necrotizing cells is characterized by pycnotic nuclei or compaction of nuclei so that they appear purple-black. The total score for each sample is the sum of the scores of all hepatocytes observed. Data for each animal is presented as a score, with a minimum score of 100 interpreted as all normal cells and a maximum score of 400 interpreted as all necrotizing cells.[16]

Rats will have their blood taken through the intracardiac route and then placed in a plain tube to make serum. The serum is prepared by incubating rat blood for 10-20 minutes, then centrifuging at 3000 rpm for 10 minutes. Blood serum was separated from the solution by taking the supernatant without touching the sediment. The serum was then put into microtubes that had been labelled. Serum albumin levels were examined using a photometer with BCG albumin reagent (Dialab). Liver histopathology scores and serum albumin levels will be statistically analyzed using the Independent t-test after the data is declared normally distributed and homogeneous ($p > 0.05$). The test results are declared significantly different if $p<0.05$. Furthermore, multivariate analysis was conducted using multivariate analysis of variance (MANOVA) to simultaneously determine the effect of independent variables on the dependent variable.

Results

The observation showed that the control group had a lower average score (157.50 ±16.99) than the chlorpyrifos group (251.50 ±17.51). The Shapiro-Wilk and Levene tests will be used to assess the normality and homogeneity of the hepatic histology scores. The significant value for both >0.05 indicates that the data is normally distributed and homogeneous, allowing further analysis using the parametric test, specifically the independent t-test. The parametric comparison test yielded a significance value of less than 0.05. As a result, the liver histopathology scores in this study varied significantly between groups.

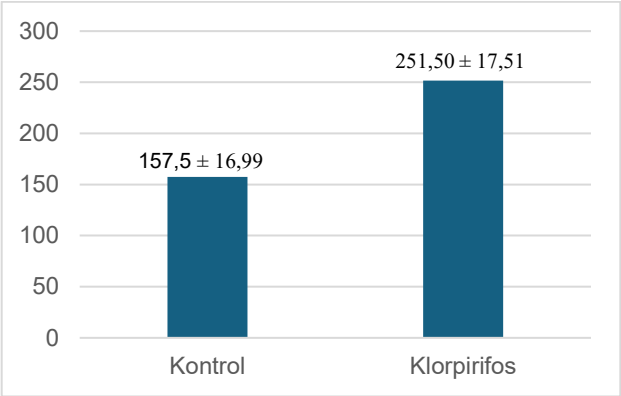
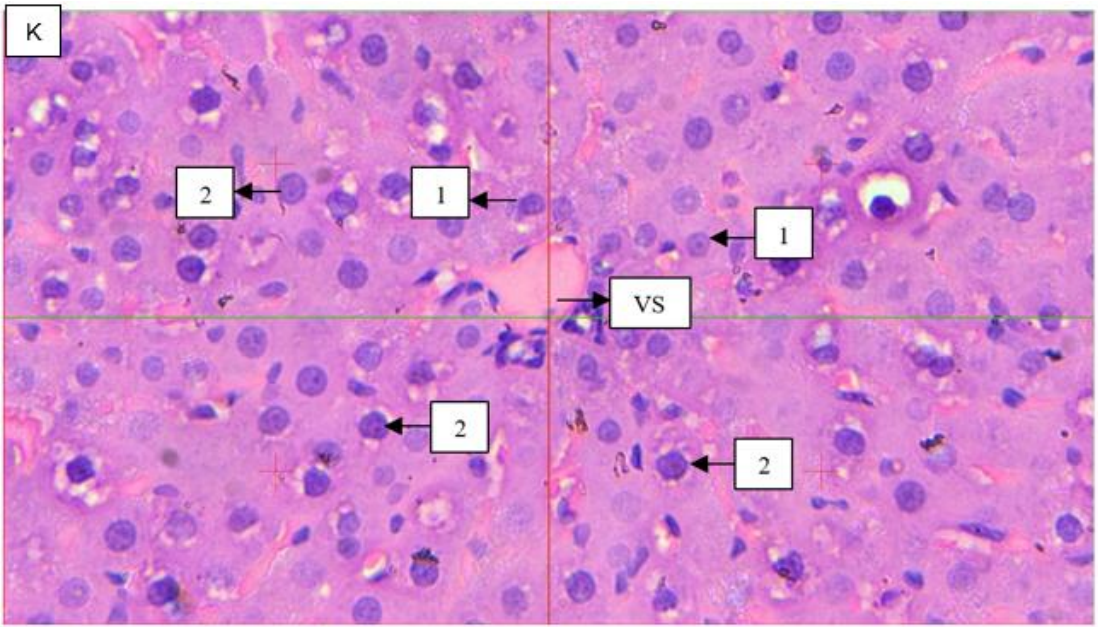


Figure 1. Average Liver Histopathology Score by the Manja Roenigk Method



.Figure 2. Histopathologic Features of the Liver Control Group
K is the control group with normal saline (+5% Tween 20). VS: Central vein;
1: Normal Hepatocytes; 2: Parenchymal Degenerated Hepatocytes

Figures 2 and 3 depict observations of the liver histopathology pictures. The majority of cells in the control group were normal in terms of parenchymal degeneration. Most cells remain normal, with spherical nuclei in the center of the cell and a uniform red cytoplasm.

Low dosages of chlorpyrifos (5 mg/kg bw) can induce hepatocytes to become larger until necrosis. The chlorpyrifos group had greater hydropic degeneration and necrosis of the cells. This demonstrates that exposure to chlorpyrifos for 7 days can cause histopathological changes in Wistar rats.

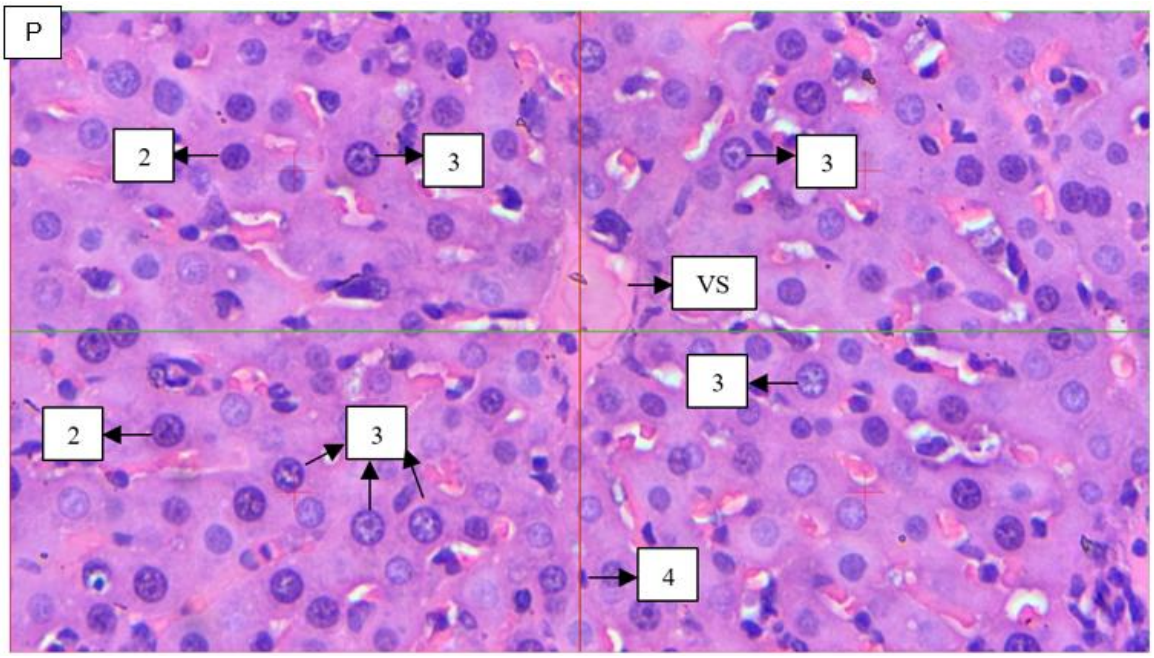


Figure 3. Histopathologic Features of the Liver Chlorpyrifos Group
P is the chlorpyrifos group with chlorpyrifos + normal saline (+5% Tween 20).
VS = Central Vein; 1: normal hepatocytes; 2: parenchymal degenerated hepatocytes;
3: Hydropic degenerated hepatocytes; 4: Necrotized hepatocytes

Serum albumin can be seen in Figure 4. The normality and homogeneity tests yielded p values greater than 0.05, indicating that the data are homogeneous and normally distributed, allowing for further use of the parametric test, the Independent t-test. In this study, the serum albumin levels of the Wistar rats in the chlorpyrifos and control groups differed significantly ($p < 0.05$). The control group's average serum albumin level was 3.03 g/dL ± 0.24, while the chlorpyrifos group was 2.09 g/dL ± 0.23. Compared with the chlorpyrifos group, the control group's average was greater. This result demonstrates that the serum albumin levels of Wistar rats can drop after 7 days of exposure to chlorpyrifos at a dose of 5 mg/kg bw.

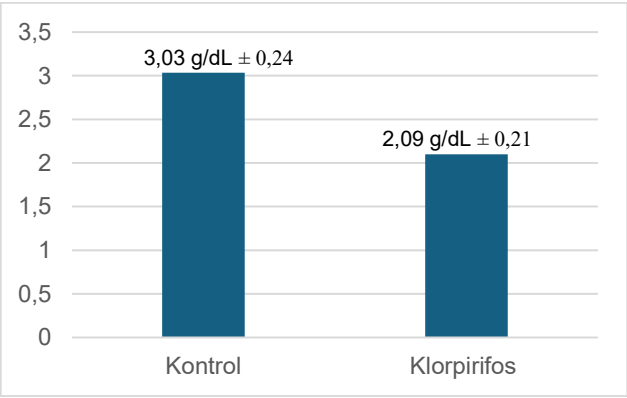


Figure 4. Albumin Serum Levels

Discussion

The chlorpyrifos exposure given in this study was at least equivalent to the maximum safety limit of the chlorpyrifos residues. According to the Food and Agriculture Organization, in 2014, the maximum limit of chlorpyrifos residue was approximately 0.5 mg/kg to 2 mg/kg. Exposure to 5 mg/kg bw of chlorpyrifos for 7 days can have a significant impact on hepatic histopathology scores and serum albumin levels of Wistar rats, whereas previous studies have tended to detect the effect of chlorpyrifos at a longer time and higher dose.

Effect of Low Dose of Chlorpyrifos on Liver Histopathology

Hepatic histopathology preparations in this study were made using the hepatic median lobe because it is the largest lobe and has the most vascularization.[17] Assessment of the hepatic histopathology score using the Manja Roenigk method was performed on zone 3 of the hepatic or centrilobular zone adjacent to the central vein. The zone has fewer nutrients, more metabolite content, and more cytochrome p450.[18]

According to the findings in the control group, there were normal hepatocytes and parenchymal degeneration. In contrast, the chlorpyrifos group discovered several hepatocytes undergoing parenchymal degeneration, hydropic degeneration, and necrosis. This result is consistent with Tanvir et al[10] findings, which reveal that exposure to chlorpyrifos for 21 days at a dose of 5 mg/kg bw can cause liver histological damage characterized by degeneration and hepatocyte infiltration in the central venous area.[10] Similarly, Zhang et al[8] discovered that 12 weeks of exposure to chlorpyrifos at a dose of 10 mg/kg bw could cause histopathological damage in the chlorpyrifos exposure group, as evidenced by increased hepatocyte cells, localized inflammation, and hepatocyte necrosis.[8] However, the duration of chlorpyrifos exposure in the previous study was more prolonged than that in this study. This shows that chlorpyrifos exposure for a shorter time, namely for 7 days, has an effect in the form of an increase in the hepatic histopathology score of Wistar rats.

The hepatic damage is the effect of chlorpyrifos administration, which can increase the level of free radicals in the body, one of which is ROS. Giving chlorpyrifos for 24 hours can cause an excessive increase in free radical production that damages cell membranes, lipids, proteins, and cellular DNA.[19] Damage to cell membranes causes disruption of ion transportation, one of which is Ca^{2+} ions.[20] Increased Ca^{2+} ion influx damages hepatic cell organelles, resulting in mitochondrial dysfunction.[19] This condition causes swelling of the hepatocyte cells.[21] Chlorpyrifos can also reduce the survival rate of macrophages, increase intracellular ROS and lipid peroxidation characterized by increased malondialdehyde (MDA) levels, and cause a decrease in antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase.[22] These three enzymes are the main enzymes that fight free radicals.[23] An increase in free radicals and a decrease in oxidant neutralizers will cause an imbalance in cells, leading to oxidative stress conditions. Oxidative stress in hepatocytes causes damage or changes in the histopathological structure of the liver.[8-10]

Effect of Low Dose of Chlorpyrifos on Serum Albumin Levels

This study showed a decrease in the average serum albumin level of Wistar rats. Therefore, it can be proven that exposure to low chlorpyrifos doses for 7 days can reduce serum albumin levels in rats. This study showed that the average serum albumin level in the chlorpyrifos group was lower than the normal level in male rats of 3.1-5.1 g/dL. In addition, based on statistical tests, serum albumin levels in this study significantly differed between the control and chlorpyrifos groups. This is in line with research by Uzun et al[24] who found that exposure to chlorpyrifos for 28 days at a dose of 5.4 mg/kg bw caused a decrease in serum albumin levels and changes in hepatic histopathology characterized by congestion in central veins and pycnotic cells in Wistar rats.[24] A retrospective study conducted by Noh et al[25] stated that of 217 patients with organophosphate poisoning, 40 patients were found with

hypoalbuminemia accompanied by respiratory failure, kidney, and hepatic damage after 24 hours of consumption of organophosphate pesticides.[25]

Chlorpyrifos that enters the body will be processed in the liver through Phase 1 with the help of cytochrome p450. The process produces more reactive and dangerous products, namely, chlorpyrifos-oxon and TCP.[9] Both substances can increase free radicals in the body, causing oxidative stress. Free radicals, namely ROS, are physiologically generated by the body to help signal transmission in metabolism, gene transcription/translation, and gene differentiation. However, continuous exposure to chlorpyrifos will increase ROS production, resulting in an imbalance between the production and degradation of free radicals that can be detrimental to cells.[23] The imbalance will affect various cellular signalling pathways due to different pathological mechanisms, including changes in the cell structure up to cell death.[26] The effect of chlorpyrifos on serum albumin levels is unknown. However, structural changes, especially in hepatocytes, are thought to cause functional disorders in the liver, one of which is the transcription process that produces mRNA.[27] Chlorpyrifos can specifically interfere with the DNA transcription process into mRNA so that there is a failure or change in the amount of albumin-coding mRNA production, which causes the body to lack albumin-forming amino acids. This condition can cause the body to lack albumin or hypoalbuminemia. Liver dysfunction can develop quickly when extensive necrosis occurs, regardless of the etiology. Furthermore, hazardous chemicals can produce liver dysfunction without parenchymal necrosis, resulting in the decreased function of hepatocytes even while they are still alive [28]

The decrease in albumin levels may also be due to chlorpyrifos affecting the kidneys.[29] However, this study did not assess the condition of the kidneys. The kidneys function as a place to dispose of dissolved substances in water. One of the chlorpyrifos compounds that will be released is TCP. The compound will also increase free radicals in the body, damaging kidney cells. This has been proven by Deng et al.[9] who showed that the administration of TCP for 28 days can cause histopathological changes in the kidneys of rats, characterized by swelling of epithelial cells, interlobular edema, focal hemorrhage, and inflammatory cell infiltration. The study also showed increased LDH and TCH associated with renal dysfunction.[9] Another study by Aung et al.[9] proved that chlorpyrifos for 180 days in rats significantly caused oxidative stress in the kidneys characterized by increased MDA levels, nephrotoxicity characterized by increased creatinine and changes in renal cell epithelial tissue.[29] Damage to epithelial cells or podocytes will cause impaired filtration of small solutes in the urine. If podocytes are damaged, there will be leakage of substances along with urine, one of which is albumin. Albumin and other proteins will come out along with urine, causing albuminuria and albumin deficiency or hypoalbuminemia.[30] Hypoalbuminemia can cause systemic edema and myocardial vascular disorders due to decreased vascular osmotic pressure. Hypoalbuminemia also interferes with the transport of drugs, bilirubin, lipid-derived hormones, and minerals.[14]

The limitation in this study is that the average control group had an albumin level of 3.03 g/dL, which is lower than the normal albumin level of male Wistar rats. However, in this study, the cause of the low serum albumin level is unknown because it was not checked before administering normal saline (+5% Tween 20). Based on research, tween does not affect proteins, including albumin.[31] Tween 20 is a stable non-ionic surfactant with a low level of toxicity that does not affect the reactivity of a compound.[32] In addition, the shortcomings of this study were that it did not further investigate the kidney damage that affects serum albumin levels.

Conclusion

Seven days of oral low-dose chlorpyrifos treatment in Wistar rats can considerably enhance liver histopathology scores while decreasing blood albumin levels. However, more research is needed

on qualitative changes in renal histology. Furthermore, more research is required to determine whether other causes induce a drop in serum albumin levels due to chlorpyrifos exposure.

Conflict of Interest

There is no conflict of interest.

Acknowledgment

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