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Resrarch Article

# Histological Study of the Liver of Albino Rats Treated with Insecticide (Chlorpyrifos)

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## Abstract

The aim of this study was to investigate the histopathological effects of Insecticide (chlorpyrifos), on the liver of albino rats. From the result obtained, the control group exhibited normal liver architecture with clear central veins, radiating sinusoids, and healthy hepatocytes. In contrast, treated groups showed varying degrees of liver damage, which were statistically significant at the $p < 0.05$ level. Group B, treated with 0.02 ml/kg of chlorpyrifos, displayed early signs of hepatocellular injury, including enlarged but diluted central veins, fatty deposits, and infiltrated cells, with significant morphological changes ($p = 0.004$). Group C, treated with 0.01 ml/kg, revealed necrotic foci and darkened nuclei, indicating severe cellular damage and compromised liver function. Group D, treated with 0.05 ml/kg, demonstrated extensive distortion in liver cells, disrupted architecture, dilated central veins, and increased fat deposits, reflecting advanced liver injury with significant differences ($p = 0.004$). Group E, treated with 0.03 ml/kg, exhibited the most severe damage, with high degrees of distortion across hepatocytes, sinusoids, and nuclei, confirming the dose-dependent hepatotoxic potential of chlorpyrifos. The results indicate that chlorpyrifos induces significant, dose-dependent liver damage in albino rats. The observed histopathological changes range from mild steatosis and cell infiltration to severe necrosis and structural disorganization. These findings underscore the critical need for stringent regulation and monitoring of chlorpyrifos exposure to mitigate its hepatotoxic effects. Further research is necessary to elucidate the underlying mechanisms of chlorpyrifos toxicity and to develop effective strategies for protecting liver health against such environmental hazards. This study highlights the potential risks associated with chlorpyrifos use and emphasizes the importance of establishing safe exposure limits and protective measures for populations at risk.

**Keywords:** histology; liver; albino rats; insecticide

## Introduction

Pesticides are substances or mixtures used to prevent, destroy, or control pests such as disease vectors, weeds, animals, and insects [1]. These active ingredients are mixed with one or more of the 900 inert materials to create the approximately 50,000 commercial pesticide preparations that are currently registered for use worldwide today [2]

Pesticides are deemed hazardous to both the environment and human life when used. The extensive use of pesticides for crops, fruits and vegetables causes serious problems on non-target organisms leading to a number of pathological and disordered biochemical processes, viz; immune-deficiency associated with dysregulation, allergies, autoimmunity and dysfunction at neuromuscular synapses [3]

Pesticides are classified into Insecticides, Weedicides or Herbicides, Rodenticides and Fungicides [4]. Insecticides are types of pesticides that are used to specifically target and kill insects and this includes organophosphates (BaytexEC50, Harcros Demro Malathion, Parathion, diazinon, Fenthion, dichlorvos, chlorpyrifos, ethion), carbamates (Baygon Fly Bait), Organochlorine (Aldrin) and Pyrethroids (Baygon mosquito coil).

Human and animal exposure to pesticide may lead to Oxidative stress [5]. Oxidative stress occurs when free radicals outnumber antioxidants. Free radicals are oxygen molecules with an odd number of electrons. Their odd number helps them to quickly react with other molecules, a process known as oxidation [6]. One or more unpaired electrons make up a free radical.

Pesticides have been found to generate ROS and oxidative damage [7].. RNS damage occurs when the cell's free radical quenching/antioxidant capacity is exceeded [8]. Pesticide-induced oxidative stress is a plausible mechanism of toxicity, according to research

Pesticides affect the cell's enzymatic and non-enzymatic antioxidant reserves, creating oxidative stress [9]

The liver is a vital organ responsible for numerous metabolic, detoxification, and synthetic functions essential for homeostasis. Structurally, the liver is composed of hepatocytes, bile ducts, and a vascular network, organized into lobules. Hepatocytes play a crucial role in detoxifying endogenous and exogenous compounds, synthesizing proteins such as albumin and clotting factors, and producing bile for digestion. The liver's role in detoxification makes it particularly susceptible to toxic substances, including insecticides like chlorpyrifos. Hepatocytes metabolize these toxins, which can lead to cellular damage if the exposure is significant or prolonged [10] Chemical-induced oxidative stress damages tissues, aging, and illness. CPF and Cd exposure lowers mitochondrial potential and increases ROS. It is important to note that occupational exposure to these pesticides occur from skin absorption and inhalations. Pesticides are exposed through mixing, loading, spraying, and incorrect handling [11].. Farmers who employ these pesticides have limited or no access to information on proper usage or handling precautions. So, they regularly ignore even the most basic hygiene and safety measures

## Materials And Methods

### Study Area

The study was carried out in Madonna University Teaching Hospital Elele, Rivers State, Nigeria. It is located in the tropics of the southern part of Nigeria. Elele town is located in Ikwerre local government area, Nigeria. Its geographical coordinates are latitude 5 6' 6" North and longitude 6 48' 51" East.

### Experimental Animals

Forty-one (41) male albino rats of eight weeks old that weighed between 130-150g obtained from the livestock breeding unit of animal friend farm, Royce Road Owerri, were used as the experimental animals. The rats were weighed and kept in cages for two weeks at the Animal House, Madonna University Elele and allowed to acclimatize with their new environment. Within these periods the animal whose life span is 2.5-3.5 years were feed with vital grower food and water. The protocol was in line with the guideline of the National Institute of Health (NIH) (NIH Publication 86-23, 1985) for laboratory animal's care and use.

### Experimental Design

In the experimental Design, the first stage is the pilot stage, which was done to a certain lethal dose of the insecticide of interest. On these sixteen (16) albinos' rats were used for this study, the rest of the twenty-five (25) rats were randomly assigned to five groups of five (5) animals each ie., A B, C, D, and E, with A serving as the control and B, C, D, E ,serving as the test groups. Prior to the time of administration of the drug, the animal was weighed to a certain equal volume of dosage were given.

The rats were weighted after one week interval and at the end of the experiments the animal were sacrificed and liver tissue sample were collected. Then tissue sections of liver were prepared and stained with Haematoxylene and Eosin and examined microscopically.

### Laboratory Procedure/ Analytical Method.

Haematoxylin & eosin (H&E) staining method

Hematoxylin and Eosin (H&E) staining is the most widely used staining technique in histopathology. The fixed tissues passed through routine histological processing techniques for the preparation of tissue slides before staining in H and E stains. Sections will be stained by H&E. This will enable preliminary presumptive histological diagnosis of tracheal cancer before further studies. The stained sections will be sent to two independent pathologists to confirm diagnosis of tracheal cancer before further discussion.

Histopathological Procedure

Tissues (spleen) was fixed in 10% formal saline and will be dehydrated in four (4) concentrations of Isopropyl alcohol, i.e. 70%, 80%, 90%, 100% for 1hr each and then cleared in xylene, then impregnation before embedded in molten paraffin wax to remove the isopropyl alcohol. Micro sections of 5micrometer using Leica RM 212 Rt. Rotary Microtome, tissues was stained using Haematoxylin and Eosin (H&E) to demonstrate general tissue structure. Tissues sectioned was examined and interpreted using Leica DM 750 binocular microscope with photomicrographic facilities and then photomicrographed by a histopathologist (Ahmed, 2016).

### Microscopy and photomicrography

The stained slides were examined using optical microscope and photomicrographs of the sections taken.

## Statistical Analysis

Numerical data obtained from this study were analyzed using Statistical Science for Social Sciences (SPSS) version 27. Data obtained for relative organ weight and bodyweight were analyzed using ANOVA followed by post hoc test. Data were considered significant at $p<0.05$.

## Result

Group A (Control): The liver architecture appeared normal with clear central veins, radiating sinusoids, and healthy hepatocytes. The hepatic muscles were open-faced and well-organized, indicating no adverse effects and representing the baseline morphology of a healthy liver. This serves as references for comparing the effects observed in the treated group

Group B: The liver showed an enlarged but diluted central vein with fatty deposits and infiltrated cells. The morphology was affected, and the nuclei appeared weak, indicating early signs of liver damage. The presence of fatty deposits (steatosis) and cell infiltration suggests the liver's initial response to the insecticide exposure, pointing towards a mild to moderate level of hepatocellular injury. The significant difference observed in the liver morphology at this concentration ($p=0.004$) highlights the early toxic effect of the insecticide.

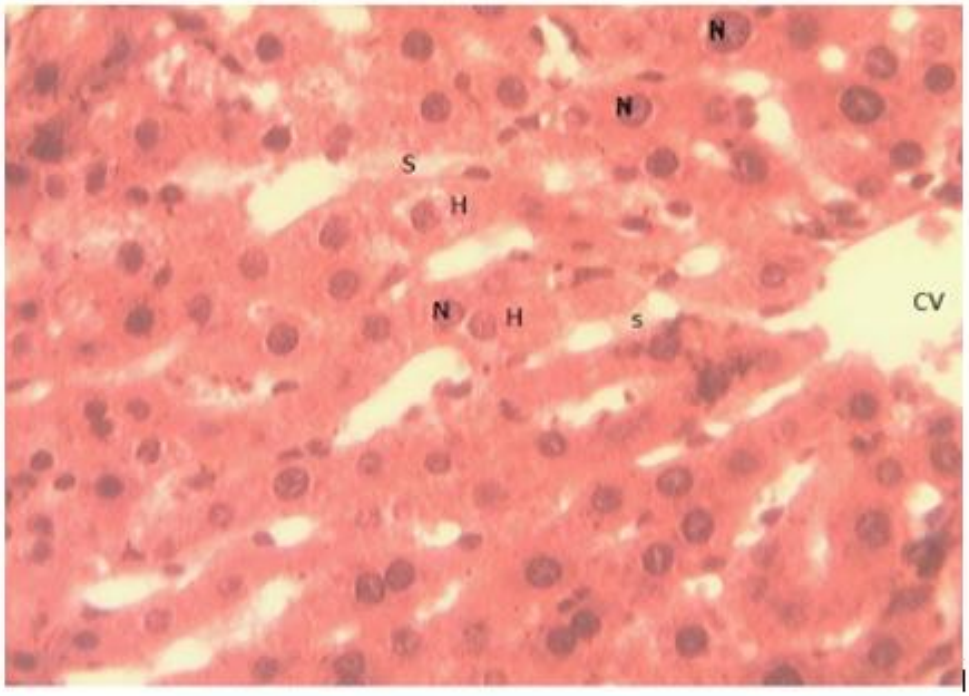
Group C: The central vein exhibited necrotic foci with darkened nuclei. Despite the normal hexagonal arrangement, the liver cells were significantly affected, suggesting notable cellular damage due to insecticide exposure. Necrosis indicates a severe level of cell death, likely resulting from the toxic effects of the insecticide, which compromised cellular integrity and function. The significant differences in histopathological changes at this concentration underscore the insecticide's potential to cause severe liver damage.

Group D: A prominent distortion in liver cells was observed, disrupting the liver architecture. The central vein was dilated and filled with fat deposits and infiltrated cells, indicating severe liver damage from the insecticide. The extensive distortion and fat deposition (steatosis) reflect a more advanced stage of liver injury, suggesting chronic exposure or higher toxicity of the insecticide. The significant differences observed ($p=0.004$) at this concentration emphasize the progressive nature of the liver damage.

Group E: The liver showed a high degree of distortion across hepatocytes, sinusoids, and nuclei. This group exhibited the most severe liver damage, indicating a dose-dependent toxic effect of the insecticide. The widespread hepatocellular damage and structural disorganization underscore the severe hepatotoxic potential of the insecticide, which led to significant alterations in liver morphology. The statistically significant differences observed at the concentration further validate the dose-dependent toxicity of the insecticide.

## Results:

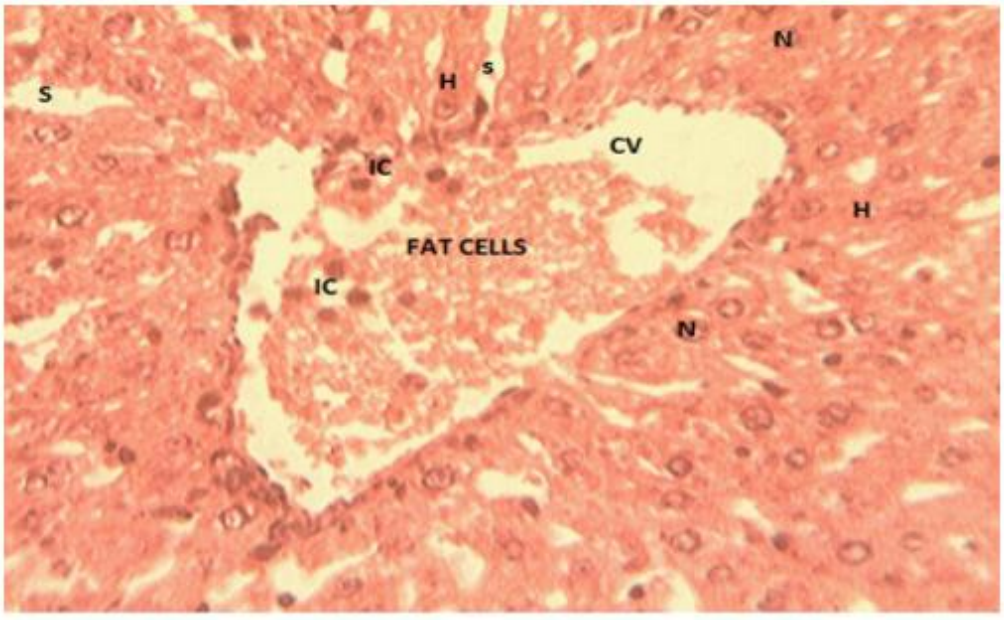
### GROUP A




**Liver X400 Magnification (magfxn)**

The Cross section of Group A Liver X400 Mgfxn stained in H&E showing a normal liver architecture, the central is clear, the sinusoids and hepatocytes radiate hexagonally and the hepatic muscles looks open faced and healthy.

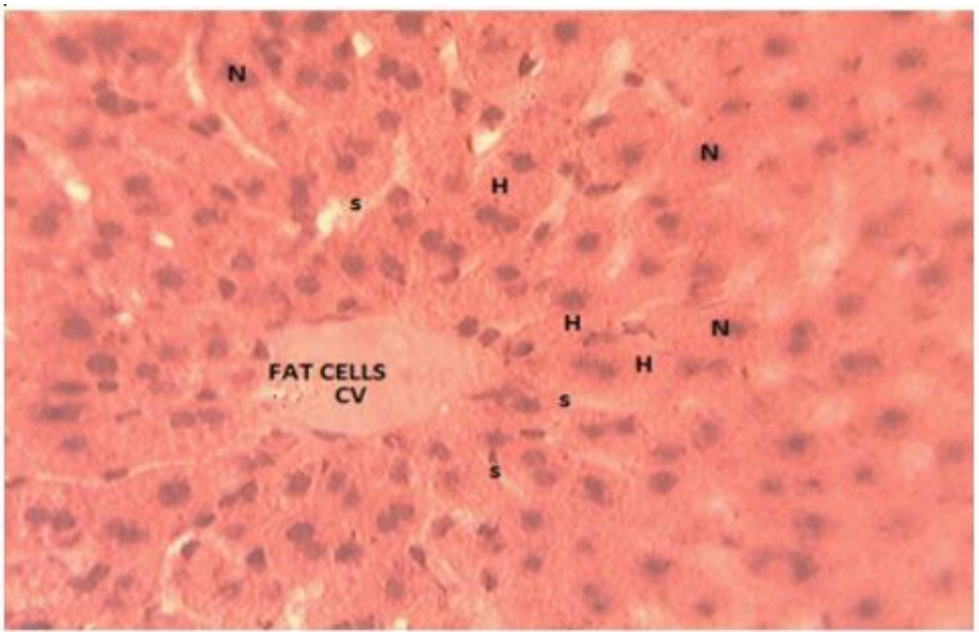
**Group B**




**LIVER X400 magfxn**

The Cross section of Group B Liver X400 Mgfxn stained in H&E showing an enlarged but diluted central vein with fatty deposits and infiltrated cells, the morphology seems affected as the nuclei look weak through open faced.

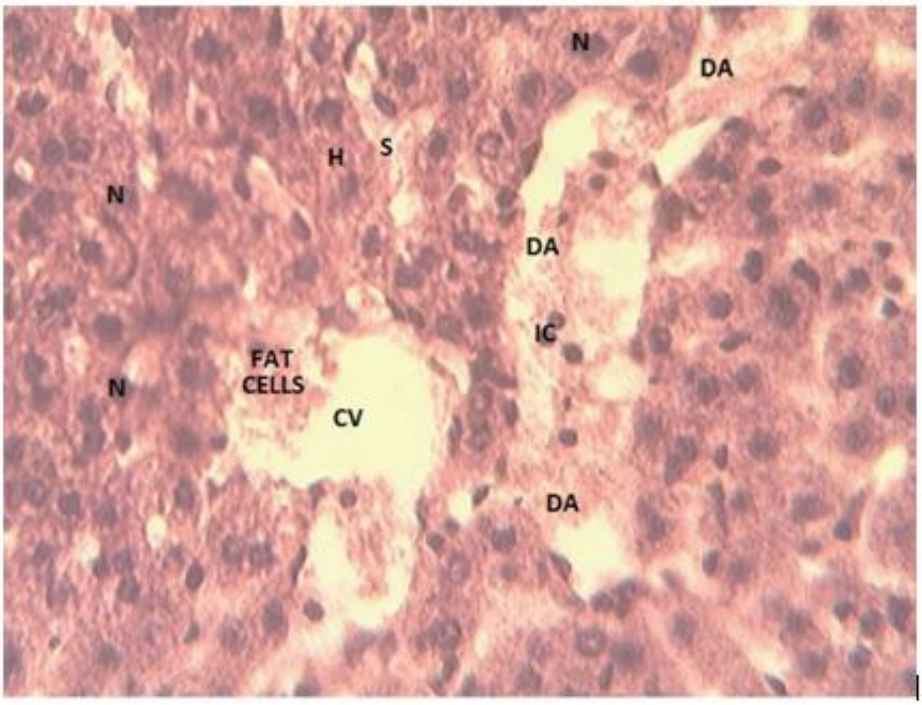
**GROUP C**




**LIVER X400 magfxn**

The Group C liver shows a central vein (cv) with necrotic foci and nuclei totally darkened though the hexagonal arrangement seems normal but the liver cells are affected.

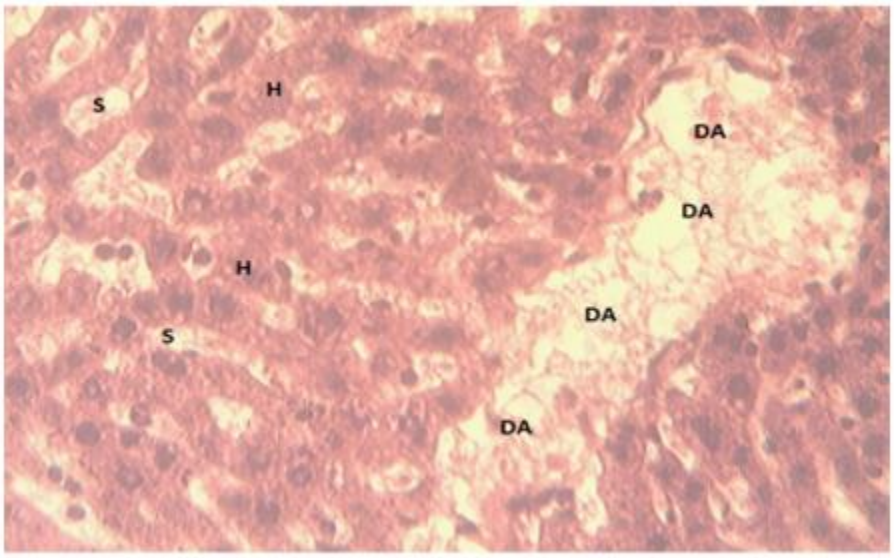
**GROUP D**




**LIVER X400 magfxn**

The Group D liver shows a prominent distortion on the liver cells distorting the liver architecture. The central vein (cv) dilating and filled with fat deposits and infiltered cells.

**GROUP E**




**LIVER X400 magfxn**

The photomicrograph of Group E Liver x400magfxn stained in H&E showing a high degree of distortion on the liver cells, the distortion cuts across the hepatocytes, the sinusoids and nuclei

## Discussion

The histological examination of liver sections under X400 magnification, stained with Hematoxylin and Eosin (H&E), reveals varying degrees of structural changes across different groups of albino rats treated with insecticide. Significant differences were observed at the $p<0.05$ level, indicating that the changes are statistically significant and not due to random variation.

The histopathological findings indicate that the insecticide causes dose-dependent liver damage in albino rats [12, 13]. The observed changes range from mild alterations, such as fatty deposits and cell infiltration, to severe damage, including necrosis and extensive structural distortion [14]. These findings are consistent with other studies such as [15, 16, 17] on the hepatotoxic effects of various insecticides, which often report similar patterns of liver injury.

The presence of fatty deposits (steatosis) in Groups B and D suggests that the insecticide may interfere with lipid metabolism, leading to the accumulation of fat within hepatocytes. This condition, if prolonged, can progress to more severe liver diseases, such as steatohepatitis and cirrhosis [18, 19]. The observed necrosis in Group C indicates that the insecticide can induce cell death, compromising liver function and potentially leading to liver failure [20, 21,22].

The most severe damage observed in Group E highlights the critical need to regulate and monitor insecticide exposure to prevent significant health risks [23]. These findings underscore the importance of establishing safe exposure limits and implementing protective measures for individuals who may be at risk of insecticide exposure.

## Conclusion

The histopathological examination of liver sections from albino rats treated with varying concentrations of insecticide reveals significant structural changes, confirming the hepatotoxic effects of the insecticide. These effects were statistically significant at the $p < 0.05$ level, underscoring the reliability of the observed differences.

Overall, the study confirms that the insecticide induces significant, dose-dependent hepatotoxic effects in albino rats. The findings highlight the necessity of regulating insecticide use to prevent severe liver damage and protect liver health. Further research is needed to elucidate the mechanisms of toxicity and develop effective strategies to mitigate these adverse effects.

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