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*Full Length Research Paper*

# Haemato-pathological effect of dichlorvos on blood picture and liver cells of albino rats

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**The effect of intraperitonal exposure of dichlorvos an organophosphate (OP) pesticide, on haematology parameters and liver pathology of Wister rats was investigated. Thirty male albino rats grouped into six (6) of five rats each were injected with 0, 3.7, 7.4, 11.1, 14.8 and 18.5mg/kg of dichlorvos (DDVP) (1 ml/kg) respectively. The haematological parameters measured were red blood cells, haemoglobin; packed cell volume, total white blood cells count and platelet levels. Histological examination of liver tissue was investigated as well. The result of the haematological parameters of the dichlorvos treated rats showed a significant decrease ($p<0.05$) in the mean values of red blood cells, haemoglobin and packed cell volume and a significant increase ($p<0.05$) in the total white blood cell count and platelet count which was dose dependent. Changes observed in the liver architecture of the treated rat tissues were feathery looks, fatty changes and centrilobular necrosis. However there was no architectural distortion observed in the liver tissue of the control rats. Dichlorvos had dose dependent target toxicity.**

**Key words:** Liver, toxicity, dichlorvos, pestiscides, haematology.

## INTRODUCTION

Organophosphates (OPs) today have become the most implicated pesticide in cases of poisoning, they are frequently used as households, garden and farmland insecticides. The importance of pesticide use in Nigeria can be understood from the fact that agriculture is a major component of the Nigerian economy. It contributes 22% of the nation's gross domestic product (GDP) and is the livelihood of nearly 60% of the country's workforce. The widespread use of pesticides in agricultural practice, public health, commerce and individual households results in acute intoxication each year. The first global estimates of the extent of pesticide poisoning were published in 1990 by the World Health Organization (WHO).

WHO estimates based on 2001 data reported that, 849,000 people die globally from self harm each year (WHO, 2002). OPs are highly toxic chemicals that kill insect and other pest by inhibiting the action of the enzyme acetylcholinesterase that functions normally by degrading acetylcholine in nerve synapses. Exposure to OP pesticides had been found to produce adverse effects in exposed populations including humans; such exposures can result in ill health and even death (Michael

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et al., 2008). The populace is inevitably exposed to OP pesticide poisoning through environmental contamination or occupational use. Occupational exposure can occur at all stages of pesticide formulation, manufacture and application.

The potential adverse impact of dichlorvos on human health is likely to be higher in developing countries like Nigeria, due to easy availability of this highly hazardous chemical product and its low risk awareness among the populace especially local farmers. Individuals can be exposed to dichlorvos through both direct and indirect routes, direct exposure occurs in farmers and individuals who personally apply pesticides in agricultural, occupational or residential settings. Indirect exposure occurs through drinking water, air, dust and foods. This represents routes of long-term low level exposures (Alavanja et al., 2004). As with other organophosphates; dichlorvos is readily absorbed through the skin, as well as by inhalation and from the gastrointestinal tract (Alavanger et al., 2004; Yurumez et al., 2007), acute illness through dichlorvos exposure is limited to the effects of cholinesterase inhibition compared to poisoning by other organophosphates.

Therefore, this present study was aimed at investigating the effects of acute dichlorvos exposure on haematology parameters and liver pathology.

## MATERIALS AND METHODS

### Test animals

A total of thirty (30) Wister albino rats (*Ratus ratus*) of 150 to 200 g average weight were used for the study. The animals were housed in polypropylene cages under hygienic conditions and were acclimatized for three weeks prior to commencement of the study. The rats were maintained on standard laboratory feed and water *ad libitum* and treated in accordance with the standard guide for the care and use of laboratory animals (NRC, 1985). There was no ethical issue; however it is relevant that the standard guide for the use of laboratory animal be followed.

### Animal treatment schedule

Rats were randomly divided into two groups: a control group (n=5) and an experimental group (n=25). The experimental group rats were further divided into five subgroups, each group representing the different dose levels of dichlorvos. The treatments were administered in the morning (8.00 am to 9.00 am) to non fasted rats. The rats were anesthetized with chloform and sacrificed within 2 h of chemical administration.

### Acute toxicity study

The acute toxicity study ($LD_{50}$) entails administering a single dose of the chemical substance (dichlorvos) intraperitoneally into the experimental rats and observing its effects over a short period, usually over 24 h (Dede et al., 1991). The effects of dichlorvos on the tested animals was observed, blood samples were taken for haematological tests. The animals were sacrificed, the liver excised and subjected to histological examination.

### Haematological investigation

A general anesthesia was achieved by inhalation of isofluorene. The rat was placed back on a dissecting board, xyphoid process was palpated and 21 G needle was inserted into the ventricle, the plunger was pulled backward slowly to allow blood sample collection into EDTA bottle for haematological investigation. The haematological parameters investigated were red blood cell (RBC), haemoglobin (HB), packed cell volume (PCV), total white blood cell (WBC) and platelet count. All samples were analyzed using ERMA INC. full automatic blood cell analyzer, model PCE- 210N.

### Histopathological examination

The Wister (albino) rats were sacrificed and the liver resected. The resected livers were fixed by placing it in plastic jars containing 10% formal aldehyde labelled accordingly. After fixation, the liver tissues were dehydrated by using increasing strength of alcohol: 70, 85, 90 and 100% in an incubation period of 1 h respectively. The tissues were cleared with xylene for 30 min, and were embedded in a mould containing liquid paraffin wax and allowed to solidify. The prepared and embedded tissue block was first trimmed and then secured to a holder which is then mounted on the microtone. The thickness of the sections was reset at 5 mm thickness and 8 to 10 sections of each liver tissue were made. The microscopic slides were labelled using diamond pen accordingly. The thin sections were carefully transferred to water at 45°C in water bath. The labelled microscopic slide were dipped into adhesive solution and slowly pulled upward, out of the solution, allowing sections to adhere to the surface. The bottom of the slide were dried and carefully blotted of excess adhesive from around the sections. The sections were allowed to dry overnight in the storage box. The sections were cleared by passing the mounted sections through the cleaning agent xylene, leaving only the tissue adhering to the slide. The sections fixed on slide were stained with Haematoxylin for 5 min and washed in running water for 30 s. Excess stain were washed in 1% acid alcohol by continuous agitation for 15 s and later washed in running tap water for 30 s. The slides were dipped into ammonia water 2 to 3 times and then washed in running tap water for 30 s. Eosin was applied for 3 to 5 min and then washed in running water for 30 s. These were dehydrated by dipping the slides in increasing concentration of alcohol of 50, 70, 95 and 100% for 2 to 3 min. Then cleared with xylene and mounted with Canada balsam. The stained sections were observed under a light microscope and photographs of the various observations were taken and saved in JPEG format as x400 magnification.

### Statistical analysis

Statistical analysis was carried out on the haematological data obtained using Microsoft Excel statistical tools (2003 version). The mean, standard deviation and standard error of mean were calculated. The values were presented as means ± standard error of mean (SEM) and compared by student's statistical test. $P<0.05$ was accepted as statistically significant level.

## RESULTS

Male albino rats treated with different doses of dichlorvos (3.7, 7.4, 11.1, 14.8 and 18.5 mg/kg), showed sluggish behaviour, restlessness, micturition, respiratory distress, convulsion and death especially at higher doses of the chemical. These symptoms are typical signs of toxicosis which

**Table 1. Toxic Acute Effect of Dichlorvos on Hematological Parameters (mean±SEM)**

| Group | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Does mg/kg | 0 (Control) | 3.7 | 7.4 | 11.1 | 14.8 | 18.5 |
| RBC $X10^{12}$/i | 7.81.0 | 7.2±1.2 $^{ns}$ | 6.9±0.5 $^{ns}$ | 5.0±0.8$^{a}$ | 4.32±1.2$^{a}$ | 3.78±1.0$^{a}$ |
| HB g/dl | 17.5±0.5 | 15.7±0.9 $^{ns}$ | 14.7±1.0$^{ns}$ | 12.8±0.6$^{a}$ | 12.0±0.8$^{a}$ | 11.5±1.0$^{a}$ |
| PCV % | 52±0.6 | 47±0.5$^{ns}$ | 45±0.8$^{ns}$ | 38 ±0.5$^{a}$ | 36±0.9$^{a}$ | 33±0.9$^{a}$ |
| WBC $X10^{9}$/i | 9.0±1.0 | 9.7±0.6 $^{ns}$ | 10.5±1.0$^{ns}$ | 13.2 ±1.5$^{a}$ | 15.5±1.2$^{a}$ | 17.0 ±1.5$^{a}$ |
| PLT $X10^{9}$/i | 40±1.0 | 42±0.6$^{ns}$ | 48±1.0$^{ns}$ | 53±1.6$^{a}$ | 17.0±1.5$^{a}$ | 65±1.5$^{a}$ |

n=5; $^{a}$P<0.05 = significant; ns – not significant; SEM = standard error of mean; n- no of rats.

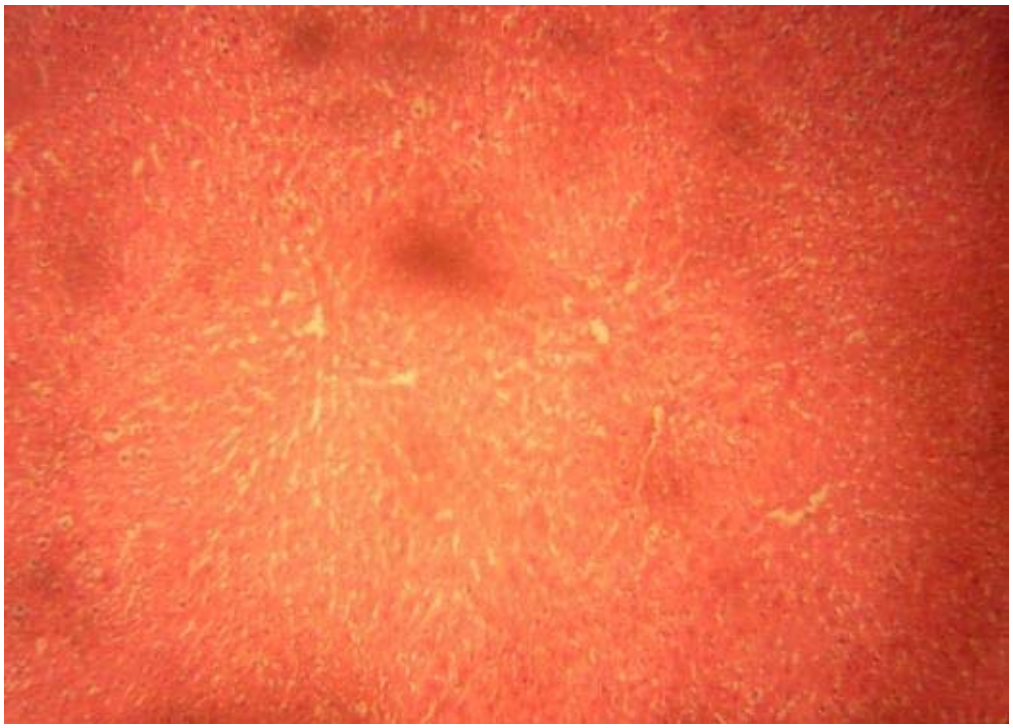

**Figure 1.** Liver histology of control Wister albino rat (0.9% saline). ×200 magnification. Observation: Normal liver architecture.

which could be attributed to inhibitory action of dichlorvos on the enzyme cholinesterase there, resulting to accumulation of acetylcholine at synapses.

## Haematological observations

Table 1 showed haematological indices RBC, HB, PCV, Total WBC and Platelet levels obtained in albino rats exposed to acute dichlorvos poisoning. Statistically dose dependent decreases were observed in the RBC, HB and PCV values obtained ($p< 0.05$) for the exposed albino rats when compared to the control rats. While statistically dose dependent increases were observed in the WBC and platelets values ($p < 0.05$) when compared with the control value.

## Histopathological observations

Pathological changes observed in architecture of the liver of rats exposed to acute dichlorvos poisoning that was dose dependent mostly from dose level 11.1 to 18.5 mg/kg. The liver of the dichlorvos treated rats (Figure 1 to 4.) showed morphological changes in the mitochondrial, areas of feathery degeneration, and steatosis. The control rat group showed normal liver architecture.

## DISCUSSION

Acute exposure to organophosphate pesticides is often accompanied with serious health hazards. It is established that many of these pesticides can produce some

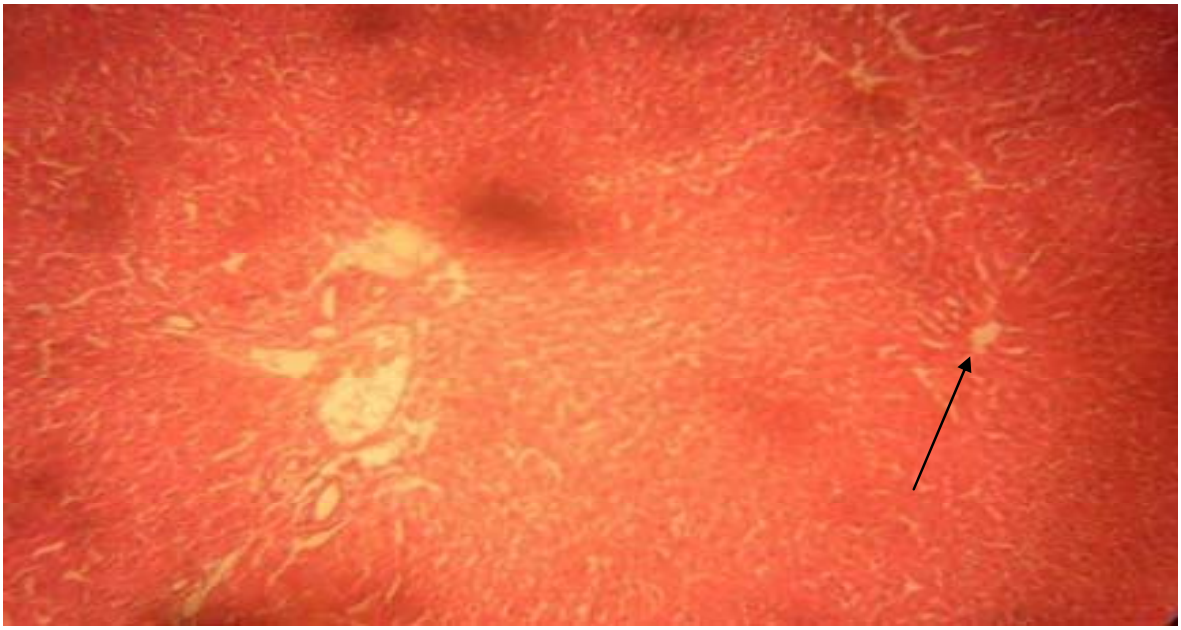

**Figure 2.** Liver histology of Wister albino rat (7.4 mg/kg dichlorvos). ×200 magnification
Observation: Slight feathery change.

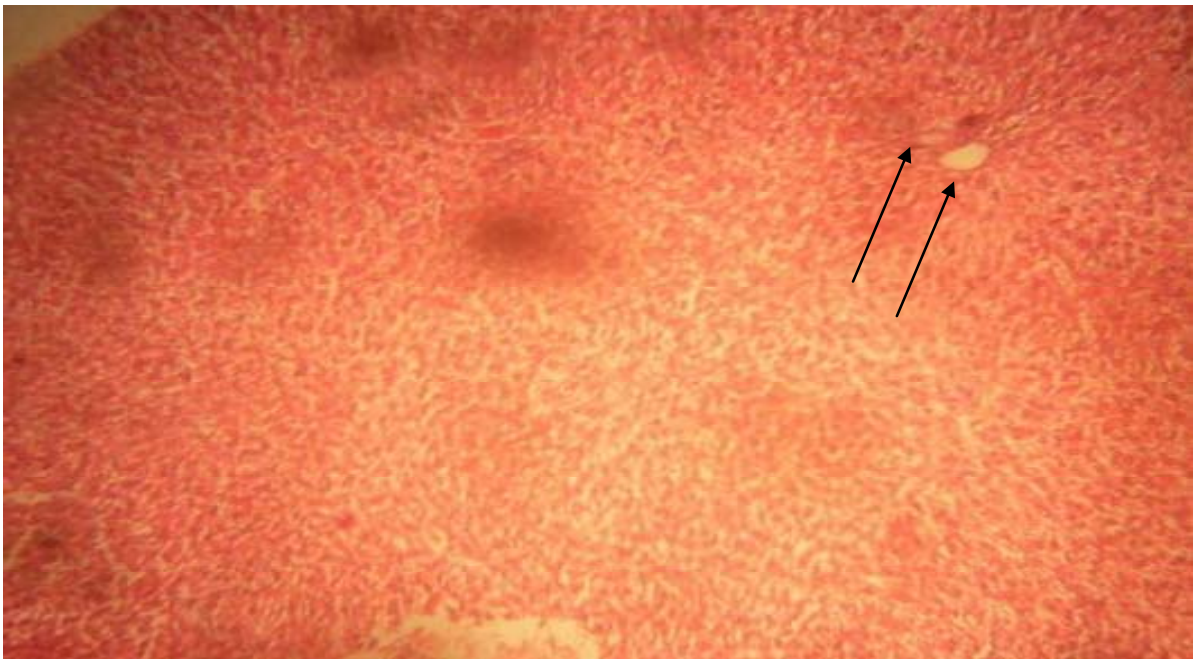

**Figure 3.** Liver histopathology of Wister albino rat ( 11.1 mg/kg dichlorvos). ×200 magnification
Observation: Mild feathery change. It represents feathery texture that is observed all over the picture.

adverse effects on the liver and other vital organs in the body through their mode of action or by the production of free radicals (Khan et al., 2005). Dichlorvos acts mainly by irreversibly inhibiting the enzyme acetylcholinesterase (AChE) at cholinergic junctions of the central nervous system (Petroianu et al., 2006) which induce oxidative stress and results to hepatotoxicity in rat (Gupta et al., 2005) Liver plays a crucial role in detoxification of harmful chemical substances, it is the site of biotransformation of many toxic compounds into less harmful products thereby

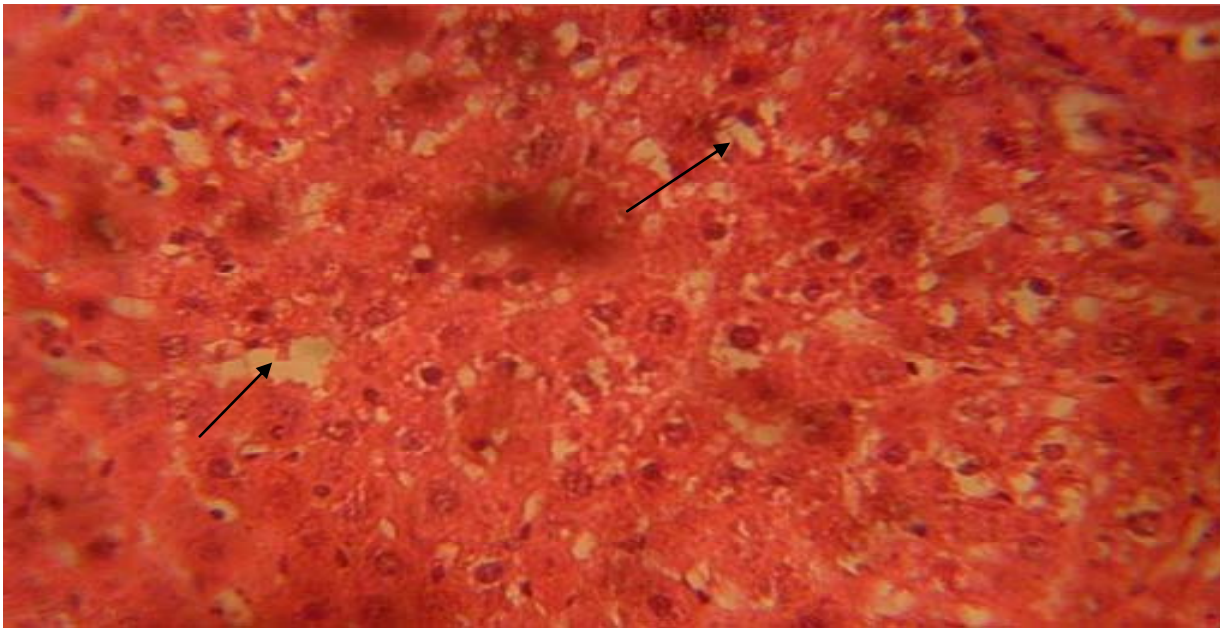

**Figure 4.** Liver histopathology of Wister albino rat ( 14.8 mg/kg dichlorvos). ×200 magnification
Observation: Liver tissue showing congestion, fatty changes and steatosis around the central vein.

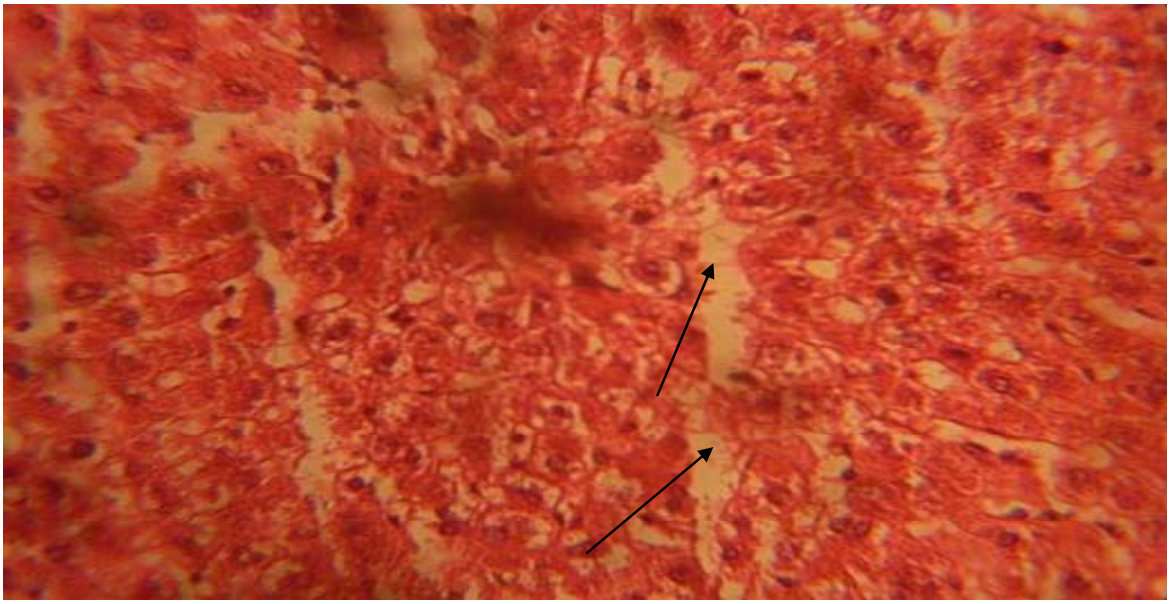

**Figure 5.** Liver histopathology of Wister albino rat ( 18.5 mg/kg dichlorvos). ×200 magnification.
Observation: Liver tissue showing centrilobular necrosis and sinusoidal dilation.

reducing their toxicity and ensuring structural stability of the body. Haematological parameters serve as important indices in the monitoring and management of health status. The lifespan of the normal red cell is 120 days, in severe haemolysis the cells survive for only few days, increased bone marrow activity may compensate temporarily for this reduction. However, when the bone marrow fails to increase the production of erythrocytes the offset for the loss anaemia develops (Lewis et al., 2001).

In the present study, acute intraperitoneal administration of varying doses of dichlorvos to the experimental rats caused anaemia as shown by the decreased levels of

haemoglobin, packed cell volume and red blood cells. The observed decreased levels of these parameters were dose dependent, the higher the concentration of dichlorvos the more its impact on the blood parameters. This finding indicates a state ofanaemia which could arise as a result of excessive destruction of erythrocytes by dichlorvos at a rate that exceeds the bone marrows capability to compensate or offset for the blood loss. These findings corroborated with those reported by Mohssen (1997) and Dede and Chike (2000). Erythrocytopenia observed in this study could be attributed to the suppressing effect of dichlorvos on erythropoiesis. The toxicity of dichlorvos on haemopoietic cells in the bone marrow could be due to metabolites of dichlorvos that are produced in relatively high concentration and act in synergistic manner to disrupt the mechanism that regulate blood cell formation. Also obtained was a dose dependent increase in the values of white blood cells and platelets count. The increase in the total WBC could be attributed to the rats' defense mechanism in response to the invading xenobiotic (dichlorvos).

The liver slides in this study as presented in Figure 1 to 5 revealed areas of fatty changes, steatosis, congestion, centrilobular necrosis, sinusoidal dilation and infiltration of the sinuses by few mononuclear cells. Similar observation has also been reported in dichlorvos treated rats in the study by Luty et al. (1998). In the present study, hepatocellular damage was more pronounced at higher doses of dichlorvos (11.1 to 18.5 mg/kg) with no effects observed at lower doses of dichlorvos poisoning and also in the control group. Study also conducted by Binukuma et al. (2010) reported considerable morphological alterations in the structure and function of the liver in male albino rats exposed to chronic dose of dichlorvos. Somia and Madiha (2012) in their study also reported abnormal size and shape of hepatic cells, massive aggregation of inflammatory cells in the portal area and hepatocytomegalocytosis in liver of mice fed for three months on faba beans treated with dichlorvos

## Conclusion

This present study demonstrated the acute toxic effects of dichlorvos on haematological parameters and histology of the rats at different dose levels. Acute exposure of the albino rats to dichlorvos poisoning induced anaemia as shown by the decrease levels of HB and PCV was observed in the current study. There was also a significant increase in the total WBC and platelet counts that was dose dependent. Furthermore, dichlorvos promoted necrosis of the liver tissue, thereby altering the normal physiological functions of the liver. From the analysis of the parameters undertaken, it can be inferred that dichlorvos is hepatoxic and also caused anaemia in the exposed rats. Prompt recognition of poisoning cases and aggressive treatment of acute intoxication is essential in order to minimize the morbidity and mortality that could arise from the use of this lethal chemical.

## Conflicts of interest

The authors declare that they have no conflicts of interest.

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