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EVALUATION OF DICHLORVOS INHALATION ON THE LIVER HISTOLOGY AND SELECTED LIVER ENZYMES ACTIVITIES IN ADULT WISTAR RATS

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ABSTRACT

Dichlorvos is one of the cheapest insecticides use to eradicate mosquitoes in Nigeria. It forms the active ingredient of the solution called *Ota piapia* or *Madarar piapia*. It binds irreversibly with acetylcholine receptors and inhibits its activity. It is metabolized in the liver and kidney. Oral ingestion of dichlorvos was commonest cause of toxicity reported however few literatures were reported on the effect of dichlorvos inhalation on the liver. The aim of this study was to evaluate the effects of dichlorvos inhalation on the liver histology and selected liver enzymes in Adult Wistar Rats. Twenty five apparently healthy Adult Wistar Rats were randomly selected and divided into five groups. Group one was used as control the rest were used as treatments for twenty eight days. The animals were sacrificed; their blood and liver tissues were collected for enzyme assay and routine histological technique respectively. Significant increase [$P < 0.05$] in the values of the Albumin, Alkaline Phosphatase, Aspartate Aminotransferase and Alanine Aminotransferase as well as variable degrees of apoptosis and steatosis were observed in dose dependent manner in the treatment groups. Dichlorvos inhalation damaged histo-architechture and altered enzymes activities of the liver.

KEYWORDS: Dichlorvos, inhalation, histology, liver enzymes.

INTRODUCTION

Dichlorvos is an insecticide and pesticides that has been used for decades. It is cheaply available, accessible and affordable under different brand names. In Nigeria dichlorvos forms the active ingredient of locally formulated insecticide *Ota – pia-pia or Madarar pia-pia*.[1] Its ability to kill organisms renders them harmful to humans and other living beings.[2,3] The compound has a wide range of both acute and chronic health effects such as cancer, neurological damage, reproductive effects, immune suppression, birth defects; and are suspected endocrine disruptors.[4,5] In addition to use as pesticides, organophosphorous compounds [OP] such as 2, 2, dichlorovinyl dimethyl phosphate; [DDVP], chlorpyrifos, paraoxon etc are also used as chemical warfare because it binds irreversible to acetylcholine receptors and cause subsequent inactivation of acetylcholinesterase, an enzyme that normally catalyzes hydrolysis of acetylcholine [ACh] at neuromuscular junctions and other cholinergic synapses.[6] Dichlorvos is rapidly absorbed through the gastrointestinal and respiratory tracts and skin, it enters human system via inhalation, dermal or oral routes, and it is metabolized by the liver and excreted by the kidney.[7,8]

The liver is a complex three-dimensional structure that plays important role in carbohydrates, proteins and lipids metabolism to maintain energy level and structural stability of body. It consists of epithelial and mesenchymal elements arranged in repetitive microscopic units called hepatic cords.[9] Hepatocytes are the smallest microscopic structures of the liver normally arranged in cords that are one or two cells thick separated by sinusoids. Presence of thicker cords suggests regenerative activity, but in the setting of a lesion, may also indicate a neoplasm.[10, 11] The hepatocyte is polygonal, measures about 25 to 40 μm and has abundant eosinophilic cytoplasm and a central nucleus. The nucleus is round or oval, and may contain glycogen, which is more common in certain conditions, such as young age, diabetes, and Wilson disease.[9]

Dichlorvos affects the antioxidant system, which plays an important role in making xenobiotics entering the body ineffective. The basic reason for this effect is that DDVP is made depending on GST which is an essential enzyme in the detoxification of the antioxidant system.[12] Researchers have found the effects of pesticides with organophosphate including dichlorvos on erythrocyte antioxidant enzymes. They also found that erythrocyte glycose-6-phosphate dehydrogenase activity decreased after the erythrocytes were affected by organophosphate pesticides, and moreover, glutathione-S-transferase [GST] and glutathione reductase activities

increased.[13] Glutathione-S-transferase activity increases in all tissue, but it's higher in liver tissues than in other tissues. Dichlorvos metabolizes in the liver using two metabolic pathways. At the end of one pathway, desmethyldichlorvos forms as glutathione dependent, and at the end of the other pathway, dimethylphosphate and dichloroacetaldehyde forms.[14]

Dichlorvos as an anticholinestrase, binds irreversibly to acetylcholine receptors and inhibits its action. Although oral ingestion of dichlorvos through contaminations of agricultural products are the major causes of its toxicity, dichlorvos toxicity may also occur by prolong exposure through inhalation. The need for maximum protection against pests and insects such as mosquitoes causes the users to get exposed into high concentrations of this chemical on daily basis especially in low income countries such as Nigeria. Since the liver metabolizes dichlorvos and kidney excretes the by-product in the urine, this may damage the structural and functional efficiency of the DDVP metabolizing organs such as the liver when exposed for a long time. Our study aims to evaluate the effects of dichlorvos inhalation on the histology of the liver and the activity of selected liver enzymes in Adult Wistar Rats.

MATERIALS AND METHOD

Procurement of Animals

Twenty five apparently healthy Adult Wistar Rats, weighed 195 – 400g were purchased from the Pharmacology Department, Aminu Kano Teaching Hospital [AKTH] Kano, Nigeria and allowed to acclimatize for two weeks in laboratory condition before experimentation. The animals were housed in well-ventilated rectangular aluminum cages [290 × 320 × 390 mm] bedded with soft saw dust and maintained under natural temperature and atmospheric condition with proper illumination of light/dark cycle. The animals had free access to food (*Vital feed*) and water *ad libitum*. The sanitary of the husbandry were maintained in accordance with the "Guide for the Care and Use of Laboratory Animals". The animals were then divided into five groups (i.e. I, II, III, IV & V), each with equal numbers.

Procurement of Chemical and Dosage Preparation

A stock solution of 1000 mg/l dichlorvos dichlorvos [Delvap Super ®] was purchased from the vendors of insecticides, pesticides and other Agro Allied Chemicals at Sabon-Gari market, Kano, Nigeria. The lethal concentration LC_{50} of dichlorvos which was reported as 15mg/m^3 by Material Safety Data Sheet [MSDS] was adopted as reference value.[15] In our study we used the sub-lethal doses equivalent to 75% (11.25mg/ m^3), 50% (7.50mg/m^3) and 25% (3.75mg/m^3) of the reference LC_{50} dose.

Experimental Design

Five poorly ventilated 1m x 1m cubed wooden boxes labeled A, B, C, D and E, each with a rectangular sliding glass pane measured about 0.2m x 0.1m for entrance at the top was constructed for the exposure of the animals. About 2 mls each of the graded solutions were drawn separately using 4 mls hypodermic syringes and then sprayed thoroughly into the boxes every day before the animals were exposed. This was to mimic the live human scenario. The animals in groups I and II were exposed into ambient air and 2 mls of 2.5 mg/m^3 ethanol whereas, those in groups III, IV and V were exposed into the boxes sprayed with 11.25 mg/m^3, 7.50 mg/m^3, and 3.75 mg/m^3 concentrations respectively. The exposure duration lasted for 2 hours in all the groups every day for twenty eight days.

Samples Collection

Twenty four hours after the last exposure, 2 mls of were randomly collected after cervical decapitation and preserved in EDTA heparinized blood containers from each group. The abdomens of the animals were then cut – opened using sharp dissection scalpel. The livers preserved in 10% formalin.

Determination of liver Enzyme Activity

The blood samples collected were centrifuge at 500 r.p.m. for 5 minute and then analyzed each for Albumin test (ALB), Alkaline Phosphatase test (ALP), Aspartate Aminotransferase (ALP) and Alanine Aminotransferase (ALT) using Varian Spectrophotometer – Cary 50 at the Bayero University Kano, Biochemistry departmental Multiuser lab.

Slide Preparation

The liver tissues collected were prepared into histological slides by routine paraffin wax histological techniques. The slides were then stained using conventional Haematoxylin and Eosin stain and viewed under *Olympus* light microscope to which fitted *Celestron*© [Digital Microscope Imager, USA] with an inbuilt 15x magnifying lens at 150 and 600 magnifications respectively.

STATISTICAL ANALYSES

The mean Albumin (ALB), Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) value of the hematological indices collected were expressed as mean±SD. One way analysis of variance (ANOVA) followed by Post – hoc (*Tukey's*) test was carried out to determine the mean differences across the groups.

RESULTS

Tables 1: One way ANOVA test for selected Liver Enzymes in Adult Wistar Rats

Variable	Mean±SD					p-value
	[I] Ambient air	[II] Ethanol	[III] 11.25mg/m^3	[IV] 7.50mg/m^3	[V] 3.75mg/m^3	
ALB	1.44±0.71	3.25±1.24*	3.13±0.81*	2.97±0.68*	2.96±0.58*	0.006
ALP	162.95±0.94	133.56±1.12*	138.51±0.41*	199.00±0.54*	60.40±0.84*	0.0001
ALT	20.17±0.69	21.84±0.73*	26.873±1.16*	37.56±0.76*	30.61±1.20*	0.0001
AST	101.65±0.75	111.23±0.29*	109.65±0.67*	155.10±1.24*	116.84±0.41*	0.0001

ALB = Albumin; **ALP** = Alkaline Phosphatase; **AST** = Aspartate Aminotransferase; **ALT** = Alanine Aminotransferase
$p < 0.05$

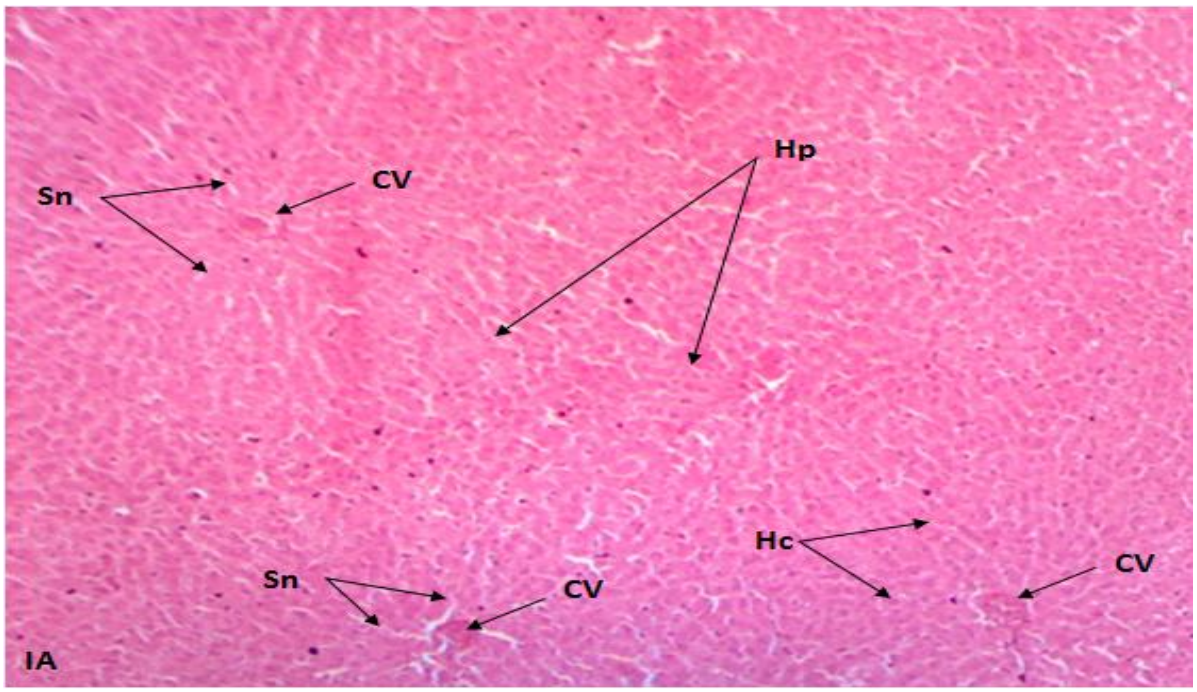

Plate IA: H&E Normal photomicrograph of Liver Exposed to ambient air as control in Adult Wistar Rat at x150 magnifications. Sn= Sinusoid; CV=Central Vein; Hp= Hepatocytes; Hc: Hepatic cord.

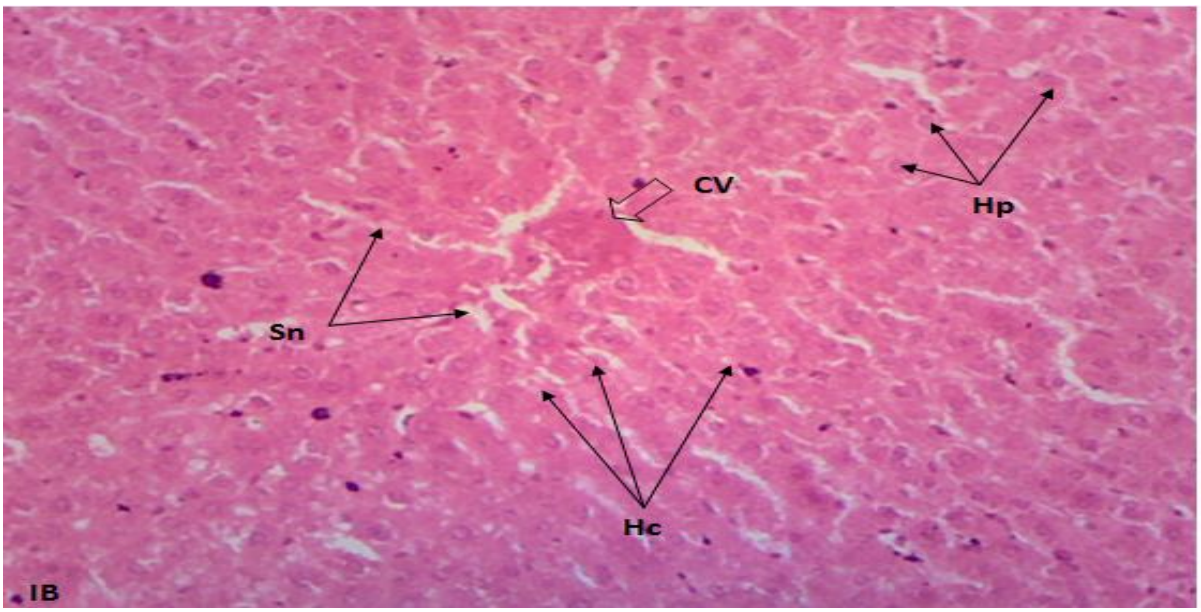

Plate IA: H&E Normal photomicrograph of Liver Exposed to ambient air as control in Adult Wistar Rat at x600 magnifications. Sn= Sinusoid; CV=Central Vein; Hp= Hepatocytes; Hc: Hepatic cords.

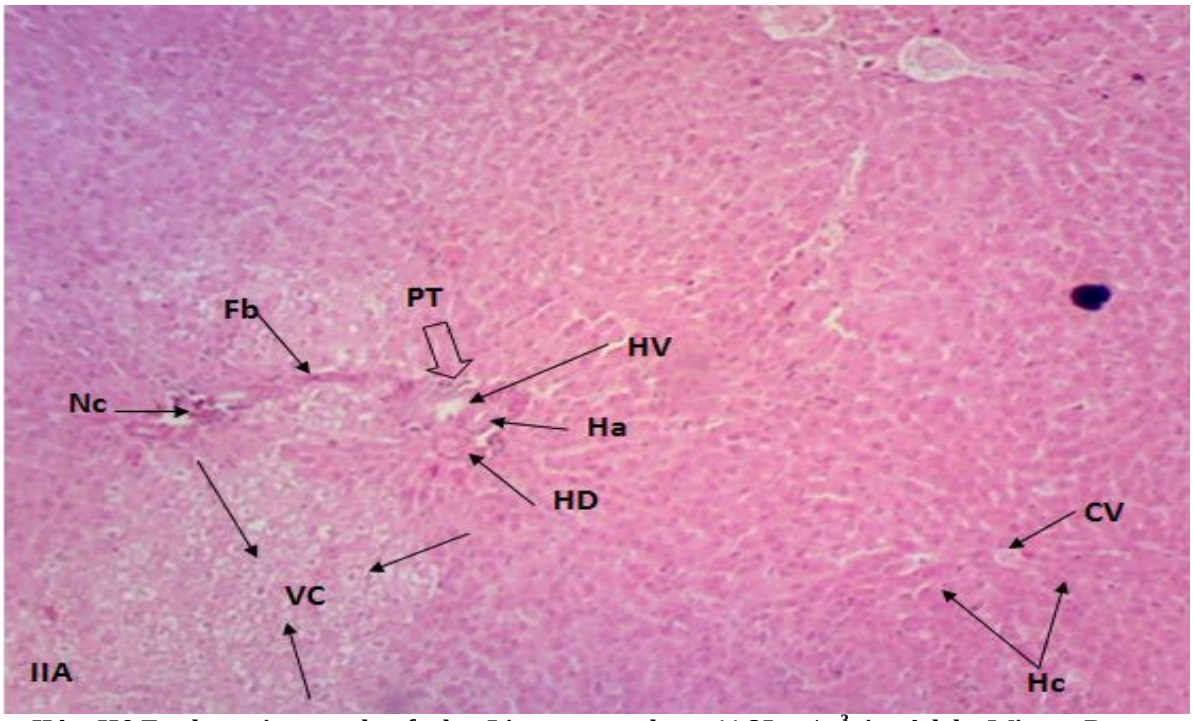

Plate IIA: H&E photomicrograph of the Liver exposed to 11.25mg/m^3 in Adult Wistar Rat at x150 magnifications. Fb= Fibrosis; Nc= Necrosis; PT= Portal triad; HV= Hepatic Vein; Ha= Hepatic artery; HD= Hepatic duct; VC= Vacuolation; CV= Central vein; Hc= Hepatic cord.

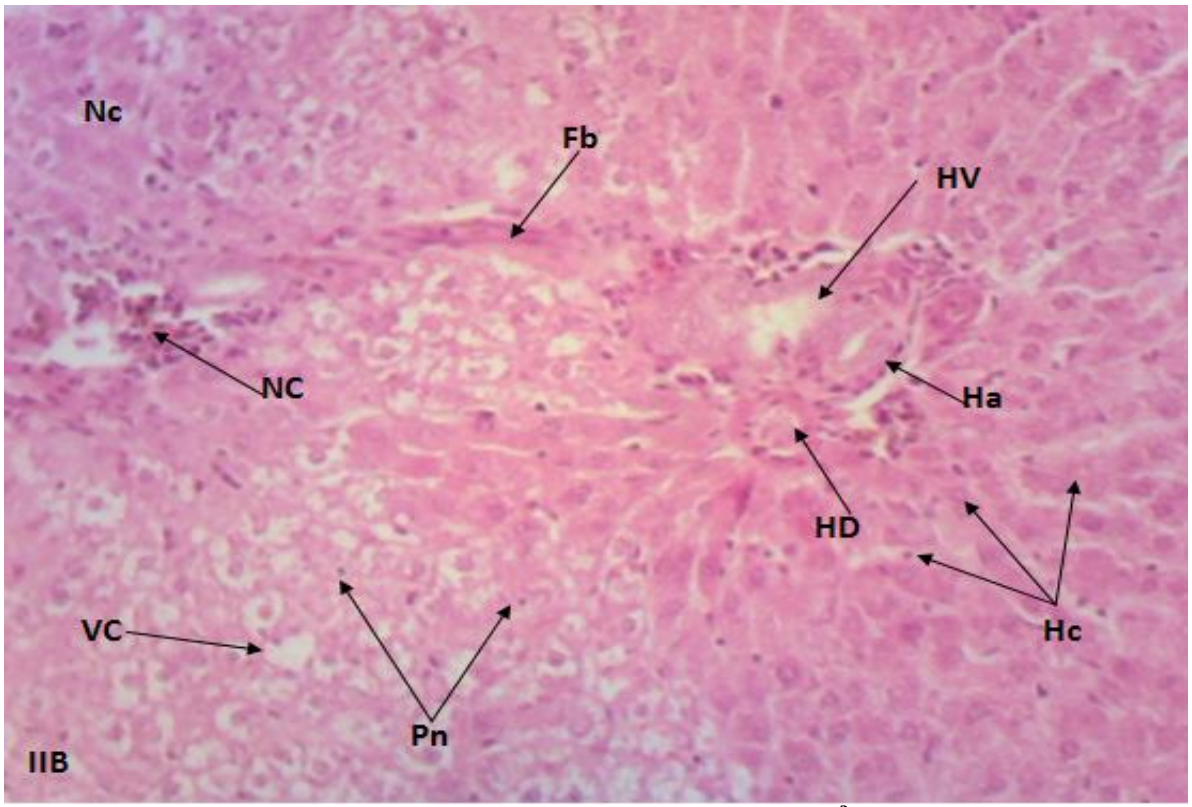

Plate IIB: H&E photomicrograph of the Liver exposed to 11.25mg/m^3 in Adult Wistar Rat at x600 magnifications. Fb= Fibrosis; Nc= Necrosis; PT= Portal triad; HV= Hepatic Vein; Ha= Hepatic artery; HD= Hepatic duct; VC= Vacuolation; Pn= Pyknosis; Hc= Hepatic cord.

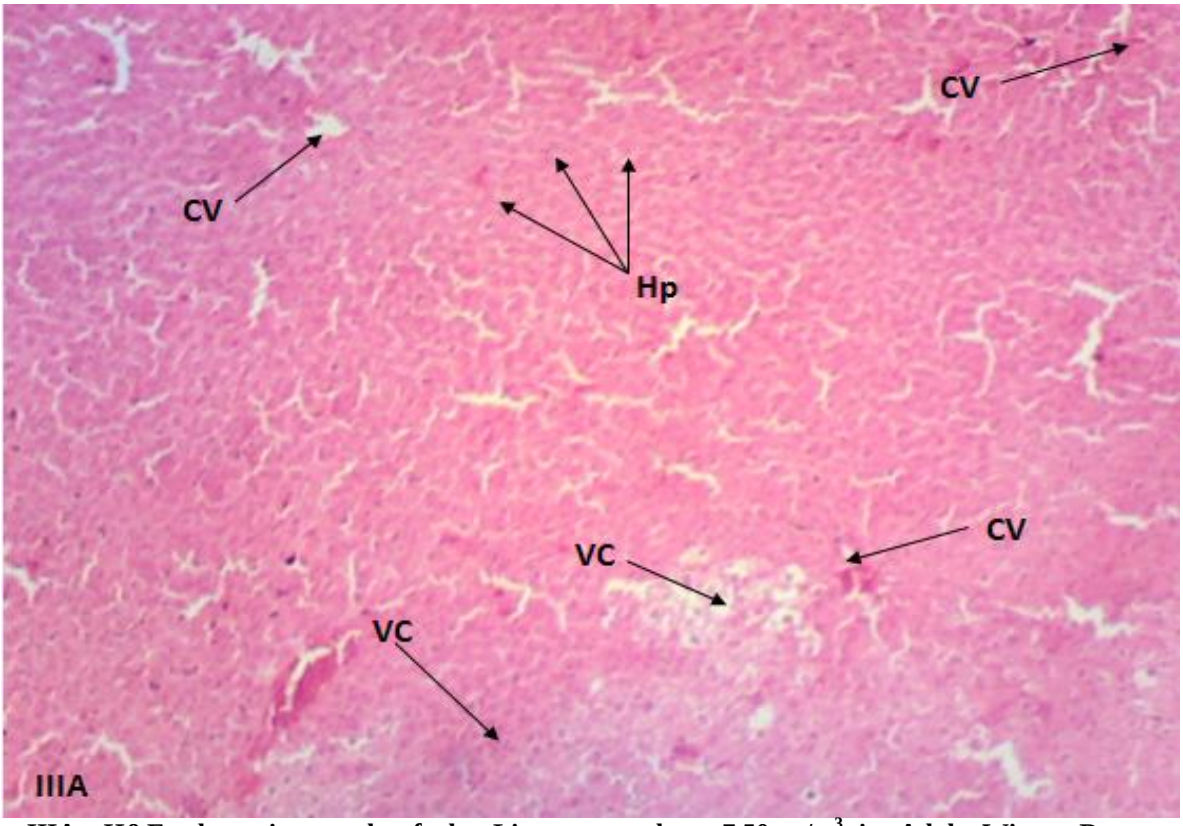

Plate IIIA: H&E photomicrograph of the Liver exposed to 7.50mg/m^3 in Adult Wistar Rat at x150 magnifications. HV= Hepatic Vein; Hp= Hepatocytes.

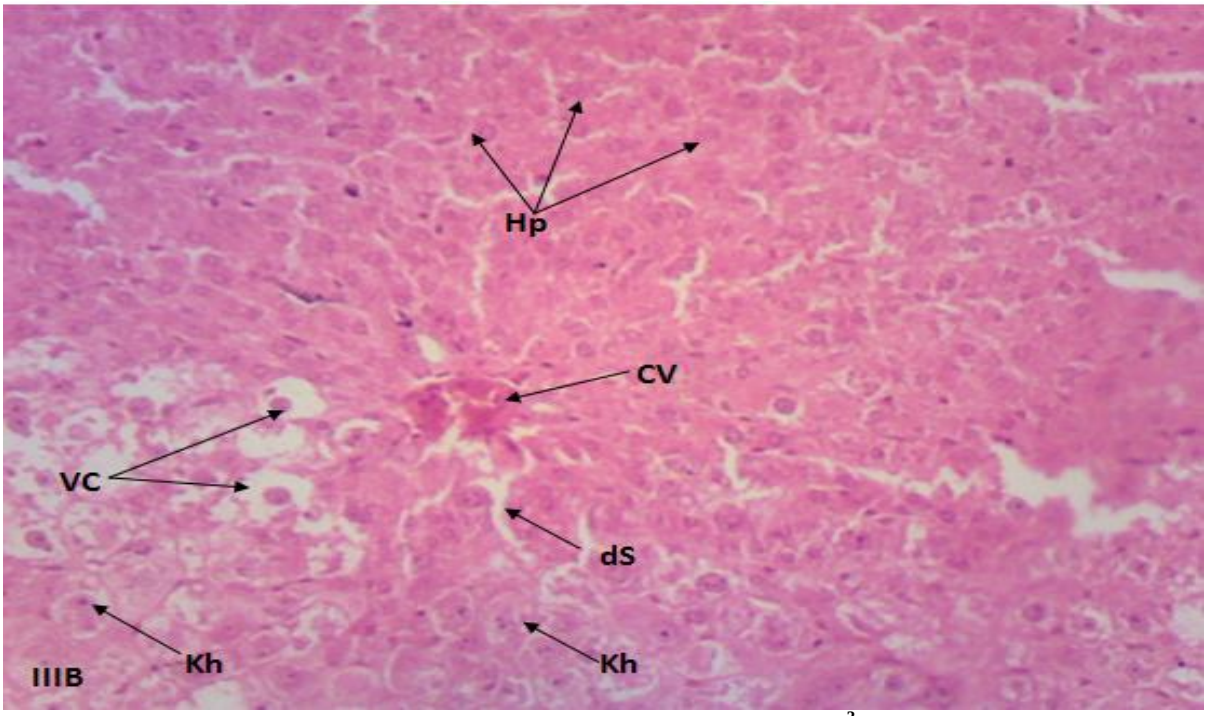

Plate IIIB: H&E photomicrograph of the Liver exposed to 7.50mg/m^3 in Adult Wistar Rat at x600 magnifications. Hp= Hepatocytes, VC= Vacuolation; dS= Dilated Sinusoids; Kh= Karyorhexis; CV= Central Vein.

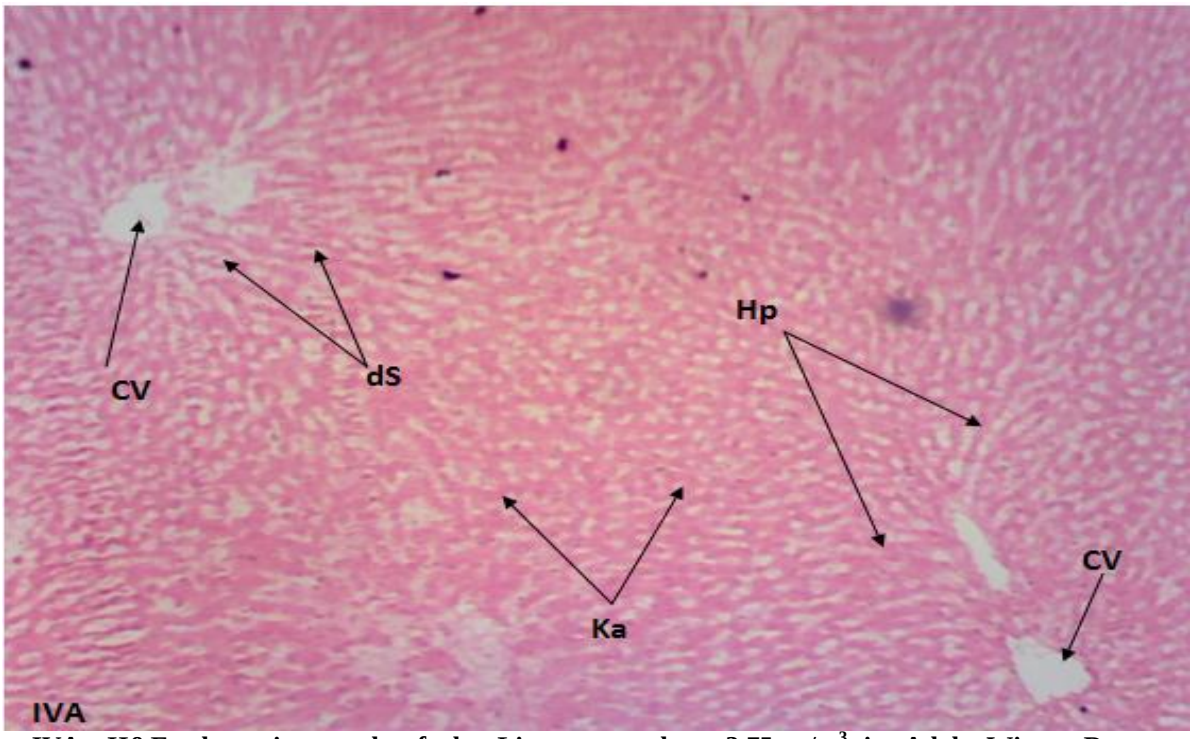

Plate IVA: H&E photomicrograph of the Liver exposed to 3.75mg/m^3 in Adult Wistar Rat at x150 magnifications. CV= Central Vein; dS= Dilated Sinusoid; Ka= Karyolysis; Hp= Hepatocytes.

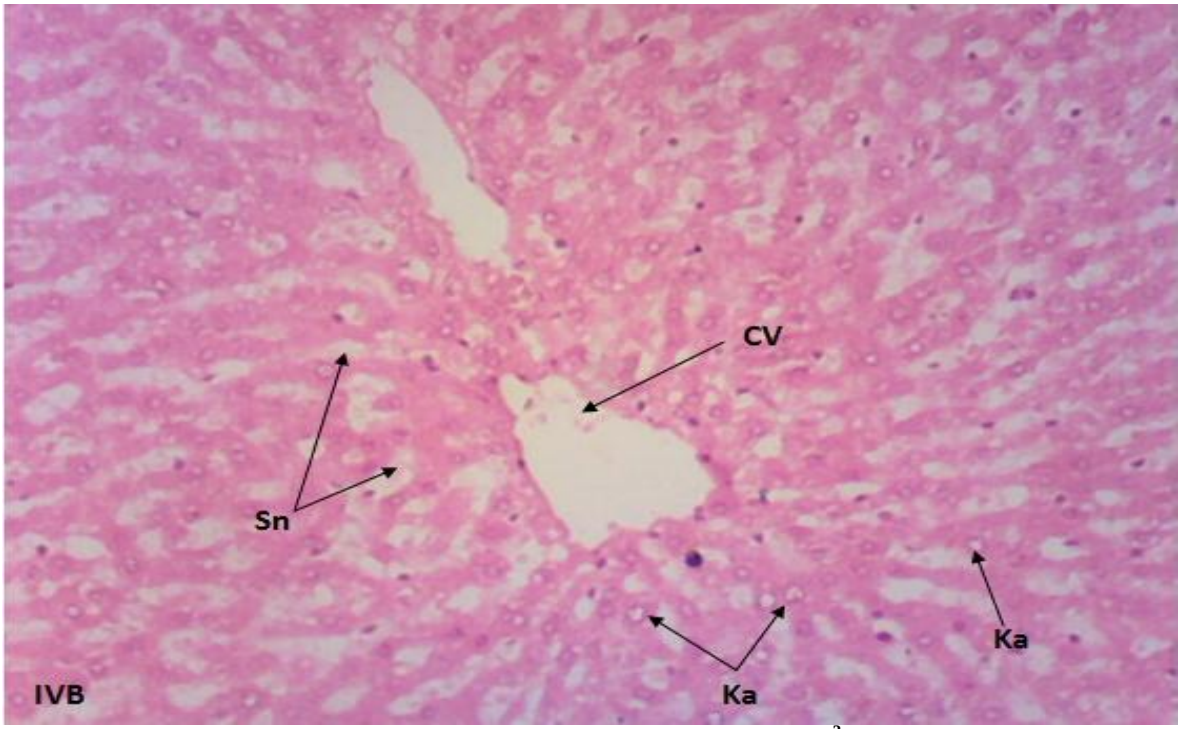

Plate IVA: H&E photomicrograph of the Liver exposed to 3.75mg/m^3 in Adult Wistar Rat at x600 magnifications. CV= Central Vein; dS= Dilated Sinusoid; Ka= Karyolysis; Hp= Hepatocytes.

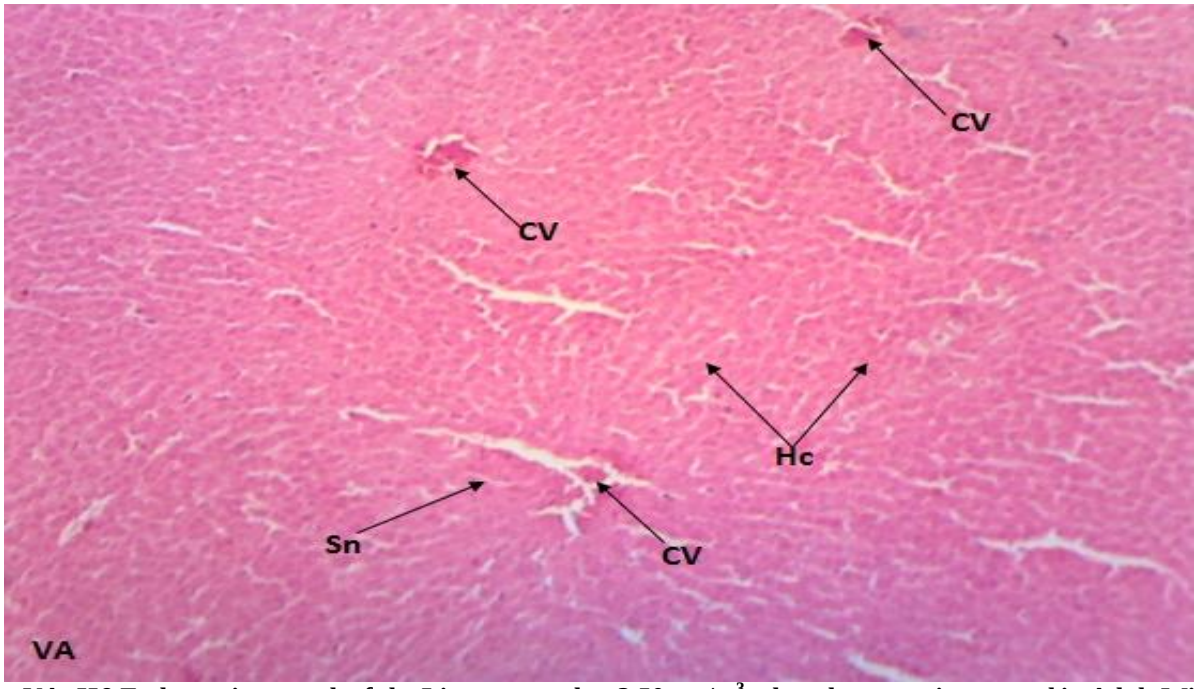

Plate VA: H&E photomicrograph of the Liver exposed to 2.50 mg/m^3 ethanol as negative control in Adult Wistar Rat at x150 magnifications. CV= Central Vein; Sn= Sinusoid; Hc= Hepatic cord.

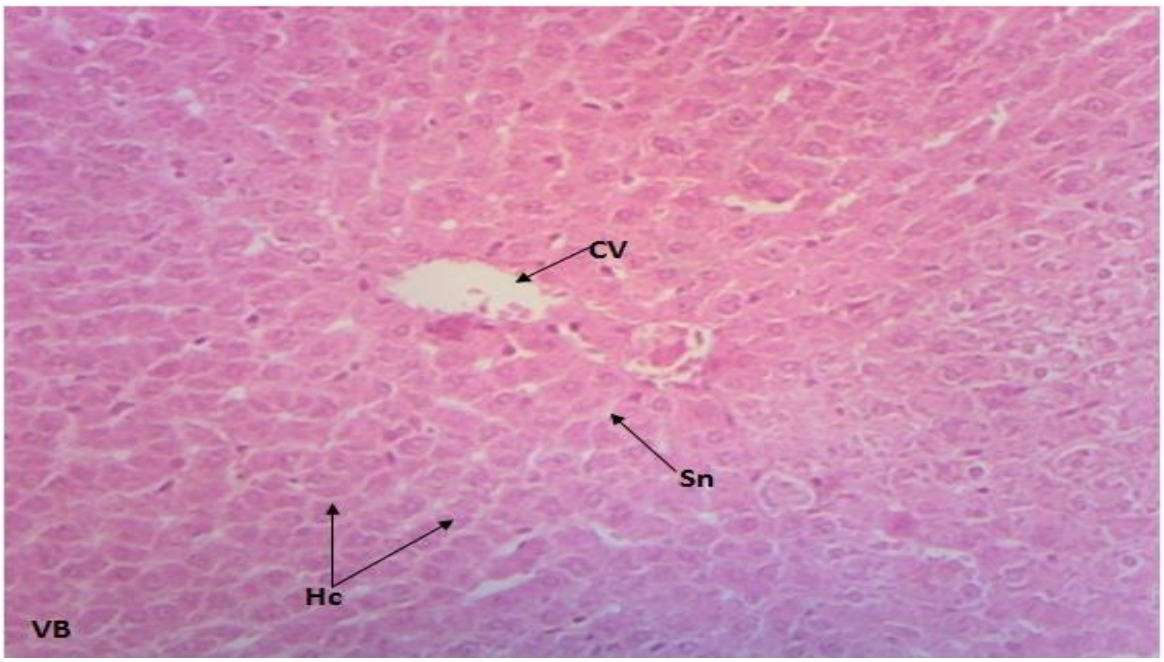

Plate VB: H&E photomicrograph of the Liver exposed to 96% ethanol as negative control in Adult Wistar Rat at x600 magnifications. CV= Central Vein; Sn= Sinusoid; Hc= Hepatic cord.

Table 1 shows one way ANOVA test for liver enzymes in Adult Wistar Rats exposed to various doses of dichlorvos in ethanol solution through inhalation. The result shows significant increase [$p< 0.005$] in the values of the ALB in the order of III > IV > V when compared with the positive control group. For the ALP, AST and ALT there was significant increase in the entire treated group when compared to normal. However the values were higher in the order of groups IV > III > V.

Plate IA shows the photomicrograph of Adult Wistar Rat exposed to ambient air at x150 magnification. The plate shows cords of normal hepatocytes [Hc] radiating from the centrally located vein [CV] flanked by radially arranged sinusoids [Sn]. At higher magnification [x600], in plate IB the hepatic cords appear rather more conspicuous separated from each other by sinusoids [Sn]. The hepatocytes nuclei appear both at euchromatic and heterochromatic stages.

The photomicrograph in Plate IIA presents a section of liver of Adult Wistar Rat exposed to 11.25 mg/m^3 dichlorvos in ethanol solution. At lower resolution [x150] the portal triad [PT] in the plate shows hepatic vein [HV], artery [Ha] and duct [HD]. The general histoarchitechture of the tissue indicates necrosis [Nc] of the central vein, patches of lipolitic vacuolation [VC], and mild element of fibrosis [Fb] of the hepatic tissue. At higher magnification, the necrosis [Nc], vacuolation [VC], and fibrosis [Fb] appeared distinct. In addition, the hepatocytes indicate mild pyknosis [Pn] of the hepatic nuclei, hepatic artery [Ha], vein [HV] and duct [HD] of the portal triad [PT].

The photomicrograph of Adult Wistar Rat exposed to 7.50 mg/m^3 at x150 magnification was shown in Plate IIIA. There were patches of mild lipolitic vacuolation [VC] of the hepatocytes. At higher magnification in plate IIIB, the vacuolations [VC] were rather more prominent with the hepatocytes nuclei mildly karyorhexic [Kh]. The central veins were slightly congested, thus bearing collapsed lumen and hence indicating mild element of steatosis.

Plate IVA shows a photomicrograph of Adult Wistar Rat liver exposed to 3.75 mg/m^3 dichlorvos in ethanol solution. It indicates mild lipolitic vacuolations of hepatocytes [VC] radiating from the central veins [CV] flanked by dilated sinusoid [dS]. At higher resolution Plate IVB, the vacuolations were more intense with some of the hepatocytes showing patches of karyolitic nuclei [Ka], dilated sinusoid [dS] and wide empty central veins [CV] with slight exudates.

The rat exposed to 2.50 mg/m^3 ethanol was presented in plate VA and VB. At lower magnification plate VA, the hepatocytes appeared normal featuring central vein [CV] and hepatic cords that flanked by sinusoid [Sn]. At higher magnification, it showed portal triad bearing dilated central vein [CV], distinct hepatic cords [Hc] and normal hepatic sinusoid [Sn].

DISCUSSIONS

Our study showed dichlorvos [DDVP], the active component of the commonly used insecticides *Ota pia – pia or Madarar pia – pia* altered the histoarchitecture of the liver and enzymes activity in Adult Wistar Rats by inhalation. These alterations were more striking in the hepatocellular arrangements of the hepatocytes and vessels in dose-dependent manner. Functionally, the liver plays important role in carbohydrates, proteins and lipids metabolism [16]. Since the liver is the major site of metabolism and is susceptible to both endo- and exo-toxins exposure, therefore biotransformation of toxic compound into less harmful form to reduce toxicity occurs in the liver [17]. The hepatocytes are the first part of the liver that is directly exposed to exo-toxins such as the dichlorvos. Therefore continuous release of dimethyl phosphate as a metabolic waste product over the period of exposure implies continuous overloading of the liver with dimethyl phosphate which might have injured the hepatocytes, thereby releasing mitochondrial and cytosolic enzymes thus, elevating the serum levels of the enzymes. This claim could be corroborated from the series of degenerations of hepatocellular architecture presented in our results. We also observed significant increase [$p<0.05$] in the serum levels of ALB, ALT and AST however, ALP decreased across the groups when compared to control group value. According to Paliwal *et al.*,[18] transaminases are proteolytic enzymes that help in protein metabolism; therefore a damaged liver will indicate high level of serum Alanine transaminases [ALT] and Aspartate transaminases [AST]. He equally stated that Aspartate transaminase is the mitochondrial enzyme, predominantly found in the liver, skeletal muscles and kidneys, and that Alanine transaminase is a cytosolic enzyme, which is more specific for the liver than Aspartate transaminase. The reasons for the significant increase in the serum level of the liver enzymes and the hepatocellular architecture could not be farfetched also from the continuous accumulations of dimethyl phosphate, DDVP by-product that could have damage the hepatocellular histology and therefore released the liver enzymes into blood circulation thus, raised the serum level.

CONCLUSION

Prolong exposure to dichlorvos through inhalation caused hepatocellular degeneration in Adult Wistar rats. These further led to significant increase [$p<0.05$] in serum hepatocellular enzymes level. We therefore recommend that those who work with or handle dichlorvos should take prudent steps to reduce duration of exposures as long-term exposure may lead to hepatocellular damage thereby increasing the serum level of liver enzymes.

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