Chronic inhalation of 2,2-dichlorovinyl dimethyl phosphate (DDVP) induces organ pathology in the adult albino rats

John O. Ogunsola[1], Olayinka A. Oridupa[2], Kehinde O. Awotunsin[2], Adebowale B. Saba[2]

[1]*Veterinary Teaching Hospital, University of Ibadan, Ibadan, Nigeria,* [2]*Department of Veterinary Pharmacology and Toxicology, University of Ibadan, Ibadan, Nigeria*

SUMMARY

The acute toxic effects of the acetyl cholinesterase-inhibiting pesticide, DDVP, following oral and dermal exposure are well recorded in literature. The ability of DDVP to easily vaporize makes the aero-nasal route a possible means of exposure, albeit chronically. This study aimed to describe the pathology, if any, of the heart, kidney and liver following chronic exposure to various concentrations of DDVP via inhalation.

Sixty male Wistar rats were divided into 6 groups (A-F) of 10 rats each. Rats in Group A were exposed to distilled water only, while rats in groups B, C, D, E and F were exposed to 20, 40, 60, 80 and 100% v/v concentrated fumes of DDVP respectively. Duration of inhalational exposure was for 90 days. The heart, liver and kidney of the rats in the groups were extracted for routine histopathology. Organ pathologies were semi-quantitatively scored and analyzed across and between the 6 groups.

Generally, lesions were of progressive severity with increasing concentrations of DDVP. Across the organs, pathology was related to altered vascular and degenerative changes. Specifically, the heart, kidney and liver showed shredding of cardiomyocytes, sloughing of renal tubular epithelial cells with dilated tubular lumina, and hepatocellular degeneration and necrosis respectively. Inflammatory changes were limited to the livers of rats exposed to 80 and 100% v/v DDVP.

It was concluded that DDVP induced altered vascular and degenerative changes following chronic exposure via inhalation. Safer alternatives to aerosolized DDVP-containing insecticides are recommended for the control of arthropod vectors in enclosures.

Key words: Dichlorvos – Heart – Histopathology – Kidney – Liver – Vascular changes

INTRODUCTION

Dichlorvos or 2,2-dichlorovinyl dimethyl phosphate (DDVP) is an organophosphate insecticide with contact, respiratory and stomach action (Ecobion, 1996). Like other organophosphate insecticides, it is neurotoxic in action, and acts on the nervous system by inhibiting the enzyme acetyl cholinesterase (AchE) (Zhao et al., 2015; Zhang et al., 2015). The action between an organophosphate insecticide and the active site in the AchE protein results in the formation of a transient intermediate complex that partially hydrolyses, leaving a stable phosphorylated and largely non-reactive inhibited enzyme that, under normal circumstances, can be reactivated only at a very low rate (Ecobion, 1996). This results in disruption of the neuromuscular system.

Dichlorvos has a high acute toxicity. The oral LD50 in rats is between 56 and 108mg/kg (ETN, 1993; Günde and Yerli, 2012; Isegbe et al., 2016). It is classified by the WHO (1989) as a Class IB,

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Corresponding author: Dr. John O. Ogunsola. Veterinary Teaching Hospital, University of Ibadan, Ibadan, Nigeria. Phone: +2347031604111.

E-mail: ogunsolajo@yahoo.com

'highly hazardous'. The dermal toxicity is similar to oral toxicity, and dermal exposure is a cause for concern (WHO, 1989). The literature is replete with reports of acute toxicity of DDVP in man (Kumar et al., 2009), companion animals (Snow, 1973), and laboratory animals (Owoeye et al., 2012, 2014) via the oral (Snow, 1973) and dermal routes (US Public Health Service, 1995).

DDVP is rapidly metabolized in the liver by esterase to dimethyl phosphate and dichloroacetaldehyde, and the former is excreted by the kidney in the urine (Garcia-Repetto et al., 1995; Somia and Madiha, 2012). Lesions seen following acute toxicity could be absent or non-specific. When present, lesions have consistently resolved around altered vascular changes. Lesions reported have included severe pulmonary edema and congestion, emphysema, bronchoconstriction with increased mucus production, marked congestion of coronary blood vessels, haemorrhages and congestion of the bowel and other organs (Snow, 1973; Luty et al., 1998), and destruction of the renal corpuscles (Owoeye et al., 2012, 2014).

Although DDVP is marked as 'very toxic by inhalation' acutely by WHO (1998), reports on its effect following inhalation of sublethal doses over a long period of time are very few (Ajiboso et al., 2012; Owoeye et al., 2012; Kemabonta and Akinhanmi, 2013). It is widely accepted that DDVP vapourizes quickly and easily (Howard, 1991). Chronic toxicity of a pesticide is assessed differently from acute toxicity (LD50). Different tests are performed on animals, which help to predict whether a pesticide will cause long-term effects. Test animals are exposed to sublethal levels of pesticides for periods ranging from about 90 days to several years. They are examined for a wide variety of toxic effects from dermal, oral and respiratory exposure (ETN, 1993). The aim of this study was therefore to assess the changes in the histopathology of the heart, liver and kidney of Wistar rats exposed to varying concentrations of DDVP by inhalation for a period of 90 days.

MATERIALS AND METHODS

Ethical approval

The experimental protocols were carried out according to the guidelines set by the University of Ibadan Animal Care and Use in Research Ethical Committee, which conforms to internationally acceptable guidelines on the ethical use of experimental animals in research.

Animals and acclimatization

Sixty male albino rats with a mean weight of about 150g were obtained from the experimental animal unit of the Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan. They were stabilized for two weeks during which they were allowed access to pelleted commercial rat feed and portable clean water ad libitum.

Exposure and study design

The pesticide, 2,2 dichlorovinyl dimethyl phosphate (DDVP) (Sniper®) was purchased from a chemical retail store in Ibadan, Nigeria. The rats were randomly divided into six groups (A-F) of ten rats each. Group A were control group that were exposed to zero percent DDVP solution. The other groups of rats were exposed to different concentrations of DDVP (as indicated in Table 1) for periods of ninety days. The improvised diffusion chamber, which was hung on one corner of the cage, was adequately secured and body contact was prevented. All the rats were allowed free access to feed and portable drinking water throughout the ninety days of the experiment.

Clinical signs

Animals in all groups were monitored for cholinergic toxicity signs such as tremors, ocular and nasal discharges, as well as increased frequency and consistency of salivation, urination and defecation.

Histopathology

At completion of exposure, animals were anaesthetized with ketamine anaesthesia (10 mg/kg intraperitoneally). The liver, heart and kidney of each animal were also harvested for routine histopathology as described by Bancroft and Gamble (2008). Succinctly, the harvested organs were fixed in 10% formalin, then dehydrated with grades of ethanol (70, 80, 90, 95 and 100%). Dehydration was then followed by clearing the samples in two changes of xylene. Samples were then impregnated with 2 changes of molten paraffin wax, then embedded and blocked out. Paraffin sections (5-6 µm) and thick transverse sections of the liver and the lung were cut using a rotary microtome and mounted on glass slides. The slides were stained with haematoxylin and eosin (H&E). Stained sections of control and treated rats were examined under the light microscope (Olympus CH, Japan) for histological alterations semi-quantitatively by two independent pathologists who were oblivious of the groups. In cases where their semi-quantitative assessments were not the same, a median point was selected. Photomicrographs were obtained with the aid of Amscope camera fitted on an Accu-scope microscope. Images were analyzed with the aid of ToupView software.

Table 1. Distribution of rats to inhalational exposure to various concentrations of dichlorvos.

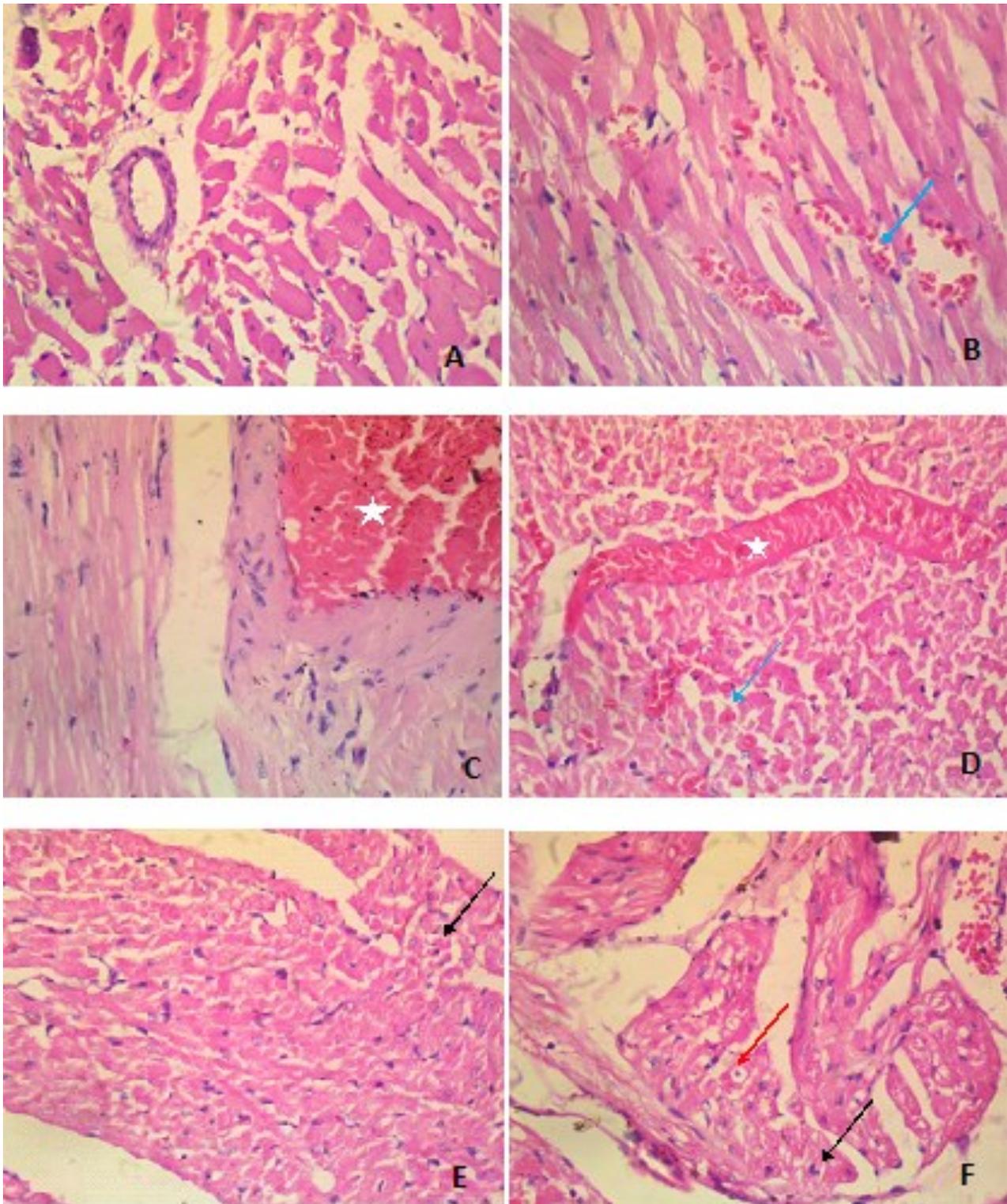| Groups	Exposure Levels/Concentration
A	0% (0ml DDVP/100mls distilled water v/v)
B	20% (20mls DDVP/80mls distilled water v/v)
C	40% (40mls DDVP/60mls distilled water v/v)
D	60% (60mls DDVP/40mls distilled water v/v)
E	80% (80mls DDVP/20mls distilled water v/v)
F	100% (100mls DDVP/0ml distilled water v/v)

Fig 1. Heart. Wistar rats with chronic inhalation exposure to DDVP concentrations (v/v) of 0% (**A**), 20% (**B**), 40% (**C**), 60% (**D**), 80% (**E**) and 100% (**F**). There are foci of congestion (star), haemorrhages (blue arrows) as well as cardiomyocyte degeneration (red arrow) and necrosis (black arrow).

Statistical analysis

Lesions at histopathology were scored semi-quantitatively as follows: 0 – absent/normal, 1+ mild, 2+ moderate, and 3+ marked by two (2) independent pathologists. These scores across individuals in the group were subjected to a measure of central tendency (mode).

RESULTS

Clinical signs of toxicity

Severe toxicity was observed after 40 days of continuous exposure of rats in group F to 100% DDVP. Cholinergic signs observed included anorexia, muscular tremors, nasal discharges, in-

creased frequency of salivation and urine staining, and diarrhoea. No adverse effects or cholinergic signs were observed in the functional, observational battery and locomotor of the rats exposed to between 20% and 80% DDVP.

Histopathology

Heart

Compared with the control group, inhalational exposure to DDVP resulted in varying degrees of degeneration and necrosis of the cardiomyocytes. There were also a few foci of detachment and shredding of the cardiac muscle. Notably, there were vascular changes resulting in varying degrees of congestion of glomerular capillary tufts and subscapular capillaries, and some foci of extravasation of the blood resulting in haemorrhages. Inflammatory aggregates were absent in heart sections of all the groups. Details are presented in Fig. 1A-F and Table 2.

Kidney

Kidney sections of rats from Groups B and C revealed mild cloudy swelling and sloughing off of renal tubular epithelium with presence of intraluminal eosinophilic tubular casts. Kidneys of rats from Groups D and E had mild to moderate congestion of the subcapsular vessels and glomerular capillary tufts, as well as moderate sloughing off of the tubular epithelium. The renal section of rats in Group F revealed multiple foci of marked sloughing off of the renal tubular epithelium in the corticomedullary junction and the medulla creating a dilated appearance for affected tubules. Also, inflammatory cellular aggregates were absent in the kidney sections of all the groups. Details are presented in Fig. 2A-F and Table 3.

Liver

Hepatic lesions present in Groups B-F included varying degrees of cord atrophy (thinning of hepatic cords with dilated sinusoids), single-cell hepatocellular necrosis, Kupffer cell hyperplasia and congestion of hepatic sinusoids. Surprisingly, only rats in Group B showed mild vacuolar change of hepatocytes. Inflammatory mononuclear cellular aggregates were only present in the liver of rats in Groups E and F. Details are presented in Fig. 3A-F and Table 4.

DISCUSSION

This study has shown that chronic inhalational exposure to varying concentrations of dichlorvos (from 20% to 100% v/v) over a period of 90 days induced histopathologic changes in the heart, kidney and liver. These changes were of progressive severity with increasing concentration, and were mostly degenerative, altered vascular flow (including congestion and haemorrhages), and to a lesser extent inflammatory.

Circulatory changes were the dominant feature with marked dilation and engorgement of capillaries and veins in dogs with oral exposure to dichlor-

vos (Snow, 1973). At histology, Snow (1973) reported subendocardial and epicardial haemorrhages. Random foci of cardiac haemorrhages as well as shredding/separation and necrosis of cardiomyocytes were observed. It is plausible that the neuropathy induced by dichlorvos causes intense vagal stimulation of the cardiac muscle, which in turn leads to rupture of engorged veins and capillaries resulting in cardiac haemorrhages.

Evidence of glomerulopathy was not found in this study. This is in contrast with studies by Owoeye et al. (2014) that reported damage to the renal cor-

Table 2. Semiquantitative assessment of histopathology of the heart in Wistar rats exposed to various levels of DDVP inhalation-induced toxicity.

	A	B	C	D	E	F
CM Degeneration	-	1+	1+	2+	2+	3+
CM Necrosis	-	-	1+	1+	1+	2+
CM Shredding	-	1+	-	1+	1+	2+
Inflammatory aggregates	-	-	-	-	-	-
Haemorrhages	N	Y	N	Y	Y	Y
Congestion	-	1+	3+	3+	-	-

CM cardiomyocyte; Y seen; N not seen; - absent; 1+ mild; 2+ moderate; 3+ marked; DDVP Exposure levels (v/v): A 0%; B 20%; C 40%; D 60%; E 80%; F 100%.

Table 3. Semiquantitative assessment of histopathology of the kidney in Wistar rats exposed to various levels of DDVP inhalation-induced toxicity

	A	B	C	D	E	F
Glomerulopathy	-	-	-	-	-	-
Tubular degeneration	-	1+	2+	1+	2+	1+
Tubular necrosis/dilation	-	-	1+	1+	2+	3+
Tubular casts	-	-	2+	-	-	-
Inflammatory aggregates	-	-	-	-	-	-
Congestion	-	-	-	-	1+	1+

- absent; 1+ mild; 2+ moderate; 3+ marked;
DDVP Exposure levels (v/v): A 0%; B 20%; C 40%; D 60%; E 80%; F 100%

Table 4. Semiquantitative assessment of histopathology of the liver in Wistar rats exposed to various levels of DDVP inhalation-induced toxicity

	A	B	C	D	E	F
Cord atrophy	-	-	2+	2+	2+	2+
Random hepatocellular necrosis	-	1+	1+	1+	2+	2+
Vacuolar change	-	2+	-	-	-	-
Kupffer cell hyperplasia	-	1+	1+	2+	2+	2+
Inflammatory aggregates	-	-	-	-	-	2+
Congestion	-	-	-	-	-	1+

- absent; 1+ mild; 2+ moderate; 3+ marked;
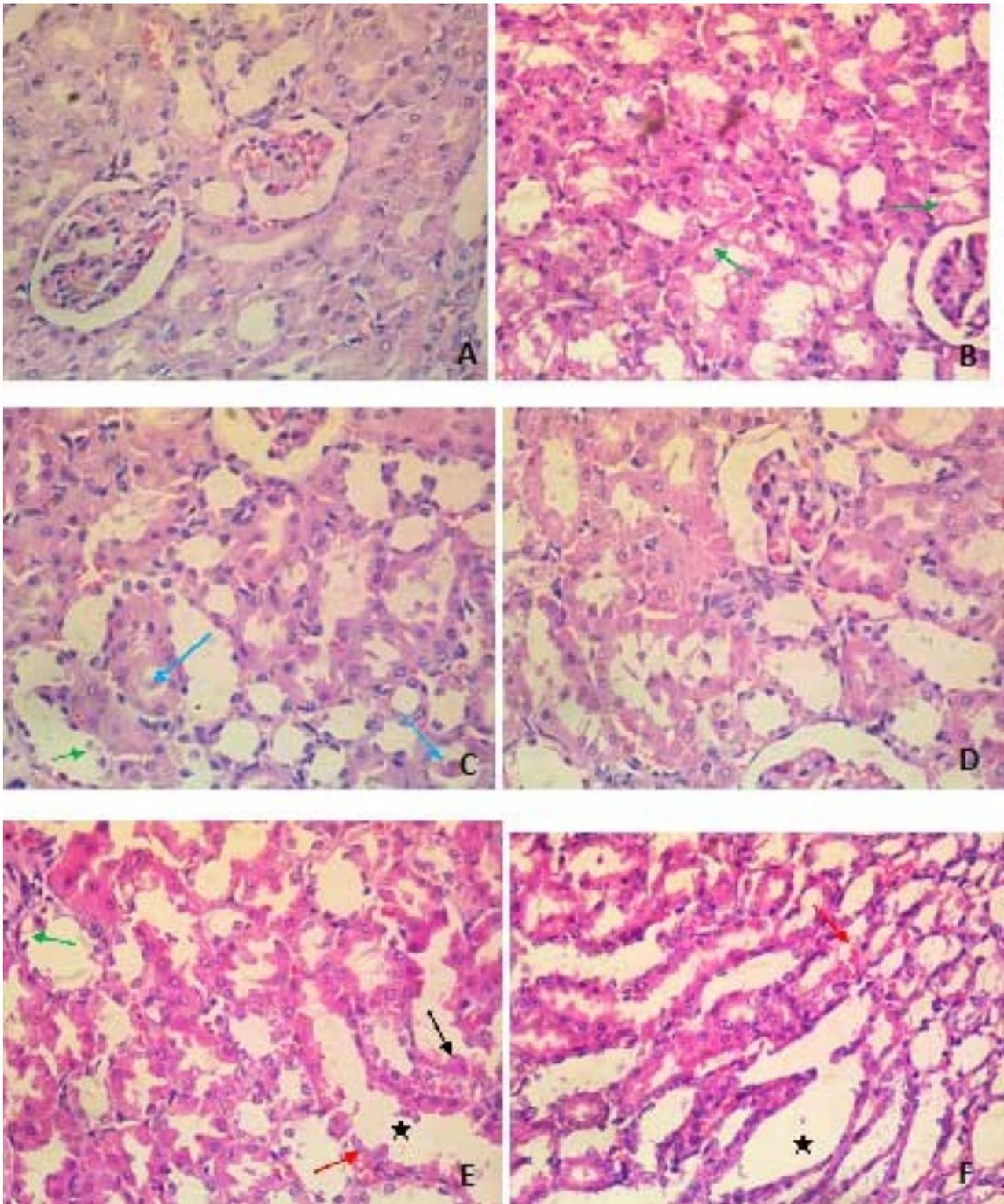DDVP Exposure levels (v/v): A 0%; B 20%; C 40%; D 60%; E 80%; F 100%

Fig 2. Kidney. Wistar rats with chronic inhalation exposure to DDVP concentrations (v/v) of 0% (A), 20% (B), 40% (C), 60% (D), 80% (E) and 100% (F). There are multiple foci of tubular casts (blue arrows), varying degrees of degeneration (green arrows) and necrosis (black arrows) of tubular epithelial cells resulting in cyst-like dilated lumens (star). There are varying degrees of vascular congestion (red arrows).

puscles. However, our findings of tubular degeneration, tubular casts, sloughing off of tubular epithelial cells and cystic-like dilation of tubules corroborated the findings of Snow (1973), Luty et al. (1998), Al-Attar (2010), Somia and Madiha (2012) and Owoeye et al. (2014).

In tandem with other authors (Snow, 1973; Luty et al., 1998; Celik et al., 2009; Binukumar et al., 2010; Owoeye et al., 2012; Somia and Madiha, 2012; Kemabonta and Ainhanmi, 2013; Ajiboso et al., 2016), dichlorvos induces various pathologies in the liver. Evidence for liver pathology following
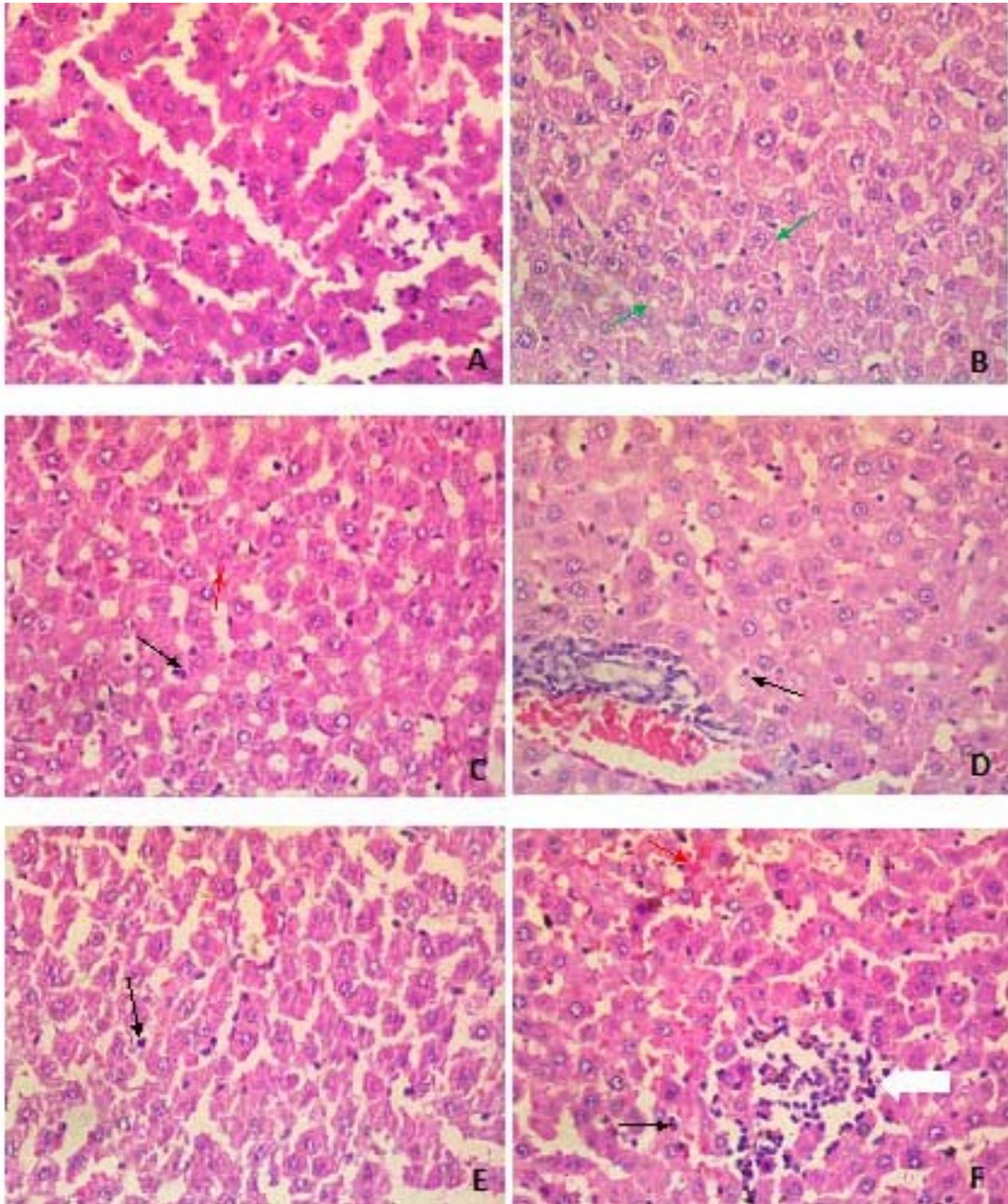
Fig 3. Liver. Wistar rats with chronic inhalation exposure to DDVP concentrations (v/v) of 0% (A), 20% (B), 40% (C), 60% (D), 80% (E) and 100% (F). There are varying degrees of cord atrophy with dilated congested sinusoids (red arrows). There are random foci of hepatocellular vacuolar change (blue arrows) and single cell hepatocellular necrosis (black arrows). Note the inflammatory aggregates (thick arrow).

acute and subchronic exposure to DDVP has been provided through haematology (Celik et al., 2009), serum chemistry (Celik et al., 2009; Kemabonta and Ainhanmi, 2013; Ajiboso et al., 2016), histology (Snow, 1973; Luty et al., 1998; Owoeye et al., 2012; Somia and Madiha, 2012), ultrastructure (Luty et al., 1998) and molecular studies (Yamano, 1996; Binukumar et al., 2010; Sharma and Singh, 2012). The liver was the only organ in our study that contained inflammatory mononuclear cellular aggregates with chronic inhalational exposure (80% and 100% v/v), apparently suggesting that

the liver might be worst hit since it is the seat of detoxification.

The basis for DDVP toxicity has been reported to be oxidative stress through generation of reactive oxygen species (Sharma and Singh, 2012). Excessive production of reactive oxygen species can lead to lipid peroxidation (Al-Attar, 2010) and damage to other macromolecules including DNA (Yamano, 1996). This is likely to be the basis for the consistent finding of cardiomyocyte, renal tubular epithelial cell and single-cell hepatocellular necrosis, associated with exposure to higher concentrations of DDVP via inhalation. Inflammation has been reported in the heart (Luty et al., 1998) and kidney (Luty et al., 1998; Al-Attar, 2010; Somia and Madiha, 2012). Surprisingly, findings from this study revealed that inflammatory cellular aggregates were restricted to the liver and absent in the heart and kidney. The role of apoptotic pathways (programmed cell death) as opposed to necrosis in the heart and kidney may be incriminated for this finding. This is supported by the finding of DDVP-induced DNA damage reported by Yamano (1996).

The toxicological method employed in this study involved neither direct spraying nor release from sprayed surfaces (active exposure), but release from non-contact perforated containers (passive exposure). Thus, an indication that DDVP has mobility potential whether sprayed or not (in use and not in use), and this mobility is dose-related (concentration dependent). This is in agreement with the volatility of DDVP described by Howard (1991) and has been employed by Ajiboso et al. (2012) in sub-chronic inhalational toxicity studies.

CONCLUSION

The results gathered from the study have shown deleterious effects on organs following chronic exposure to DDVP fumes. Chronic inhalation remains an often neglected or overlooked route of exposure to organophosphate pesticides such as DDVP. This study is meant to create awareness of the toxicity of DDVP pesticide following passive or active exposure, and the hazardous effects on individuals in Nigeria as a result of poor handling. It is concluded that users of DDVP-containing pesticides are at risk of developing organ damage following prolonged active or passive exposure to such pesticides if appropriate protective measures for the aero-nasal route exposure are not provided.

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