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Effects of Pre-treatment with Aqueous and Methanolic Leaf extracts of *Morinda lucida* (Benth) on Dichlorvos-Induced Toxicity in Balb/c mice

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Abstract

The pesticide Dichlorvos, also known as DDVP, is an organ ophosphate insecticide widely used in agriculture, institutional and industrial sites, homes, and on pets, that is, acutely toxic to animals including humans. DDVP is used by farmers in Nigeria although listed by WHO as a possible carcinogen. *Morinda lucida* is a Nigerian medicinal plant known to exhibit strong antioxidant activity and therefore may alleviate DDVP induced damage in cells. This study was designed to assess the potential of pre-treatment of Balb/c mice with *Morinda lucida* in reducing dichlorvos induced cell damage. Six groups of mice, each having five mice, were orally administered with the following: Group A— 0.1ml of 0.9% v/w Normal saline; Group B—0.1ml of 10mg/kg of DDVP only. Groups C, D, E and F were pre-treated with 0.1ml of 300 and 500mg/kg aqueous and methanol extracts of *Morinda lucida* leaves respectively and after 14days, with 0.1ml of 10mg/kg DDVP for five days. Haematological parameters, activities of Serum biochemical and antioxidant enzymes Malondialdehyde (MDA), Catalase (CAT), Superoxide dismutase (SOD) and Glutathione superoxide hydrogenase (GSH) were measured to evaluate tissue damage by the pesticide. Compared with the Dichlorvos (only) group, mice pre-administered with *Morinda lucida* showed significantly ($P<0.05$) enhanced antioxidant activity, haematological and serum biochemical parameters. *Morinda lucida* reduced tissue damage resulting from dichlorovos and therefore, can be used to ameliorate Dichlorvos toxicity.

Effets du prétraitement par les feuilles aqueuses et méthanoïques d' extraits *Morinda lucida* (Benth) sur la toxicité induite par le dichlorvos chez des souris Balb /c

Résumé

Les dichlorvos de pesticides, également connu sous le nom DDVP, est un insecticide organophosphoré largement utilisé dans l'agriculture, les sites institutionnels et industriels, des maisons et il est si toxique aux animaux ainsi bien que aux humains. DDVP est utilisé par les agriculteurs au Nigeria et classifie par WHO comme un carcinogen. *Morinda lucida* est une plante médicinale connue pour présenter une

forte activité antioxydante et c'est possible qu'il peut améliorer les dégâts de DDVP dans les cellules. Cette étude a été conçue pour évaluer le potentiel du prétraitement des souris Balb / c avec des dommages cellulaires induite par *Morinda lucida* par la réduction de dichlorvos. Six groupes de souris, ayant chacun cinq souris ont été administrés par voie orale avec les éléments suivants: Groupe A 0,1 ml de 0,9% p / v de solution saline normale, Groupe B, 0,1 ml de 10 mg / kg de DDVP seulement. Les groupes C, D, E et F ont été pré-traitées avec 0,1 ml de 300 et 500 mg / kg de methanol et d'extraits aqueux des feuilles de *Morinda lucida,* respectivement, et après 14 jours, avec 0,1 ml de 10 mg / kg pendant 5 jours DDVP. Les paramètres hématologiques, biochimiques et activités sériques des enzymes antioxydantes et le malondialdéhyde (MDA), la catalase (CAT), la superoxyde dismutase (SOD) et de glutathion superoxyde hydrogénase (GSH) ont été mesurées pour évaluer des dommages aux tissus par le pesticide. En comparaison avec le groupe unique de Dichlorvos, les souris pré-administrés avec *Morinda lucida* ont montré de manière significative (P <0,05) l'activité hématologiques et biochimiques ainsi que de paramètres de serum. *Morinda lucida* réduit les lésions tissulaires résultant d'dichlorovos et cela peut être utilisé pour améliorer la toxicité dichlorvos.

Introduction

Pesticides are the most effective means of pest eradication all over the world and their application is often the first line of action in pest management, but their abuse has reached an alarming rate resulting in adverse effects on both target and non-target organisms including humans (Saxena and Saxena, 2010). Therefore, pesticides have become a major source of concern as water, and soil pollutants. 2, 2-dichlorovinyldimethyl phosphate (DDVP) known as Dichlorvos is a synthetic organophosphate pesticide, and has been classified by the World Health Organization (WHO) as highly hazardous and toxic; Dichlorvos is sold under many trade names including Nopest®, Vapona®, Atgard®, Nuvan®, Sniper®, and Task®, among others. In Southwest Nigeria, there is evidence that Dichlorvos is the major active pesticide ingredient of locally produced *Ota-piapia* which is commonly hawked around and used for domestic purpose to kill insects. It is also used for agricultural purposes to control insects on crops and stored produce, and also to treat external parasitic infections in farmed fish, livestock, and domestic animals (Musa *et al.,* 2010). These are the possible routes of exposure. Generally, in humans, organophosphate poisoning causes the disruption of nervous processes (e.g., mental retardation) and some physiological processes which affects a large number of organs (Foley, *et al.,* 2006).

Historically and up till today, all cultures around the world have relied on medicinal plants for health care, and thus medicinal plants have formed the basis of many traditional systems of medicine (Babar, *et al.,*2013). In Africa, the use of traditional medicine is still widespread with an estimated 80% of the population consulting traditional healers (Eastman, 2005; Ogbole, *et al.,*2014). Plant-derived substances, collectively termed "phytonutrients," or "phytochemicals," are becoming increasingly known for their antioxidant activity. *Morinda lucida* (Benth) commonly known as Brimstone tree belongs to the family Rubiaceae. It is a medium sized tree about 15m tall with scaly grey bark, short crooked branches and glabrous foliage. Leaves are opposite, simple and entire. It is adapted to tropical climates and distributed across the western part of Africa. The major constituents of *M. lucida* extract have been reported to be anthraquinones and alkaloids as two novel anthraquinols (oruwalol and oruwal) and 18

anthraquinones have been isolated from the stem (wood and bark) (Lawal *et al.,* 2012). In addition, polyphenols such as tannins and flavonoids, and saponosides have also been isolated (Lawal *et al.,* 2012). Also, two known triterpenic acids (Ursolic and oleanolic acids) were isolated from the leaves (Richard, *et al.,* 2006; Lawal *et al.,* 2012).

Morinda lucida is one of the four most used plants in Nigerian ethno-medicinal preparations for the treatment of fever (Adeyemi, *et al.,* 2014). It is also used to treat diseases and ailments such as malaria, arthritis, rheumatism, leprosy, pulmonary troubles, diabetes, hypertension, cerebral congestion, dysentery, stomach-ache, ulcers, jaundice, leprosy and gonorrhea (Burkill, 1985; Akinloye and Balogun, 2012; Lawal, *et al.,* 2012).

For decades, the screening of medicinal plant materials for their therapeutic values has continued to represent potential sources of new effective medicines. Besides, evidence from epidemiological studies suggest that high consumption of fruits and vegetables is linked to reduced risk of developing most oxidative stress–induced diseases (Dani *et al*., 2008; Wasson *et al*., 2008; Atrooz, 2009). Therefore, the aim of this research is to investigate the pre-treatment effects of aqueous and methanol extracts of *Morinda lucida* before Dichlorvos-induced toxicity in Balb/C mice by assessing haematological changes, serum biochemical enzymes and antioxidant enzymes markers changes, and liver histopathology of the Balb/C mice.

Materials and Methods

Pesticide

NOPEST® (Dichlorvos) produced by NINGBO-Agrostar Industrial Co. Ltd, China with NAFDAC Reg. No. A5-0330 and marketed by Unique Agrochemicals Limited, Lagos, Nigeria, was obtained from an Agrochemical shop in Ibadan, Oyo State, Nigeria.

Plant Collection and Identification

Whole Fresh leaf samples of *Morinda lucida* used in this study were collected from a garden at Oluwatedo Estate, Aduloju, Bodija, Ibadan. The plants were identified and authenticated at the Herbarium of the Department of Botany, University of Ibadan, Nigeria.

Preparation of plant extracts

Fresh *Morinda lucida* leaves were harvested, cleaned to remove extraneous matter and air-dried at room temperature for three weeks. The dried leaves were then coarsely milled with a mechanical grinder.

Aqueous Extract

50g of milled *M. lucida* was soaked in 200ml of distilled water for 48 hours. Method of Ojewunmi *et al.* (2013) was modified; the mixture was filtered using a muslin cloth and then Whatman® filter paper 1. The filtrate was concentrated with a rotary evaporator and 11.64g of dried mass was obtained and stored in a refrigerator at 4^0C till it was ready to be used.

Methanolic Extract

50g of milled *M. lucida* was soaked in 200ml of methanol for 48 hours after which it was filtered using a muslin cloth and then Whatman® filter paper 1. 15.38g of dried mass was obtained and then stored in a refrigerator at 4^0C till it was ready to be used.

Experimental Animals

Thirty (30) three to four week old Balb/C mice with average weight of 24g were obtained from the research animal house of International Institute of Tropical Agriculture (IITA), Oyo road, Ibadan and housed under standard conditions in cages at the animal house of the Department of Zoology, University of Ibadan. They were acclimatised for 14 days in a 12/12hour light/dark schedule. The mice were fed with pelletized growers mash (Ladokun Feeds Ltd.) and water *ad libitum* throughout the period of the experiment.

Administration of Leaf Extracts and Exposure to Dichlorvos

Administration of leaf extracts and DDVP was oral with an oral canula. All the groups except the control groups were first administered the leaf extracts for 14 days before the exposure to DDVP for 5 days. The mice were randomly distributed into the following six groups, with five mice in each group:

Group A: **(negative control)**: Administered 0.1ml of 0.9% v/w Normal saline,

Group B: Administered 0.1ml of 10mg/kg of DDVP only.

Group C: Administered 0.1ml of 300mg/kg aqueous extract of *Morinda lucida* leaves and afterwards, 10mg/kg DDVP.

Group D: were administered 0.1ml of 500mg/kg aqueous extract of *Morinda lucida* leaves and afterwards, 10mg/kg DDVP.

Group E: were administered 0.1ml of 300mg/kg methanolic extract of *Morinda lucida* leaves and afterwards, 10mg/kg DDVP.

Group F: were administered 0.1ml of 500mg/kg methanolic extract of *Morinda lucida* leaves and afterwards, 0.1ml of 10mg/kg DDVP.

Twenty-four hours after the final oral administration, the mice were sacrificed and blood for haematological, biochemical and antioxidant enzymes analyses was collected from each mouse. The liver was taken out for histopathological examination.

Experiments were performed in accordance with NIH guidelines for the care and use of animals.

Haematology

Blood samples were obtained from orbital sinuses and collected in EDTA bottles via a capillary tube inserted into the medial canthus of the eye at 30^0 angle to the nose. A little pressure was applied with the thumb to puncture the tissue into the orbital sinus from where blood was collected in a capillary tube. After the required volume of blood was collected, the capillary tube was gently removed and the blood transferred into the EDTA bottles. Bleeding was stopped by applying gentle finger pressure.

The following haematological parameters were then analysed at the Clinical Pathology Laboratory, Veterinary Pathology Department, University of Ibadan; packed cell volume (PCV), haemoglobin (HB), red blood cell (RBC), white blood cell (WBC), platelets, lymphocytes, Neutrophils, Monocytes, and Eosinophils.

Serum biochemistry

Blood was collected in plain tubes by the same method described above and analysed immediately in the Clinical Pathology Laboratory, Veterinary Pathology Department, University of Ibadan for Aspartate Transaminase (AST) and Alanine Transaminase (ALT) enzymes.

Antioxidant enzymes

Blood collected in EDTA tubes was centrifuged to obtain the plasma which was analysed for antioxidant enzymes– Malondialdehyde (MDA), Catalase (CAT), Superoxide dismutase (SOD) and Glutathione superoxide hydrogenase (GSH) at K & D analytical laboratory, Oyo road, Ibadan.

Histopathology

The mice were sacrificed by cervical dislocation and dissected to obtain the livers, which were preserved in 10% neutral formalin for histopathology examination in labelled universal bottles. The histopathology of the livers was done at the Clinical Pathology Laboratory, Veterinary Pathology Department, University of Ibadan.

Statistical Analysis

The statistical package (SPSS®) version 20.0 was used to carry out the statistical analyses and the Mean ± SEM was determined. One way analysis of variance (ONE WAY ANOVA) was used to determine the statistical differences between the groups for least significance difference (LSD) and Duncan's test. $P< 0.05$ significance level was used.

Results

Packed Cell Volume (PCV)

The lowest PCV was recorded in group B — DDVP (22.0%) — and was significantly lower ($P<0.05$) than in all other groups. PCV was numerically higher in group D (Aq500) — 30.0%), when compared with group A (Negative control) — 29.5%, however, Groups E (Meth 300) — 30.3% and F (Meth500) — 30.7% had significantly higher values ($P<0.05$) than all other groups (Table 1).

Haemoglobin level

The haemoglobin level in group B — DDVP (7.15g/dl) was significantly lower ($P<0.05$) than in all other groups. Groups D —Aq500 (10.1g/dl), E — Meth 300 (10.5g/dl) and F — Meth500 (10.2g/dl) had significantly higher haemoglobin levels when compared with group A — Negative control (9.9g/dl). Table 1.

White blood cells

Group A (negative control) had the highest WBC values which was significantly higher when compared with other groups (4.63±0.03). Lowest WBC count was in group C (2.65±1.15) and was significantly lower when compared with other groups. WBC count in the DDVP group was not significantly different ($p \leq 0.05$) from other treatment groups except group C, which had a lower value (Table 1).

Red blood cells

RBC count in DDVP group (4.10±0.02) was significantly lower than all the other groups. The highest value was in Group D (Aq 500) and was significantly higher than the values in all other groups (Table 1).

There were no significant differences ($p \leq 0.05$) in the following parameters — Lymphocytes, Platelets, Neutrophils, Monocytes and Eosinophils (Table 1).

Liver Enzyme Analyses

Aspartate aminotransferase (AST)

The DDVP group (320.5±4.5) had significantly higher AST level ($P<0.05$) compared to the negative control (111.5±5.50) and other treatment groups- Aq300 (197.0±12.0); Aq500 (217.7±2.91); Meth300 (223.7±11.6) Meth500 (201.0±11.8) (Table 2).

Alanine aminotransferase (ALT)

The value of ALT for the DDVP-challenged group (234.0) was significantly higher ($P<0.05$) when compared with the negative control group (89.0) and other treatment groups- Aq300 (117.5); Aq500 (125.0); Meth300 (130.0); Meth500 (112.0) (Table 2).

Antioxidant Enzymes

Malondialdehyde (MDA)

The DDVP group had significantly higher ($P<0.05$) MDA (22.3±1.17nmol/ml) when compared to the control (0.77±0.08nmol/ml) and all treatment groups- Aq300 (4.65 nmol/ml), Aq500 (1.94 nmol/ml), Meth300 (1.36 nmol/ml), Meth500 (0.93 nmol/ml) (Table 3).

Catalase (CAT)

The DDVP group had significantly higher ($P<0.05$) CAT (322.2±3.9 u/mg) when compared to the control (199.7±2.27 u/mg) and all treatment groups- Aq300 (213.4±3.41u/mg), Aq500 (221.82±2.53 u/mg), Meth300 (220.45±7.67u/mg), Meth500 (205.15±2.56u/mg). Table 3.

Superoxide dismutase (SOD)

There was no significant difference ($P>0.05$) in SOD levels between all groups. However, the DDVP group had a numerically lower value (17.0 spe/min) when compared with the control (35.0spe/min) and treatment groups- Aq300 (29.5 spe/min); Aq500(34.3 spe/min); Meth300 (39.3 spe/min); Meth500 (30.0 spe/min) (Table 3).

Glutathione superoxide hydrogenase (GSH)

At $P<0.05$, GSH level was significantly lower in the DDVP group (0.34±0.16) compared with all other groups. However, when compared with the control (4.3), all treatment groups had significantly lower values (Aq300- 3.1; Aq500- 3.3; Meth300- 3.5; Meth500- 2.8) (Table 3).

Table 1: Effects of Oral Administration of Different Doses of Aqueous and Methanolic Extracts of *Morinda lucida* on Haematological Parameters after Exposure to Dichlorvos

Group	PCV (%)	Hb (g/dl)	WBC $X10^3$ (µL)	RBC	Lymphocytes (%)	Plateletsx10^4 (µL)	Neutrophils (%)	Monocytes (%)	Eosinophils (%)
A (Control)	29.50±0.50^{bc}	9.90±0.50^{ab}	4.63±0.03^{b}	4.52±0.05^{ab}	63.50±0.50^{a}	5.55±0.15^{a}	30.00±1.00^{a}	2.50±0.50^{a}	3.0±0.00^{a}
B (DDVP Only)	22.00±1.00^{a}	7.15±0.2^{a}	3.33±0.3^{ab}	4.1±0.02^{a}	58.00±1.00^{a}	3.55±0.15^{a}	24.50±1.50^{a}	2.00±1.00^{a}	1.5±0.50^{a}
C (Aq300 + DDVP)	26.00±1.00^{ab}	9.00±0.4^{b}	2.65±1.15^{a}	4.51±0.18^{ab}	63.50±2.50^{a}	4.2±1.8^{a}	32.00±3.00^{a}	2.00±1.00^{a}	2.5±0.50^{a}
D (Aq500+ DDVP)	30.00±1.15^{bc}	10.1±0.6^{b}	4.06±0.32^{ab}	5.29±0.30^{b}	63.67±5.24^{a}	6.60±0.72^{a}	31.67±5.21^{a}	2.33±0.67^{a}	2.0±0.58^{a}
E (Meth300+ DDVP)	30.33±1.45^{c}	10.5±0.6^{b}	3.25±0.52^{ab}	5.19±0.29^{b}	65.33±3.28^{a}	6.00±1.91^{a}	32.33±3.93^{a}	1.33±0.33^{a}	1.0±0.58^{a}
F (Meth500+DDVP)	30.67±1.20^{c}	10.2±0.6^{b}	3.53±0.46^{ab}	4.7±0.33^{ab}	66.00±4.04^{a}	6.40±1.58^{a}	29.67±4.48^{a}	2.33±0.33^{a}	2.0±1.0^{a}

Means having the same alphabet in the same column are not significantly different (P<0.05)

Table 2: Variations in Liver Enzyme Values of Mice in Groups A to F

Group	AST	ALT
A (Control)	111.5±5.50^{a}	89.0±2.0^{a}
B (DDVP Only)	320.5±4.5^{c}	234.0±9.0^{c}
C (Aq300 + DDVP)	197±12.0^{b}	117.5±10.5^{b}
D (Aq500+ DDVP)	217.7±2.91^{b}	125.0±2.9^{b}
E (Meth300+ DDVP)	223.7±11.6^{b}	130.0±9.2^{b}
F (Meth500+ DDVP)	201±11.8^{b}	112.0±8.3^{ab}

Means having the same alphabet in the same column are not significantly different (P<0.05)

Table 3: Variations in Antioxidant Enzyme Values in DDVP Challenged Mice Pre-treated with Extracts of *Morinda lucida* Leaves

Group	MDA	CAT	SOD	GSH
A (Control)	0.77±0.08^{a}	199.7±2.27^{a}	35.0±2.0^{bc}	4.3±0.2^{c}
B (DDVP Only)	22.3±1.17^{c}	322.21±3.9^{d}	17.0±2.0^{a}	0.34±0.16^{a}
C (Aq300 + DDVP)	4.65±0.25^{b}	213.4±3.41^{abc}	29.5±0.5^{b}	3.1±0.5^{b}
D (Aq500+ DDVP)	1.94±0.43^{a}	221.82±2.53^{cd}	34.33±7.9^{bc}	3.3±0.25^{b}
E (Meth300+ DDVP)	1.36±0.07^{a}	220.45±7.67^{bc}	39.33±11.1^{cd}	3.5±0.12^{bc}
F (Meth500+ DDVP)	0.93±0.02^{a}	205.15±2.56^{ab}	30.0±3.0^{b}	2.8±0.26^{b}

Means having the same alphabet in the same column are not significantly different (P<0.05)

Liver Histopathology

There was no visible lesion in the hepatocytes of the control group (Plate 1).

However, the DDVP group showed random single-cell hepatocellular necrosis, with a few foci of inflammatory cellular aggregates (Plate 2). Aq300 showed evidence of regenerating hepatocytes with marked Kupffer cell hyperplasia (Plate 3).

Aq500 showed random single-cell necrosis of hepatocytes with mild Kupffer cell hyperplasia (Plate 4).

As shown in Plate 5, the single-cell hepatocellular necrosis and Kupffer cell hyperplasia of Meth300 are mild with closely-packed hepatic plates.

Meth 500 also showed mild Kupffer cell hyperplasia with the hepatocytes undergoing mitosis (Plate 6).

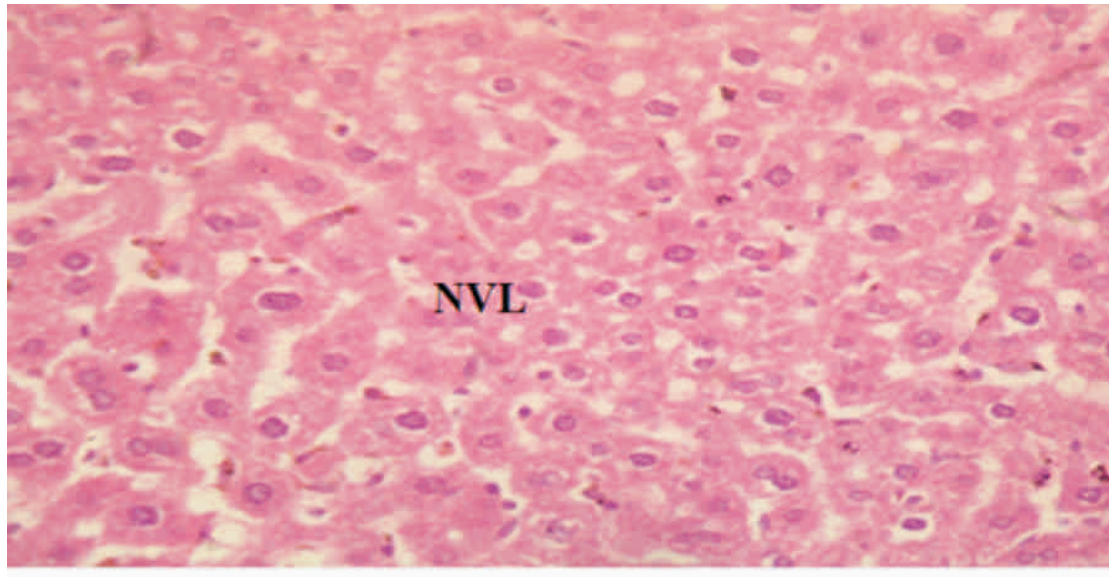


Plate 1: (Group A- Control)
Photomicrograph of the liver showing hepatocytes with no visible lesion
NVL — No Visible Lesion

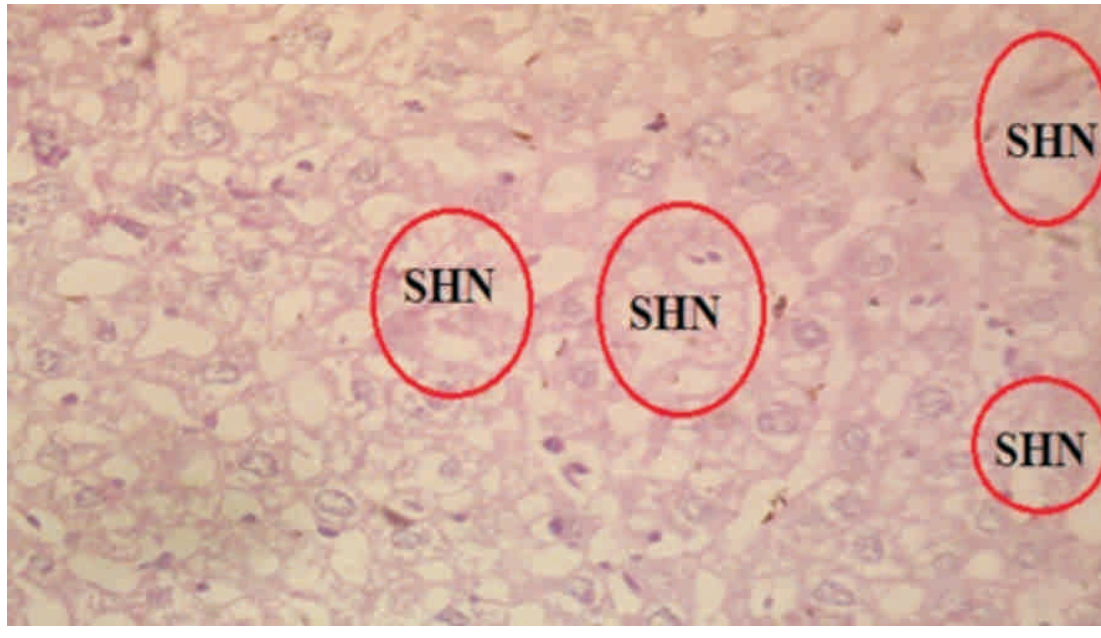
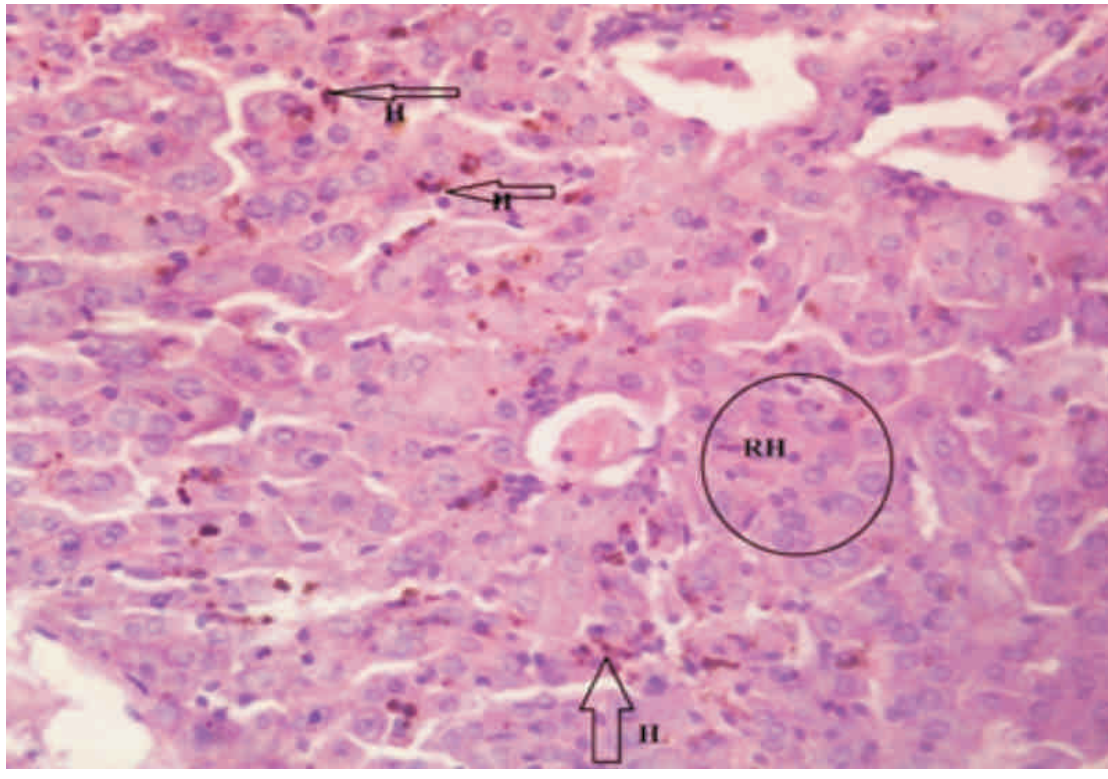
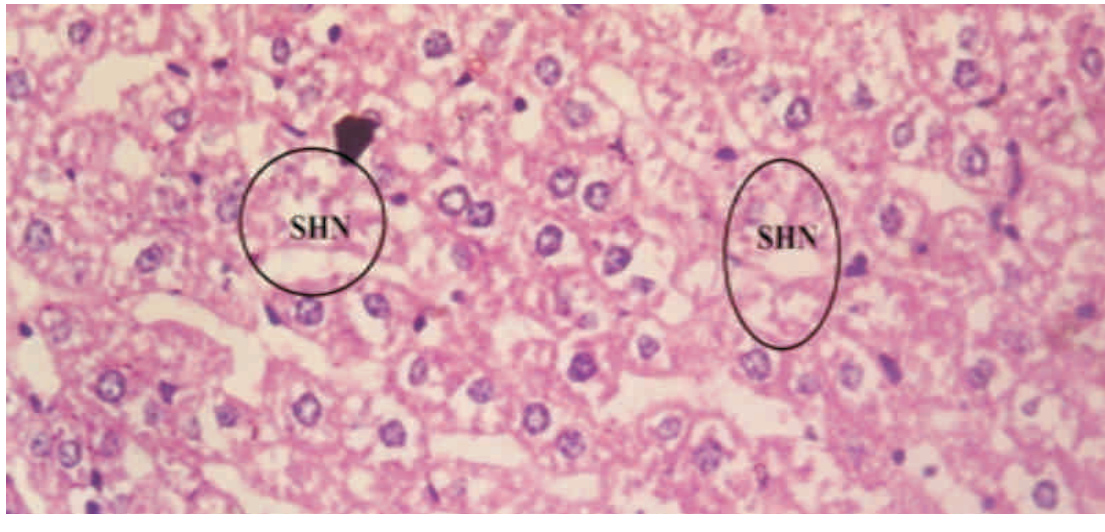


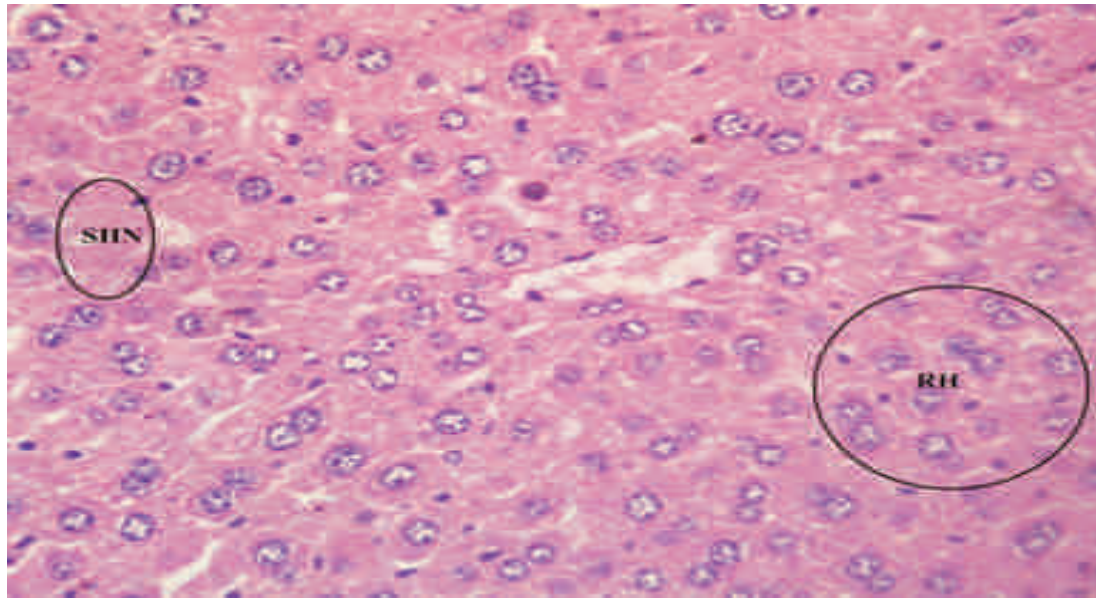
Plate 2: (Group B — DDVP Group)
Photomicrograph of the liver showing random, mild, single-cell hepatocellular necrosis, a few foci of inflammatory cellular aggregates and multifocal thinning of cords
SHN- Single-cell hepatocellular necrosis



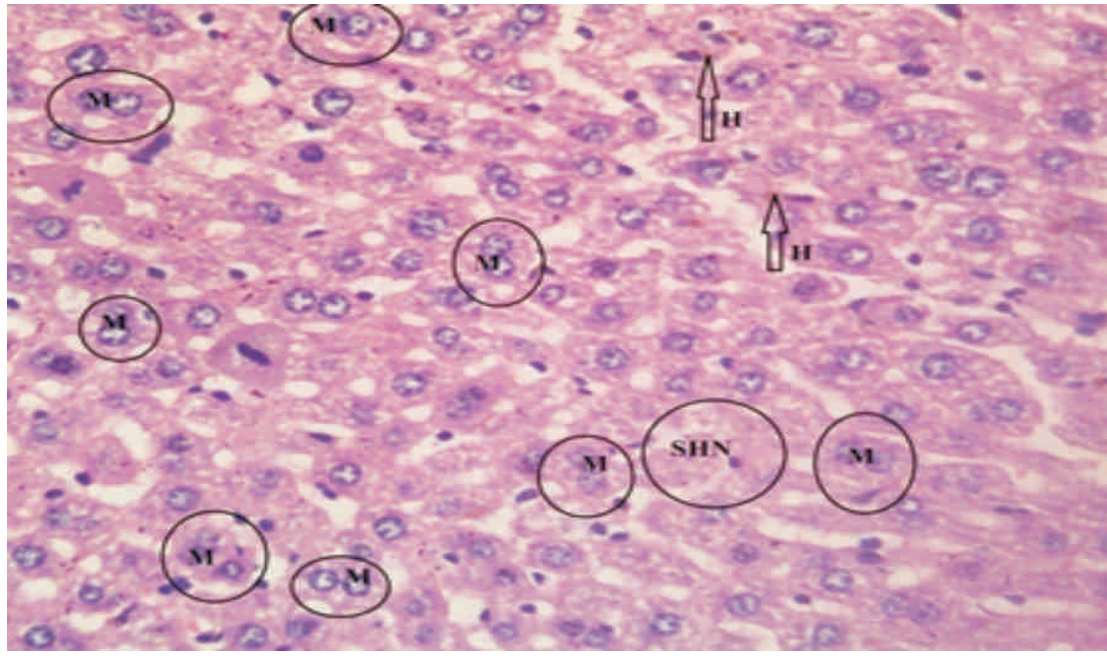
(C- Aq300)
Plate 3: Photomicrograph of the liver showing marked Kupffer cell hyperplasia with Kupffer cells containing golden brown pigments; closely packed hepatic plates with evidence of regenerating hepatocytes.
H- Hyperplasia of Kupffer cells
RH- Regenerating Hepatocytes



(D- Aq500)
Plate 4: Photomicrograph of the liver showing widespread, moderate vacuolar change of hepatocytes; there are a few foci of random single-cell necrosis of hepatocytes; mild Kupffer cell hyperplasia
SHN- Single-cell Hepatocellular Necrosis



SHN- Single-cell Hepatocellular Necrosis
(E- Meth300)
Plate 5: Photomicrograph of the liver showing mild random single-cell hepatocellular necrosis; mild Kupffer cell hyperplasia; closely-packed hepatic plates
SHN- Single-cell Hepatocellular Necrosis
RH- Regenerating Hepatocytes



F- Meth500)
Plate 6: Photomicrograph of the liver showing a few foci of hepatocytes undergoing mitosis [middle left of photomicrograph]; mild Kupffer cell hyperplasia
M- Mitosis
H- Hyperplasia of Kupffer cells
SHN- Single-cell Hepatocellular Necrosis

Discussion

Oxidative stress results from an imbalance between free radicals and the body's antioxidant defence systems; resulting in red blood cell dysfunction, platelet destruction and tissue injury which may affect the functions of blood cells as well as the coagulation system(Hamed, 2016).This dysfunction is evident in the significant decreases observed in the PCV and haemoglobin and red blood cells (RBC) values of the DDVP group (PCV- 22%; RBC- 4.1; Hb- 7.15). The significant increases in the PCV, RBC and Haemoglobin values of the extracts- 500mg/kg aqueous (PCV- 30%; RBC- 5.29; Hb- 10.1), 300mg/kg methanolic (PCV- 30.3%; RBC- 5.19; Hb- 10.5) and 500mg/kg methanolic (PCV- 30.7%; RBC- 4.7; Hb- 10.2)- showed that the pre-exposure treatment with the extracts improved haematological values at these doses. However, the insignificant difference between the DDVP-challenged group and 300mg/kg aqueous extract (PCV- 26%; RBC- 4.51; Hb- 9.0) showed that the dosage may be insufficient to counter oxidative stress. Enzyme activity levels in body fluids can reflect leakage from cells due to cellular injury (Arneson and Brickell, 2007).The activities of ALT and AST enzymes are sensitive biomarkers directly implicated in the extent of hepatic damage and toxicity (Heikall *et al.,* 2013). The significant increases in the plasma ALT and AST levels in the DDVP-challenged group (ALT- 234.0; AST- 320.5) indicates hepatocellular damage which is common with dichlorvos toxicity as reported by Blair *et al.,*(1976). Elevation in the activities of AST and ALT can be associated with hepatocellular necrosis (Duncan *et al.,* 1994). However, the pre-exposure treatment with *M. lucida* extracts (aqueous and methanolic) significantly reduced the ALT level (Aq300- 117.5; Aq500- 125.0; Meth300- 130.0; Meth500-112.0) and AST level (Aq300- 197.0; Aq500- 217.7; Meth300- 223.7; Meth500- 201.0) in all treated groups. Glutathione hydrogenase (GSH), Superoxide dismutase (SOD), Catalase (CAT) enzymes make up the principal system against reactive oxygen species (ROS) (Noori, 2012). GSH, an important naturally occurring antioxidant prevents free radical damage by conjugating with chemicals (Heikal *et al.,* 2013), and the significant decrease in its level in DDVP-challenged group (0.34) shows that it has conjugated with dichlorvos making it less available in circulation. GSH levels of the pre-treated groups were thus, significantly higher (Aq300- 3.1; Aq500-3.3; Meth300- 3.5; Meth500-2.8). SOD, the first enzyme involved in the antioxidant defences against ROS by dismutation of superoxide anion (O^{2-}) to hydrogen peroxide (H_2O_2)(Mohieldin *et al.,* 2011) reduced. However, the pre-treated groups (Aq300- 29.5; Aq500- 34.3; Meth300- 39.3; Meth500-30.0) showed improvement in SOD level with 300mg/kg methanolic group having the highest increase (even than the negative control- 35.0). Catalase (CAT), the second enzyme involved in the antioxidant defences against ROS by conversion of H_2O_2 to H_2O and O_2 (Mohieldin *et al.,* 2011) increased significantly in the DDVP-challenged group (322.2). MDA is a stable metabolite of the free radical-mediated lipid peroxidation cascade serving as marker of lipid peroxidation (Mohieldin *et al.,* 2011). Its significant increase in the DDVP-challenged group (22.3) relative to the negative control group (0.77) showed the high biotransformation rate of the dichlorvos in the liver. However, the pre-treated groups (Aq300- 4.65; Aq500- 1.94; Meth300-1.36; Meth500- 0.93) showed large decreases in MDA level. The single-cell hepatocellular necrosis seen in the liver of the DDVP group showed the damaging effect of the dichlorvos on the liver as there was no visible lesion in the liver of the control group. In the livers of the pre-treated groups, there were still some foci of single-cell hepatocellular necrosis though mild and random. However, they showed evidence of regenerating hepatocytes (by mitosis) with marked Kupffer cell hyperplasia which was not seen in the DDVP group. When these Kupffer cells were activated by dichlorvos they induced hepatocytes proliferation (Roberts *et al.,* 2007).

Conclusion

The haematology, serum biochemistry, antioxidant enzymes and histopathology assays showed that pre-treatment of test animals with aqueous and

methanolic leaf extract of *Morinda lucida* before exposure to DDVP has ameliorative effects on tissue damage by DDVP. The methanolic extracts were more potent in this effect than the aqueous extracts. This is probably because alcohol extracts more phytochemicals from plants than water. However, caution should be taken with dosage since higher dosages may be toxic.

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