



Ameliorative Effects of Vitamins C and E on Haematotoxicity and Spleen Histopathology Induced by Dichlorvos Insecticide in Female Wistar Rats

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Received 21 Oct 2022, Revised 27 June 2023, Accepted 30 June 2023 Published June 2023

DOI: <https://dx.doi.org/10.4314/tjs.v49i2.21>

Abstract

This research aimed to assess the toxicological effects of dichlorvos insecticide (2,2-dichlorovinyl dimethyl phosphate or DDVP) on haematological indices of female Wistar rats and to investigate the antioxidant properties of vitamins C and E in ameliorating haematotoxicity. The rats were divided into 6 groups; control-water, control-oil, dichlorvos group, dichlorvos + vitamin C group, dichlorvos + vitamin E group and dichlorvos + vitamin C + E group. Dichlorvos and vitamins were orally administered to rats with an interval of half an hour between treatments. The control-oil group was given 2 ml corn oil and the control-water group was given water adlib. The treatments were done for 28 consecutive days and blood samples were taken by cardiac puncture. The following haematological parameters were analysed: RBC, WBC, Hb, PCV, MCV, MCH, MCHC and THR. Bone marrow smears were prepared on 14th and 28th days for various blood stem cells evaluation and spleen tissues for histopathology. There was a significant decrease ($p < 0.01$) in RBC counts, PCV, MCV and MCH values, whereas the WBC, THR counts and MCHC values increased significantly ($p < 0.01$) in dichlorvos treated rats as compared to the controls and dichlorvos + vitamins co-treated groups. There were no significant differences in blood parameter counts between controls and dichlorvos + vitamins co-treated groups. In the bone marrow smear of the dichlorvos treated group, there was increased number of megakaryocytes and mature neutrophils. In conclusion, findings from the current study revealed that, vitamins C and E supplements were capable of mitigating the haematotoxic effects induced by dichlorvos insecticide in Wistar rats.

Keywords: Dichlorvos toxicity, Blood parameters, Spleen, Vitamin C, Vitamin E.

Introduction

Pesticide applications have become inevitable all over the world due to increased crop destruction caused by various pests (Oerke 2006). In most African countries where the economy is dependent on agriculture, the use of pesticides is necessary to ensure increased crop production (Holy et

al. 2015). Pests are of different types including insects, herbs, fungi and rodents (Pimentel 2005). They usually lead to crop losses and spread of diseases to other non-targeted organisms (Oerke 2006). Due to increased crop losses and destruction of properties caused by pest invasions, pesticides were introduced (Tawatsin et al.

2015). Since the 1950s the use of pesticides in agriculture has led to improvements in crop production and animal yields hence the economic growth (Aktar et al. 2009, Ojo et al. 2016, Rajabu et al. 2017). Pesticides are of different categories depending on the types of pests they control (insecticides, fungicides, herbicides and rodenticides) or on their chemical compositions such as organophosphates, organochlorines, carbamates and pyrethroids (Aktar et al. 2009, Sinyangwe et al. 2016, Rajabu et al. 2017, Yadav and Devi 2017). Organophosphate pesticides are highly toxic and therefore very effective in controlling agricultural pests (Holy et al. 2015).

Dichlorvos (2,2-dichlorovinyl dimethyl phosphate, commonly abbreviated as an DDVP) is the mostly used organophosphate insecticide in controlling insect pests in households, public health programs and other agricultural settings (Celik et al. 2009). It is very effective in the control of aphids, spider mites, mushroom flies, caterpillars, white flies, thrips, mosquitoes, termites and cockroaches (Kanu et al. 2016). It is also used in treatment of parasitic worm infections in livestock, dogs and even humans (Celik et al. 2009). Regardless of their importance in agricultural and public health programs, organophosphate pesticides are harmful chemicals to humans and other non-targeted organisms. They pose health problems leading to ecological imbalance especially when used uncontrollably (Ajao et al. 2017, Celik et al. 2009). Sharma and Singh (2012) revealed that, pesticides generate reactive oxygen species (ROS) which have the property of stimulating oxidative stress as the main cause of oxidative modifications of proteins, lipids and DNA of living organisms. Prolonged use of pesticides may lead to adverse immunological, reproductive, developmental as well as neurological effects (Ige et al. 2021). Dichlorvos, like other organophosphate pesticides is reported to cause health hazards to exposed populace that may result into death (Holy et al. 2015). The effects induced by dichlorvos poisoning are more rapid compared to other organophosphate pesticides and symptoms

are rapidly recovered due to its quick degradation and removal from the organism's body (Chedi and Aliyu 2010, Imam et al. 2018).

The exposure to dichlorvos insecticide may be by dermal absorption, inhalation or ingestion (Chedi and Aliyu 2010, Agina et al. 2017, Ige et al. 2021). Due to its volatility, dichlorvos can easily be inhaled by people living near waste sites or those using it at home (Okoroiwu and Iwara 2018). People with impaired liver functions, reduced pulmonary function and convulsive disorders are at great risk from dichlorvos exposure (Chedi and Aliyu 2010). The adverse effects of dichlorvos are highly pronounced in developing countries where it is widely used due to its low cost and limited knowledge on its effects to human health (Holy et al. 2015). Dichlorvos is also evidently used in vegetable farming in Tanzania due to the fact that, about 2.86% of its residues were found in ready-to-eat vegetables in Arusha (Kiwango et al. 2018). The mode of action of dichlorvos pesticide is by inhibiting the acetylcholinesterase enzyme whose function is to hydrolyze acetylcholine neurotransmitter in nerve synapses (Ige et al. 2021). The acetylcholinesterase enzyme blockage leads to accumulation of acetylcholine neurotransmitter in nerve presynaptic spaces that impedes nerve functions (Okoroiwu and Iwara 2018). Sunkaria et al. (2012) reported that, dichlorvos has another mechanism of imparting its toxicity; when microglial cells of central nervous system (CNS) get activated by cellular damage induced by organophosphate compounds; they secrete neurotoxic particles such as nitric oxide (NO) that destroy cells and hence cause DNA alteration. Following dichlorvos poisoning, the symptoms include nausea, vomiting, abdominal cramps, bleeding, blurred vision, diarrhoea, coughing and chest discomfort, involuntary muscle contraction, burning sensation, bloody or runny nose and headache and if poisoning is severe it will affect the central nervous system (Chedi and Aliyu 2010, Imam et al. 2018). Dichlorvos has been classified as a possible carcinogenic compound to humans (Koutros et al. 2008).

However, antioxidant compounds such as vitamin C and vitamin E have been reported to have protective properties against different toxins such as pesticides (Dirican and Kalender 2012). They do protect the cells by preventing oxidative damage caused by these toxic substances (Ryan et al. 2010). Vitamins C and E are natural antioxidants that prevent the increase in free radicals caused by oxidative stress in various cells and tissues (Jin et al. 2014). Ismiyati et al. (2015) reported that, hydrophilic antioxidants such as vitamin C and flavonoids and lipophilic antioxidants such as vitamin E and carotenoids have the ability to prevent the development of atherosclerosis and cardiovascular disease by controlling redox steps in disease progression. They reduce chromosomal aberrations by binding to free radicals (Pala et al. 2016). Vitamin E is a lipid-soluble antioxidant; it resides in the bi-lipid membranes and therefore maintains their stability and prevents lipid peroxidation in cellular membranes. Vitamin C is water soluble and usually found in cytosol and extracellular fluids that helps in neutralizing ROS and therefore reduces oxidative stress (Ryan et al. 2010, Uzun and Kalender 2011, Jin et al. 2014). Pala et al. (2016) proved that vitamins C and E were capable of protecting cells against radiation induced by hysterosalpingography (HSG) by reducing cellular degeneration in the endometrium. Vitamins C and E also suppressed cell apoptosis caused by DDT cytotoxicity in hepatic cells (HL-7702 cells) (Jin et al. 2014). It has been suggested that, the use of both, vitamins C and E have resulted to better antioxidant effects than using either of the vitamins alone (Ryan et al. 2010).

Blood is the major tissue involved in transport of respiratory gases, nutrients, hormones, waste materials for their removal and also fights against various infections in the body. Blood parameters are good indicators of organisms' health status through full blood picture (Bezerra et al. 2017). When the values of blood parameters are abnormal, it indicates the presence of disorders like

cardiovascular disease, immune disease or cancer (Kelada et al. 2012). The health status of an organism is reflected in the blood parameters including the bone marrow that can be used for toxicity indication (Ajao et al. 2017). Information on the effects of dichlorvos insecticide on hematological parameters in rats is still limited. Therefore, current study assessed the effects of orally administered dichlorvos insecticides on haematological parameters, spleen immunohistochemistry as well as the bone marrow smear cytology in Wistar rats. The study also evaluated the effects of vitamins C and E supplements in mitigating the toxicity.

Materials and Methods

Experimental procedure

A total of 72 female Wistar rats (*Rattus norvegicus*) with an average body weight of 146 g were acclimatized for two weeks to laboratory conditions at the College of Veterinary Medicine and Biomedical Sciences of the Sokoine University of Agriculture (SUA). The rats were randomly divided into 6 groups of 12 animals each. Two groups served as controls and the other four were experimental groups as shown in Table 1. The dichlorvos (2,2-dichlorovinyl-dimethyl phosphate, AMVACTM, Netherlands, B.V.) an organophosphate insecticide of 500 g/l was used in this study. Dichlorvos and Vitamin C (Elys Chemical Industries, Kenya) and vitamin E (Geltec Inc., USA) were administered daily by oral gavage between 9.00–10.00 am for 28 days. In the control animals, one group was given water adlib and the second with 2 ml corn oil as a vehicle since it was used to dissolve dichlorvos insecticide and vitamin E prior administration (Uzun and Kalender 2011). In vitamin treated groups, vitamin C and vitamin E were dissolved in water and corn oil, respectively. At the end of the treatments, all the animals were anaesthetized with chloroform before dissection. All ethical guidelines of the University of Dar es Salaam were adhered to during the study.

Table 1: Experimental set-up of dichlorvos exposure to Wistar rats

Group	DDVP 10 mg/kg bwt./day	Vitamin C 25 mg/kg bwt./day	Vitamin E 50 mg/kg bwt./day
Control (water)	-	-	-
Control (oil)	-	-	-
DDVP	+	-	-
DDVP + Vitamin C	+	+	-
DDVP + Vitamin E	+	-	+
DDVP + Vitamins C + E	+	+	+

Haematological analysis

Blood was collected by cardiac puncture in heparinized tubes and the haematological parameters for both the control and experimental groups were obtained using automated haematology analyzer (MS4s, MeletSchloesing Laboratories, France). The haematological parameters analysed included white blood cells (WBC), red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and thrombocytes (THR).

Bone marrow smear and spleen histopathology

Rats were dissected and spleen was resected for histopathological studies. Bone marrow from femur bone was aspirated by a syringe and smeared on a clean labelled slide. The smear was dried and stained by Giemsa stain and observed under light microscope (x200) for different precursor/stem cells. Spleen resection and bone marrow smear were done on day 14th and 28th of the experiment.

Immunohistochemistry

Spleen tissues were fixed in Bouin's fluid, dehydrated, embedded in histological wax and sectioned using a rotary microtome (Baird & Tatlock Ltd, UK). Thin sections of 7 μ were deparaffinized in xylene, rehydrated in ethanol series to phosphate-buffered saline (PBS). They were immersed in 0.3% v/v hydrogen peroxide in water for 20 min at room temperature (RT) to inhibit activities of endogenous peroxidase enzymes. The sections were then washed (3 x 5 min) in 0.01 M PBS, pH 7.4 followed by incubation with

10% normal goat serum in PBS for 30 min at room temperature (RT) to block non-specific binding. To detect single stranded DNA (ssDNA) in cells degenerating by apoptosis, the sections were incubated with polyclonal rabbit anti-ssDNA antibody (DakoCytomation, Code No 18731) diluted at 1:200. For negative control, 1% bovine serum albumin in PBS was applied in place of anti-ssDNA antibody. The tissues were then incubated for 24 h in a dark, humid chamber at 4 °C. Sections were washed (3 x 5 min) in PBS, followed by incubation for 30 min at room temperature (RT) with biotinylated goat anti-rabbit IgG (MP Biomedicals, Inc., Germany). Sections were washed (3 x 5 min) in PBS before incubation with streptavidin-peroxidase conjugate for 30 min at RT. The sections were then incubated with 0.05% 3,3'-diaminobenzidine-tetrahydrochloride, 0.01% hydrogen peroxide and 0.05 M Tris-HCl, pH 7.6 to visualize binding sites for 3–5 min. Reaction was stopped in distilled water then dehydrated through a graded alcohol series, cleared and mounted in dibutylphthalate polystyrene xylene (DPX). Binding sites were assessed using Olympus BH-2 microscope fitted with Olympus camera.

Statistical analysis

The data for haematological parameters were presented as mean \pm standard deviation. The data were tested for normality and found to be non-parametric and therefore subjected to Generalized Linear Model (GLM) and one way analysis of variance (ANOVA). The statistical significance was set at $p < 0.05$.

Results

General observations after treatment

On the second day of treatment, the animals in the dichlorvos treated group showed some cholinergic signs such as muscular tremors, diarrhoea, fur erection and sluggishness. Trembling took about half an hour after which the animals settled and appeared normal again. The rats were also scratching around the mouth a sign of itching. There were no major differences in body weight of animals between groups though in dichlorvos treated group, the animals had the least body weight but not significant as compared to that of the control and other three experimental groups. In the dichlorvos and vitamins co-treated groups, muscular tremors were not severe and lasted shortly after vitamins administration. The control group animals appeared normal throughout the experiment.

Haematological parameters observations

After 28 days, it was apparent that exposure to dichlorvos had hazardous effects on the haematological parameters of female Wistar rats as shown in Table 2. There was a significant ($p < 0.01$) decrease in RBC, PCV, MCV, and MCH values in dichlorvos treated group compared to controls and vitamins plus dichlorvos co-treated groups. There was no significant decrease of Hb concentrations in the dichlorvos treated group as compared to controls and vitamins plus dichlorvos co-treated groups. Significant ($p < 0.01$) increase in MCHC values, WBC and THR counts were observed in dichlorvos treated group as compared to control and dichlorvos plus vitamins co-treated groups. There were no significant differences in the haematological parameters between controls and dichlorvos plus vitamins co-treated groups.

Table 2: Haematological parameters of female Wistar rats exposed to dichlorvos insecticide for 28 days. Values are expressed as Mean \pm SD (n = 6)

Parameters	Control Water Adlib	Control Oil 2 ml	DDVP 10 mg/kg/b.wt	DDVP + Vit C 10 + 25 mg/kg/b.wt	DDVP + Vit E 10 + 50 mg/kg/b.wt	DDVP + Vit C + Vit E 10 + 25 + 50 mg/kg/b.wt
WBC ($\times 10^9/L$)	6.5 \pm 2.2	6.5 \pm 2.3	10.4 \pm 4.1***	6.2 \pm 1.9	6.9 \pm 1.8	5.6 \pm 1.9
RBC ($\times 10^{12}/L$)	7.1 \pm 0.7	7.1 \pm 0.5	5.9 \pm 1.1**	7.0 \pm 0.7	7.2 \pm 0.5	7.3 \pm 0.8
Hb (g/dl)	13.2 \pm 1.6	12.9 \pm 0.6	12.2 \pm 1.3	13.2 \pm 0.9	12.9 \pm 1.2	13.2 \pm 0.9
PCV (%)	46.4 \pm 4.6	46.6 \pm 2.7	42.9 \pm 4.9**	47.3 \pm 3.4	46.8 \pm 5.7	47.2 \pm 5.8
MCV (fl)	62.9 \pm 3.1	60.1 \pm 4.5	54.1 \pm 7.2**	63.8 \pm 4.5	62.9 \pm 4.5	63.8 \pm 4.4
MCH (pg)	17.9 \pm 2.7	17.1 \pm 1.7	16.5 \pm 2.4*	18.5 \pm 2.7	18.4 \pm 2.5	19.9 \pm 3.5
MCHC (g/dl)	23.1 \pm 5.5	23.9 \pm 5.6	26.6 \pm 2.4**	23.4 \pm 4.5	23.3 \pm 4.3	24.4 \pm 6.5
THR ($\times 10^9/l$)	840 \pm 12	876 \pm 92	1079 \pm 48**	912 \pm 64	910 \pm 65	900 \pm 62

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, WBC-White Blood Cells, RBC-Red Blood Cells, Hb-Haemoglobin, PCV-Packed Cell Volume, MCV-Mean Cell Volume, MCH-Mean Cell Haemoglobin, MCHC-Mean Cell Haemoglobin Concentration, THR-Thrombocytes, Vit-Vitamin.

Bone marrow smears cytology

Compared to the control groups, bone marrow smear of dichlorvos treated group showed an increased number of megakaryocytes and large number of band and mature neutrophils (Figure 1 C). Reduced number of erythroblasts and a few small lymphocytes were also observed. In dichlorvos and vitamin C co-treated groups, the increased number of early and late erythroblasts was apparent. Mature neutrophils and lymphocytes were also seen (Figure 1 E). There was also an increased number of early and late erythroblasts in dichlorvos plus vitamin E co-treated group and the dichlorvos plus vitamins C plus E co-treated group. Normoblasts, band neutrophil, metamyelocytes, polymorphocytes and lymphocytes were also observed (Figure 1 F).

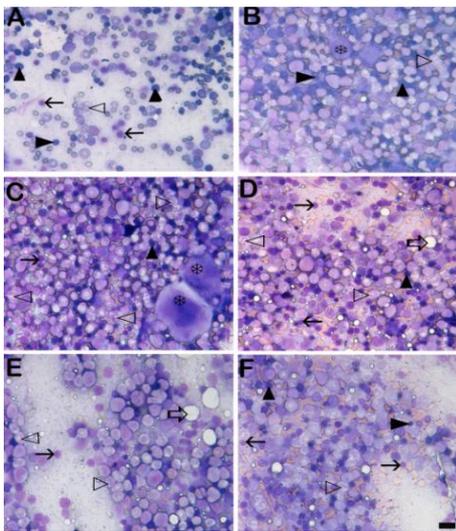


Figure 1: Bone marrow Giemsa stained smears showing various cells. **A:** Control water: showing early and late erythroblast and normoblast (arrows); band neutrophils and neutrophilic myelocyte (open arrow head) and lymphoblast (arrow heads). **B:** Control oil group showing the presence of band neutrophils (open arrow head), lymphoblast (arrow heads) and megakaryocytes (asterisks), the largest cells with abundant cytoplasm and multilobulated

nuclei. **C:** DDPV treated group showing large number of band neutrophils and some mature neutrophils with multilobulated nuclei (open arrow head) and a few small lymphocytes (arrow heads) with dark blue nucleus. Also, megakaryocytes (asterisks) were present. **D:** DDPV + Vitamin C treated group showing early and late erythroblasts, normoblast (arrows); band neutrophil and metamyelocytes and polymorphocyte (open arrow head) and lymphocytes (arrow heads) with dark blue nucleus. **E:** DDPV + Vitamin E treated group showing early and late erythroblasts, normoblast (arrows), band neutrophil and metamyelocytes and polymorphocyte (open arrow heads) and lymphocytes (arrow heads) with dark blue nucleus. **F:** DDPV + Vitamins C + E treated group showing early and late erythroblasts, normoblast (arrows), band neutrophil and metamyelocytes and polymorphocyte (open arrow head) and lymphocytes (arrow heads) with dark blue nucleus. Note the presence of Adipocytes (open arrows) (D and E). Magnification x200.

Immuno-histochemical findings

Detection of immunoreactivity labelling of ssDNA, a marker of apoptotic cells was consistently observed from spleen sections of rats treated with dichlorvos (Figure 2 E and F). These DNA positive cells were shown as brown deposits resulting from 3,3'-diaminobenzidine (DAB) peroxidase reaction (Figure 2). The reaction appeared to be intense in the red pulp and more intense in the white pulp of dichlorvos treated rats spleen section. The intensity of brown deposits decreased in sections from rats treated with DDPV followed by vitamin C (Figure 3 G and H) and vitamin E (Figure 3 I and J) supplementation. In spleen sections from rats supplemented with vitamins C and E, the reaction appeared much reduced indicating the preventive effect of vitamins against dichlorvos induced damage (Figure 3).

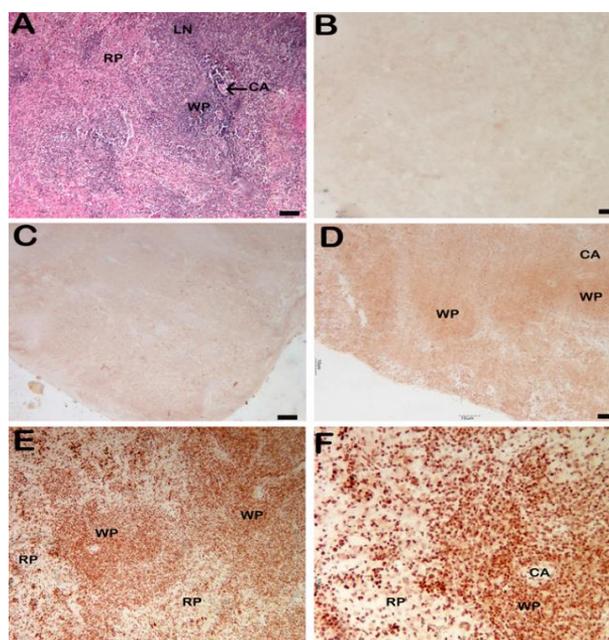


Figure 2: Tissue sections of rat spleen. A: Haematoxylin & Eosin stained spleen sections showing normal structures. B: Negative control incubated with normal serum in the place of ssDNA antibody showing absence of brown deposit. C and D: Control rats given water and corn oil showing very few spots of brown deposit. E and F: Rats treated with dichlorvos (DDPV) showing intense dark brown deposit of DAB throughout the spleen in both red and white pulp surrounding the central artery (CA). RP: red pulp; WP: white pulp. Scale bar: 100 μ m.

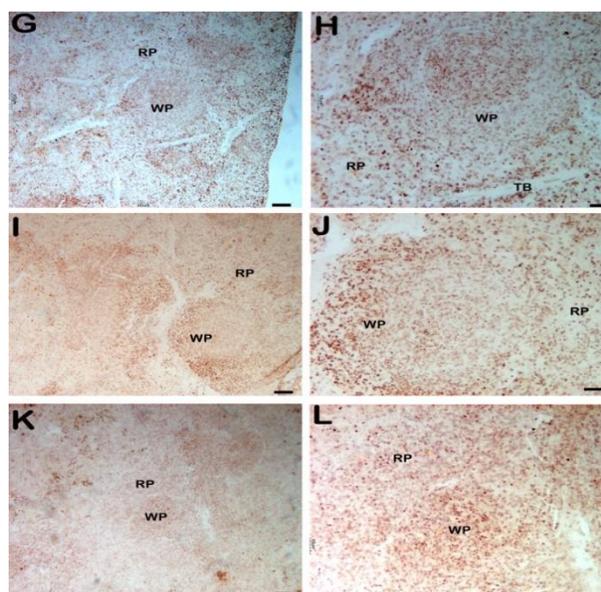


Figure 3: Tissue sections of spleen for rats treated with dichlorvos (DDPV) and supplemented with vitamin C (G and H), vitamin E (I and J), and co-treated with both vitamins C and E (K and L) showing reduced dark brown deposit of DAB throughout the spleen in red pulp (RP) and white pulp (WP), TB : Trabeculae. Scale bar: 100 μ m.

Discussion

The results from this study revealed that dichlorvos had toxicological effects on Wistar rats' haematology. This organophosphate insecticide caused significant changes in haematological parameters, spleen histology and bone marrow smear cytology. It can be proposed that, changes in haematological parameters are related to the effects of dichlorvos on the bone marrow and on the spleen histopathology. It was evident that in dichlorvos treated group, except haemoglobin, all other blood parameters were altered significantly compared to control groups and those co-treated with vitamins C and E. In dichlorvos treated group, the RBC, PCV, MCV and MCH values were significantly lower as compared to control and dichlorvos plus vitamins C and E co-treated groups. The MCHC values of the dichlorvos treated group were significantly higher than that of the control and dichlorvos plus vitamins co-treated groups. This indicates that, dichlorvos has a property of causing anaemia in exposed rats. These findings are in agreement with that reported by Agina et al. (2017) and Kanu et al. (2016). The significant decline in RBC, PCV, MCV and MCH suggested that there was RBC destruction by dichlorvos insecticide which was beyond the production capacity of the bone marrow. Therefore, the decrease in RBC, PCV, MCV and MCH parameters indicated that, dichlorvos is capable of inducing anaemia. Agina et al. (2017) suggested that, the decrease in RBC, MCV, PCV and MCH may be caused by suppression of erythropoiesis and disintegration of RBCs in the circulation. Furthermore, Holy et al. (2015) found the decrease in RBCs, PCV and Hb after intraperitoneal exposure to dichlorvos and suggested that the decrease of erythrocytes exceeded the bone marrow's capacity to compensate for the loss. The increase in the values of MCHC suggests that dichlorvos caused severe loss of permeability and shrinkage of RBCs which led to increased cellular Hb concentrations. Therefore, the continuous application of dichlorvos insecticide can lead to severe anaemia in

living organisms. The findings from this study showed that, in groups that were given dichlorvos with vitamins C, E or combination of the two altogether had no significant effect on haematological parameters as compared to the group treated with dichlorvos alone. In addition, there was also a significant increase in WBCs in dichlorvos treated groups that suggests severe leukocytosis. The increased WBCs implies that, the animals were stressed when given dichlorvos that could have caused some inflammation or injuries to different organs and therefore bodies responded by amplifying the immune system. These findings concur with that reported by Agina et al. (2017) and Ige et al. (2021) who found elevation in WBC counts after dichlorvos treatments in albino rats and explained the increase as a result of immobilization of the immunological system or the shift of leukocytes pool from the spleen to the peripheral circulation. Holy et al. (2015) reported the increase in WBCs after intraperitoneal exposure of rats to dichlorvos insecticide and clarified the increase as a result of defensive mechanism in response to inflammation. Celik et al. (2009) also reported leukocytosis in Wistar rats exposed to dichlorvos and explained it as the normal reaction of the body against any new substance that tends to alter physiological processes. The thrombocytes count was also found to be significantly higher in dichlorvos treated group than in control and dichlorvos + vitamins co-treated groups. This implies that, the insecticide caused some injuries to treated rats which led to intravascular bleeding and therefore prompted the bone marrow to produce more thrombocytes to enhance clotting. These findings correspond to that reported by Holy et al. (2015) who found the dose dependent increase in platelet counts in albino rats after intraperitoneal exposure to dichlorvos. Likewise, thrombocytosis in albino rats was apparent following exposure to dichlorvos insecticide (Kanu et al. 2016).

There were no significant differences in WBCs, RBCs, PCV, Hb, MCV, MCH and MCHC values between the dichlorvos plus vitamins C, E or both vitamins C and E co-

treated and control groups. This proves that, vitamins C and E have strong ameliorative properties towards the dichlorvos toxicity. Several studies have proved the antioxidant nature of vitamins C and E in removing or reducing the destructive effects caused by different xenobiotic materials. El-Shenawy et al. (2009) confirmed that, vitamin E supplement was capable of preventing the oxidative stress effects induced by diazinon in the mice liver. Oularbi et al. (2017) found the reduction of emamectin benzoate residue concentrations in the liver of emamectin benzoate plus vitamin C treated rats compared to those treated with emamectin benzoate only therefore, vitamin C aided in the reduction of hepatotoxicity caused by emamectin benzoate in rats. Furthermore, Ebuehi et al. (2012) reported the reduction in the blood lead concentrations, hepatic damage and brain oxidative stress in rats after vitamins C and E oral administration. Furthermore, Owoye et al. (2014) found that, vitamins C and E were able to ameliorate the dichlorvos induced toxicity in hippocampus of rats. In the study performed by Oral et al. (2006) on endometrial damage and apoptosis induced by dichlorvos insecticide, it was confirmed that, the combination of vitamins C and E were capable of improving the histological effects induced by dichlorvos on the rats' endometrium. The findings from the current study confirm the ameliorative nature of vitamins C and E by recovering the haematotoxicity of dichlorvos insecticide. The haematological values in the dichlorvos plus vitamins co-treated groups were nearly equal to control groups, therefore vitamins C and E had a protective potential against insecticide induced toxicity. Similar results of reduced dichlorvos toxicity were obtained when the two vitamins C and E were fed up together.

The bone marrow is a major haematopoietic and lymphoid tissue. This tissue is a potential area for chemicals exposure and therefore its assessment for toxicity is very essential (Travlos 2006). The bone marrow is also affected by xenobiotic materials such as pesticides leading to its

failure in blood production. In the current study, the bone marrow smear of dichlorvos treated group showed an increased number of megakaryocytes, mature neutrophils and lymphocytes. This suggests that, the insecticide caused injuries or blood vessels distortion that led to bleeding. The increased lymphocytes count implies that dichlorvos caused stimulation of the immune system due to its toxic property. The neutrophil increment is possibly due to haemorrhage, excitement or inflammation. The increased amount of megakaryocytes in the bone marrow smear implies that, there were some vascular bleeding which forced bone marrow to increase megakaryocyte production and the latter helps in platelet production so as to stop bleeding. Travlos (2006) clarified the increase in megakaryocytes as a response to anaemia or due to over consumption or destruction of platelets. This implies that dichlorvos may have destructive effects on platelets. Vinayakamurthy et al. (2017) also found that, there was an increment in megakaryocytes in most cases of iron-deficiency anaemia.

Spleen is a lymphoid organ that is directly connected to blood circulatory system. Adhering to its functions of RBC and platelets storage, breakdown of defective RBCs and immune system activation, any destruction in its morphology may have effects on its functions. In the present study, spleen was seen to be highly affected when exposed to dichlorvos. This was indicated by presence of brown deposits in both red and white pulp of the spleen, the intensity was more pronounced on the white pulp in dichlorvos treated group which denoted cellular apoptosis. It is clear that, dichlorvos exposure resulted in the increased amounts of WBCs (leukocytosis) and the decrease in RBCs counts, PCV, MCV and MCH values were obvious indicating the anaemic conditions. The spleen blood vessels are lined by B-lymphocytes for memory, so when blood moves through the spleen, it is checked by T-lymphocytes for any new agent and if detected, it is taken to B-lymphocytes for matching and if mismatches, the B-cells divide to produce more antibodies against the

antigen and this may be the reason for the increased WBCs. There was an intense apoptosis in the red and white pulps of the spleen and since the spleen is the RBCs bank, this means that, dichlorvos led to destruction of the stored RBCs which then became defective and therefore engulfed and digested by microphages lining the spleen blood vessels. This left the rats' bodies with no replacement of RBCs after bone marrow intoxication. This finding is in harmony with the findings reported by Ekanem et al. (2015) who found the decrease in PCV and Hb counts and an elevation in leukocytes following spleen toxicity induced by lead acetate. The intensity of brown deposits decreased in dichlorvos treated groups supplemented by vitamins C and E as singly or altogether and this indicates the protective nature of these vitamins as anti-oxidants. Aldahmash and El-Nager (2014) found the same results that, vitamin C treatment followed by administration of lead acetate brought some improvements to rats' liver and spleen histological structure and this shows protective nature of these vitamins as antioxidants. The antioxidant power of vitamins C and E are due to their ability to scavenge free radicals which are sources of increased ROS. Vitamin E is reportedly a powerful antioxidant; it is capable of donating its hydrogen atoms to free radicals making them stable compounds, then the unstable vitamin E will be reduced to its original stable form by vitamin C and the cycle continues (Higgins et al. 2020, Traber and Stevens 2011).

Conclusion

The findings of this study revealed that, dichlorvos causes significant toxicological alterations in blood parameters which include erythrocytopenia, leukocytosis and thrombocytosis whose effects were ameliorated by the intake of vitamins C and E as antioxidants. The populace should therefore be educated on the hazardous effects induced by dichlorvos and other pesticides and be advised on eating foods rich in antioxidants like fish, vegetables and fruits so as to amplify their immune systems.

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