ORAL ADMINISTRATION OF VITAMIN C ATTENUATES PRO-INFLAMMATORY CYTOKINES IN DICHLORVOS-INDUCED TOXICITY IN RATS

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Abstract

Background of the Study: Dichlorvos (DCLV) is a widely used organophosphate insecticide. Its exposure in humans has been linked to several adverse health effects. The harmful impact caused by dichlorvos on human health has increased the demand for protective measures against its exposure. Aim: This study, therefore, aimed to investigate the effect of vitamin C (VIT C) on pro-inflammatory cytokines, tumour necrosis factor-alpha and interleukins (TNF-α and IL-6) and acetylcholinesterase activity (AChE) linked to dichlorvos.

Materials and Methods: Rats were divided into three groups (8 animals/group); The first group served as a control (received corn oil), group 2 animals were orally treated with 2mg kg−1 body weight DCLV and group 3 animals received DCLV plus VIT C (2mg kg−1 body weight) for 21 days after which the blood and the brain were removed. Thereafter, plasma, red blood cell and lymphocytes were separated, and the biochemical parameters were determined spectrophotometrically. Results: Inhibition of AChE activities caused by DCLV in the RBC (65.8%) and the brain (55.8%) was significantly ($p<0.001$) reversed by Vitamin C treatment. Similarly, the treatment could mitigate the DCLV-induced increase in IL-6 and TNF-α concentrations in the rats' plasma, lymphocyte, and brain. Conclusion: This study suggests that vitamin C can attenuate brain damage induced by dichlorvos in rats.

Keywords: Vitamin C, Dichlorvos, Pro-inflammatory cytokines, Acetylcholinesterase, Organophosphate, Insecticides, Inflammation, Tnf-alpha, and Interleukin-6.

1.0 Background

The indiscriminate use of dichlorvos and its effects on human is one of the significant public health concerns. Dichlorvos (2, 2-dichlorovinyldimethyl phosphate; DDVP) is an organophosphate insecticide widely used in pest control in developing countries, especially Nigeria. Its trade names include *Sniper*, *Dedevap, Nogos, Nuvan, Phosvit, Vapona, Daksh* and *Otapiapia* (Owoeye et al., 2012). Organophosphate pesticides have been discovered in the soil, water bodies, vegetables, grains and other food products (Cho et al., 2011). Dichlorvos is readily available, cheap and effective. According to World Health Organization, dichlorvos is classified as a Class IB, 'highly hazardous chemical (Chemicals & Organization, 2010). Its route of exposure may be through ingestion, inhalation and skin contact (Chaudhary et al., 2014).

Previous studies have linked dichlorvos exposure to several adverse health effects, namely neurotoxicity

(Wani et al., 2014), hepatoxicity (Ajiboye, 2010; Ogutcu et al., 2008), oxidative stress (El-Demerdash, 2011a, 2011b), reproductive effect (Okamura et al., 2005; Oral et al., 2006; Oya et al., 2017), nephrotoxicity (Hou et al., 2014) and cardiotoxicity (Anand et al., 2009; Saka et al., 2020). The dichlorvos toxicity is mainly irreversible inhibition of neural acetylcholinesterase (AChE), resulting in the accumulation of acetylcholine in the cholinergic synapse (Pereira et al., 2014; Wang et al., 2004).

Pro-inflammatory cytokines (IL-6 and Tnf-α) are mainly produced by activated macrophages in response to inflammatory stimuli. They are involved in the up-regulation of host reactions to inflammation, infections, and neuropathic pains (Dinarello, 2000; Gao et al., 2015; Zhang & An, 2007). Chronic inflammation plays a role in tumor initiation. Approximately 25% of all cancers are caused by chronic inflammation (Perwez Hussain & Harris, 2007). Some epidemiologic studies have established a positive relationship between circulating concentrations of pro-inflammatory cytokines and the risk of lung cancer (Pine et al., 2011; Zhou et al., 2012). Up-regulation of pro-inflammatory cytokines caused by dichlorvos toxicity has also been linked to lung cancer (Cho et al., 2011; He et al., 2018). Globally, lung cancer is the leading cause of cancer-related deaths (Cokkinides et al., 2005; Siegel et al., 2014), hence an urgent need for its management.

Vitamin C, a potent water-soluble antioxidant, has been shown by previous studies to have protective effects against dichlorvos toxicity. *In vitro* and *in vivo* systems exposed to pesticides have also verified that vitamin C has an anticancer and antimutagenic property (Castillo et al., 2000; Durak et al., 2009). Dichlorvos-induced oxidative stress in human erythrocytes was shown to be suppressed by vitamin C treatment (Eroglu et al., 2013). Another study carried out by Owoeye et al. showed an ameliorative effect of Vitamins C and E on the dichlorvos-induced histopathological changes in the lung and liver of rats (Owoeye et al., 2012). Also, the endometrial damage and apoptosis induced in the rat by dichlorvos were reversed by treatment with vitamins C and E (Oral et al., 2006). However, studies on the protective effect of vitamin C on dichlorvos-induced pro-inflammatory cytokines (IL-6 and Tnf-α) are scanty.

The present study was carried out to investigate the protective effect of vitamin C on dichlorvosinduced toxicity caused by inhibition of acetylcholinesterase (AChE) activity and up-regulation of pro-inflammatory cytokines (IL-6 and Tnf-α) concentration in wistar rats.

2.0 Materials and Methods

2.1 Chemicals and Reagents

The organophosphorus compound dichlorvos was obtained from Saro Agrosciences Limited, Nigeria. Streptozotocin was procured from Sigma-Aldrich Chemical Co. (Germany). Vitamin C was purchased from a standard pharmaceutical store, Newton Pharmacy Limited Lagos, Nigeria. All other chemicals and Kits used were of analytical grade.

2.2 Animals

Twenty-four adult male Wistar rats with an average body weight of 100g were purchased from the College of Veterinary Medicine, the Federal University of Agriculture, Abeokuta, Nigeria, and kept at the animal house of the Department of Biochemistry, Lagos State University. The animals were regularly exposed to a 12h light-dark cycle at a controlled temperature (22 ± 2°C) and fed with a standard pellet diet and water *ad libitum.* The rats were acclimatized to their new surrounding for 14 days before treatments.

2.3 Animal Treatments

The rats were randomly divided into three groups: the control group, dichlorvos group, and dichlorvos + vitamin C group. The toxic agent was administered twice daily (morning and evening). The control group received corn oil at a dose of 0.5ml/kg body weight of the rats twice daily. The treated group received dichlorvos at 2mg per kg body weight twice daily in corn oil. While the treated + vitamin C group received 2mg per kg body weight dichlorvos and vitamin C orally twice daily.

2.4 Measurement of Body Weights

The animal body weight was recorded daily using a mechanical scale with an accuracy of ±0.1g before and after

treatment with dichlorvos and vitamin C. Thiscontinued every day in all the groups for the period during which the animals were induced with dichlorvos and treated with vitamin C.

2.5 Animal Blood and Organ Collection

At the end of the 21 days of induction and treatment, the rats fasted overnight before sacrifice. The animals were anaesthetised with light anaesthesia (5% ketamine and 2% xylazine), and the blood were collected by cardiac puncture into heparinised bottles. The brain was removed from the animals for biochemical analysis. Blood samples were centrifuged to separate the plasma and the red blood cells. All samples were stored at -20℃ until analysed. The lymphocyte was separated from other blood components using the *Ficoll* method (Boyle & Chow, 1969).

3.0 Biochemical Analyses

3.1 Acetylcholinesterase activity

According to Ellman et al. (1961), acetylcholinesterase activity was determined by hydrolysing acetylcholine. The increase in absorbance is monitored every 30secs for 3mins at 420nm at 37°C with the use of a spectrophotometer. The incubation mixture was mixed by inversion and it contained 25µl of 5, 5′-dithionitrobenzoic acid (DTNB), 5µl of acetylthiocholine iodide (ATC), 650µl 0f acetylcholine buffer and 5µl of sample.

3.2 IL- and TNF-α concentration

Quantitative *in vitro* analysis of IL-6 and TNF-α in plasma and lymphocyte was done using anti-rat IL-6 and TNF-α ELIZA kits according to the manufacturer's instruction.

3.3. Protein determination

Protein concentration in red blood cell, brain and plasma was determined using Bradford method, where bovine serum albumin served as the standard (Bradford, 1976).

3.4. Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (version 6.0). All results were expressed as the mean ± standard mean error of Mean.

4.0 Results

Table 1 shows the effect of dichlorvos and vitamin C treatments on the body weight, brain weight, and relative brain weight of control and experimental animals at the end of the 3rd week of treatment. There was a significant difference in the body weight of treated animals when compared with that of control. The body weight of animals in the treatment (dichlorvos) group increased significantly (17.9%) than that of the control group at the end of the 3rd week of treatment. Also, a significant increase was observed in the bodyweight of animals treated with dichlorvos + vitamin C compared with that of the control group. This suggests that dichlorvos has no adverse effect on the bodyweight of dichlorvos-induced animals. However, a significant decrease was observed in the bodyweight of animals treated with dichlorvos + vitamin C (11.2%) compared with animals treated with only dichlorvos (17.94%). Furthermore, the brain weight of the control group (1.51 ± 0.03), when compared with that of the treatment group (1.54 ± 0.03) and treatment + vitamin C (1.60 ± 0.05) group, showed a significant increase. No substantial increase or decrease was observed in the treated animals' relative weight compared with the control group.

Depicted in figure 1 is the acetylcholinesterase acidity in plasma, red blood cell and brain after exposure to the treatment group. A significant difference in acetylcholinesterase (AChE) activity in the plasma, the red blood cell, and the brain was observed across the groups (figure 1). In the plasma, dichlorvos exposure caused a significant increase in AChE activity compared with the control. This increase was markedly lowered by vitamin C treatment, although not to the basal level. In contrast, AChE activity in red blood cell was significantly inhibited by dichlorvos exposure. The activity was, however, restored to the control level by co-treatment with

vitamin C. In the brain, dichlorvos exposure significantly reduced AChE activity when compared with the control. Though Vitamin C treatment ameliorated the loss of activity, this was not reversed to the control level (figure 1). A decrease in AChE activity in the brain of animals treated with dichlorvos in this study demonstrated the inhibitory effect of dichlorvos on AChE, which is a neurotoxic effect of dichlorvos (Wang et al., 2004).

Figures 2 and 3 show the effect of dichlorvos and co-treatment with vitamin C on TNF-α and IL-6 concentrations in the control and treated groups. In the plasma, lymphocyte and brain of experimental animals, TNF-α concentrations were significantly increased in the dichlorvos-treated animal group compared with the control group. Treatment with vitamin C mitigated this effect in all the compartments but was only totally reversed in the lymphocyte. The highest TNF-α concentration was detected in the brain of the dichlorvos-treated group compared with the plasma and the lymphocyte (figure 2).

Similarly, the IL-6 concentrations were significantly increased in the dichlorvos-treated groups' plasma, lymphocyte, and brain. The lymphocyte of the dichlorvos-treated group showed the highest concentration of IL-6 compared with the plasma and the brain. This increase in IL-6 was significantly reduced upon treatment with vitamin C in all three compartments (figure 3).

Table 1 Effect of administration of vitamin C on the body and brain weight of dichlorvos-induced toxicity in rats

Experimental Groups	Bodyweight (g)			Absolute Brain weight (g)	Relative Brain weight (g)
	Initial	Final (3rd week)	%Change		
Control	209 ± 12.2[a]	226 ± 8.61[a]	8.13 ± 0.29[a]	1.51 ± 0.03[a]	0.007 ± 0.0003[a]
Dichlorvos	223 ± 2.88[a]	263 ± 1.70[b]	17.94 ± 0.41[b]	1.54 ± 0.03[a]	0.007 ± 0.0002[a]
Dichlorvos + Vitamin C	223 ± 2.88[a]	248 ± 5.06[a]	11.21 ± 0.76[a]	1.60 ± 0.05[a]	0.007 ± 0.0004[a]

Each value represents the mean±S.E.M. of 8 rats. Values within a row with different alphabets are significantly different from each other, P < 0.05, compared to control.

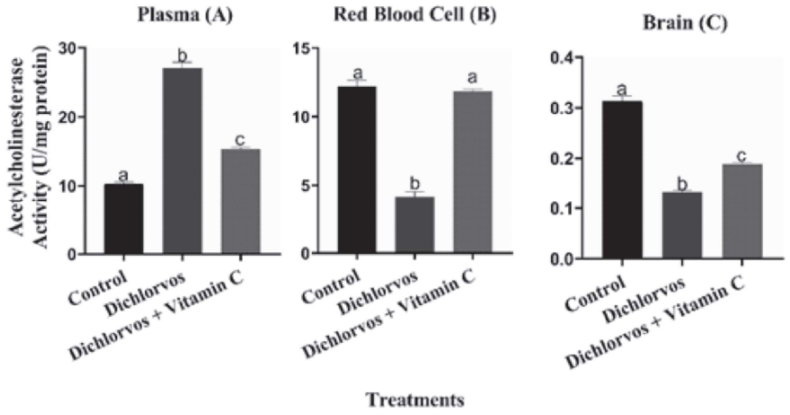

Figure 1: Effect of administration of vitamin C on the acetylcholinesterase activity in the plasma (1A), red blood (1B) and brain (1C) of dichlorvos-induced toxicity in rats. Each bar represents the mean ± S.E.M. of 8 rats. Bars with different alphabets are significantly different at $p < 0.05$

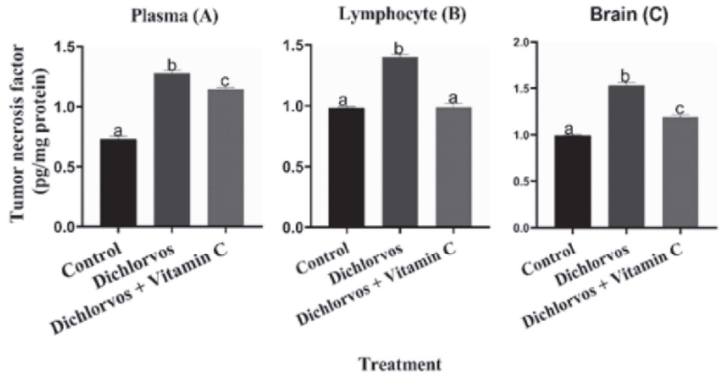

Figure 2: Effect of administration of vitamin C on the TNF-α in the plasma (2A), red blood cell (2B) and brain (2C) of dichlorvos-induced toxicity in rats. Each bar represents the mean ± S.E.M. of 8 rats. Bars with different alphabets are significantly different at $p < 0.05$

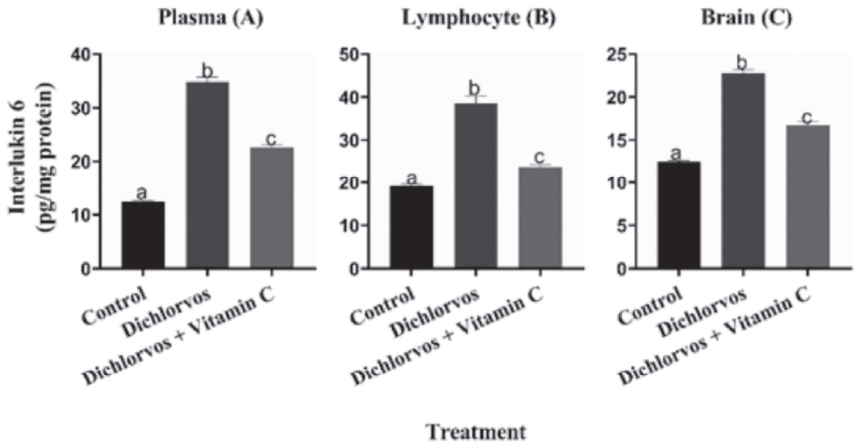

Figure 3: Effect of administration of vitamin C on the TNF-α in the plasma (3A), red blood cell (3B) and brain (3C) of dichlorvos-induced toxicity in rats. Each bar represents the mean ± S.E.M. of 8 rats. Bars with different alphabets are significantly different at $p < 0.05$

4.0 Discussion

This study investigated the protective effect of vitamin C on dichlorvos-induced toxicity using a rat model. Dichlorvos, an organophosphate pesticide, exerts its neurotoxic effect mainly by irreversibly inhibiting AChE activity leading to the accumulation of acetylcholine in the cholinergic synapse. Acetylcholinesterase is an enzyme that catalyses the breakdown of the neurotransmitter acetylcholine to acetate ion and choline. It is found at the neuromuscular junction, the synapse between nerve cells and muscle cells. Thus, it is a crucial regulator of neuronal processes. Acetylcholinesterase increase has been attributed to both muscarinic and nicotinic toxicity. (Khan et al., 2009). In this study, a decrease in AchE activity compared with the control was observed in the brain and red blood cells of rats exposed to dichlorvos. This agrees with Aminu *et al.* and Srivastava et al.; Imam et al., 2018; Srivastava & Shivanandappa, 2011) who reported a significant decrease in AChE activities in various brain regions of animals following dichlorvos exposure. Also, a reduction in AChE activity in red blood cell, as observed in this study, agrees with a similar study reported by Ochigbo et al. (2017), where a reduction in serum AChE activity of rats exposed to dichlorvos was observed. Other studies carried out by Okamura et al and He et al. demonstrated decreased AChE activity in red blood cell of animals after dichlorvos exposure (He et al., 2018; Okamura et al., 2005). The AChE

activity inhibition observed in this study agrees with one of dichlorvos' mechanism of actions (Wang et al., 2004). AChE activity induced by dichlorvos in the brain and red blood cells increased after rats were treated with vitamin C in this study. This suggests a protective ability of vitamin C against dichlorvos-induced neurotoxicity, which could result from its antioxidant property.

On the other hand, no reduction in AChE activity was observed in the plasma of rats exposed to dichlorvos in this study; instead, an increase in the activity was observed. This is contrary to the result in a study by Okamura et al. (2005), where AChE activity in plasma showed a significant decrease. Also, a study by Duysen *et al.* revealed an inhibition in plasma AChE activity of mice treated with dichlorvos (Duysen & Lockridge, 2011). The contradiction in plasma AChE activity could have resulted from differences in route of exposure, duration and dose of dichlorvos exposure. In this study, rats were exposed to dichlorvos via oral administration. In contrast, subcutaneous exposure to dichlorvos was investigated in other studies.

A significant increase in body weight and brain weight of animals treated with dichlorvos compared to control was observed in this study. The possible cause of this weight gain could be the disruption of endocrine systems, which is one of the hallmarks of environmental chemicals and pesticides like dichlorvos (Birnbaum & Fenton, 2003; Palioura et al., 2011; Tabb & Blumberg, 2006).

TNF-α and IL-6 are the pro-inflammatory cytokines investigated in this study. Pro-inflammatory cytokines are produced mainly by activated macrophages. They regulate host responses to infection, inflammation, and trauma, worsening pathological disorders (Dinarello, 2000; Gao et al., 2015). Ample evidence that pro-inflammatory cytokines contribute to the process of pathological pain has been provided by several studies (J. DeLeo, 1996; J. A. DeLeo et al., 1996; Ramer et al., 1998; Wagner & Myers, 1996; Woolf et al., 1997). Pro-inflammatory cytokines have also been associated with the risk of lung cancer (He et al., 2018; Pine et al., 2011; Zhou et al., 2012). TNF-α and IL-6 concentrations were significantly increased in the dichlorvos-treated animal group when compared with the control group. This is consistent with previous studies where pro-inflammatory cytokines, including TNF-α and IL-6, were significantly increased in animals exposed to dichlorvos (Binukumar & Gill, 2010; He et al., 2018; Sunkaria et al., 2014). In this study, pro-inflammatory cytokines concentrations induced by dichlorvos exposure in animals were significantly reduced following vitamin C treatment. This is indicative of the protective effect of vitamin C against dichlorvos toxicity related to pro-inflammatory cytokines.

5.0 Conclusion

In the present study, we have investigated the link between acetylcholinesterase activity and pro-inflammatory cytokines and dichlorvos-induced toxicity. However, as demonstrated by the result of this study, these harmful effects could be ameliorated with vitamin C treatment.

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