

# Ameliorative Potentials of Vitamin C against Inhaled Dichlorvos Lung Toxicity of Wistar Rats

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## Abstract

**Introduction:** Dichlorvos (DDVP), an active ingredient of Sniper insecticide, is commonly used in Nigeria to control insects by killing them thereby preventing the spread of diseases. However, it can be highly toxic to humans when inhaled into the respiratory system which could elicit oxidative stress and cause respiratory infections. Vitamin C is known to be an anti-oxidant, capable of inhibiting oxidative stress. This study was aimed at investigating the ameliorating effect of vitamin C on dichlorvos-induced toxicity of the rat lung. **Materials and methods:** Forty (40) male wistar rats (weighing 150 – 200g) were recruited and randomly grouped into five (5) groups of eight (8) rats each. A group was the control; two groups were exposed to graded concentrations of DDVP while the last two groups were treated with vitamin C. Body weights were obtained before and after the period of 21-day exposure. After 21 days, histopathological and biochemical analysis were carried out to examine the level of toxicity of DDVP and effect of vitamin C treatment on the lung tissues. **Results:** Rat groups treated using vitamin C administration had significant improvements in body weights compared to DDVP-exposed groups. MDA levels as well as CATA and SOD activities increased significantly on vitamin C-treated rat groups ( $p < 0.05$ ). Post treatment of rat groups with vitamin C showed that the lung histoarchitecture significantly improved. **Conclusion:** It can be concluded that Vitamin C could be a supplementary remedy in organophosphate (dichlorvos) poisoning through inhalation.

**Keywords:** Dichlorvos, oxidative stress, vitamin C, Sniper, organophosphate poisoning.

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## INTRODUCTION

Insecticides are chemical substances used to control insects by killing them or preventing them from spreading diseases thereby causing harm to humans [1-3]. In Nigeria, the need to reduce the prevalence of diseases such as malaria, typhoid fever, among low-income households has resulted in the usage of cheaper insecticides as they are cheaper to produce and purchase, and easily accessible [4]. Dichlorvos (DDVP), an organophosphate, is a predominant pesticide used in domestic control of insects in developing countries [5]. Sold using the brand name, Sniper, it is predominantly used as an insecticide due to its effectiveness in killing insects [6, 7]. Because dichlorvos is highly volatile, inhalation is the most common route of exposure [8].

Organophosphates have been shown to induce toxicity of the nervous system by inhibiting acetyl cholinesterase activity [9]. Also, it has been postulated that organophosphate pesticides produce oxidative stress in different tissues through the formation of reactive oxygen species (ROS) [10-12]. In the respiratory system, experimental studies have shown that exposure to organophosphates could cause lesions such as pulmonary edema and congestion, as well as emphysema and broncho-constriction [7, 13].

Vitamin C is one of the potent reducing agents in biological systems, working as a scavenger of oxidizing free radicals and harmful oxygen derived species, such as hydroxyl radical, hydrogen peroxide ( $H_2O_2$ ), and singlet oxygen [14 -16]. Therefore, this study aimed at investigating the ameliorating effect of

vitamin C on dichlorvos-induced toxicity of the rat lung tissue.

## MATERIALS AND METHODS

### Experimental design

For the purpose of achieving this study, an ethical clearance was applied for and obtained (with reference number UPH/CEREMAD/REC/MM78/049) from the Research Ethics Committee of the University of Port Harcourt. Experimental protocols were carried out in accordance to the guidelines set by the University of Port Harcourt Animal Care and Use in Research Ethical Committee, which conforms to internationally acceptable guidelines on the ethical use of experimental animals in research. Sniper was purchased from the Dooka Pharmacy located opposite University of Port Harcourt Teaching Hospital, Alakahia, Port Harcourt. Ascorbic acid was obtained from the same pharmacy shop. Forty (40) male wistar rats (weighing 150 – 200g) were recruited for this research and were randomly placed into five (5) groups of eight (8) rats per group. A box-like cage measuring 40cm x 40cm x 15cm made of Perplex glass was used as inhalation chambers for the experimental rats. Group 1 (the control group) was administered *ad libitum* for 21 days. Group 2 was exposed to dichlorvos inhalation (20ml DDVP/80ml distilled water – v/v) for 21 days for 4 hours per day at room temperature. Group 3 was exposed to dichlorvos inhalation (40ml DDVP/60ml distilled water – v/v) for 21 days for 4 hours per day at room temperature. Group 4 was exposed to dichlorvos inhalation (20 ml DDVP/80ml distilled water – v/v) for 4 hours per day at room temperature followed by administration of ascorbic acid (160mg/kg) once daily for 21 days. Group 5 was exposed to dichlorvos inhalation (40 ml DDVP/60ml distilled water – v/v) for 4 hours per day at room temperature followed by administration of ascorbic acid (160mg/kg) once daily for 21 days.

### Biochemical analysis

Upon completion of the experiment, 10ml of blood was taken from the rats of each group and subjected to biochemical analysis. The following assays were analyzed;

#### Assessment of catalase (CAT) activity

The activity of catalase in blood plasma was determined using hydrogen peroxide and peroxidase reagent containing peroxidase and a chromogen system according to Aebi [17]. This method is based on the peroxide removal by catalase. At the end, the reaction was stopped by the peroxidase reagent and consequent reduction of remaining hydrogen peroxide to form a chromophore which was recorded at 505 nm.

#### Assessment of superoxide dismutase (SOD) activity

The activity of SOD in blood plasma was determined using the nitroblue tetrazolium (NBT) method described by Nishikimi *et al.* [18]. This method

is based on the ability of enzyme to inhibit the reduction of NBT by superoxide radicals. The reduction of NBT by superoxide radicals to blue formazan was followed at 560 nm.

#### Determination of malondialdehyde (MDA) level as a lipid peroxidation marker

Malondialdehyde (MDA) was determined in blood serum according to Ohkawa *et al.* [19]. In this method, thiobarbituric acid (TBA) reacts with MDA at 95°C for 30 min. The resultant product was measured at 530 nm.

#### Histopathological examination

At completion of exposure, animals were anaesthetized with chloroform. Animals were sacrificed and lungs from all rat groups were harvested for routine histopathology procedure. These lungs were fixed in 10% formaldehyde, and then hydrated with grades of ethanol (75%, 90%, 95% and 100%). Dehydration was then followed by clearing the samples in two changes of xylene. Samples were then impregnated in molten paraffin wax, then embedded and blocked out. Paraffin sections of 5µm thick were cut using a sledge microtome and mounted on glass slides and stained with H&E staining method. The stained sections were morphologically evaluated and the pictures of the slides compared. Photomicrographs were obtained with the aid of an Amscope camera fitted on an Accu-scope microscope.

## STATISTICAL ANALYSIS

Data was analyzed using Statistical Package for Social Sciences (SPSS IBM version 23.0). Values were expressed as mean ± standard deviation in descriptive statistics. One-way analysis of variance (ANOVA) was used to analyze the difference between the groups followed by least significant difference (LSD) post-hoc test. Confidence interval was set at 95% and therefore P<0.05 was considered significant.

## RESULTS

### Effect on changes in rat body weight

Table 1 below shows the mean (initial) body weight of the rat groups before the start of the experiment. The control group had a mean body weight of 173.5±5.3g, while 20ml DDVP and 40ml DDVP rat groups had 179.5±5.1g and 182.0±6.4g, respectively. While the vitamin C-treated rat groups (20ml DDVP + 2ml Vitamin C and 40ml DDVP + 2ml Vitamin C) had mean body weights of 178.5±5.7g and 180.0±6.5g, respectively.

After a 21-day inhalation exposure period, there was a significant decrease in the mean body weights of the rat groups exposed to inhalation of 20ml and 40ml DDVP solutions (162.0±4.2g and 153.0±3.7g). Rat groups that were treated using vitamin C administration had significant increase in body

weights compared to DDVP-exposed groups (164.5±5.6g and 166.0±4.3g).

**Effect of vitamin C on DDVP-induced biochemical changes**

In table 2, the MDA levels as well as the activities of CATA and SOD were decreased significantly in the rat groups that were exposed to various concentrations of DDVP ( $p < 0.05$ ). However, the CATA and SOD activities were increased significantly upon the treatment of rat groups with vitamin C ( $p < 0.05$ ). MDA levels also increased in like manner to oxidative activities.

**Effect of vitamin C on rat lung histopathology**

Histological observations from the lung tissue of control group showed normal tissue organization of lung parenchyma. The presence of the bronchial lumen and associated alveolar spaces are clearly seen. However, there were notable signs of bronchio-dilation of bronchial lumen and presence of aggregations of bronchus associated lymphoid tissues (BALT). Post treatment of rat groups with vitamin C showed that the lung histoarchitecture significantly improved. There were observable reductions in the aggregation of lymphoid tissues.

**Table-1: Effect of vitamin C on DDVP-induced rat body weight**

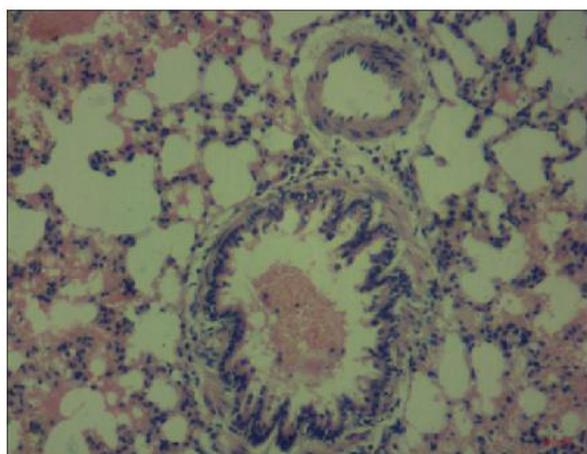
Groups	Mean initial body weight (g)	Mean final body weight (g)
Control (feed and water only)	173.5±5.3	190.0±6.7
20ml DDVP	179.5±5.1	162.0±4.2
40ml DDVP	182.0±6.4	153.0±3.7
20ml DDVP/2ml Vit. C	178.5±5.7	164.5±5.6
40ml DDVP + 2ml Vit. C	180.0±6.5	166.0±4.3

**Table-2: Effect of vitamin C on DDVP-induced rat lung oxidative stress markers**

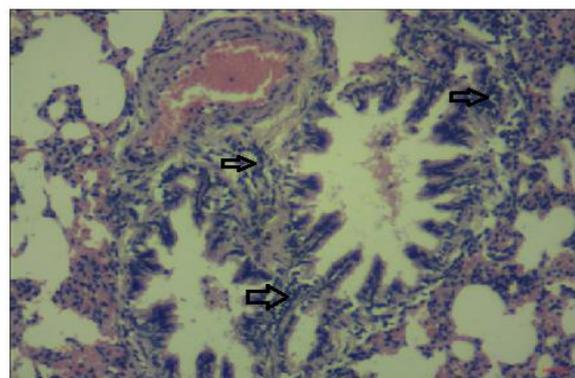
Group	CATA(u/l)	SOD(u/ml)	MDA(nmol/ml)
Negative group	1.78±0.93	0.69±0.05	0.16±0.02
20ml DDVP	2.06±0.30*	0.63±0.03	0.37±0.02*
40ml DDVP	2.52±0.27*	0.90±0.05*	0.65±0.03*
20ml DDVP + 2ml Vit. C	1.86±0.66*	0.57±0.04*	0.29±0.06*
40ml DDVP + 2ml Vit. C	2.43±0.67*	0.82±0.16*	0.59±0.10*

Each value represents mean ± SD, Values marked with asterisk (\*) differ significantly from the control group ( $p < 0.05$ ).

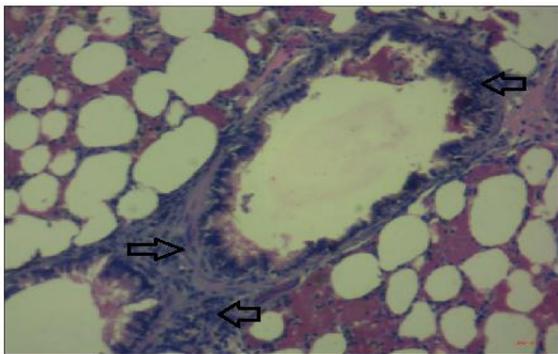
CATA = Catalase, SOD =Superoxidase dismutase, MDA = Malondialdehyde.



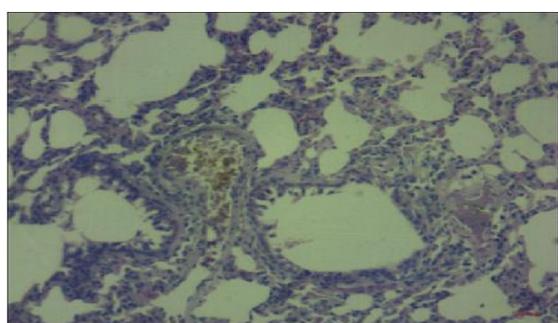
**Fig-1: Photomicrograph of rat lung tissue of control group (x40)**



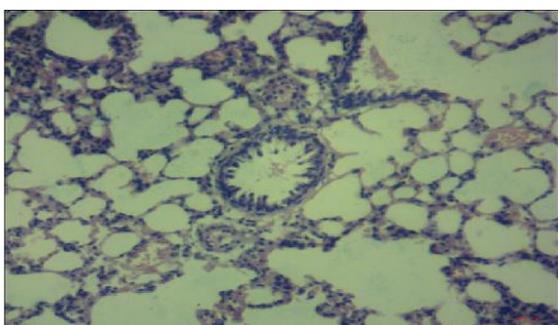
**Fig-2: Photomicrograph of rat lung tissue (exposed to 20ml DDVP/80ml distilled water). Mild signs of bronchio-dilation and aggregations of bronchus associated lymphoid tissues (BALT) as shown by the arrows (x40)**



**Fig-3: Photomicrograph of rat lung tissue (exposed to 40ml DDVP/60ml distilled water). Visible signs of bronchio-dilation and aggregations of bronchus associated lymphoid tissues (BALT) shown clearly by the arrow signs (x40)**



**Fig-4: Photomicrograph of Vitamin C-treated rat lung tissue (exposed to 20ml DDVP/80ml distilled water). Mild signs of bronchio-dilation and reduction in aggregations of bronchus associated lymphoid tissues (BALT) (x40)**



**Fig-5: Photomicrograph of vitamin C-treated rat lung tissue (exposed to 40ml DDVP/60ml distilled water). Little or no signs of bronchio-dilation and moderate reductions in aggregations of bronchus associated lymphoid tissues (BALT) (x40)**

## DISCUSSIONS

The present study was undertaken to analyze the toxicological effects of inhalation of DDVP (Sniper) on the lungs of wistar rats and check whether Vitamin C (ascorbic acid) could possibly ameliorate the toxic effect. To the best of the researchers' knowledge, there have been arguably no works that have been done to investigate the protective effect of Vitamin C on DDVP-induced lung toxicity.

This study showed that there was a significant decrease in the mean body weights of the non-treated

groups that were administered graded levels of DDVP compared to that of the control group. However, the rat body weights of the treated groups exhibited a significant increase compared to the non-treated groups. A similar study by Zhang *et al.* [20] also showed from their study that upon the administration of Acetamiprid, an insecticide, there was also a reduction in the body weights of the experimental mice. This reduced weight loss could be best explained as occurring due to increased oxidative stress markers. The treatment with ascorbic acid inhibited the oxidative stress activities of these markers, thereby improving the weight gain of treated groups.

Also, this study showed that the MDA levels as well as the activities of CATA and SOD were increased significantly in the rat groups that were exposed to various concentrations of DDVP ( $p < 0.05$ ). A similar study conducted by Achudume *et al.* [21] on the effect of Dichlorvos and Parquat on oxidative markers showed that while SOD levels increased due to organophosphate toxicity, CAT and MDA levels were inhibited. The present findings done in this study indicate that free radicals will increase the levels of SODs and CAT in the body due to their increasing roles in fighting off increased exposure to free radicals. In another similar study carried out by Almaeen and Ibrahim (2018) on the toxic effect of cyclophosphamide on lung antioxidative parameters, it was observed that there was a decrease in the enzymatic activities of CATA and SOD levels. This study showed that vitamin C helped increase the anti-oxidative activities of CATA and SOD levels. This is in line with the study done by Almaeen and Ibrahim [22] which showed that Vitamin C ameliorated the effect of cyclophosphamide on pulmonary toxicity of wistar rats.

The results showed the control tissue having normal architecture and organization devoid of peribronchiolar collagen proliferation, excessive dilation of the bronchial lumen and aggregation of lymphoid cells, showing distinctively the bronchioles and blood vessels. However, groups exposed to high concentration of DDVP showed signs of toxicity such as peribronchiolar collagen proliferation, excessive dilation of the bronchial lumen and aggregation of lymphoid cells. In line with Owoeye *et al.* [7], there were observed signs of extension in the basal associated lymphoid tissue (BALT) for rats exposed for one, four and five weeks. Upon the administration of vitamin C, there were noted changes in lung architecture compared to untreated groups.

## CONCLUSION

It can be concluded that ascorbic acid (Vitamin C), due to its anti-oxidative property, could be a supplementary remedy in organophosphate (dichlorvos) poisoning through inhalation.

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## CONFLICT OF INTEREST

Both authors declare that there is no competing interest.

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