THE POTENTIAL PROTECTIVE EFFECTS OF ASCORBIC ACID AGAINST DICHLORVOS-INDUCED HEPATOTOXICITY

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ABSTRACT

BACKGROUND: The reliance on commonly used organophosphates for agricultural purposes still poses significant health risks to local agricultural farmers. **OBJECTIVE**: This study investigated the hepatoprotective effects of ascorbic acid against dichlorvos-induced liver toxicity using animal models. **METHODOLOGY**: After dividing twenty-five (25) Wistar rats randomly into five groups, group 1 (control) was exposed to clean air at room temperature while groups 2 and 3 were exposed to 10ml and 20ml dichlorvos (DDVP) solutions, respectively. Groups 4 and 5 were exposed to their respective 10ml and 20ml dichlorvos (DDVP) solutions and subsequently administered with 2ml of ascorbic acid orally per day. The exposures were conducted in well-ventilated chambers at 4 hours daily for 21 days. Body weight, serum liver enzyme markers such as alanine transaminase (ALT), aspartate transaminase (AST), albumin (ALB) and total bilirubin (TB) levels, and liver histopathology were evaluated upon completion of the 21-day exposure. **RESULTS**: Study results demonstrated that DDVP-exposed rats showed significant changes in body weight and liver enzyme levels, and severe histopathological liver damage. On the contrary, observations from rat groups treated with ascorbic acid revealed significant body weight increases, lower levels of AST, ALT, and TB while ALB levels increased. Liver histomorphology showed reduced necrosis and central vein congestions, as well as decreased hepatocytic distortions and cytoplasmic vacuolations. **CONCLUSION**: Study findings suggest that ascorbic acid could have hepatoprotective benefits against DDVP-induced liver toxicity, highlighting its potential therapeutic applications in ensuring liver health due to pesticide exposure.

Keywords: Dichlorvos, organophosphates, liver, toxicity, ascorbic acid

INTRODUCTION

Organophosphates (OPs) pesticides are a class of chemicals widely used in agriculture for pest control due to their effectiveness and relatively low cost **(Ojo, 2016; Oguh et al., 2019).** OPs function primarily as acetylcholinesterase (AChE) inhibitors responsible for breaking down acetylcholine – a neurotransmitter that transmits signals in the

nervous system. When OPs inhibit AChE, acetylcholine accumulates at nerve endings, resulting in persistent activation of specific organ systems **(Kaur et al., 2014; Aroniadou-Anderjaska et al., 2023).** This overstimulation can result in a range of symptoms, from mild to severe, depending on the level of human exposure to these chemicals. However, several evidence have suggested that different routes of administration could lead to significant variations in the absorption, distribution, metabolism, and excretion of these chemicals thereby affecting the extent of liver damage and the overall toxicity profile of the compounds **(Saafi et al., 2011; Ayed-Boussema et al., 2012; Beydilli et al., 2015).**

However, their use has raised significant concerns regarding human health, particularly due to their toxicological effects **(Hart et al., 2022).** Moreover, agricultural workers often lack access to proper safety equipment and training, increasing their risk of exposure. In many cases, these workers may not be aware of the potential dangers associated with the pesticides they handle, leading to unsafe practices and increased health risks **(Ojo, 2016).** OPs can lead to the generation of reactive oxygen species (ROS), in turn causing significant hepatotoxic effects **(Sharma et al., 2014).** Studies have shown that exposure to OPs like chlorpyrifos and malathion increases markers of oxidative stress in liver tissues **(Goel et al., 2005; Lasram et al., 2014).** These chemicals can cause instabilities of these markers, in turn reducing the liver's ability to neutralize ROS **(Zhu et al., 2012; Arauz et al., 2016).**

Furthermore, OPs can inhibit enzymes involved in detoxification and metabolism that could lead to liver damage due to the accumulation of toxic metabolites **(Badr, 2020; Mali et al., 2023).** For instance, chlorpyrifos has been shown to downregulate CYP3A expression, which is crucial for the metabolism of various endogenous and exogenous compounds **(Rouimi et al., 2012).** Some histopathological studies have revealed that exposure to OPs can lead to significant liver damage, characterized by necrosis, and inflammation **(Badr, 2020; Sharma and Sangha, 2014; Li et al., 2022).** These changes are often accompanied by elevated serum liver enzymes, indicating hepatocellular injury – although the severity of these histopathological alterations depends on the duration and level of exposure.

It is important to understand that in real-life settings, most agricultural workers that utilize OPs for agricultural practices are usually predisposed to them through inhalation **(Boonupara et al., 2023).** When these chemicals are usually inhaled, they enter the respiratory system and diffuse through the pulmonary capillaries to enter the bloodstream. Upon mixing with the blood, they are transported via systemic circulation and enter the liver through the arterial blood supply **(Priyadharshini et al., 2017; Sobolev et al., 2022).** Because of this knowledge, there is a need to improve literature on the effects of these chemicals on the liver. In addition, recent research has been tailored towards providing efficient therapeutic approaches to mitigate liver damage from OP inhalation exposure. Therefore, the goal of the current study was to investigate ascorbic acid's potential for ameliorating dichlorvos-induced hepatotoxicity after subacute exposure.

MATERIALS AND METHODS

Ethical Considerations

An ethical clearance was obtained from the Research Ethics Committee of the University of Port Harcourt (reference number UPH/CEREMAD/REC/MM78/049). The procedures for the experiments followed the rules set by the University of Port Harcourt Animal Care and Use in Research Ethical Committee. In line with the regulations for the protection of animals used in scientific procedures in the Directive 2010/63/EU, proper handling of cages was done and the conditions under which animals are kept were favorable throughout the research process. These rules are in line with the ethical use of animals in research that is accepted around the world.

Sample Collection/Identification

Chemicals used in this study, including dichlorvos or DDVP (commercially available as Sniper) and ascorbic acid, were purchased from a local pharmaceutical store, situated in Port Harcourt, Rivers State, Nigeria.

Experimental Design

This was a randomized controlled trial (RCT) study design that involved the use of twenty-five (25) adult male Wistar rats. Rats were divided into five (5) experimental groups containing five (5) each. In line with **Festing et al. (2006),** the resource equation method based on the degrees of freedom (E) was used to determine the suitable rat sample size for the study based on two criteria, the number of groups (k) and the number (n) of rats per group. Therefore, E was calculated as; thus, $kn - k = (25 - 5) = 20$. Since the value of E is 20, it was considered sufficient to use five (5) rats per group.

An inhalation chamber made of Perplex glass, measuring 40 cm x 40 cm x 15 cm, was used for experimental rats during exposure periods. In line with literature as reported by **Akang et al. (2012)** and **Awotunsin et al. (2019)**, the average lethal concentration for dichlorvos inhalation is 50 ml DDVP/50ml distilled water. The groups were designed as follows;

Group 1 (Control): Rats not exposed to DDVP and were administered food and water ad libitum.

Group 2: Rats were exposed to a concentration of 10 ml DDVP/90 ml distilled water.

Group 3: Rats were exposed to a concentration of 20 ml DDVP/80 ml distilled water.

Group 4: Rats were exposed to a concentration of 10 ml DDVP/90 ml distilled water, followed by administration of 2 ml of ascorbic acid (160 mg/kg) once daily.

Group 5: Rats were exposed to a concentration of 20 ml DDVP/80 ml distilled water, followed by administration of 2 ml of ascorbic acid (160 mg/kg) once daily.

All exposures were conducted in the well-ventilated chambers for 4 hours daily over a 21-day period. The exposure chambers were designed to allow the volatile components of DDVP to evaporate and saturate the environment.

Evaluating Serum Biochemical Indices

On the final day of exposure, the rats were sacrificed, and their blood samples were collected using EDTA bottles. Later, blood centrifugation was done using a refrigerated centrifuge to obtain plasma and then analyzed in the laboratory to assess levels of serum liver enzyme markers. The determination of both serum alanine transaminase (ALT) and aspartate transaminase (AST) activities were conducted using the **Reitman and Frankel (1957)** method. Also, serum albumin levels were determined using a dye binding technique that utilizes the ability of albumin to form a stable, blue-colored complex with bromocresol green dye, and serum total bilirubin concentration was determined using the dimethylsulphoxide method **(Tietz et al., 1994).**

Histopathological Evaluation

After the exposure period, the animals were anesthetized with chloroform and sacrificed to harvest their livers for histopathological analysis. Prior to being embedded in paraffin wax, the livers were dried using varying ethanol concentrations, fixed in 10% formaldehyde, and cleaned with xylene. Thin sections (5 µm thick) were cut, mounted on glass slides, and stained with hematoxylin and eosin (H&E). The stained sections were examined under a light microscope, and photomicrographs were taken for detailed comparison.

Statistical Analysis

IBM version 23.0 of the Statistical Package for the Social Sciences (SPSS) and Microsoft Excel 2019 edition were used to analyze the data. The values were presented in tabular style. Rat body weight variations between the baseline

and end weights were analyzed using a paired t-test, and group differences in body weight and serum biochemical markers were compared using a one-way analysis of variance (ANOVA). A significant p-value was defined as one with a confidence level of less than 0.05.

RESULTS

Rat Body Weight Alterations

Table 1 presents the differences between initial (before exposure) and final (after exposure) mean body weights of the rat groups during the 21-day inhalation exposure. The control group exhibited initial and final body weights of 173.6g and 190.0g. Rats administered with 10 ml DDVP showed initial and final body weights of 181.3g and 161.5g, respectively and those administered with 20 ml DDVP had mean body weights of 179.7g and 153.0g, respectively. The final body weights differed significantly ($p < 0.05$) from the initial weights for both 10ml DDVP and 20ml DDVP-induced groups. However, the groups treated with ascorbic acid for both 10 ml DDVP and 20 ml DDVP-treated groups showed that their final mean body weights differed significantly ($p < 0.05$) from both their corresponding initial body weights and their final body weights for both their DDVP-exposed groups (171.0g and 164.5g respectively).

Changes in Serum Liver Profile Markers

As seen in **Table 2**, AST levels of the control group (14.50u/l) were significantly lower compared to both 10ml DDVP and 20ml DDVP-exposed groups (28.50u/l and 32.00u/l) respectively. ALT levels of the control group (4.65u/l) were significantly lower compared to both 10ml DDVP and 20ml DDVP-exposed groups (7.50u/l and 10.80u/l) respectively. Total bilirubin levels of the control group (3.75mg/dl) were significantly lower compared to both 10ml DDVP and 20ml DDVP-exposed groups (5.80mg/dl and 6.20mg/dl) respectively. There were no significant differences between serum albumin levels of the control group and the DDVP-exposed groups. However, the groups treated with ascorbic acid for both 10 ml DDVP and 20 ml DDVP-treated groups showed significant decreases ($p < 0.05$) from their corresponding DDVP-exposed groups as shown in the table.

Changes in Histopathology of the Liver

The control group liver tissues **(A)** showed normal architecture, while rats exposed to 10ml DDVP **(B)** and 20ml DDVP **(C)** exhibited mild to moderate histopathological changes, including central vein congestion, reduced sinusoidal spaces, necrosis, and distorted hepatocytes. Conversely, 10ml DDVP **(D)** and 20ml DDVP-treated **(E)** livers displayed improved liver architecture, with reduced necrosis and hepatocyte distortion **(Figure 1).**

Table (1): Rat body weight changes upon DDVP exposure and ascorbic acid treatments

Groups	Initial body weight (g) (Mean ±Standard deviation)	Final body weight (g) (Mean ±Standard deviation)
Control	173.6±5.3	190.0±6.7
10ml DDVP	181.3±4.7	161.5±5.0#
20ml DDVP	179.7±5.1	153.0±4.2#
10ml DDVP + 2ml Ascorbic Acid	184.3±5.3	171.0±4.7*#
20ml DDVP + 2ml Ascorbic Acid	178.6±5.7	164.5±5.6*

represents significant mean difference between initial and final body weights of all groups
* denotes highest significant difference in DDVP-treated group comparison with its corresponding DDVP-exposed groups

Table (2): Changes in serum liver profile markers upon DDVP exposure and ascorbic acid treatments

Group	AST(u/L) (Mean ±SD)	ALT(u/L) (Mean ±SD)	TB (mg/dL) (Mean ±SD)	ALB (g/dL) (Mean ±SD)
Control	14.50±2.95	4.65±1.39	3.75±0.78	36.50±0.71
10ml DDVP	28.50±4.95*	7.50±3.96*	5.80±0.99*	36.00±4.24
20ml DDVP	32.00±19.80*	10.80±7.35*	6.20±2.62*	34.00±2.83
10ml DDVP+ 2ml Ascorbic acid(160mg/kg)	14.50±2.12#	4.35±0.49#	3.25±0.64#	39.50±3.54
20ml DDVP+2ml Ascorbic acid (160mg/kg)	17.50±6.36#	5.50±2.12#	3.65±1.20#	37.00±1.41

* indicates significant mean difference of DDVP-exposed group in comparison with control group

signifies highest significant difference in DDVP-treated group comparison with its corresponding DDVP-exposed groups.

AST = Aspartate aminotransferase, ALT = Alanine transaminase, TB = Total bilirubin, ALB =Albumin, SD = Standard deviation

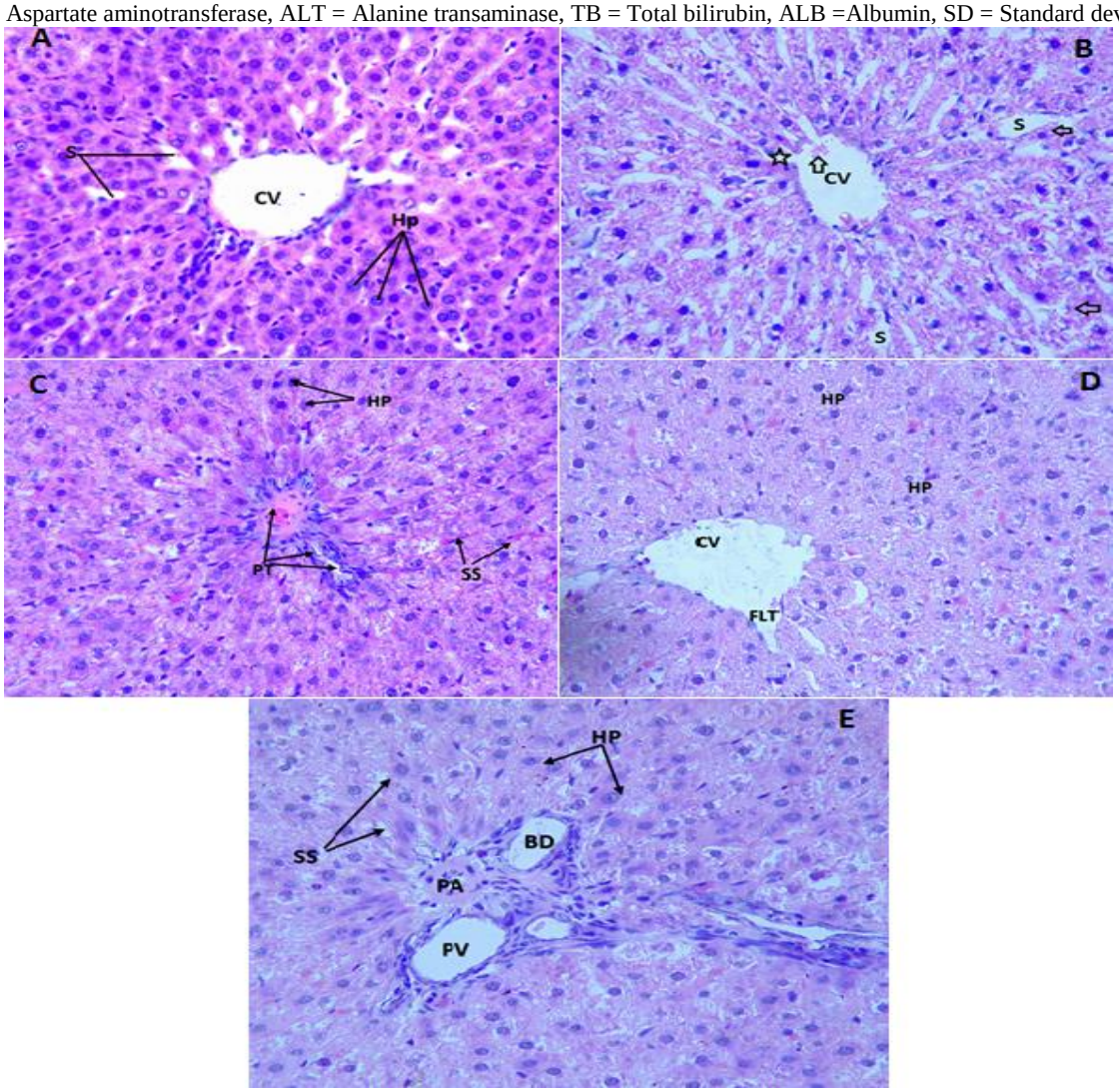

Figure (1): Liver photomicrographs showing the morphological changes in the histo-architecture of the rat groups (**A** – **E**). Group **A** (control group) shows central vein (CV), associated with normal hepatocytes (HP), and clear sinusoidal spaces (S). Group **B** (10ml DDVP-exposed) liver tissue shows central vein (CV) congestions (up-arrow) with lesser hepatocytes (back arrows), notable vacoulations (star-shaped), and less-organized sinusoids (S). Group **C** (20ml DDVP-exposed) liver tissue shows portal triad (PT), hepatocytes (HP) and intervening sinusoids (SS) are not well-organized. Group **D** (10ml DDVP-exposed+2ml ascorbic acid-treated) liver tissue shows with improved liver histo-architecture, depicted by lesser congestion of central vein (CV), reduced hepatocyte (HP) distortion, and mild fragmented liver tissue (FLT). Group **E** (20ml DDVP-exposed) liver tissue shows normal hepatocytes (HP), visible sinusoidal spaces (SS), uncongested portal vein (PV), and well-arranged portal artery (PA) and bile duct (BD).

DISCUSSIONS

Organophosphate (OP) insecticides are known to cause declines in the body weights of experimental animals. A study by **Selmi et al. (2018)** reported that the sub-acute exposure of prepubertal male mice to malathion reduced the body weight – which is in line with the present study. The present study findings suggested that ascorbic acid alleviated the adverse effects of DDVP on body weight, thereby enhancing the health status of the rats. **Uzunhisarcikli and Kalender (2011)** found statistically significant reductions in body weights when the rat groups treated with methyl parathion (initial and final weights of 324.13g and 304.78g, respectively) and methyl parathion plus vitamin (initial and final weights of 326.43g and 310.80g, respectively) were compared to the control group (initial and final weights of 326.78g and 347.57g, respectively) at the conclusion of the fourth week of exposure. Conversely, **Ogutcu et al. (2006)** had earlier shown that there was no statistically significant change observed in the body weights at the end of the 4th week when vitamin E plus diazinon-treated group (initial and final weights of 243.12g and 232.12g, respectively) was compared to diazinon-treated group (initial and final weights of 243.12g and 230.12g, respectively). Factors such as dosage of administration and duration of exposure could enable some organophosphates to induce more oxidative stress than others, as well as their different responses to antioxidant vitamins in experimental rats.

In this study, rat groups predisposed to graded DDVP concentrations exhibited significant elevated levels of liver enzymatic activities of AST, ALT, and TB while ALB levels decreased signifying moderate signs of liver damage because of induced oxidative stress. Increased AST and ALT levels usually reflect damage to the liver cell membrane thereby causing leakage of intracellular hepatic enzymes. According to **Uzunhisarcikli and Kalender (2011),** at the end of the fourth week of exposure, the respective methyl parathion- and methyl parathion plus vitamin-treated groups in comparison to the control group showed statistically significant increases in the activities of ALT (85.17u/L, and 76.00u/L versus 65.50u/L), and AST (92.83u/L, and 87.50u/L versus 71.17u/L) but decreased in albumin levels (3.33g/dL, and 3.82g/dL versus 4.37g/dL). Similar results from the study by **Adeoti et al. (2017)** also indicated that persistent exposure to several pesticides that were studied showed significant rises in the concentrations of liver marker enzymes.

Rats exposed to DDVP exhibited mild to moderate histopathological changes, including central vein congestion, reduced sinusoidal spaces, necrosis, and distorted hepatocytes – all indicative of liver damage and induction of oxidative stress. In line with the findings of this present study, exposure to several OPs studied in various related literature brought about histomorphological observations such as hepatocyte and cytoplasmic vacuolizations **(Sharma and Sangha, 2014; Cobilinschi et al., 2020),** sinusoidal dilatations **(Sharma and Sangha, 2014; Ezzi et al., 2016; Ramadan et al., 2022),** necrosis **(Sharma and Sangha, 2014),** mononuclear cell infiltrations **(Sharma and Sangha, 2014; Cobilinschi et al., 2020),** and congestions of central vein and portal triad **(Ezzi et al., 2016; Cobilinschi et al., 2020; Ramadan et al., 2022).**

On the other hand, DDVP-exposed rats treated with ascorbic acid in this present study displayed improved liver architecture, with reduced necrosis and hepatocyte distortion, suggesting the positive role of ascorbic acid in mitigating DDVP-induced liver damage. The role of ascorbic acid in protecting the liver against severe toxicity is synonymous with multiple related studies. Compounds such as zinc and green tea polyphenols were described to remediate hepatotoxicity in chlorpyrifos-exposed animals **(Abed and Alkalby, 2021).** Also, studies done by **Al-Attar and Al-Taisan (2010), Beydilli et al. (2015)** and **Abedi et al. (2021)** reported that compounds such as vitamin E, selenium, and certain herbal extracts have demonstrated the ability to mitigate oxidative damage in diazinon-exposed animals. Similarly, selected herbal extracts were confirmed to lessen the inflammatory responses in dimethoate-exposed animals **(Saafi et al., 2011).**

CONCLUSION

The research concluded that ascorbic acid provides significant hepatoprotective effect against dichlorvos-induced liver toxicity in adult Wistar rats. The protective effects of ascorbic acid are substantiated by observed improvements in body weight, reductions in liver enzyme levels, and the amelioration of histopathological alterations in liver tissues. These results underscore the potential of ascorbic acid to counteract the detrimental impacts of dichlorvos and similar environmental toxins, indicating its therapeutic promise in safeguarding liver health from oxidative damage caused by pesticide exposure.

RECOMMENDATIONS

More comparative research with other antioxidants or natural substances that have hepatoprotective properties could help to bolster ascorbic acid's efficacy. Also, to widen its potential as a detoxifying agent, researchers should investigate whether ascorbic acid could provide more hepatoprotections against other types of environmental toxins, heavy metals, or industrial chemicals.

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CONFLICT OF INTERESTS

Authors declared that there were no conflicts of interests

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AUTHOR CONTRIBUTIONS

We state that all authors collectively contributed to this research. **JSH** wrote the study protocol, supervised the data analysis, and wrote the first draft of the manuscript. **EEK** managed the literature research, acquired animals, and conducted the experiment. **GNA** acquired the animals, and tools used for experiments, as well as conducted the experiment. **AIO** assisted in acquisition of animals, and tools used for experiments, as well as conducted the experiment. **OMA** managed literature research, monitored the experiment, and performed statistical analysis of data. All authors read and approved the final manuscript for submission.

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