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Original Research Article

Protective effect of vitamin c against nephrotoxicity in dichlorvos induced male albino rats

***Josiah S. Hart and Lekpa K. David**

Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria

***Corresponding Author E-mail: mypublicationmail@gmail.com**

Abstract

In the recent decades, we have witnessed the continuous increase in the use of pesticides such Dichlorvos worldwide which is due to the rapid growth in food production and the attempt in controlling vector-borne diseases as this has in turn led to the harmful effect on the environment and humanity. There is little knowledge surrounding the effect of this compound on the kidney and how protective measures like the administration of vitamin C can be used to curb its harmful side effect such as oxidative stress. Hence, this study was undertaken to analyze the toxicological effect of inhalation of DDVP (Sniper) on the kidney oxidative stress parameters of wistar rats and check whether Vitamin C (ascorbic acid) could possibly ameliorate the toxic effect. The research design is experimental which intends to investigate the ameliorative effects of ascorbic acid (vitamin C) on dichlorvos-induced toxicity on the liver of albino wistar rats. For the purpose of achieving this, an ethical clearance was applied for and obtained from the Research Ethics Committee of the University of Port Harcourt. Forty (40) albino male wistar rats were recruited for this research and were randomly placed into eight (8) groups of five (5) rats per group. A box-like cage measuring 40cm x 40cm x 15cm (6 in number) that was made of Perplex glass was used as inhalation chambers for the experimental rats for groups 3 – 8, data analysis done using SPSS version 25. On administration with 40ml DDVP/60ml distilled water of dichlorvos solution via inhalation for 28 days, there was degeneration of renal corpuscles (as marked by stars) which suggests necrosis. As a result of this necrotic event, there are marked signs of cyst-like dilated lumens (arrows) (Figure 4c). There were observable signs of normal kidney tissue architecture and arrangement, the renal corpuscle (RC) and the cortical labyrinth (CL) of this tissue were clearly seen (Figure 4d). The study clearly showed that vitamin C had a protective effect against the nephrotoxicity induced by this compounds (DDVP) on the rats.

Keywords: Protective effect, Vitamin C, Nephrotoxicity, Dichlorvos, and Male Rats.

INTRODUCTION

Pesticides are substances that are meant to control pest. A pesticide is defined as a chemical agent used to destroy or control pests (Bedient et al., 1964). Dichlorvos also known as DDVP (2,2-dichlorovinyl dimethyl phosphate) is an organophosphate insecticide/pesticide. It is a synthetic Organophosphate acetylcholinesterase inhibitor and probable mutagen, neurotoxin and reproduction toxicant that is used as a pesticide (National

Center for Biotechnology information, 2021). It combines both contact and stomach action and has marked vapor action. Dichlorvos due to its wide spectrum of pest killing is a predominantly used pesticide, as it is majorly used in domestic insect control, pest control in green houses to protect crops and also in veterinary medicine for animals. Dichlorvos as a house insecticide kills insects like cockroach, ant, bedbugs, termites etc. As a greenhouse pesticide, dichlorvos is effective against mushrooms flies, aphids, spider mites, caterpillars, thrips and whiteflies which are pests to plants and animals in the farm. Dichlorvos is officially registered and/or approved for use all over the world. In Nigeria, DDVP is commonly produced and used as an effective and potent insecticide (Akang et al., 2012). It is traded under names such as DDVP, Dedevap, Nogo, Nuvan, Phosuit, Vapona, Sniper and Daksh (Akang et al., 2012; Singh et al., 2020). Although the trade name used for this study is Sniper. Dichlorvos being an effective insecticide for pest control has been discovered to also harmful humans as it is believed to cause oxidative stress to its victims when misused.

Dichlorvos irrespective of its positive side of curbing issues of pest invasion has been reported to be poisonous not only to pest but also humans too. Over the recent years, dichlorvos poisoning has become a case of much concern as it is believed to be linked to a lot of deaths and pathologies over previous and recent years. Irrespective of this, dichlorvos being a very effective and active organophosphate insecticide is on a high side of demand and use making it readily accessible to individuals in demand. Dichlorvos is more common in underdeveloped countries like Nigeria unlike in most western countries where it is either banned or highly restricted to ordinary individuals

Vitamins are organic molecules that are essential micronutrients which an organism needs in small quantities for the proper functioning of its metabolism. Vitamins, any of several organic substances that are necessary in small quantities for normal health and growth in higher forms of animal life (Baigent and Carpenter, 2021). In general, all vitamins functions are of catalytic or regulatory nature, facilitating or controlling vital chemical reactions in the body's cells. With exception of vitamin C (ascorbic acid), all of the water-soluble vitamins have a catalytic function.

Vitamin C, a water-soluble, carbohydrate-like substance that is involved in certain metabolic processes of animals (Britannica, the Editors of Encyclopedia, 2021). Vitamin C (1-ascorbic acid) is a water-soluble macronutrient required for multiple biological functions (Pehlivan, 2017). It is an essential enzyme cofactor for several enzyme in the post-translational hydroxylation of collagen, biosynthesis of carnitine, conversion of neurotransmitter dopamine to norepinephrine, peptide amination and in tyrosine metabolism. It is also an antioxidant that helps protection against infection and non-absorption. Vitamin C is one of the potent reducing agents and scavenger of free radicals 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 (Arrgoni and De Tullio, 2002; Hacisevki, 2009). Vitamin C is necessary for metabolism and oxidative stress protection, with this vitamin C is a good protective agent against the adverse effect of dichlorvos poisoning. Hence, this study was done to analyze the protective effect of vitamin C against nephrotoxicity of dichlorvos in dichlorvos induced male albino rats.

There are reports on similar subject and related matters by other authors in other regions of the country and in international communities (Awotunsin et al., 2019; Reitman and Frankel, 1957; Tietz, 1976; Biod and Sirota, 1948; Ogutcu et al., 2008; Dirican and Kalender, 2012; Ojo et al., 2014; Nasr et al., 2016; Kalender et al., 2004; Husain et al., 2004; El-Demerdash, 2004; George and Chandrakasan, 2000; Elmas et al., 2005).

MATERIALS AND METHODS

Research Design

The research design is experimental which intends to investigate the ameliorative effects of ascorbic acid (vitamin C) on dichlorvos-induced toxicity on the liver of albino wistar rats. For the purpose of achieving this, an ethical clearance was applied for and obtained from the Research Ethics Committee of the University of Port Harcourt. Forty (40) albino male wistar rats were recruited for this research and were randomly placed into eight (8) groups of five (5) rats per group. A box-like cage measuring 40cm x 40cm x 15cm (6 in number) that was made of Perplex glass, was used as inhalation chambers for the experimental rats for groups 3 – 8.

Administration Schedule

The groups will be arranged as thus;
Group 1 (the negative control) was administered ad libitum for 21 days.
Group 2 (the positive control) was administered 160mg/kg ascorbic acid once daily for 21 days.
Group 3 was exposed to Dichlorvos inhalation (10 ml DDVP/90 ml 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/80 ml distilled water – v/v) for 21 days for 4 hours per day at room temperature
Group 5 was exposed to Dichlorvos inhalation (40 ml DDVP/60ml distilled water – v/v) for 21 days of 4 hours per day at room temperature.
Group 6 was exposed to Dichlorvos inhalation (10 ml

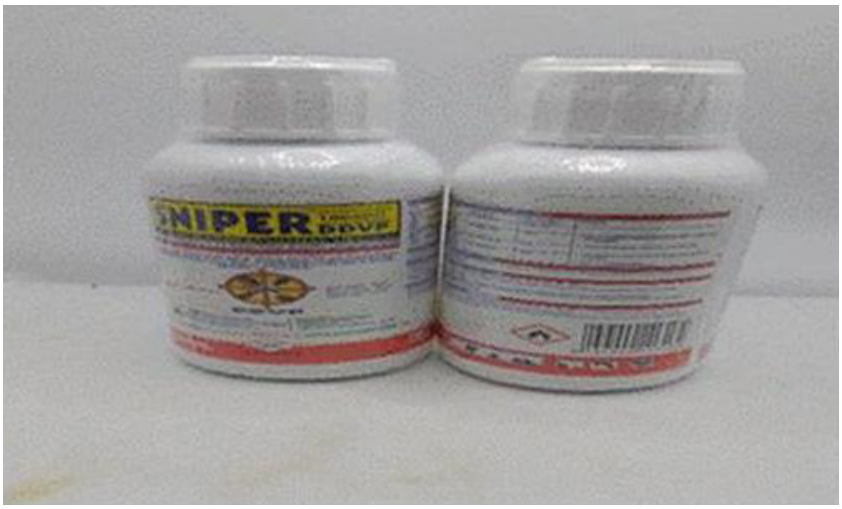

Figure 1. Showing Dichlorvos in Sniper.

DDVP/90ml 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 7 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 8 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.

Sample Size and Sampling Techniques

The sample size was calculated using the Resource equation method as described by Charan and Kantharia (2013) to determine the minimum sample size for experimental animal study.
E = (no. of animals in group × no. of groups) – no. of groups.
E = (5 × 8) - 8 = 40 – 8 = 32
Since the calculated degree of freedom for this study is 32 (which is more than the recommended degree of freedom of 20), the sample size in this study is acceptable.

Sample Collection/Identification

Dichlorvos is the active ingredient of the insecticide, Sniper. 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. (Figure 1)

Acute Toxicity for Dichlorvos Inhalation

According to previous studies, experimental animals are exposed to the test substance for a minimum of four (4) hours and are monitored for a short-term period of 14 days and a long-term period of 28 days. Animals that die during the study will be autopsied. At the end of the study, animals are sacrificed and observed for pathological changes.
In line with studies done by Akang et al. (2012) and Awotunsin et al. (2019) the average lethal concentration for dichlorvos inhalation is 50 ml DDVP/50ml distilled water.

Animal Acclamitization and Handling

The animals that were used are forty (40) male albino wistar rats weighing 100g-160g and were bred in the Pharmacology Department Animal House, Choba campus, University of Port Harcourt. They were grouped into eight groups of five animals each and left to adapt to the environment for fourteen (14) days. They were kept in standard cages and maintained in standard laboratory condition at an average room temperature of (25 ± 2^{o}C) with relative humidity (55-64%) and light and dark conditions (12/12h). They were given standard diet and water *ad libitum***.** Animal ethics and proper handling methods were closely abided. The bedding of the cages (sawdust) was changed daily and the cage also washed and disinfected weekly. The feed, Top Feeds Premier Feeds (Broiler finisher) manufactured by Premier Feed Mills Co. Ltd. (A subsidiary of Flour Mills Nig. Plc., Lagos State) were purchased at Choba, Port Harcourt. They were stabilized for one week during which they were

Figure 2a and b. Animal Acclamitization.

allowed access to commercial rat feed and portable clean water ad libitum. (Figure 2a and b)

Assessment of Serum Biochemical Markers

10ml of blood was taken from the animals in every group for determination of Kidney Function Test, and serum lipid profile. Serum alanine transaminase, aspartate transaminase levels were measured by Reitman and Frankel method Reitman and Frankel, 1957) alkaline phosphate, Na^+ and k^+ levels were estimated using colorimetric end point method, (Tietz, 1976) while urea and creatinine levels were estimated by modified methods based on diacetylmonoxime reaction and Jaffe's reaction respectively (Biod and Sirota, 1948) on standard diagnostic test kits.

Histopathological Analysis

At completion of exposure, animals were anaesthetized with chloroform. Animals were sacrificed and organs like the liver, heart, kidneys, lungs, testes, brain, and spleen were harvested for routine histopathology procedure. The harvested organs from all groups 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 Am-scope camera fitted on an Açu-scope microscope. (Figure 3a and b)

Statistical Analysis

Inferential and descriptive statistical methods were implored in analyzing the data generated from the experiment, and the values for each group were presented as a mean ± standard error of each mean. One-way analysis of variance (ANOVA) was used to analyze the difference between the groups followed by least significant difference (LSD) pot-hoc test. Confidence interval was set as 95% and therefore $p<0.05$ was considered significant.The entire statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 23, manufactured by International Business Machine Corporation (IBM) in Amonk, New York.

Ethical Approval

The ethics committee of the University of Port Harcourt gave approval before the commencement of the study. In addition, the gate keepers of the community gave a verbal approval for the study to be done in the community. Afterwards, an informed consent was gotten from the respondents and assurance of confidentiality was given to the respondents.

RESULTS

Clinical signs of toxicity

During this study, the signs that were observed in the non-treated rat groups include, miosis (constricted

Figure 3a and b. Dissection and preservation of tissues.

Table 1. Descriptive statistics of rat body weights of the various groups.

Groups	Mean Initial body weight (g)	Mean final body weight (g)
Negative Control	173.6±5.3	190.0±6.7
Positive Control (2ml Vit C)	176.2±6.0	191.1±4.5
10ml DDVP	181.3±4.7	161.5±5.0
20ml DDVP	179.7±5.1	138.0±4.2
40ml DDVP	182.4±6.4	153.0±3.7
10ml DDVP + 2ml (160mg/kg) ascorbic acid	184.3±5.3	171.0±4.7
20ml DDVP + 2ml (160mg/kg) ascorbic acid	178.6±5.7	164.5±5.6
40ml DDVP + 2ml (160mg/kg) ascorbic acid	180.4±6.5	166.0±4.3

DDVP = Dichlorvos

pupils), frequent urination, difficulty in breathing, increased defecation, redness of the eye, reduce food intake, and in few cases, death.

As shown in Table 1 above, the rat body weights for the various groups 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 negative control (161.5g, 138g and 153g, in comparison to 190g). However, the rat body weights of the treated groups exhibited a significant increase compared to the non-treated groups (171g, 164.5g and 166g).(Table 2 and 3)

There was a significant increase in the levels of potassium, urea and creatinine levels in some of the non-treated groups as compared to the negative control ($p < 0.05$). However, upon the administration of vitamin C (2ml) in the treated groups, there were significant decreases in these serum electrolyte concentrations ($p < 0.05$) in comparison to the negative control and non-treated groups.

Effect of DDVP and Vitamin C on the Histoarchitecture of the kidney

Figure 4 - Normal histological kidney (group 1) tissue architecture showing the glomerulus (G) and Bowman's capsule (BC) as well as presence of medullary rays.
Figure 5 - The rat kidney treated with vitamin C only (group 2) showing almost normal histology similar to negative control.
Figure 6 - Kidney of rat group exposed to 10ml/90ml

Table 2. Effects of DDVP and vitamin C on the serum profile of albino wistar rats.

Group	AST(u/l)	ALT(u/l)
Negative group	59.30±14.95	18.66±10.39
2ml Vit. C only	57.00±10.41	16.82±0.92
10ml DDVP	68.53±7.95*	27.50±3.96*
20ml DDVP	71.32±6.80*	25.87±3.35
40ml DDVP	76.50±8.02	31.25±0.78*
10ml DDVP + 2ml Vit. C	68.62±10.12*#	28.37±2.49*
20ml DDVP + 2ml Vit. C	66.70±7.36*	23.53±2.12*
40ml DDVP + 2ml Vit. C	72.07±8.71*	29.81±1.06*

Each value represents mean ± SD, Values marked with asterisk (*) differ significantly from the -ve control (*p<0.05), those marked with (#) differ significantly from the +ve control (#p<0.05). **AST** = Aspartate aminotransferase, **ALT** = Alanine aminotransferase, **T.B** = Total bilirubin, **ALB** = Albumin, **DDVP =** Dichlorvos

Table 3. Effects of DDVP and Vitamin C on serum electrolyte concentrations.

Group	K(mmo/l)	Na(mmo/l)	Ur(mmo/l)	Cr(mmo/l)
Negative group	3.90±0.14	122.50±3.54	3.50±0.14	71.00±1.41
2ml Vit. C only	3.93±0.49*	133.00±1.41*	3.55±0.78*	74.00±15.56*
10ml DDVP	2.77±0.35*	111.58±4.95*	4.20±1.41*	77.46±29.70
20ml DDVP	3.34±1.20	108.05±19.80	3.65±0.78	80.21±17.68
40ml DDVP	4.51±0.49*#	103.12±14.14	3.55±0.07	83.58±1.41
10ml DDVP + 2ml Vit. C	2.80±1.13*#	120.61±17.68	4.10±0.99	75.32±19.80*#
20ml DDVP + 2ml Vit. C	2.85±0.64*#	112.15±11.31*#	3.80±0.71*#	76.05±15.56*#
40ml DDVP + 2ml Vit. C	3.97±0.57*#	107.80±4.24*	3.60±0.85*#	80.50±16.26*

Each value represents mean ± SD, Values marked with asterisk (*) differ significantly from the -ve control (*p<0.05), those marked with (#) differ significantly from the +ve control (#p<0.05). **K** = Potassium, **Na** = Sodium, **Ur** = Urea, **Cr** = Creatinine.

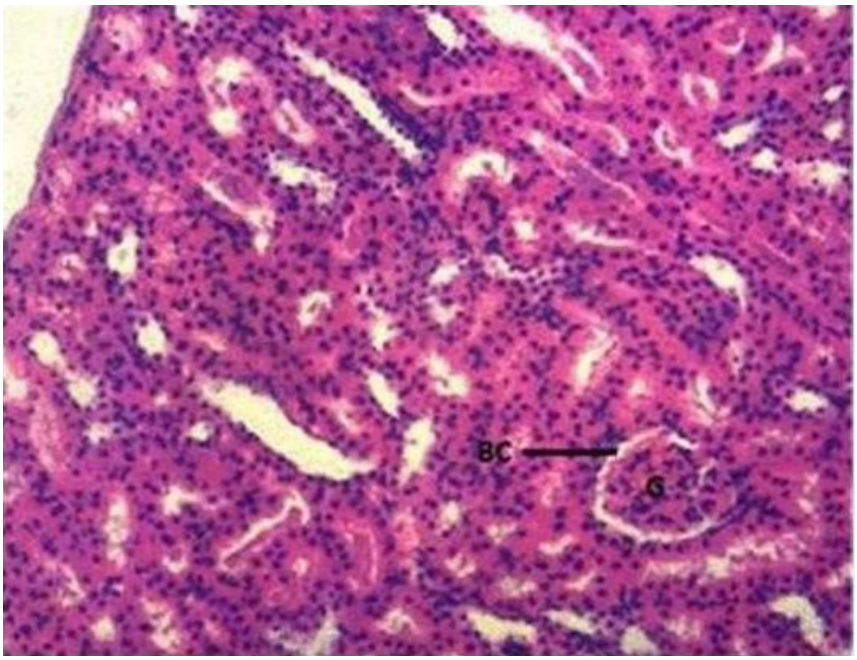

Figure 4. Normal histological kidney (group 1) tissue architecture showing the glomerulus (G) and Bowman's capsule (BC) as well as presence of medullary rays.

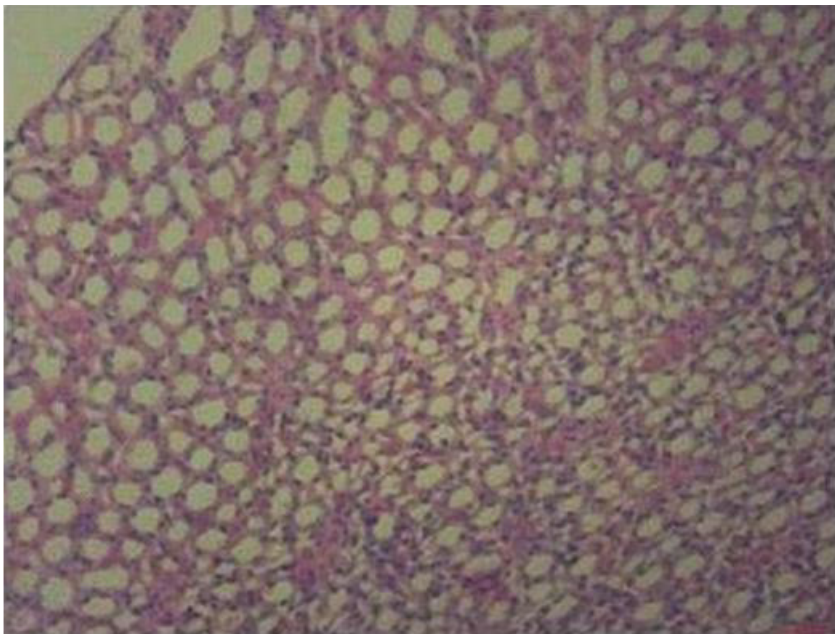

Figure 5. The rat kidney treated with vitamin C only (group 2) showing almost normal histology similar to negative control.

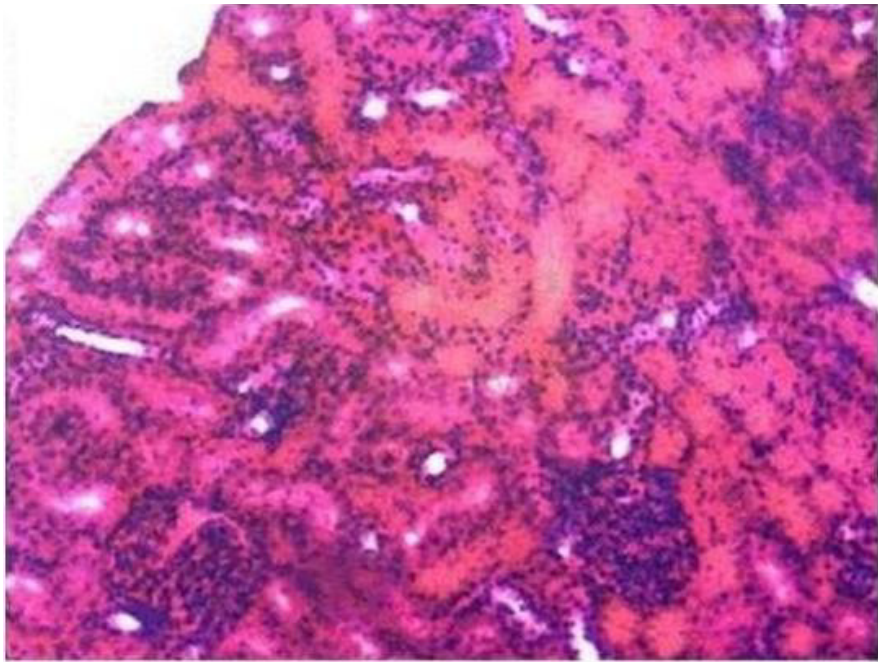

Figure 6. Kidney of rat group exposed to 10ml/90ml DDVP solution showing signs of congestion of renal blood vessels.

DDVP solution showing signs of congestion of renal blood vessels.

Figure 7 - Kidney of rats exposed to 20ml/80ml DDVP solution showing high levels of cytoplasmic vacuolations as well as necrosis of nephrocytes.

Figure 8 - Rat kidney of group exposed to 40ml/60ml DDVP solution shows congestion of glomerular tufts, tubular vacuolations as a result of necrosis.

Figure 9-Kidney of vitamin-C treated rats (10ml/90ml DDVP solution) showing minor signs of congestion of glomerular tufts, with fewer cytoplasmic vacuolations.

Figure 10 - Kidney of vitamin C-treated rats that inhaled

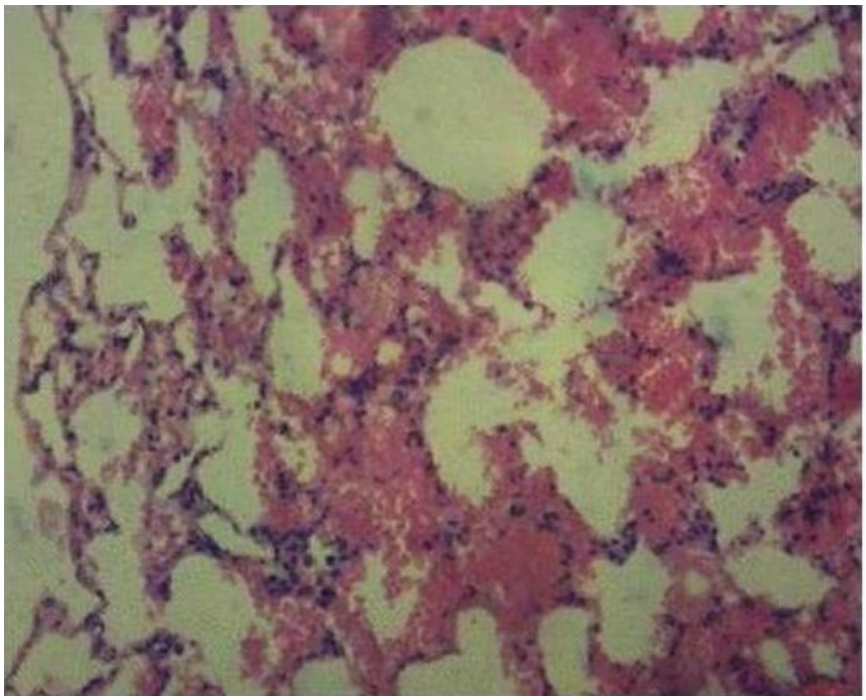

Figure 7. Kidney of rats exposed to 20ml/80ml DDVP solution showing high levels of cytoplasmic vacuolations as well as necrosis of nephrocytes.

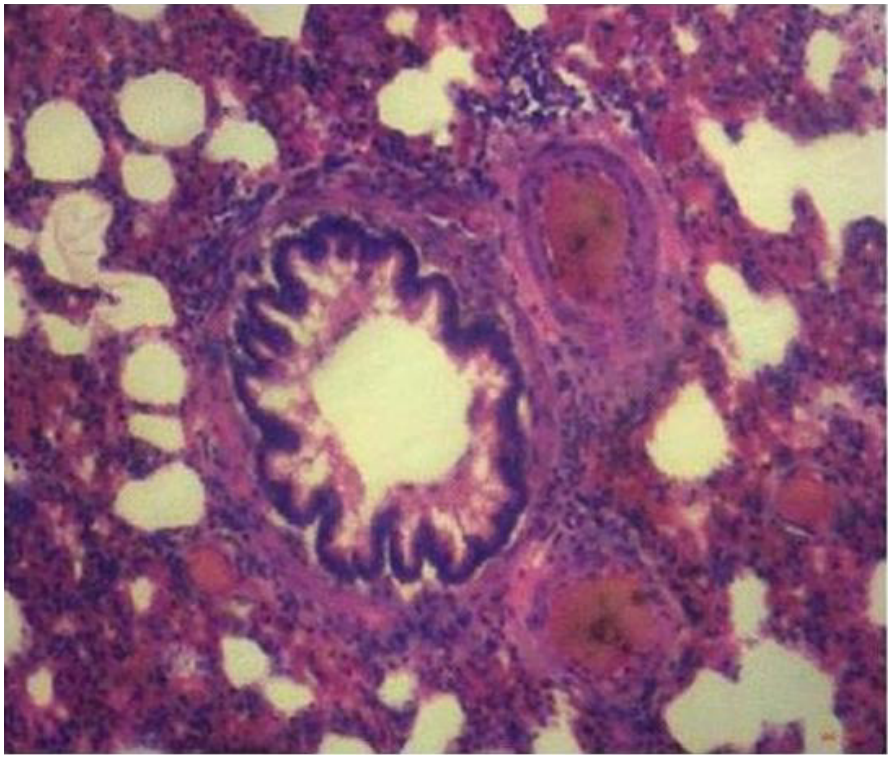

Figure 8. Rat kidney of group exposed to 40ml/60ml DDVP solution shows congestion of glomerular tufts, tubular vacuolations as a result of necrosis.

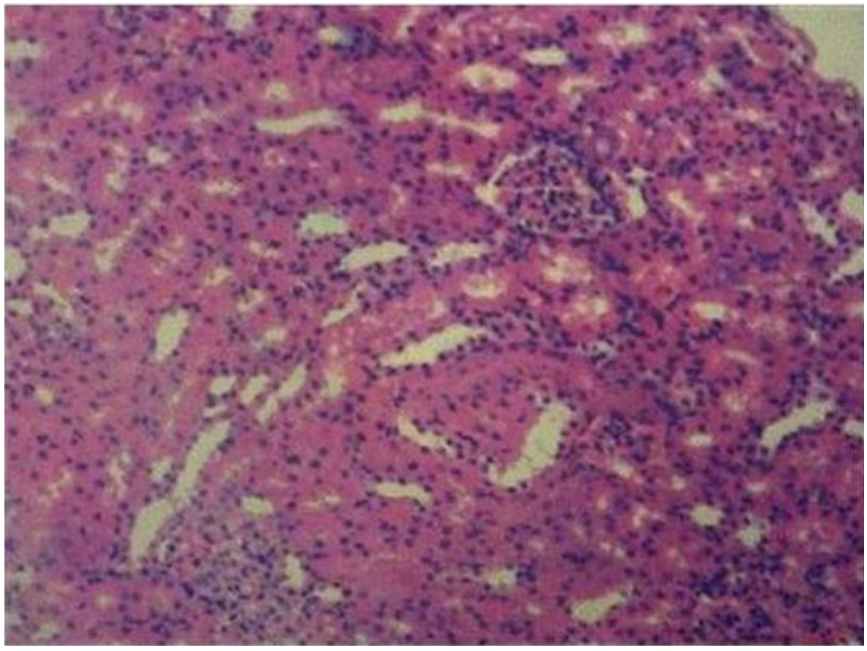

Figure 9. Kidney of vitamin-C treated rats (10ml/90ml DDVP solution) showing minor signs of congestion of glomerular tufts, with fewer cytoplasmic vacuolations.

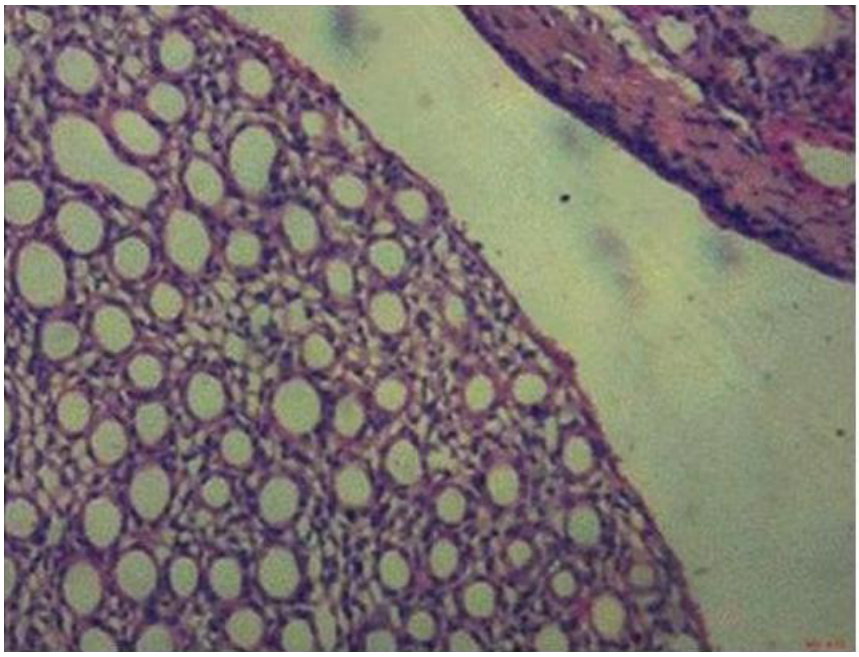

Figure 10. Kidney of vitamin C-treated rats that inhaled 20ml/80ml DDVP solution showing reduced signs of necrosis of renal tubular epithelial cells.

20ml/80ml DDVP solution showing reduced signs of necrosis of renal tubular epithelial cells.

Figure 11 - Kidney of vitamin C-treated rats that inhaled 40ml/60ml DDVP solution showing a lesser congestion of glomerular tufts compared to the untreated group.

DISCUSSIONS

Summary of results

The control specimen showed that renal corpuscles were intact as well as the medullary rays found around the

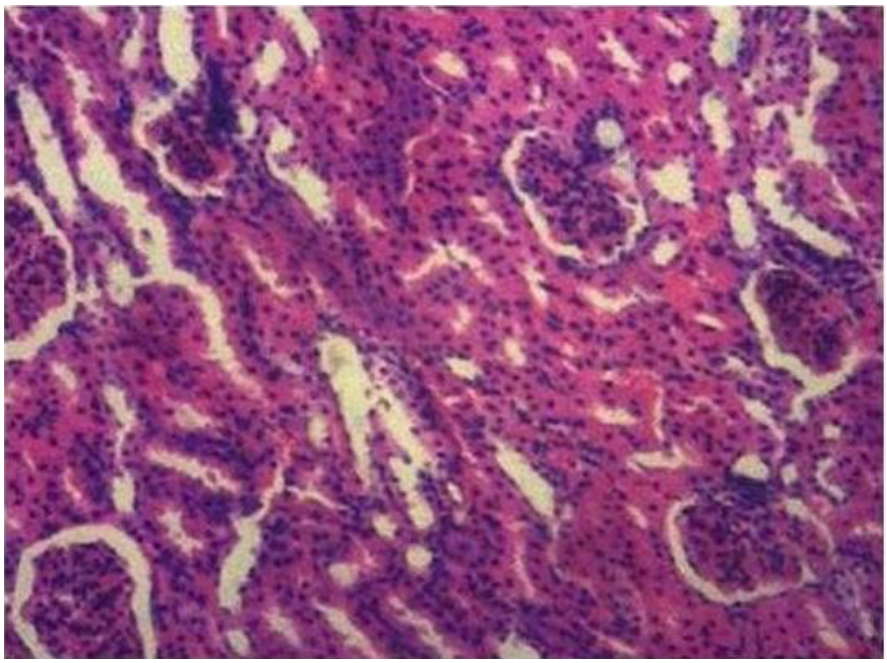

Figure 11. Kidney of vitamin C-treated rats that inhaled 40ml/60ml DDVP solution showing a lesser congestion of glomerular tufts compared to the untreated group.

renal corpuscles (figure 4a).Kidney tissue following the administration of a medium concentration (20ml DDVP/80ml distilled water) of dichlorvos solution via inhalation for 28 days showed notable signs of toxicity which includes: distortion / destruction of renal corpuscles (as marked by arrows), and the presence of dilated convoluted tubules (figure 4b). On administration with 40ml DDVP/60ml distilled water of dichlorvos solution via inhalation for 28 days, there was degeneration of renal corpuscles (as marked by stars) which suggests necrosis. As a result of this necrotic event, there are marked signs of cyst-like dilated lumens (arrows) (figure 4c).There were observable signs of normal kidney tissue architecture and arrangement, the renal corpuscle (RC) and the cortical labyrinth (CL) of this tissue were clearly seen (figure 4d).

Implications

Effect of DDVP on rat body weight

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 negative control (161.5g, 138g and 153g, in comparison to 190g). However, the rat body weights of the treated groups exhibited a significant increase compared to the non-treated groups (171g, 164.5g and 166g). A similar study has proven upon the administration of dichlorvos, an insecticide, there was also a reduction in the body weights of the experimental mice (Ogutcu et al., 2008; Dirican and Kalender, 2012). This could have occurred due to increased oxidative stress markers of the kidney like ALT, AST, urea and creatinine. The treatment with ascorbic acid inhibited the oxidative stress activities of these markers.

Effect of Vitamin C on Antioxidant Parameters and Markers Enzymes in DDVP-Induced Nephrotoxicity in Rats

The results from this study showed that there was decreased Alanine aminotransferase (ALT) and Aspartate (AST) aminotransferase activities in the non-treated groups (10ml DDVP and 20ml DDVP groups) compared to the negative control group ($p < 0.05$). Treatment with Vitamin C resulted in significant protection of the kidney, as indicated by reductions in the elevated levels of ALT and AST. A similar study by Ojo et al. (2014) showed from their study that the oral exposure for 14 consecutive days induce highly significant effect ($p < 0.001$) at high exposure dose and slight significant effect ($p < 0.05$) at low exposure dose in both genotoxicity and cholinesterase assays. On treatment with Alstonia boonei (a stem bark known to have similar antioxidant effect as Vitamin C), there was significant protection of the kidney from the indicative elevation of ALT and AST in blood serum. ALT and AST are well known biomarkers that can used in proper evaluation of the protective effect of vitamin C in nephrotoxicity induced by dichlorvos.

Effects of Vitamin C on Serum protein, Urea and Creatine in DDVP-Induced Nephrotoxicity in rats

There was significant decrease in the levels of serum total protein in the dichlorvos induced group compared with the control group. However, levels of this compound in the serum were significantly increased in dichlorvos treated group when compared with the dichlorvos control group. Levels of urea and creatine in the serum of the dichlorvos group were significantly high when compared with the control group. However, levels of serum urea and creatine were significantly decreased in dichlorvos treated groups that were administered vitamin C compared to those not administered vitamin C. This finding was also reported by Nasr et al. (2016).

Urea, uric acid and creatine levels are kidney function parameters (Kalender et al., 2004). Urea being an end product of protein catabolism there its increase can be related to increased protein catabolism in the body and/or can be referred to kidney dysfunction. The levels of urea in the plasma of rats are tested as indicators for kidney function (Husain et al., 2004; El-Demerdash, 2004). In this study, urea level was increased which is attributed to the toxic effect of dichlorvos.

Uric acid is the end product of purine catabolism and can reduce oxidative stress by scavenging various reactive oxidative oxygen species (George and Chandrakasan, 2000; Elmas et al., 2005). Serum uric acid has been discovered to increase in portion to decrease in creatine clearance and this goes with the increase in renal tubule-interstitial damage. In this study uric acid increase may be related to either increase protein degradation, which is involved in uric acid formation, or the toxic effect of dichlorvos on the kidneys.

Creatinine excretion is dependent almost on the process of glomerular filtration and it has been discovered that increase in serum creatinine level could be due to impairment of the glomerular function and tubular damage in kidney. In the present study, creatinine may be strongly related to kidney damage due to toxic effect of dichlorvos on the kidney.

Effect of DDVP and Vitamin C on the histology of the kidney

The micrographs of the kidney from this present study showed that the tissues of rats that were not treated with dichlorvos had their renal corpuscles intact as well as the medullary rays found around the corpuscles. The DDVP-induced rats (mild concentration) showed that the kidney tissues had notable signs of nephrotoxicity which includes; distortion/ destruction of renal corpuscles, and dilated convoluted tubules. The DDVP-induced rats (high concentration) showed signs of degeneration of renal corpuscles (suggesting necrosis) with marked signs of cyst-like dilated lumens. On the other hand, the vitamin C treated DDVP-induced kidney tissues had a normal kidney architecture and arrangement like that of the control group as the renal corpuscles and the cortical labyrinth were visible on the micrograph. This confirms the nephroprotective activity of vitamin C as a significant recovery of damage as decreased necrosis was evident against dichlorvos induced oxidative damage in the kidney of the rats. The study clearly showed that vitamin C had a protective effect against the nephrotoxicity induced by this compounds (DDVP) on the rats.

CONCLUSION

The study has shown that the administration of dichlorvos with increasing concentration had increasing nephrotoxicity. However, the administration of the vitamin C with DDVP showed normal kidney architecture and arrangement as that of the control group. The study showed that vitamin C had a protective effect against the nephrotoxicity induced by DDVP on the rats.

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COMPETING INTERESTS

There is no conflicting or competing interest.

AUTHORS' CONTRIBUTIONS

Author A 'JSH' designed the study wrote the protocol, Author B 'LKD' did the study analyses and statistical analysis. Author A 'JSH' wrote the first draft of the manuscript. Author B 'LKD'managed the literature search. Both authors read and approved the final manuscript.

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