The effect of Dichlorvous (Sniper) Inhalation on the Histology of the Lungs of Adult Male Wistar Rats

A. E. Anyabolu¹, D. N. Ezejindu² and B. N. Obinwa*²

¹Department of Medicine, Faculty of Medicine, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra state, Nigeria.
²Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

Authors’ contributions

This work was carried out in collaboration among all authors. Author BNO handled the analyses of data, edited and wrote the final draft. Author AEA designed the study and wrote the protocol. Author DNE managed the literature searches and wrote the first draft. All authors participated in the experiment and tissue processing and also read and approved the final draft.

ABSTRACT

The organophosphate, dichlorvous (Otapia-pia) formulated in varying concentrations as insecticides is utilized by several individuals in most remote places of Nigeria due to its affordable value and accessibility. However, this present study is conducted to investigate the adverse effect of the exposure of this substance on the respiratory system (lungs) of male albino wistar rats. Twenty (20) albino wistar rats comprising of all males weighing between 150-230g were divided into four groups (A-D) of five animals each. Group A received only water, feed, and served as the control. Group B was exposed to dichlorvous inhalation 3hrs daily for a period of two weeks; Group C was exposed to dichlorvous inhalation 6hrs daily for a period of two weeks; while Group D was exposed to dichlorvous inhalation 10hrs daily for a period of two weeks. Twenty four hours after the last exposure, the animals were sacrificed by cervical dislocation and dissected. The lungs were weighed and fixed in 10% formal saline for histological studies. The body weight of the experimental groups decreased insignificantly when compared with the control group. The lungs weight increased significantly when compared to the control groups. Histological observation revealed a moderate to severe effects on the lungs with severe consolidated inflammatory
**Keywords:** Dichlorvous; sniper; lung; histology.

1. BACKGROUND OF THE STUDY

Over the years, people in most remote places especially in the Northern, Northeastern and South-south states of Nigeria make use of dichlorvous in varying ways and quantities as insecticides and pesticides. In 1955, it was discovered that crystalline trichlorfon, an organophosphate insecticide, gave off a vapor which was capable of killing insects [1].

Dichlorvous (2,2-dimethyl dichlorovinyl phosphate, DDVP), is therefore, an organic synthetic chemical that is commonly formulated in varying concentration (1 – 10%) as otapia-pia in Nigeria and other developing countries in tropical Africa [2]. It is not naturally occurring in the environment but industrially manufactured and is reported to be made of several chemicals such as toluene, cumene, 1,2,3-Trimethylbenzene, decane, undecane, dodecane, 11,12-dibromo-tetradecan-1-ol acetate, pentadecane, and 2,2-dichlorovinyl dimethyl phosphate being the major component [1,3]. The potential adverse impact of dichlorous on the human health is likely to be more pronounced in developing countries of Africa like Nigeria, possibly because it is readily available and its risk of low awareness among the populace especially local farmers. An individual can be exposed to dichlorous directly or indirectly, direct exposure occurs in farmers and individuals who personally apply pesticides in agricultural, occupational, or residential settings while indirect exposure occurs through drinking water, air, foods and dust [4]. Dichlorous is highly toxic by inhalation, dermal absorption and ingestion. Because dichlorous is volatile, inhalation is the most common route of exposure. Compared to poisoning by other organophosphates, dichlorous causes a more rapid onset of symptoms, which is often followed by a similarly rapid recovery [5]. Dichlorous is rapidly absorbed through the gastrointestinal and respiratory tracts and skin; it enters human system via inhalation, dermal or oral routes [6]. Following exposure by any route, other systemic effects may begin within a few minutes or be delayed for up to 12 hours. These may include pallor, nausea, vomiting, diarrhea, abdominal cramps, headache, dizziness, eye pain, blurred vision, constriction or dilation of the eye pupils, tears, increased salivation, perspiration, and mental confusion [5]. In severe cases, there may also be involuntary defecation or urination, psychosis, unconsciousness, convulsions and coma [7]. Death may be caused by respiratory failure [5].

Dichlorous can bind to molecules such as DNA and for this reason; several studies reviewed by the Environmental Protection Agency (EPA) have shown that dichlorous is a mutagenic agent. It has also been classified as a possible human carcinogen by EPA after many tests carried out on rats and mice. Chronic exposure to dichlorous in male rats will cause fluid to build up in the lungs (pulmonary edema) and lung hemorrhage [8].

The lungs are the primary organs of the respiratory system in humans and many other animals including a few fish and some snails. Their function is specifically to extract oxygen from the atmosphere and transfer it into the bloodstream, and thereafter release carbon dioxide from the bloodstream into the atmosphere, in a process of gaseous exchange. The tissue of the lungs can be affected by a number of diseases, including pneumonia and lung cancer, chronic obstructive pulmonary disease including chronic bronchitis and emphysema, all of which can be related to smoking or exposure to harmful substances such as coal dust, asbestos fibers, crystalline silica dust and dichlorous. Fruton et al., reported that the lung is rich in hydrolytic activity [9]; experiments carried out by Schmidt et al., shows that exposure of dichlorous concentration of 0.8 and 1.8 μg/l for 3 days reduced acetylcholine activity in the bronchial tissues but did not elicit any changes in blood acetylcholine activity although higher concentrations (4.3 μg/l) induced a decline in ACHE activity in the blood [10]. This efferent cholinergic mechanism has been found to be at
least partly responsible for maintenance of bronchospasm and hyper secretion in chronic obstructive diseases of the respiratory system [11].

Dichlovous otherwise called Otapia-pia has been shown from previous studies to have alternative effect on the lungs; therefore, this study is aimed at investigating the effect of the exposure to dichlovous on the histology of the lungs of adult male Wistar rat, thus, evaluating the extent of the side effects of this chemical (if any) within the space of exposure.

2. MATERIALS AND METHODS

2.1 Location and Duration of the Study

This study was carried out at Anatomy Department, Nnamdi Azikiwe University, Nnewi Campus, Anambra State Nigeria. The rats were made to acclimatize for a period of two weeks after which the test substance was administered for two weeks; the entire experiment lasted for four weeks.

2.2 Materials Used for the Study

The materials used included; A Nigerian local dichlovous (1%) preparation (Otapia-pia), standard cages, rats, 4x4cm sized foam, normal growers mesh, water, weighing balance, cartons, disposable glove, dissecting set, white paint bucket, cotton wool, beakers, conical flask, feeding troughs, plastic dish for liquid reagent, automatic microplate washer, light microscope (compound microscope), slides and 10% formal saline.

2.3 Collection of Material

The dichlovous also known as sniper (DDVP) was purchased from a local market of chemicals at Nkwo Nnewi, Anambra State, Nigeria. The chemical was completely sealed to avoid loss of its strength and concentration.

The feed used was normal growers mesh a product of Premier Feed Mills Co. Limited (A subsidiary of Flour Mills Nigeria Plc.) in Sapele, Delta State, Nigeria.

2.4 Experimental Animals and Design

The research was done with 20 adult albino Wistar rats comprising of all males weighing between 150-230g. The rats were bought from a local farm at Nnewi and moved to the site of the experiment. The weight of each rat was measured using an analytical weighing balance. They were fed with feeds and water for a period of two weeks to enable them acclimatizes with their new environment before the experiment began. During the period of acclimatization, the rats were fed with normal grower’s mesh of known weight of about 100g daily and given water, after which they were randomized into 4 groups of 5 rats per group. The group A served as the Control group while groups B, C, and D served as the Test groups.

2.5 Extraction of the Organs and Collection of Samples

At completion of exposure, the rats were sacrificed by cervical dislocation, the lungs were then harvested, weighed and fixed in 10% formal saline to maintain normal physiological conditions for its histological processing.

2.6 Tissue Processing

Tissue sections were produced by normal histochemical methods of fixation, dehydration, clearing, impregnation, embedding, sectioning, mounting, and staining. The micrographs of relevant stained sections were subsequently taken with a photomicroscope.

2.6.1 Fixation

After weighing the organ, a small part was cut from it and immediately fixed in 10% formal saline in order to preserve the various constituents of the cells in their normal micro anatomical position and to prevent autolysis and putrefaction.

Fixation is a very important step in tissue processing as it does not only protect the tissue from autolysis and putrefaction but also hardens the tissue to withstand other chemicals applied in subsequent treatments and for easy handling.

2.6.2 Dehydration

After fixation, the tissue were transferred and dehydrated in ascending grades of alcohol (50%, 70%, 90%, 95% and 100% or absolute alcohol once for 2hours each but twice in absolute alcohol).

The tissue was placed in ascending grades of alcohol to prevent distortion and distortion to the cell structure would have happened if directly placed in absolute alcohol. However, sufficient
time was allowed in absolute alcohol to enable complete dehydration.

2.6.3 Clearing

The tissue was cleared twice in Xylene for 1 to 2 hours each time. This is to avoid over exposure in the clearing agent, which will make them brittle. Xylene was used as the clearing agent as it does not only remove alcohol but is equally miscible with paraffin used in embedding.

2.6.4 Impregnation

The tissues were placed in molten paraffin wax at a constant temperature of 56°C (3°C above the melting point of paraffin wax used) in an oven and were passed through two changes of paraffin wax in the oven, 4 hours each. This was done to replace the clearing agent or antemedium with molten paraffin wax and can also be referred to as Infiltration. The tissue were subsequently removed from the oven and embedded in paraffin wax.

2.6.5 Embedding

Embedding is a process of burying a tissue in molten paraffin wax. The paraffin becomes a solid firm structure when it is cold. This forms a support medium for the tissue during microtomy. The tissues were then immersed in molten paraffin wax at a constant temperature of 36°C to 60°C in an oven of paraffin bath changing it twice for 2-4 hours each time. They were left to cool and solidify in metallic embedding molds. The tissue blocks obtained were casted on to the wooden blocks for sectioning.

2.6.6 Sectioning

This was done using a Rotatory Microtome. The tissue blocks were mounted on wooden blocks. With the microtome knife and blocks positioned accurately, sections were made at 5 microns each. The ribbons of sections were floated in warm water bath (37°C) to straighten them. The best ribbons were picked with forceps and placed on albuminised slides. The slides were labeled using diamond pencils and transferred to a slide rack. They were then placed in an oven to keep the specimens warm before staining.

2.6.7 Staining

The tissues were stained using Ehrlich’s Haematoxylin and Eosin stains. The staining procedure is as follows:

- The slides treated with paraffin wax were cleared in xylene for 3 minutes, and were rehydrated in descending grades off alcohol; absolute alcohol for 2 minutes to remove xylene, 90% alcohol for 2 minutes, 70% alcohol for 2 minutes, 30% alcohol for 2 minutes, and then rinsed in water for 1 minute each.
- The tissues were stained by immersing them in aqueous solution of haematoxylin for 30 minutes, and then rinsed in water to remove excessive stains. The tissues were subsequently differentiated in 1% acid alcohol for 1minute. This process called Bluing gave the tissues their characteristic blue background.
- The tissues were then stained in aqueous eosin for 10 minutes.
- The tissues were now immersed in ascending grades of alcohol as follows; 50%, 70%, 90% and absolute alcohol for 1 minute each and then cleared in xylene for 1 minute.

Staining gives contrasting colors to different elements of the cells or tissue thus making them conspicuous and easy to study.

2.6.8 Mounting

The slides were removed from the rack through their edges with the aid of forceps and placed on the filter papers. Blotting was done in one direction on the filter papers using the index finger and few drops of xylene were placed on the slides to make them wet. A drop of Dibuty Phthalate Xylene (DPX) mountant was placed on the slide which was laid in the middle to minimize the likelihood of trapping air bubbles. The slides were quickly inverted over cover slip and then brought down horizontally until the mountant made contact.

3. RESULTS

3.1 Physical and Behavioral Changes

As observed during the period of acclimatization, the albino Wistar rats were healthy, with fine smooth skin hairs, clear pinkish eyes and normal skin color with a profound increased in size and weight.

After commencement of the experiment, group A showed no clinical signs while the remaining groups had labor breathing, staggering, coughing, bulging eyes, and sluggish motion and a remarkable decrease in food intake which was more pronounced in group D resulting in decreased weight.
3.2 Body Weight Changes

The control group recorded an increase in body weight as against the test groups that lost weight when the initial and final weight of the groups were compared as shown in Table 1.

3.3 Organ Weight Changes

A significant increase in relative organ weight was recorded in the test groups when compared to the control as shown in Table 2.

3.4 Histological Finding

An exposure time dependent histoarchitectural changes/damage was observed in the lungs of the test groups as opposed to those of the control group that turns out normal.

Fig. 1. Pictures of wistar rats showing inflamed eyeballs

Table 1. Shows the effect of the hyper inhalation of dichlorous (2, 2-dichlorovinyl dimethyl phosphate) on body weight

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>MEAN ±SEM</th>
<th>P-VALUE</th>
<th>t-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A Initial</td>
<td>155.75 ±2.17</td>
<td>0.20</td>
<td>1.64</td>
</tr>
<tr>
<td>Group A Final</td>
<td>170.00 ±4.56</td>
<td>0.016*</td>
<td>3.786</td>
</tr>
<tr>
<td>Group B Initial</td>
<td>168.75 ±4.27</td>
<td>0.40</td>
<td>-0.97</td>
</tr>
<tr>
<td>Group B Final</td>
<td>162.50 ±3.23</td>
<td>0.27</td>
<td>-1.36</td>
</tr>
<tr>
<td>Group C Initial</td>
<td>191.25 ±4.27</td>
<td>0.001*</td>
<td>3.786</td>
</tr>
<tr>
<td>Group C Final</td>
<td>182.50 ±3.23</td>
<td>0.12</td>
<td>-2.18</td>
</tr>
<tr>
<td>Group D Initial</td>
<td>216.25 ±6.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group D Final</td>
<td>202.50 ±3.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data were analyzed using student dependent t-test and values were considered significant at P<0.05. *P<0.05 means significant. Result from the table above showed that there was a non-significant (*p>0.05) increase in the body weight in group A, when comparing the Initial weight (155.75±2.17) to the Final weight (170.00±4.56).

Group B showed an insignificantly (*p>0.05) slight decrease in the body weight, when comparing the Initial weight (168.75±4.27) to the Final weight (162.50±3.23). Group C, showed a non-significant (*p>0.05) decrease in the body weight, when comparing the Initial weight (191.25±4.27) to the Final weight (182.50±3.23). Group D showed a non-significant (*p>0.05) decrease in the body weight, when comparing the Initial weight (216.25±6.25) to the Final weight (202.50±3.23).

Table 2. Shows the effect of the hyper inhalation of dichlorous (2, 2-dichlorovinyl dimethyl phosphate) on the Relative weights of the lungs of wistar Rats

<table>
<thead>
<tr>
<th>Organ Weight</th>
<th>Mean ±SEM Relative Weight(g)</th>
<th>P-Value</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lungs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>1.40 ±0.25</td>
<td>0.82</td>
<td>0.016*</td>
</tr>
<tr>
<td>Group B</td>
<td>1.51 ±0.030</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>1.68 ±0.120</td>
<td>0.93</td>
<td>0.001*</td>
</tr>
<tr>
<td>Group D</td>
<td>2.17 ±0.33</td>
<td>1.07</td>
<td>3.786</td>
</tr>
</tbody>
</table>

Data were analyzed using One way ANOVA followed by Post HOC Fisher’s LSD multiple comparison, and data were considered significant at P<0.05. *P<0.05 means significant and P>0.05 means not significant. Result from the table above showed a significant (*p<0.05) increase in the Relative lung weight in group B (0.92), C (0.93) and D (1.07) when compared to the control group A (0.82).
Fig. 2. Photomicrograph A section presents lungs not exposed to sniper (X400). (H/E) shows normal section of lungs with well circumscribed alveoli space (CAS), Blood vessel (BV) and endothelial cells (EC).

Fig. 3. Photomicrograph B section presents lungs exposed to low dose of sniper for 14 days (X400). (H/E) shows mild effect with onset of tissue necrosis (TN) and congestion of blood vessel (CBV).

4. DISCUSSION

From time immemorial, various types of organophosphates have been used domestically and industrially as both pesticides and insecticides [12]. However, the use of dichlorvous, traded under different names such as otapia-pia, Nuvan, Rambo and Sniper which has been selected as the best control measure against insects and pests due to its affordability and accessibility [13] and its adverse effect on human health is likely to be more pronounced in developing countries of Africa like Nigeria, possibly because of its low risk of awareness among the populace especially the local farmers [14]. Dichlorous generally is readily absorbed in the body of mammals via all routes of exposure [15].

Previous studies reported the toxicity of dichlorous after oral and dermal administration on animal tissue. Luty et al.; Sharma & Singh in
their studies reported the generation of reactive oxygen species in the mechanism of dichlorvous which causes irreversible impairment of DNA, and exhibits different types of neoplasms namely; adenomas of the exocrine pancreas, mononuclear cell leukemia, and squamous cell papilloma of the fore stomach in male and female Fischer rats [16-17]. Reports show that there were alterations in the microanatomy of the liver of rats exposed to dichlorvous, revealing the massive necrosis of hepatocytes which are replaced with fibrous tissues. Another study by Brown et al., demonstrated the acute toxicity of dichlorvous on haematological parameters, inducing anemia, decreasing blood volume and packed cell volume, increasing the total white blood cell and platelet count [18] and Tela et al., showed that prolonged exposure to dichlorvous is injurious to the architecture of renal cortex which can result into renal failure [19].

Fig. 4. Photomicrograph C section presents lung exposed with medium dose of sniper for 14 days (X400). (H/E) shows necrotic effect with mild chronic consolidated focal necrosis (FN) and focal area of tissue

Fig. 5. Photomicrograph D section presents lung exposed with high dose of sniper for 14 days (X400). (H/E) shows severe effect with severe chronic consolidated focal necrosis (FN) and focal area of hemorrhage (H). The overall features are consistence with (lobar pneumonia)
In this study, 20 adult albino rats comprising of all males were used as working models to evaluate the effect of the long exposure to dichlorvous on the histology of the lungs. The result of this study revealed that the inhalation of dichlorvous caused some characteristic physical changes in adult wistar rats as evident in the reduction of physical activity, bulging eyeballs and shivering actions.

Observation of the body weight differences in all groups reveals increase in body weight of animals in group A. This could be physiological as the only substance they were exposed to was water and feed. A non-significant body weight decrease was observed in the experimental groups B, C and D. The decrease in body weight of animals in group D could be as a result of the daily ten hours duration in which they are exposed to dichlorvous when compared with the control and other groups. This is probably as a result of loss of appetite, weakness and reduction of food intake by the animals in the group. This result agrees with a previous study [20] which reported a significant reduction in body weight when overweight adult rats were exposed to dichlorvous inhalation. The groups that were exposed to dichlorvous inhalation daily for three and six hours, groups B and C respectively, showed an insignificant decrease at P>0.05 in body weight which differ from the control group. This is also in line with work done by Tela et al., who reported reduction in body weight when overweight adult rats were exposed to dichlorvous inhalation [19].

The relative organ weight result also showed salient differences in the groups. There was a significant (P<0.05) relative increase in the lung weight in groups B, C and D animals exposed to 3 hours, 6 hours and 10 hours of dichlorvous inhalation respectively when compared with the control group A. The significant increase in the relative lung weight was irrespective of the fact that there was total body weight loss. This could have been pathological and one may deduce that the increase in the relative lung weight of these groups was not growth but inflammation.

The significant increase in the relative organ weight was in respect to the fact that there wasn’t any growth but inflammation that could occur resulting to a total body weight loss. This could have been as a result of the negative effect of dichlorvous inhalation causing death of tissues and hindering blood circulation.

Histopathological findings of the present study indicated a normal basic architecture of the lung showing well circumscribed alveoli spaces, endothelial cells and bronchioles in the control group A. The group B animals exposed to dichlorvous inhalation for 3hrs shows mild aggregates of inflammatory cells otherwise, it is said to be normal with a well circumscribed alveoli spaces, endothelial cells and bronchioles as seen in photomicrograph 2, while in photomicrograph 3 and 4 representing the groups C and D animals exposed to dichlorvous inhalation for 6hrs and 10hrs respectively caused moderate to severe effect on the lungs with a severe consolidated inflammatory exudates and a moderate intra-alveoli hemorrhage, and severe dilation of alveolar septa as an evidence of severe emphysematous change. This could be suggestive of the negative influences of dichlorvous on the lungs. The effects observed could be said to be time dependent, as the degree of the effects was seen to be progressive with the increase in the hourly periods the animals were subjected to inhaled the vapor of dichlorvous. This did not agree to the work carried out by Luty et al., who reported a mild generalized congestion of the peri-bronchiolar and inter-alveolar septum [21] but similar to the work carried out by Owoeye et al., who reported the development of the widening of the intercellular septa with histiocytes as well as hyperemic foci of intra-alveolar space and extravasation to the lumen of lung alveoli [22].

The effect observed could be said to be time dependent, as the degree of the effects was seen to be progressive as the periods of exposure of the animals to dichlorvous inhalation increases.

5. CONCLUSION

The result of this present study suggests that the exposure to dichlorvous inhalation has a significant damaging effect on the lungs architecture, ranging from moderate to severe effects on the lungs with a severe consolidated inflammatory exudates (discharge of fluids from pores), a moderate intra-alveoli hemorrhage, and a severe dilation of alveolar septa which is evident as emphysematous changes. The increase in these features was observed as the periods of exposure increases, thus, it is exposure time dependent. Therefore, the results
of this investigation have demonstrated that the exposure to dichlorvos (otapia-pia) affects the lungs, marked by progressive loss of elasticity, and eventual rupture of the alveoli, accompanied by labored breathing, husky cough, thus influencing their normal physiologic mechanisms.

6. RECOMMENDATION

Following the result of this study, we recommend the need for relevant health agencies in Nigeria to create awareness on the effect of the exposure to dichlorvos on the histology of the lungs for public health importance. Further in-depth research should be carried out on the effect of dichlorvos inhalation on vital organs like pancreas, liver, kidney and adrenal gland.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was obtained from the ethical committee, Faculty of Basic Medical Science, Nnamdi Azikiwe University, Nnewi Campus Anambra State.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES