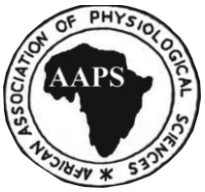
https://dx.doi.org/10.4314/jaaps.v12i1.4



J. Afr. Ass. Physiol. Sci. 14(1): 40-54 June 2024

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences

https://www.ajol.info/index.php/jaaps

Research Article

Ameliorative Effects of *Moringa oleifera* Aqueous Leaf Extract on Dichlorvos-Induced Interstitial Pneumonitis in Wistar Rats

Mabel B. Ehi-Omosun* and Felicitas E. Egbase

Departments of Anatomy and Medical Laboratory Sciences, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria.

Keywords:

Moringa oleifera; dichlorvos; insecticide; Interstitial pneumonitis; Lungs; histoarchitecture; respiratory diseases; exterminators

* Address for Correspondence:
Email:
mabel.ehi-omosun@uniben.edu

https://orcid.org/009-0000-5612-8466.

ABSTRACT

Background: Insecticide poisoning is the most common cause of lung diseases among people with insecticide-related occupations which has significant morbidity and mortality if left untreated. This study aimed to investigate the effects of aqueous leaf extract of *Moringa oleifera* on dichlorvos-induced interstitial pneumonitis in the adult Wistar rat.

Methods: Thirty (30) adult Wistar rats (male and female) weighing between 240 g and 270 g were divided into five (5) groups of six (6) rats per group. Group A rats were placed on feed and water only. Group B rats were exposed to only 2 puffs of dichlorvos (10 ml/puff) at a concentration of 100 mg/m3, administered at 10 am daily for 30 minutes via inhalation. Group C rats received 500mg/kg body weight per day (BWT/D) of Moringa oleifera. Groups D and E rats were exposed to dichlorvos via inhalation and received 250mg/kg BWT/D (low dose) and 500mg/kg BWT/D (high dose) of Moringa oleifera respectively. The *Moringa oleifera* dosages were given for 30 consecutive days via an orogastric tube. The weights of the animas in each group were taken and recorded weekly and the difference noted. At the end of the 30th day exposure, the animals were euthanized under chloroform anaesthesia and the lungs were harvested and processed for histological examination. The obtained data were analyzed using the one-way Analysis of Variance, with level of significance set at <0.05.

Results: Exposure to dichlorvos did not affect body weight in rats, but it did cause significant changes in blood parameters, including decresed lymphocytes and red blood cells, and disruptions of other red cell indices, indicating haematoogical toxicity. This suggests that dichlorvos may have effects of the bood and haematoogical system, even if it doesn't affect body werght. The histological sections of the lungs of rats in Group A, C, D and E showed normal histoarchitecture of the lungs. There were observable histological variations in the lung histoarchitecture of the exposed rats (Group B) which include bronchiolar haemorrhage, alveolar haemorrhage and interstitial infiltrates of inflammatory cells (evidence of interstitial pneumonitis).

Conclusion: It was concluded that Moringa oleifera had an ameliorative effect on dichlorvos-induced interstitial pneumonitis in Wistar rats. Moringa oleifera is therefore valuable in combating interstitial pneumonitis.

https://dx.doi.org/10.4314/jaaps.v12i1.4

1. Introduction

Moringa oleifera, commonly known as Moringa is a member of the *Moringaceae* family (Kumari *et al.*, 2021). It is a species of flowering plant that is cultivated in Asia, China, South East Asia and the Tropics (Luseba *et al.*, 2016; Saleh and Rahman, 2019). Moringa is characterized by its feathery, fern-like leaves, fragrant white flowers, and long, slender seed pods. *Moringa oleifera* has been used for centuries in traditional medicine and culinary practices in various regions, particularly in Asia and Africa. The leaves, pods, roots, bark, flowers and seeds are valued for their nutritional and medicinal properties, and are used to treat various ailments, such as inflammation, fever, and respiratory problems (Gupta *et al.*, 2016; Kumari *et al.*, 2021).

A review of the literature reveals the potential benefits of *Moringa oleifera* on lung health, as discussed beow:

- *Moringa oleifera* leaf extract has been found to have anti-inflammatory properties, which can help alleviate symptoms of asthma and other lung diseases.
- The seed and leaf extracts of *Moringa oleifera* have been observed to be safe for consumption and offer enhanced immunity and pulmonary protective potential when taken in appropriate doses.
- The aqueous and methanolic extracts of the leaves and stem of *Moringa oleifera* have been found to have elevated oxygen-radical scavenging activity
- The leaf extracts of *Moringa oleifera* have been found to have the highest 2,2-Diphenyl-Picrylhydrazyl (DPPH) and Ferric Reducing Ability of Plasma (FRAP) activities, and leaf and seed extracts have exhibited anti-inflammatory activities.
- The leaf, stem and root extracts of *Moringa oleifera* have been found to have antimicrobial properties, which can help combat lung infections.

Moringa oleifera oleifera was choosen as a potential therapeutic agent for dichlorvos-induced lung injury due to its well-documented antioxidant and anti-inflammmatry properties, which align with the need to counteract the oxidative stress and inflammation caused by dichlorvos toxicity, and this study aims to fufil the current knowledge gap by investigating its specific efficacy, haematolgical and body weight impact in this context.

Moringa oleifera may exert its protective effect against interstitial pneumonitis by inhibiting inflammation and oxidative stress through its antioxidant and anti-infammatory compounds, such as isothiocyanates, curcubitanes, triterpenoids and flavonoids, which may reduce lung tissue damage and modulate immune responses.

Moringa oleifera contains the toxic alkaloid cucuvesine, which has been found to also have anti-allergenic, antipyretic and anti-inflammatory effects (Luseba *et al.*, 2016). Phytochemical constituents of *Moringa oleifera* include: flavonoids, saponins, phenols, steroid, coumarine, curcubitane, triterpenoids and glucosinolates (Kumari *et al.*, 2021). These compounds are notable for their diverse biological activities and have been reported to possess antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, which may contribute to the plant's adaptogenic and immune-modulating effects, highlighting the potential of *Moringa* oleifera as a valuable resource for nutraceutical and pharmaceutical applications. Literature reports that *Moringa oleifera* leaves can be utilized in the therapy o fever, asthma, chest pain, cough and catarrh. Scientists have opined that the active principles which confer antipyretic, anti-inflammatory, antitussive and soothing effects on the plant are the curcubitanes and triterpenoids (Kumari *et al*., 2020; Brautigam, 2012).

Dichlorvos is an organophosphate widely used as insecticides to control household pests, in public health, and protecting stored products from insects (Eddleston *et al.*, 2016; Calistus *et al.*, 2016; Eteng, 2018; Chukwu and Ubosi, 2016; Dietz *et al.*, 2014). Pest eradicators,

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exterminators and pest control technicians are often exposed to dichlorvos daily for hours during the course of their work. Breathing the fumes of dichlorvos insecticide can occur during fumigation, while working directly with dichlorvos or using equipment sprayed with dichlorvos (El-Hilaly *et al.,* 2019; Ade-Ademilua and Obalola, 2018; Baccarelli *et al.,* 2014). Dichlorvos contains an organic chemical, dimethoate-cyflutrinas the active ingredient which is toxic to humans with significant morbidity and mortality if left untreated (Zidan *et al.,* 2019; Gizywa-Celinska *et et.,* 2019; Iden, 2015; Adewole *et al.,* 2017; Ahmed *et al.,* 2019).

Previous studies have shown that exposure to dichlorvos fumes causes respiratory allergy and shortness of breath in experimental animals (Eddleston *et al.,* 2016; Isam *et al.,* 2020; Kwan *et al.,* 2014; Edokpa and Ikelegbe, 2012). Dichlorvos vapor is a severe respiratory and skin irritant (Cheesbrough, 2016; Fletcher 2017; Mohamed *et al.,* 2016). It exerts its toxicity by inhibiting acetylcholinesterase, leading to acetycholine accumulation, hyperstimulation, and disruption of neural function, causing neurotoxicity, respiratory failure, and metabolic perturbations. This results in a range of symptoms, from coughing, eheezing, tachypnea, pulmonary osdema, tremors, and muscle weakness to paralysis and respiratory faiure which can be fatal (Mark *et al.,* 2016; Elham *et al*., 2019; Gizaw *et al.,* 2016). Hence, the objective of this paper was to evaluate the effects of aqueous leaf extract of *moringa oleifera* on dichorvos-induced interstitial pneumonitis in adult Wistar rats.

Interstitial pneumonitis, also known as ivterstitial pneumonia, is a condition characterized by inflammationand damage to the interstitial tissue of the lungs, which iv the area between thealveoli and the blood vesses. This can lead it scarring and thickening of the lung tissue, making it difficiltfor oxygen to pass into the bloodstream (Mark *et al.,* 2016; Iyawe and Ebomoyi, 2017; Drake *et al.,* 2015; Ganapathy *et al.,* 2019; Autifi *et al.,* 2015). Interstitial pneumonitis can be caused by vafious factors, including exposure to toxins or chemical, radiation therapy, viral or bacterial infections and autoimmune disorders (Harrison, 2014; Moore and Dalley, 2014; George *et al*., 2014*;* Ayres, 2015). Symptoms may include shortness of breath, cough, fatigue, chest pain, and difficulty breathing (Cheesbrough, 2016; Iyawe *et al.,* 2014). If left untreated, interstitial pneumonia can lead to serios complications, such as respiratory failure or pulmonary fibrosis (Heyder *et al.,* 2016; Amedu and Salami, 2018; Abdul-Wahab *et al.,* 2016). Beside dichorvos insecticide fumes, other airborne particles such as gasoline fumes, silica dust and coal dust have been linked to interstitial pneumonitis (Ibe *et al.,* 2014).

1.1 Objectives of the Study:

The objectives were to;

1. Investigate the effects of aqueous leaf extract of *Moringa oleifera* on the weight of Wistar rats.
2. Determine haematological parameters in rats exposed to dichlorvos
3. Determine histological features in the lungs of Wistar rats exposed to dichlorvos via inhalation.
4. Investigate the effects of *Moringa oleifera* on dichlorvos-induced interstitial pneumonitis in the adult Wistar rat.

2. Materials and Methods

2.1 Plant Materials

The *Moringa oleifera* leaves used in this study were collected from the University of Benin Farm Project, located in Benin City, Nigeria. The plant material was authenticated by botanists at the Department of Plant Biology and Biotechnology, Faculty of Sciences, University of Benin, Benin City, Edo State, Nigeria. A voucher specimen (voucher number: UB/MPI/008) was lodged at the department's herbarium for future reference.

2.2 Extract Preparation

Moringa oleifera leaves were oven-dried at 40^{o}C after air-drying for 7 days. The dried leaves were then grounded using a 2018 model mechanical grinder (Dozenmann, U.S.A). The cold maceration method was used to extract the powdered material by soaking 500g of the powdered *Moringa oleifera* leaf in 1litre of water for 24 hours at room temperature (Okonkwo *et*

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al., 2015). The cold maceration method was selected for extracting active compounds from *Moringa oleifera* leaves due to its ability to yield high-quality extracts with minimal degradation of phytochemicals. This technique enables the extraction of a wide range of bioactive compounds, including flavonoids, saponins, and terpenoids which are known to contribute to the medicinal properties of *Moringa oleifera*. Furthermore, cold maceration is a simple and scalable method that can be easily replicated in a laboratory setting, making it an attractive choice for our research.

The soaked *Moringa oleifera* was filtered with the aid of cotton wool. Using evaporating dishes, the filtrate was concentrated over a hot water bath to obtain 20 g concentrated jellylike extract of *Moringa oleifera* leaf which was then transferred into plain specimen bottles for storage in a refrigerator at 4^{o}C. Acute oral toxicity of the extract was evaluated. Appropriate doses of the extract were then made by diluting with distilled water into 250mg/kg body weight and 500mg/kg body weight which were administered to the rats orally.

2.3 Experimental animals

Thirty (30) adult Wistar rats (both sexes) of 240g-270g in weight were purchased from the Animal House, Department of Anatomy, University of Benin, Nigeria and were utilized for this experiment. The animals were randomly assigned into 5 cages, with 6 rats per cage, and housed under standard conditions. The housing conditions were as follows:

- Number per cage: 6 rats
- Bedding: Wood shavings
- Lighting: 12-hour ight/dark cyce
- Humidity: 50-60%
- Ambient temperature range: 25-28^{0}C

The housing conditions were designed to minimize stress and ensure the comfort and well-being of the animals. The animals were left to acclimatize for 2 weeks before commencement of the experiment. During this period, they were allowed access to standard animal feed manufactured by Bendel Flour Mill, Ewu, and clean water *ad libitum*. The suppier's details are as follows:

- Supplier: Bendel Flour Mill
- Location: Ewu, Edo State, Nigeria
- Product: Standard Animal Feed (Pellet Form)
- Batch number: BMI/EWCB/009
- Expiration date: May 2024

We ensured that the feed was used within the recommended shelf life to maintain its nutritional value

2.4 Ethical Consideration

Ethical approval was obtained from the Research Ethics Committee of the College of Medical Sciences, University of Benin, Nigeria (The approval number obtained is CMS/REC/2012/302). Each animal procedure was carried out in accordance with approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for research (Buzek and Chastel 2010).

2.5 Induction of Interstitial Pneumonitis

Interstitial pneumonitis was induced in the test animals by exposure to dichlorvos at 100mg/m^{3}, delivered as 2 puffs (10 m/puff) via a handheg sprayer in a fume distributor gass-chamber FDGC), eith daily 1-hour exposure for 30 days (Okonkwo *et al.*, 2015). The dichlorvos was manufactured by:

- Company name: DZT Chemicals
- Address: 208 Industrial Estate , Sango-Ota, Ogun State, Nigeria.
- Product name: Dichlorvos insecticide
- Product Code: DVN-027
- Batch number:DCN/8947/008CN/002
- Manufacturing date: Dec. 2021
- Expiration date: Dec. 2024

We ensured that the concentration and dose of dichlorvos used in this study were accurate and reproducible, and we provide these details to facilitate replication by other researchers.

A pilot study was done on the 28th day of the experiment which confirmed interstitial pneumonitis.

https://dx.doi.org/10.4314/jaaps.v12i1.4

2.6 Experimental design

In this study, 30 animals were divided into 5 groups comprising of 6 rats per-group. To minimize the impact of environmental factors on our study outcomes, we implemented the following controls:

- Housing: Rats were housed in groups of 6 per cage, with identical cage, size, bedding materials, and ventilation.
- Feeding: All rats received the same standardized feed, manufactured by Bendel Flour Mill, and had access to clean water *ad libitum*.
- Lighting: The rats were exposed to a standardized 12-hour light/dark cycle, with controlled lighting intensity and wavelength.

These controls ensured that any differences observed between groups were due to the experimental treatment and not to environmental factors.

We employed a randomized block design to divide the 30 rats into five groups of six rats each. To minimize bias, we:

- Used a computer-generated randomization program to assign rats to groups.
- Ensured that the groups were matched for relevant variables such as age, sex, and and body weight.
- Used a blinded procedure for group assignment and treatment administration.
- Ensured that the researchers involved in data collection and analysis were unaware of the group assignments.

By taking these measures, we aimed to ensure the validity of our study results.

Group A rats which served as control received standard feed and clean water *ad libitum*. Group B rats were exposed to dichlorvos only via inhalation. Group C rats received 500mg/kg body weight per day (BWT/D) of *Moringa oleifera.* Group D rats were exposed to dichlorvos via inhalation and received 250mg/kg BWT/D of *Moringa oleifera.* Group E rats were exposed to dichlorvos insecticide via inhalation and received 500mg/kg BWT/D of *Moringa oleifera*. We selected the dosages of *Moringa oleifera* for groups D and E based on previous studies that have demonstrated the safety and efficacy of this pant extract in animal models.

Specifically:

- Group D received 250mg/kg body weight/day of *Moringa oleifera* which is equivalent to the dosage used in a previous study that showed significant antioxidant activity (Kumari et al., 2021).
- Group E received 500mg/kg body weight/day of *Moringa oleifera* which is equivalent to the dosage used in a previous study that showed significant anti-inflammatory activity (Brautigam, 2012).

We choose these dosages to investigate the potential dose-resonse relationship of *Moringa oleifera* on interstitial pneumonitis in rats. Our selection of these dosages was also guided by the results of our pilot study, which suggested that these dosages were wel-tolerated and effective in reducing inflammation in rats.

We calculated the volumes of *Moringa oleifera* to be administered based on the body weight of the Wistar rats as follows:

Group D: 250mg/kg body weight = 0.98 ml/kg (based on a concentratin of 250mg/ml).

Group E: 500mg/kg body weight = 1.96 ml/kg (based on a concentratin of 500mg/ml).

Therefore, the vomumes of *Moringa oleifera* administered per kg body weight were 0.98 ml/kg for group D and 1.96m/kg for group E. The dosages were given for 30 consecutive days via an orogastric tube. The weights of the animals in each group were taken weekly and the difference between them and previous weights were noted.

2.7 Method of Termination and Sample Collection

After the 30th day of exposure to dichlorvos insecticide, the animals were weighed and then euthanized under chloroform anaesthesia. Blood samples were collected through cardiac puncture into anticoagulant (Ethylene Diamine Tetraacetic Acid) bottle for haematological analysis and the lung of each rat was excised and fixed in 10% formal saline for 24 hours before the histological

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procedures. The tissues were trimmed to about 3-5 mm thick sections and processed according to the method of Drury and Wallington (1980). The trimmed tissues were histologically processed manually for microscopy using the foowing methods:

- Fixation: Tissues were fixed in 10% neutral buffered formalin (P^H 7.4) for 24-48 hours at room temperature using a Fisherbrand fixation container.
- Embedding: Tissues were embedded n paraffin wax (PW) using a Leica TP1020 tissue processor with the following settings: temperature 58 ^{0}C, vacuum 100 mbar, and time 12 hours.
- Tissue staining: Tissues were stained with Hematoxylin and Eosin (H&E) using a Thermo Scientific Gemini tissue stainer with the following settings: temperature 37 ^{0}C, time 30 minutes, and staining solution Hematoxylin and Eosin (cataog nmber 124567). By using H&E stain, we were abe to visualize tissue morohology and atchitecture, and subsequently evaluate histological changes and their correlation with experimental results.

Histological sections were examined under a Leica DM750 research microscope with a digital camera (Leica ICC50) attached. Photomicrographs of the tissue sections were taken at magnification of x40.

White blood cells, lymphocytes, red blood cells, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, and platelets were analysed using an auto-analyzer (2006 model, manufactured by Hoddlier and Stoughton Group of company, London with a recognized biochemical kit (2010 model, Diagnostic Merck, London).

2.8 Statistical analysis

Statistical analysis was carried out with Statistical Software Package, Microsoft Excel, 2010 and Statistical Package for Social Sciences (S.P.S.S.) version 20. Results were presented as Mean (X) ± Standard error of mean (SEM). The one way analysis of Variance (ANOVA) was used to determine the significance of the difference in means at 95% confidence interval. $P \leq 0.05$ was considered significant.

3. RESULTS

The two doses of *Moringa oleifera* extract demonstrated different efficacy profiles in protecting against dichlorvos induced ung damage.

Specifically:

- The lower dose (250mg/kg) activated cells of the mononuclear phagocyte system, indicating an immune response to the toxicant. This suggests that the extract at this dose immunomodulatory effects.enhancing the body's natural defense mechanisms.
- In contrast, the higher dose (500mg/kg) showed interstitial infiltrates of infammatry cells, indicating a mure pronounced inflammatory response. This suggests that the extract at this dose has anti-inflammatory effects, potentially mitigating the harmful effects of dichlorvos.

These findings indicate that the efficacy of *Moringa oleifera* extract in protecting against dichlorvos-induced lung damage is dose-dependent, with the lower dose providing immunoprotection and the higher dose exerting anti-inflammatory effects.

Changes in body weights of the animals in all the experimental groups are presented in Table 1. It was observed that there was no significant difference in body weights of the rats in the various groups exposed to dichlorvos though there was a slight decrease.

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Table 1: Change in Body Weights of the Rats in all the Experimental Groups.

Groups	**Initial Body Weight (Grams)**	**Final Body Weight (Grams)**	**P-value**
Control (Group A)	168.00±19.90	195.00±19.22	0.158
Dichlorvos (Group B)	214.67±13.30	211.67±17.46	0.580
Extract only (Group C)	184.00±18.04	192.00±16.70	0.062
Dichlorvos + low dose) extract (Group D)	186.00±18.77	182.33±17.61	0.134
Dichlorvos + high dose (Group E)	176.67 ±11.86	175.67±16.75	0.605

n=6; Values are Mean ± S.E.M

As shown below in **Table 2,** haematological analysis of whole blood shows that dichlorvos decreased some haematological parameters such as lymphocytes, red blood cells, mean cell volume and mean cell haemoglobin concentration.

Table 2: Comparison of Haematological Parameters in all the Experimental Groups

Groups/Tests	**CONTROL**	**GROUP B**	**GROUP C**	**GROUP D**	**GROUP E**	**P Value**
WBC count (10^3/uL)	9.43 ± 0.89	6.43 ± 1.11	7.80 ± 0.77	8.13 ± 0.31	6.73 ± 0.99	0.145
LYM (%)	8.93 ± 0.85	6.10 ± 1.06*	7.25 ± 0.59	7.65 ± 0.29	6.38 ± 0.90	0.115
RBC count (10^6/uL)	6.76 ± 0.35	5.56 ± 0.40*	6.88 ± 0.20	6.69 ± 0.15	6.44 ± 0.08	0.863
HGB (g/dl)	12.90 ± 0.86	12.03 ± 0.78	12.50 ± 0.44	12.30 ± 0.21	12.08 ± 0.30	0.776
HCT **(%)**	39.63 ± 2.31	38.23 ± 1.60	40.02 ± 1.73	39 00 ± 1.02	37.75 ± 0.84	0.709
MCV (Fl)	58.63 ± 0.57	56.47 ± 1.69*	59.53 ± 1.04	58.48 ± 1.01	58.73 ± 0.57	0.882
MCH (pg)	19.00 ± 0.27	17.30 ± 0.17	18.10 ± 0.26	18.38 ± 0.13	18.70 ± 0.28	0.079
MCHC (%)	32.50 ± 0.32	28.33 ± 0.87*	30.55 ± 0.29	31.50 ± 0.45	31.93 ± 0.44	0.116
PLT (10^3/uL)	904.00 ±18.87	851.33± 21.99	393.00 ± 32.85	724.50±10.75	813.00±15.66	0.567

Values are Mean ± S.E.M *Significnty different from the control group

KEY: White blood cells (WBC), Lymphocytes (LYM), Red blood cells (RBC), Haemoglobin (HGB), Haematocrit (HCT), Mean cell volume (MCV), Mean cell haemoglobin (MCH), Mean cell haemoglobin concentration (MCHC), Platelets (PLT).

The histopathological examination of lung tissues from the different treatment groups revealed distinct reatures:

Group A (Control): Normal tissue architecture was observed, with intact aveoli, bronchioloalveolar lymphoid tissue, bronchial artery, and interstitial space, indicating no signs of damage or inflammation. These findings demonstrate that the lung tissue in Group A rats is histopathologically normal, providing a control reference point for the study.

Group B: (Dichlorvos-treated): Severe damage was evident, characterized by bronchiolar haemorrhage, alveolar haemorrhage, and heavy

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interstitial infiltrates of inflammatory cells, indicating a robust inflammatory response to the toxicant.

GroupC: (500mg /kg *Moringa oleifera* extract only): Normal architecture of alveoli, bronchial artery, bronchial vein, and terminal bronchiole was observed, with activated cells of the mononuclear phagocyte system, indicating an enhanced immune response to the extract.

Group D (250mg /kg *Moringa oleifera* extract + dichlorvos):

- Normal architecture of aveoli, bronchial artery, bronchia vein, and terminal bronchioles, indicating no damage to the lung tissue.
- Activated cells of the mononuclear phagocyte system, suggesting an immune response to the extract.

Group E (500mg/kg *Moringa oleifera* extract + dichlorvos):

- Normal architecture of alveoli, and respiratory bronchiole, indicating no damage to the lung tissue.
- Interstitial infiltrates of inflammatory cells, suggesting a mild inflammatory response to the higher dose of the extract.

These findings suggest that *Moringa oleifera* extract exerts a protective effect against dichlorvos-induced lung damage, with the higher dose showing enhanced immunoprotection and anti-inflammatory effects.

Figure 1 is a photomicrograph of a section of rat's lung in the control group (Group A) (H&E at x 40) magnification showing normal tissue architecture of alveoli (A), bronchiolo alveolar lymphoid tissue (BL), bronchial artery (BA), respiratory bronchiole (RB) and interstitial space (IS).

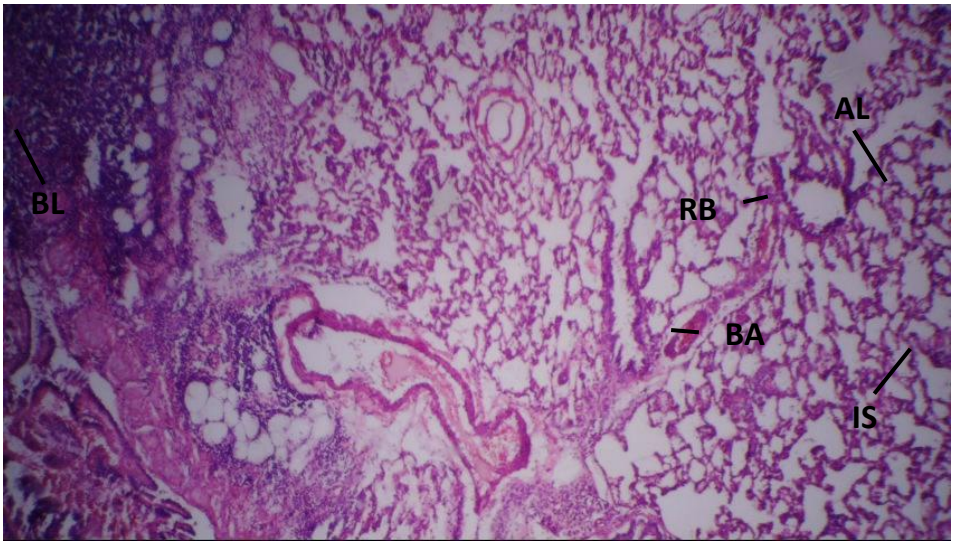

Figure1: Rat lung. Control. Composed of normal tissue architecture: bronchiolo-alveolar lymphoid tissue (BL), alveoli (AL), bronchial artery (BA), respiratory bronchiole (RB), interstitial space (IS): H&E x 40

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Figure 2 is a photomicrograph of a section of rat's lung exposed to dichlorvos only (Group B) (H&E at x 40) magnification showing: bronchiolar haemorrhage (BH), alveolar haemorrhage (AH), and heavy interstitial infiltrates of inflammatory cells IC).

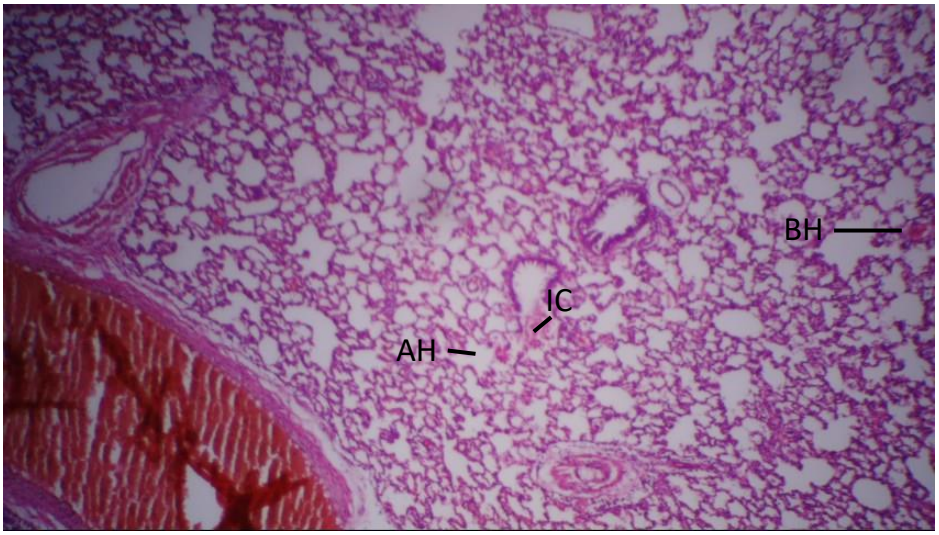

Figure 2: Rat lung given Dichlorvos only showing: bronchiolar haemorrhage (BH), alveolar haemorrhage (AH), interstitial infiltrates of inflammatory cells (IC): H&E x 40

Figure 3 is a photomicrograph of a section of rat's lung given 500mg extract only (Group C) (H&E at x 40) magnification showing normal architecture of alveoli (AL), terminal bronchiole (TB) and activated mononuclear phagocyte tissue (MP).

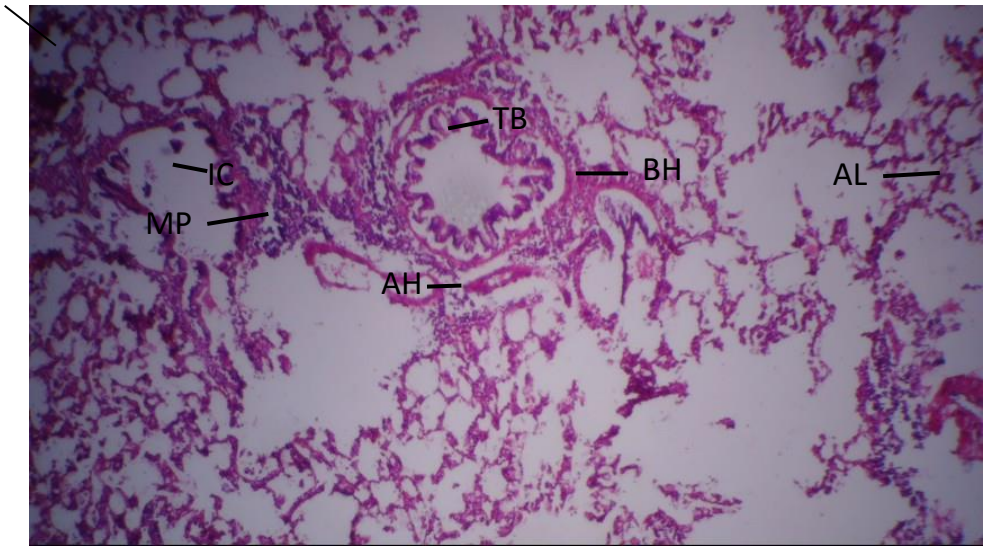

Figure 3: Rat lung given 500mg/kg extract only showing normal architecture: activated mononuclear phagocyte tissue (MP), alveoli (AL), terminal brochiole (TB): H&E x 40

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Figure 4 is a photomicrograph of a section of rat's lung given 250mg/kg extract and exposed to dichlorvos (Group D) (H&E at x 40) magnification showing: normal architecture of alveoli (AL), bronchial artery (BA), bronchial vein (BV), terminal bronchiole (TB) and activated cells of the mononuclear phagocyte system (MP)

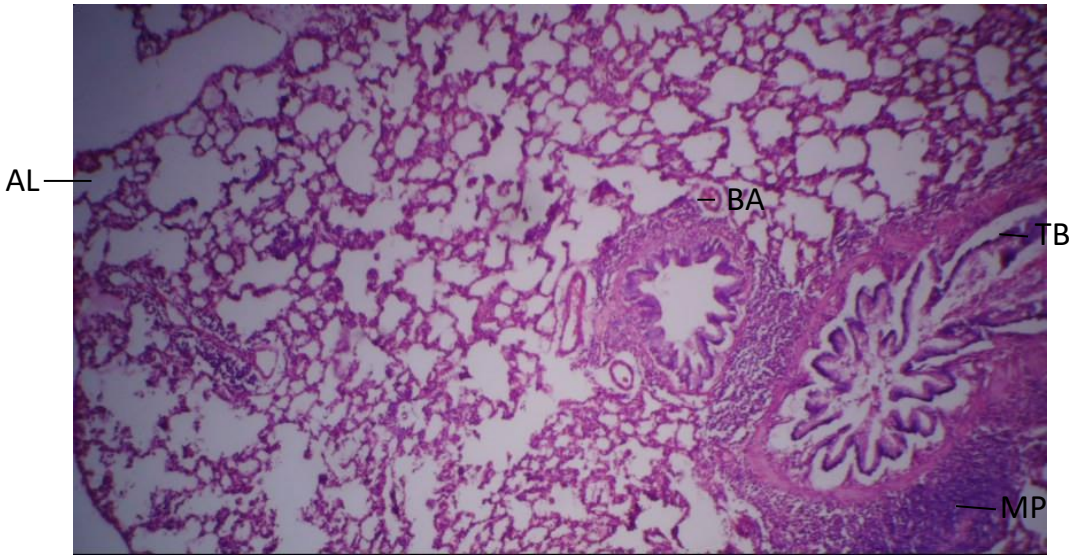

Figure 4: Rat lung given 250mg/kg extract + Dichlorvus showing normal architecture: alveoli (AL). bronchial artery (BA), activated cells of the mononuclear phagocyte system (MP), terminal bronchiole (TB): H&E x 40

Figure 5 is a photomicrograph of a section of rat's lung given 500mg/kg extract and exposed to dichlorvos (Group E) (H&E at x 40) magnification showing: normal architecture of alveoli (AL), respiratory bronchiole (RB) and interstitial infiltrates of inflammatory cells (IC).

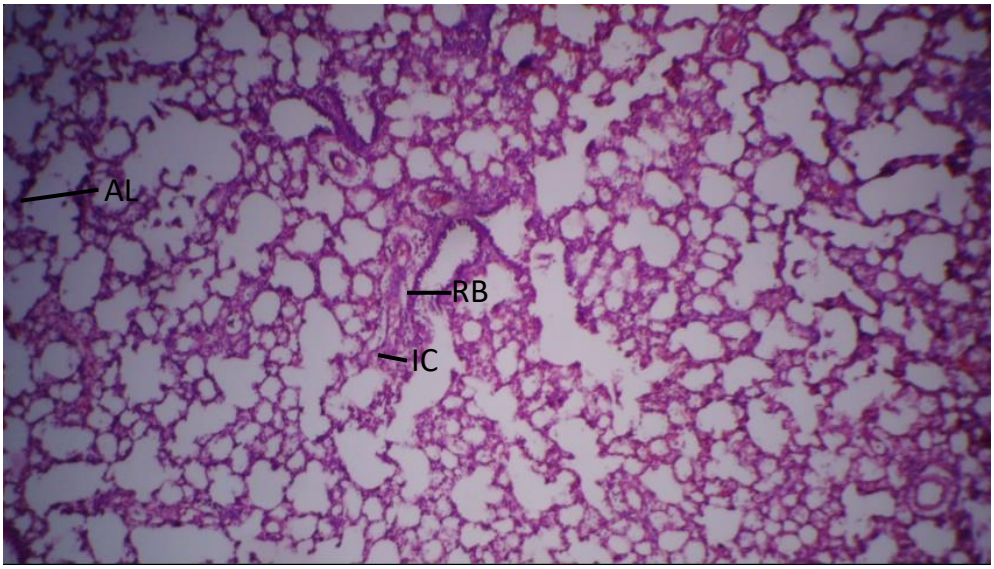

Figure 5: Rat lung given 500mg/kg extract + Dichlorvus showing normal alveoli (AL), normal respiratory bronchiole (RB), interstitial infiltrates of inflammatory cells (IC): H&E x 40

https://dx.doi.org/10.4314/jaaps.v12i1.4

4. DISCUSSION

Moringa oleifera has been reported to have various medicinal uses. Literature reports that it has analgesic, anti-tussive and anti-inflammatory properties. Against this background, this study was conducted to evaluate the effects of aqueous leaf extract of *Moringa oleifera* on dichlorvos-induced interstitial pneumonitis in Wistar rats.

Findings of the study for haematological parameters showed that dichlorvos causes derangements in haematological parameters such as lymphocytes (immunity cells), red blood cells, mean cell volume and mean cell haemoglobin concentration (Table 2). The lymphopenia observed in this study (Table 2) may have been due to the immune response to the inhaled dichlorvos fumes in the lungs as observed in the histopathological slides (Figure 2).. Reduced values of red blood cells and other red cell indices such as mean cell volume and mean cell haemoglobin concentration (Table 2) were also observed in the exposed animals and this concurs with a similar work done by Okonkwo *et al.,* 2015; Tayor et al., 2014; Elgailani and Alsakka, 2016). These haematological derangements indicate diseases and pathological symptoms of a variety of maladies including microcytic hypochromic anaemia and increased susceptibility to infections which are capabe of compromising the health of the research animals and may ultimate lead to mortality.

Observations based on photomicrography show that dichlorvos caused bronchiolar haemorrhage, alveolar haemorrhage and interstitial infiltrates of inflammatory cells (evidence of interstitial pneumonitis) in the exposed rats (Group B, Figure 2). There was florid activation of the lung tissue of the rats that that were exposed to dichlorvos alone which occurred as a result of the body sensing a foreign body (excess accumulation of dichlorvos fumes) leading to the activation of lymphoid tissues to get rid of it. As shown in Figure 3 (Group C) *Moringa oleifera* had no negative effects on the histology of the lungs as the alveoli, terminal bronchioles and bronchial artery were found to be histologically normal in the rats that were administered only the extract. Low doses of *Moringa oleifera* caused normal architecture of the alveoli, bronchial artery, terminal bronchiole and activated cells of the mononuclear phagocyte system (Figure 4). *Moringer oleifera* showed a protective effect against dichlorvos-induced lung injuries. Interstitial pneumonitis was completely prevented and the accumulated dichlorvos particulate matters were cleared. The phytochenicals present in *Moringa oleifera,* particularly flavonoids, saponins, and alkaloids, likey contributed to its protectibe effects against dichlorvos-induced lung damage. Flavonids, for instance, may have reduced inflammartion by inhibiting pro-inflammatory cytokines and scavenging free radicals, while saponins may have modulated immune responses and reduced oxidative stress.

The key phytochemicals in *Moringa oleifera,* including flavonoids and saponins, have been shown to exhibit anti-inflammatory and antioxidant properties, mhich mar have contributed to the observed protective effects. Specifically, favonoids have been demonstrated to reduce oxidative stress by scavengind free radicas, while saponins have been shown to modulate immune responses and reduce inflammation.

Our findings are consistent with previous studies that have demonstrated the protective effects of *Moringa oleifera* against toxicant-induced lung bpdamage. For example, a study by Brautigam (2012) found that *Moringa oleifera* extract reduced lung inflammation and oxidative stress in mice exposed to cigarette smoke.

The phytochemicals in *Moringa oleifera* may interact with specific cellular pathways or molecular targets to reduce lung inflammation, including the Nuclear Factor Kappa B (NF-KB) and Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2 pathways, which regulate inflammatory responses and antioxidative defenses. Further studies are needed to elucidate the precise mechanisms involved.

The different doses of *Moringa oleifera* extract used in this study may have influenced the effects on the outcome measured by modulating the bioavailability and pharmaco kinetics of the phytochemicals. Higher doses may have resulted in greater bioavailability and enhanced protective effects.

https://dx.doi.org/10.4314/jaaps.v12i1.4

Qur findings suggest that *Moringa oleifera* extracts may have potential therapeutic applications in treating or preventing lung diseases caused by toxin exposure. However, further studies are needed to evaluate the feasibility, safety, and efficacy of using *Moringa oleifera* extracts in humans. Additionally, the optimal dosage and duration of treatment need to be determined.

5. Conclusions

The results of this study have important implication frr the treatment of interstitial pneumonitis, as they demonstrate the potential of *Moringa oleifera* to mitigate the harmful effects of dichlorvos on the lung tissue. The observed effects of *Moringa oleifera* are likely due to its anti-inflammatory and antioxidant properties which may involve the inhibition of pro-infammatory cytokines, scavenging of free radicals, and enhancement of cellular defense mechanisms.

The practical applications of these findings are significant, as they suggest that *Moringa oleifera* may be used as a complementary therapy to aeviate the symptoms of interstitial pneumonitis, improve lung function, and enhance the quality of life for patients suffering from this disease. Furthermore, the dosage-dependent effects of *Moringa oleifera* observed in this study suggest that it may be possible to optimize its therapeutic efficacy through careful dosing regimens.

5.1 The Novel Aspects of This Study

- Our study reveals nove aspect, including the first demonstration of the protective effects of *Moringa oleifera* dichlorvos-induced interstitial pneumonitis in adult Wistar rats.
- Notably, our findings show that *Moringa oleifera* extract significantly reduced lung inflammation and oxidative stress, suddesting a potential therapeutic role in mitigating toxicant-induced lung damage.
- The study's results aso highlight the novel finding that *Moringa oleifera* extract modulates the NF-KB and Nrf2 pathways, ehich are critical regulators of inflammation and antioxidant responses.
- Furthermore, our research demonstrates the novel application of *Moringa oleifera* extract as a potential adjunct therapy for the treatment of interstitial pneumonitis, a debilitating respiratory disease.
- Overall, our study provides new insights into the therapeutic potential *Moringa oleifera* extract and its mechanisms of action, which have important implications for the development of novel treatments for toxicant-induced lung diseases.

6. Recommendation

Advanced studies should be conducted to develop *Moringer oleifera* into an effective therapeutic agent for interstitial pneumonitis, elucidating the underlying mechanisms, the efficacy and safety of different doses and identifying the active ingredients responsible for its therapeutiv effects.

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