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**Effects of Aqueous Leaf Extract of *Moringa oleifera* on Spray Paint-Induced Lung Damage in Wistar Rats**

EHI-OMOSUN, Mabel B. \* and AJUFOH, Zion C.

Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Nigeria.

Author for Correspondence\*: mabel.ehi-omosun@uniben.edu/+234-704-994-8914/ORCID ID: <https://orcid.org/009-0000-5612-8466>. <https://dx.doi.org/10.4314/sokjmls.v9i4.27>**Abstract**

Hydrocarbon poisoning is the most common cause of lung diseases among people with spray paint-related occupations which has significant morbidity and mortality if left untreated. The aim of this study was to investigate the effects of aqueous leaf extract of *Moringa oleifera* on spray paint-induced interstitial pneumonitis in the adult Wistar rats. Thirty (30) adult Wistar rats (male and female) weighing between 238 g and 268 g were divided into five (5) groups of six (6) rats each. Group A rats were administered feed and water only. Group B rats were exposed to 2 puffs of spray paint (10 ml/puff) at a concentration of 100 mg/m<sup>3</sup>, administered at 10 am by inhalation via fume distributor glass-chamber of dimensions 32.5 cm<sup>3</sup> × 32.5 cm<sup>3</sup> × 16.5 cm<sup>3</sup> for 1 hour daily. Group C rats received 500mg/kg body weight per day (BWT/D) of *Moringa oleifera*. Groups D and E rats were exposed to 2 puffs of spray paint (10 ml/puff) at a concentration of 100 mg/m<sup>3</sup>, administered at 10 am by inhalation via fume distributor glass-chamber of dimensions 32.5 cm<sup>3</sup> × 32.5 cm<sup>3</sup> × 16.5 cm<sup>3</sup> for 1 hour daily and received 250mg/kg BWT/D (low dose) and 500mg/kg BWT/D (high dose) of *Moringa oleifera* respectively. The dosages of the extract were given for 30 consecutive days via an orogastric tube. The weights of the animals in each group were taken and recorded weekly and the difference noted. At the end of the 30<sup>th</sup> day exposure, the animals were euthanized under chloroform anaesthesia and the lungs were harvested and processed for histological examination. Also, blood samples were collected through cardiac puncture into plain specimen bottles for haematological analysis. The

obtained data were analyzed using the one-way Analysis of Variance, with level of significance set at <0.05. The findings of the study for body weight showed a significant increase (P<0.05) in body weight of the animals in all experimental groups (Groups A-D). The control group (A) showed higher weight gain compared to the exposed groups (B-D). There were significant changes in blood parameters, including decreased lymphocytes and red blood cells, and disruptions of other red cell indices, indicating haematological toxicity. This suggests that spray paint may have effects on the blood and haematological system, and body weight. 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 severe alveolar dilation, severe vascular ulceration and congestion, interstitial haemorrhage, and interstitial inflammatory cell infiltrates (evidence of interstitial pneumonitis). Given similar biological responses, these effects of spray paint on rats can be reasonably extrapolated to humans, highlighting the importance of protective measures for individuals occupationally exposed to spray paint. This study demonstrates that *Moringa oleifera* has a protective role in combating spray paint-induced interstitial pneumonitis, characterized by reduced severity of alveolar dilation, vascular ulceration, and interstitial inflammation, in Wistar rats.

**Keywords:** *Moringa oleifera*; Spray paint; Hydrocarbon poisoning; Histoarchitecture; Lungs; Interstitial pneumonitis; Spray painters

## Introduction

Spray paint is a type of paint that is delivered in a fine, atomized mist from a pressurized can or container (Ojo, 2020). Spray paint is typically made up of a mixture of pigments, solvents, propellants and additives (Ibrahim *et al.*, 2016). Painting and coating workers in the architectural field, automotive workers, marine workers, industrial wood workers and metal coil coating workers are often exposed to spray paint fumes daily for hours during the course of their work. Spray paint contains organic chemicals, viz., toluene, ethylbenzene, propylene glycol, methyl isocyanate and acrylic acid as the active ingredients which are potentially deadly to humans, requiring timely treatment to prevent severe illness and death (Ojo, 2020). The most reported occupational hazards for spray painters are respiratory allergy, shortness of breath and other respiratory illnesses (Ibrahim *et al.*, 2016). Signs and symptoms of spray paint poisoning include headache, dizziness, fatigue, coughing, chest pain, wheezing and tachypnea (Mark *et al.*, 2016).

Spray paint contains Volatile Organic Compounds (VOCs), such as solvents, thinners, and propellants, which can evaporate quickly, releasing toxic fumes. Inhalation of these fumes can cause interstitial pneumonitis and other respiratory problems (Mark *et al.*, 2016). Moreover, spray paint can trigger inflammation in the lungs, airways, and other tissues, exacerbating conditions like asthma and chronic obstructive pulmonary disease (Harrison, 2014).

Interstitial pneumonitis is a condition characterized by inflammation and damage to the interstitial tissue of the lungs. This can lead to scarring and thickening of the lung tissue, making it difficult for oxygen to pass into the bloodstream (Autifi *et al.*, 2015; Drake *et al.*, 2015; Mark *et al.*, 2016; Iyawe and Ebomoyi, 2017). Interstitial pneumonitis can be caused by various factors, including exposure to toxins or chemicals, radiation therapy, viral or bacterial infections and autoimmune disorders (Harrison, 2014; Moore and Dalley, 2014; Ayres, 2015). Symptoms may include shortness of breath, cough, fatigue, chest pain, and difficulty breathing (Iyawe *et al.*, 2014; Cheesbrough, 2016). Untreated interstitial pneumonitis can progress to serious complications such as pulmonary fibrosis and potentially life-threatening respiratory failure (Ibe *et al.*, 2014; Abdul-Wahab *et al.*, 2016; Amedu and Salami, 2018).

*Moringa oleifera*, commonly known as Moringa, belongs to the *Moringaceae* family (Kumari *et al.*, 2021). It is a species of flowering plant that is cultivated in China, South East Asia, and the Tropics (Brautigam, 2012; Gupta *et al.*, 2016). 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, asthma, chest pain, cough and catarrh and other respiratory problems (Kumari *et al.*, 2021).

The literature suggests that *Moringa oleifera* may have beneficial effects on lung health. Its leaf extract has anti-inflammatory properties, which can help alleviate symptoms of asthma and other lung diseases. The seed and leaf extracts are also safe for consumption and offer enhanced immunity and pulmonary protection (Brautigam, 2012). Moreover, the plant's extracts have been found to have high antioxidant and oxygen-radical scavenging activities, as well as anti-inflammatory and antimicrobial properties, which can help protect against lung infections and diseases. *Moringa oleifera* was chosen as a potential therapeutic agent for spray paint-induced lung injury due to its well-documented antioxidant and anti-inflammatory properties, which align with the need to counteract the oxidative stress and inflammation caused by spray paint toxicity. This research aims to bridge the current knowledge gap by investigating its specific efficacy, haematological and body weight impact in this context.

*Moringa oleifera* contains the toxic alkaloid cucurbitacin, 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, steroids, coumarins, 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. *Moringa oleifera* may exert its protective effect against interstitial pneumonitis by inhibiting inflammation and oxidative stress through its antioxidant and anti-inflammatory compounds, such as isothiocyanates, curcubitanes, triterpenoids and flavonoids, which may reduce lung tissue damage and modulate immune responses. Scientists have opined that the active principles which confer antipyretic, anti-inflammatory, antitussive and soothing effects on the plant are the curcubitanes and triterpenoids (Brautigam, 2012; Kumari *et al.*, 2021). Hence, the present study investigated the effects of aqueous leaf extract of *moringa oleifera* on spray paint-induced lung damage in adult Wistar rats.

## Materials and Methods

**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/006) was lodged at the department's herbarium for future reference.

**Extract Preparation:** *Moringa oleifera* leaves were oven-dried at 40°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 (Brautigam, 2012). The cold macerated 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*. Additionally, 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°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.

**Experimental animals:** Thirty (30) adult Wistar rats (both genders) of 238g-268g in weight were purchased from the Animal House, Department of Anatomy, University of Benin, Nigeria and were utilized for this experiment. The rats were housed in cages with 6 per cage, wood shavings bedding, a 12-hour light/dark cycle, 50-60% humidity, and an ambient temperature of 25-28°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 supplier, Bendel Flour Mill, situated in Ewu, Edo State, Nigeria, delivered Standard Animal Feed in pellet Form. The relevant details are: batch number: BMI/EWCB/009 and expiration date May 2024. We ensured that the feed's nutritional value remained uncompromised by using it within the recommended time frame.

**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).

### **Induction of Lung Damage (Interstitial Pneumonitis):**

Interstitial pneumonitis was induced in the test animals by exposure to spray paint at  $100\text{mg}/\text{m}^3$ , delivered as 2 puffs (10 m/puff) via a handheld sprayer in a fume distributor glass-chamber FDGC), with daily 1-hour exposure for 30 days (Ojo, 2020). The spray paint was manufactured by Berger Paint Nigeria Limited, located at 208 Industrial Estate, Sango-Ota, Ogun State, Nigeria. The product details are: Product Name – Spray paint, Product Code - BPN-017, Batch number - BPN/8947/008CN/002, Manufacturing date - November 2022, and Expiration date - November 2024. We ensured that the concentration and dose of spray paint 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 28<sup>th</sup> day of the experiment which confirmed interstitial pneumonitis.

**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 rigorous controls. Rats were housed in standardized conditions, with six per cage, identical cage, size, bedding materials, and ventilation. A uniform diet was ensured by providing the same standardized feed from Bendel Flour Mill, and with unrestricted access to clean water. Additionally, a controlled 12-hour light/dark cycle was maintained, with standardized lighting intensity and wavelength, to further reduce environmental variability. 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 employed a rigorous methodology, utilizing a computer-generated randomization program to assign rats to groups. We ensured that the groups were well-matched for critical variables, including age, sex, and body weight. Additionally, we implemented a blind procedure for group assessment and treatment administration, and maintained researcher blinding throughout data collection and analysis, to prevent any potential bias. 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 *spray paint* only via inhalation. Group C rats received 500mg/kg body weight per day (BWT/D) of *Moringa oleifera*. Group D rats were exposed to *spray paint* via inhalation and received 250mg/kg BWT/D of *Moringa oleifera*. Group E rats were exposed to *spray paint* 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 plant extract in animal models. Specifically, Group D was administered 250mg/kg body weight/day of *Moringa oleifera*, a dose previously shown to exhibit significant antioxidant activity (Kumari *et al.*, 2021). Group E received 500mg/kg body weight/day of *Moringa oleifera*, a dose previously demonstrated to have significant anti-inflammatory effects (Brautigam, 2012). We chose these dosages to investigate the potential dose-response 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 well-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 concentration of 250mg/ml) while Group E: 500mg/kg body weight = 1.96 ml/kg (based on a concentration of 500mg/ml). Therefore, the volumes 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.

**Method of Sacrifice and Sample Collection:** On the 30<sup>th</sup> day of exposure to *spray paint*, the animals were weighed and subsequently euthanized under

chloroform anaesthesia. Blood samples were collected through cardiac puncture into anticoagulant (Ethylene Diamine Tetra acetic Acid) bottles for haematological analysis and the lung of each rat was excised and fixed in 10% formal saline for 24 hours before the histological procedures. The tissues were trimmed to about 5 $\mu$  (5 micrometer) thick sections and processed according to the method of Drury and Wallington (1980). The trimmed tissues underwent manual histological processing for microscopy involving fixation in 10% neutral buffered formalin (P<sup>H</sup> 7.4) for 24-48 hours at room temperature, followed by embedding in paraffin wax using a Leica TP1020 tissue processor at 58 °C, 100 mbar, and 12 hours. Subsequently, tissues were stained with Hematoxylin and Eosin (H&E) using Thermo Scientific Gemini tissue stainer at 37 °C for 30 minutes. This H&E staining enabled visualization of tissue morphology and architecture, allowing for the evaluation of histological changes and their correlation with experimental findings. Histological sections were examined under a Leica DM750 research microscope with a digital camera (Leica ICC 50) 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 analyzed using an auto-analyzer (2006 model, manufactured by Hoddler and Stoughton Group of company, London with a recognized biochemical kit (2010 model, Diagnostic Merck, London).

**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)  $\pm$  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 < 0.05 was considered significant.

### Results

The efficacy of *Moringa oleifera* extract in protecting against spray paint-induced lung damage differed significantly between the two doses tested. *Moringa oleifera* extract demonstrated dose-dependent efficacy in protecting against spray paint-induced lung damage. At the lower dose (250mg/kg), the extract exhibited immunomodulatory effects, activating cells of the mononuclear phagocyte system and enhancing the body's natural defense mechanisms. Conversely, the higher dose (500mg/kg) showed anti-inflammatory effects, marked by interstitial infiltrates of inflammatory cells, which may mitigate the harmful effects of spray paint. These findings indicate that the efficacy of *Moringa oleifera* extract in protecting against spray paint-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 below. Body weight analysis revealed a significant increase in all the groups, but the magnitude of weight gain was significantly reduced (P<0.05) in the spray paint-exposed groups compared to the control group, suggesting spray paint's inhibitory effect on weight gain.

**Table 1: Changes in body weights of the rats in all the experimental groups:**

Groups	Initial body weight (Grams)	Final body weight (Grams)	Weight Difference	P-value
Group A (Control)	5.60 ± 0.68	7.74 ± 0.60	0.94 ± 3.34	0.000
Group B (Spray paint Exposure only)	0.60 ± 0.19*	0.18 ± 0.09*	0.80 ± 0.14	0.000
Group C (Extract only)	3.42 ± 0.16*	5.36 ± 0.10*	0.68 ± 2.20	0.000
Group D (Low dose of extract + Spray paint)	0.38 ± 0.16*	0.04 ± 0.18*	0.58 ± 0.10	0.000
Group E (High dose of extract + Spray paint)	0.28 ± 0.16*	0.02 ± 0.116*	0.50 ± 0.04	0.000

n=6; Values are Mean ± S.E.M; The P-value speaks to the difference between the initial and final weight.

As shown below in Table 2, haematological analysis of whole blood shows that spray paint 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 Experimental Groups**

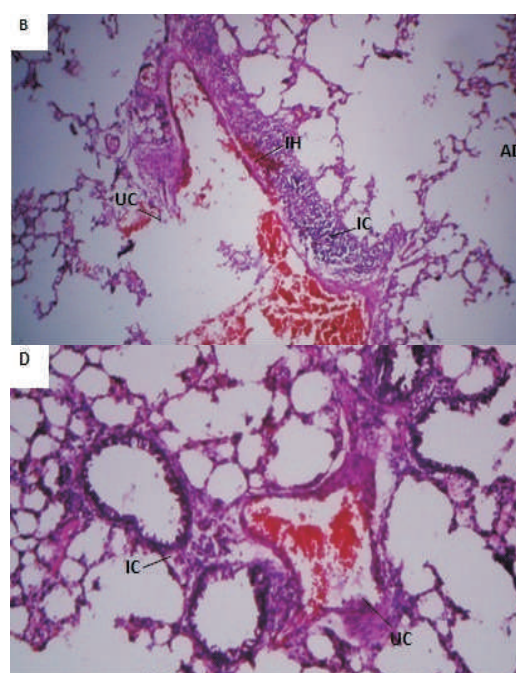
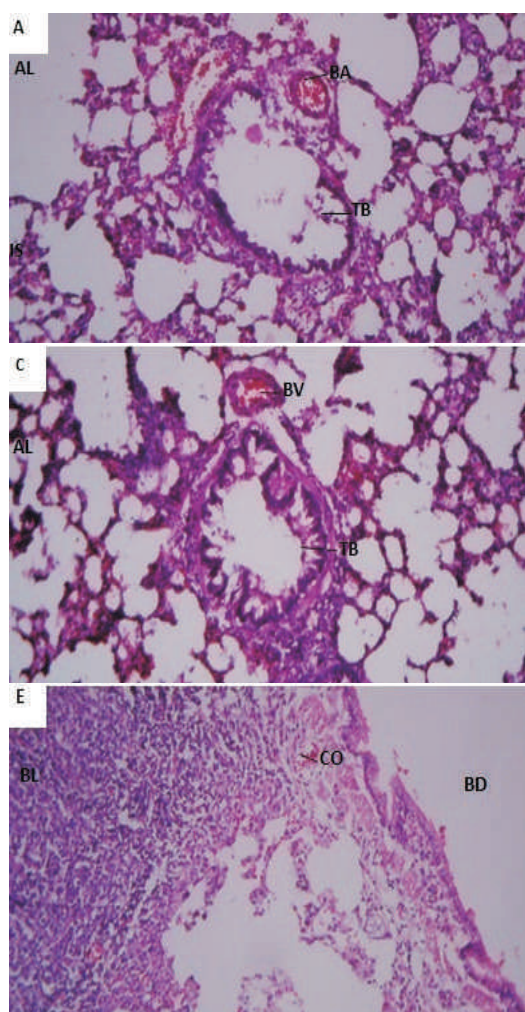
Groups/Tests	Group A	Group B	Group C	Group D	Group E	P-Value
WBC count (10 <sup>3</sup> )	9.43 ± 0.89	6.43 ± 1.11	7.80 ± 0.77	8.13 ± 0.31	6.73 ± 0.99	0.145
LYM (%)	8.93 ± 0.85	6.10±1.06*	7.25 ± 0.59	7.65 ± 0.29	6.38 ± 0.90	0.115
RBC count (10 <sup>6</sup> /ul)	6.76 ± 0.35	5.56± 0.40*	6.88 ± 0.20	6.69 ± 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±1.61*	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 <sup>3</sup> /uL)	904.00±18.89	851.33±21.9	393.00±32.	724.50±10.7	813.00±15.6	0.567

Values are Mean ± S.E.M \*Significantly 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 findings in lung tissues varied across the different treatment groups. In Group A (Control), normal tissue architecture was observed, with intact alveoli, 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. In Group B (Spray paint-treated), severe damage (interstitial pneumonitis) was evident, characterized by bronchiolar haemorrhage, alveolar haemorrhage, and inflammation of the interstitial tissue (heavy interstitial infiltrates of inflammatory cells), indicating a robust inflammatory response to the toxicant (spray paint). In Group C (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. The

histological examination of lung tissues from Group D (250mg /kg *Moringa oleifera* extract + spray paint) revealed well-preserved lung architecture, with no evidence of damage. Furthermore, the presence of activated mononuclear phagocyte system cells suggests an immune response to the extract. In Group E (500mg/kg *Moringa oleifera* extract + spray paint), the lung architecture remained intact, but with signs of a mild inflammatory response, characterized by interstitial infiltrates of inflammatory cells, likely due to the higher dose of the extract. These findings suggest that *Moringa oleifera* extract exerts a protective effect against spray paint-induced lung damage, with the higher dose showing enhanced immune-protection and anti-inflammatory effects. These findings of this study on the effects of spray paint on these rats can be extrapolated to humans, particularly spray painters, who should take measures to minimize their exposure to spray paint.



**Figure 1: Representative photomicrographs of H&E sections of the lungs of the experimental animals at the end of study:** 'A' shows the Group A (Control group) showing normal alveoli 'AL', interstitial space 'IS', normal bronchial artery 'BA' and terminal bronchiole 'TB'; 'B' shows Group B exposed to 100 mls of spray paint only via inhalation daily for 30 days showing severe alveolar dilation 'AD', severe vascular ulceration and congestion 'UC', interstitial haemorrhage 'IH' and interstitial inflammatory cell infiltrates (IC); 'C' shows Group C administered 500mg/kg body weight per day of *Moringa oleifera* aqueous leaf extract daily for 30 days showing normal architecture of alveoli 'AL', normal bronchial vein 'BV' and normal architecture of terminal bronchiole 'TB'; 'D' shows Group D exposed to 100 ml of spray paint fumes via inhalation and received 250mg/kg body weight of *Moringa oleifera* aqueous leaf extract daily for 30 days showing normal architecture of vascular ulceration and congestion 'UC' and interstitial infiltrates of inflammatory cells 'IC'; 'E' shows Group E exposed to 100 ml of spray paint fumes via inhalation and received 500mg/kg body weight of *Moringa oleifera* aqueous leaf extract daily for 30 days showing florid activation of the bronchioloalveolar lymphoid aggregate, 'BL', bronchiolar dilation 'BD' and active vascular congestion 'CO'.

### Discussion

*Moringa oleifera* has been found to possess multiple medicinal uses and therapeutic potentials. 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 spray paint-induced interstitial pneumonitis in Wistar rats.

The findings of the study for body weight showed a significant increase ( $P < 0.05$ ) in body weight of the animals in all experimental groups (Groups A-D). The control group (A) showed higher weight gain compared to the exposed groups. Inhalation of spray paint fumes triggered a systemic inflammatory response in the exposed animals, leading to pulmonary toxicity, which likely impacted their overall health and contributed to reduced weight (Ibrahim *et al.*, 2016). The implications of these results are crucial for the development of innovative weight management strategies in humans.

Findings of the study for haematological parameters (Table 2) showed that spray paint causes derangements in haematological parameters such as lymphocytes (immunity cells), red blood cells, mean cell volume and mean cell haemoglobin concentration. The lymphopenia observed in this study may have been due to the immune response to the inhaled spray paint 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. Our finding is consistent with previous reports (Taylor *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 capable of compromising the health of the research animals and may ultimately lead to mortality.

Observations based on photomicrography show that spray paint caused bronchiolar haemorrhage, alveolar haemorrhage and interstitial infiltrates of inflammatory cells (evidence of interstitial pneumonitis) in the exposed rats (Group B, Figure 1). There were observable histological variations in the lung histoarchitecture of the exposed rats (Group B) which include severe alveolar dilation, severe vascular ulceration and congestion, interstitial haemorrhage, and interstitial inflammatory cell infiltrates (evidence of interstitial pneumonitis). This florid activation of the lung tissue of the rats that were exposed to spray paint alone occurred as a result of the body sensing a foreign body (excess accumulation of the spray paint fumes) leading to the activation of lymphoid tissues to get rid of it. As shown in Figure 1 (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 1). *Moringa oleifera* showed a protective effect against spray paint-induced lung injuries. Interstitial pneumonitis was completely prevented, and the accumulated spray paint particulate matters were cleared. The phytochemicals present in *Moringa oleifera*, particularly flavonoids, saponins, and alkaloids, likely contributed to its protective effects against spray paint-induced lung damage. Flavonoids, for instance, may have reduced inflammation 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, which may have contributed to the observed protective effects. Specifically, flavonoids have been demonstrated to reduce oxidative stress by scavenging free radicals, 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 damage. 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.

These findings suggest that *Moringa oleifera* extracts may have potential therapeutic applications in treating or preventing lung diseases caused by spray paint 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. Our findings on the impact of spray paint fumes on these animals can be reasonably extrapolated to humans, suggesting a need for protective measures to safeguard human health, particularly the Automotive painters, Industrial coating technicians, Construction painters, Artists/Decorators, Aerospace painters, Marine painters, Furniture finishers, Sign painters' Special effects technicians and Restoration specialists. Preventing spray paint fumes-related interstitial pneumonitis and its complications requires a multi-faceted approach. This includes adhering to proper safety protocols, such as wearing personal protective equipment like face masks, face shields, goggles, hand gloves, boots and coveralls, to minimize exposure. Regular medical check-ups are also crucial, especially for painting company workers and those in related occupations, to mitigate occupational health risks. Additionally, raising public awareness through regular information campaigns can help prevent health hazards. Finally, adopting modern machines and technologies in painting companies, particularly in developing countries can reduce environmental spray paint fumes emissions, further minimizing risks.

### Conclusion

*Moringa oleifera* seems to have ameliorative effects against spray paint-induced vascular ulceration, interstitial haemorrhage, inflammation of the lung interstitial space, activation of lymphoid tissue and alveolar dilation. These effects were seen to be inversely proportional to the dosage as the results were more desirable at higher doses (enhanced immune-protection and anti-inflammatory effects) and could be compared to the control group. *Moringa oleifera* is therefore valuable in combating interstitial pneumonitis.

### Key Innovations of this Research

This study makes several innovative contributions, including the first-ever evidence of *Moringa oleifera*'s protective effects against interstitial pneumonitis caused by spray paint-exposure in adult Wistar rats. Remarkably, our findings show that *Moringa oleifera* extract significantly reduces lung inflammation and oxidative stress, suggesting a potential

therapeutic role in mitigating toxicant-induced lung damage. Additionally, our study highlights the novel finding that *Moringa oleifera* extract modulates the NF-KB and Nrf2 pathways, critical regulators of inflammation and antioxidant responses. Our research also demonstrates the potential application of *Moringa oleifera* extract as an adjunct therapy for treating interstitial pneumonitis, a debilitating respiratory disease. This research reveals new avenues for understanding the therapeutic properties of *Moringa oleifera* extract and its modes of action, with significant implications for the development of novel therapies for lung diseases triggered by toxicants.

### Recommendation

Based on our findings, we strongly suggest that *Moringa oleifera* be explored and developed as a potential therapeutic option for the management of interstitial pneumonia.

### Conflict of Interest Declaration

The authors, Mabel Bilu EHI-OMOSUN and Zion Chukwudi AJUFOH hereby declare no potential conflicts of interest in this manuscript.

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