PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (6) 1159-1164 June 2023

Effects of Aqueous Leaf Extract of *Kalanchoe pinnata* on Bifenthrin-Induced Injury in the Lungs of the Adult Wistar Rat

***EHI-OMOSUN, MB; ETUNIM, SC**

Departments of Anatomy and Physiology, School of Basic Medical Sciences, College of Medical Sciences University of Benin, Benin City, Nigeria

**Corresponding Email address: mabelehiomosun@gmail.com; Tel: +2348033551796*
Co-Author Email: successetunim@gmail.com

ABSTRACT: The aim of this study was to investigate the effects of aqueous leaf extract of *Kalanchoe pinnata*on Bifenthrin-induced injury in the lungs of adult Wistar rats. The 30 adult Wistar rats weighing between 250g and 280g that were used in this research were divided into 5 groups of 6 rats per group. The haematological outcome showed that Bifenthrin caused some derangements in haematological parameters especially lymphocytes and basophils, haemoglobin and red blood cells with associated reticulocytosis. Histologically, marked bronchiolar dilation, marked alveolar dilatation and severe vascular ulceration were observed in the rats exposed to bifenthrin only. *Kalanc hoepinnata* showed no negative effects on the histology of the lungs as the alveoli and the bronchial artery of the rats treated with *Kalanchoe pinnata* extract were found to be histologically normal when compared with the control. In conclusion, *Kalanchoe pinnata* has ameliorative effects against bifenthrin-induced injury in the lung of Wistar rats and its effect is inversely proportional to dosage. It is more potent at low doses.

DOI: **https://dx.doi.org/10.4314/jasem.v27i6.15**

Cite this paper as: EHI-OMOSUN, M. B; ETUNIM, S. C. (2023). Effects of Aqueous Leaf Extract of *Kalanchoe pinnata* on Bifenthrin-Induced Injury in the Lungs of the Adult Wister Rat. *J. Appl. Sci. Environ. Manage.* 27 (6) 1159-1164

Dates: Received: 17 May 2023; Revised: 08 June 2023; Accepted: 16 June 2023 Published: 30 June2023

Keywords: *Kalanchoe pinnata*; bifenthrin exposure; interstitial pneumonitis; Wister Rat

Kalanchoe pinnata (*K. pinnata)* also known as the Air Plant, Life Plant, or Miracle Leaf is a succulent sub-shrub plant of the *Crassulaceae* Stonecrop family (Jasmeet *et al.,* 2008). It is distinctive for the profusion of miniature plantlets that form on the margins of its leaves, a trait it has in common with the other members of the *Bryophyllum* section of the *Kalanchoe* genus (Michelle *et al.,* 2006). It is native to Madagascar and has become naturalized in tropical and subtropical areas (Ojewole, 2005; Ernst *et al.,* 2018). *Kalanchoe pinnata* contains the toxic alkaloid, kalanchosine, which has been found to also have anti-allergenic and antipyretic effects (Mahamood and Patil, 2002). Phytochemical constituents of *Kalanchoe pinnata* include: triterpenes, steroids, phenanthrene, flavonoids, flavones, chalcones, taraxasterol, phenolic acids, caffeic acids, syringic acids, malic, oxalic and ferulic acids, flavonoids, terenoids, terpenes, tannis and saponins (Kamboj *et al.,* 2010).The leaves of *Kalanchoe pinnata*were well recognized by herbalists in Esan Central Local Government Area of Edo state, Nigeria for treating cough, allergic reactions and difficult breathing in their adult, obese clients. The leaves are crushed and the resulting liquid can be used to treat nasal congestion, cough, difficult breathing, skin diseases and pruritus. Scientists have opined that the active principles which confer antitussive and soothing activities on the plant are the terpenes, and saponins (Anooj *et al.,* 2009; El-Hilaly *et al.,* 2004).The lungs are a pair of respiratory organs situated in the thoracic cavity. Each lung is a pinkish cone-shaped organ situated on either side of the mediastinum (Drake et al., 2014). The left lung is smaller than the right lung and it has just two lobes while the right lung has three (Moore and Persaud, 2016). The respiratory tract is the passages through

**Corresponding Email address: mabelehiomosun@gmail.com*

which air enters and leaves the alveoli. It is divided into 2 parts; upper and lower respiratory tract. The upper respiratory tract comprises the larynx, laryngopharynx, oropharynx, nose and nasal passages while the lower respiratory tract is composed of the trachea, bronchi, bronchioles and alveoli (Harrison, 2014).Interstitial pneumonitis is a group of disorders that cause progressive scarring of lung tissue (Islam *et al.,* 2020).Interstitial pneumonitis may be caused by long-term exposure to harzardous materials or irritating substances, resulting in an inflammation of the alveoli which then makes it difficult for oxygen to pass through the alveoli into the bloodstream Mark *et al.,* 2006). Bifenthrin is an insecticide in the pyrethroid family (Supratman *et al.,* 2000). Pyrethroids are manmade version of pyrethrins, which come from chrysanthemum flowers (Enoch *et al* 2014). First introduced in 1983, bifenthrin is a broad-spectrum insecticide, miticide, and termicide (Petrovska, 2012). These insecticides are used indoors and outdoors to control many pests like ants, termites, cockroaches, ticks, mole crickets, stink bug, armyworms, and many other invasives. Bifenthrin contains plant-derived chemical called diethyltoluamide as the active ingredient which is toxic to humans (Mark *et al.,* 2006). Previous studies have shown that synthetic bifenthrin such as melathion, methomyl and chlorpyrifos caused dyspnoea, haematological toxicity and lung damage in experimental animals (Supratman *et al.,* 2000). Signs and symtoms of bifenthrin poisoning include respiratory allergy, fast or irregular breathing, inflammation, cough, catarrha and dyspnoea (Mark *et al.,* 2006). Hence, the objective of this paper was to evaluate the effects of aqueous leaf extract of *Kalanchoe pinnata*on bifenthrin-induced injury in the lungs of adult Wistar rats.

MATERIALS AND METHOD

Kalanchoe pinnata leaves were harvested from the University of Benin Farm Project, Benin City. The plant was identified at the herbarium of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria. The leaves were thoroughly washed to remove sand particles after which they were taken to the Pharmacology Department for preparation of the extract. *Kalanchoe pinnata* leaves were chopped into little bits and allowed to dry at room temperature. The dried leaves were pounded using wooden mortar and pestle and milled into fine powder in an electric blender. Five hundred grams (500g) of the powder was soaked in 2litres of distilled water for 24 hours. The mixture was filtered with white filter paper and the residue was separated from the filtrate. The filtrate was concentrated using rotary evaporator at the department of Pharmacognosy, University of Benin, Benin City, Nigeria. The crude extract was then preserved in plain specimen bottles.

Qualitative analysis of each phytochemical constituent of *Kalanchoe pinnata*was done using Gas chromatography. Phytochemical constituents of *Sansevieria trifasciata* include: triterpenes, steroids, phenanthrene, flavonoids, flavones, chalcones, taraxasterol, phenolic acids, caffeic acids, syringic acids, malic, oxalic and ferulic acids, flavonoids, terenoids, terpenes, tannis and saponins (Anooj *et al.,* 2009; Kamboj *et al.,* 2010).Acute oral toxicity of the extract was evaluated**.** Appropriate doses of the extract were made by diluting with distilled water into 200mg/kg body weight and 400mg/kg body weight which were administered to the rats orally.

Experimental Animals: Thirty (30) adult Wistar rats of either sex weighing between 250g and 280g were used for this study. The animals were allowed to acclimatize for a period of 2 weeks before commencement of the experiment. During this period they were allowed access to standard animal feeds (Vital Growers' Feed, manufactured by Bendel Flour Mll, Ewu, Edo state Nigeria) and clean water *ad libitum.* Ethical approval was obtained. 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). Bifenthrin poisoning was induced by exposing the test animals to bifenthrin via inhalation in a fume distributor glass-chamber (FDGC) 1hour daily for 30 consecutive days (Supratman *et al.,* 2020). A pilot study was done on the 28thday of the experiment which confirmed interstitial pneumonitis.

Experimental Design: 30 adult Wistar rats weighing between 250g and 280g were randomly assigned into a control group (Group A) and four treatment groups (B, C, D and E) comprising of six (6) rats per group. Group A rats which served as control received 1ml of distilled water daily to compensate for stress of administration procured in the test groups. Group B rats were exposed to 15mls of bifenthrin daily via inhalation. Group C rats were treated with 400mg/kg body weight per day (BWT/D) of *kalanchoe pinnata* leaf extract. Group D rats were treated with 200mg/kg BWT/D o*f kalanchoe pinnata* leaf extract and were exposed to 15mls of bifenthrin via inhalation. Group E rats were treated with 40000mg/kg BWT/D of *kalanchoe pinnata* leaf extract and were exposed to 15mls of bifenthrin via inhalation. The dosages were given for 56 consecutive days via orogastric method. The weight of the animal in each group was taken and

recorded every 7 days so as to get the cumulative weight required for experimental use.

Method of Sacrifice and Sample Collection: At the end of the 8th week, the animals were euthanized under chloroform anaesthesia; a midline incision was made through the ventral wall of the thorax of the rats to access the lungs. The lungs were harvested and immediately fixed in 10% formal saline for24 hours before the histological analysis. The tissues were trimmed to about 3-5mm thick sections and processed according to the method of Drury and Wallington (1980) and then histologically assessed using the following methods: fixation, embedding and tissue staining for microscopy. Histological sections were examined under Leica DM750 research microscope with a digital camera (Leica ICC50) attached. Photomicrographs of the tissue sections were taken at various magnifications i.e. x40 and x100.

Lymphocytes, red blood cells, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, basophils and reticulocytes 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).

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.

RESULTS AND DISCUSSION

As shown in Table 1, there was no significant difference in body weight of the rats in the various groups exposed to bifenthrin though there was a slight decrease in body weight of the rats that were exposed to birenthrin only (Group B) which concurs with previous work (Mahamood and Patil, 2002).

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

Groups	Initial Body Weight	Final Body Weight	P-Value
Control (Group A)	168.00±19.90	195.00±19.22	0.158
Bifenthrin exposure only (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
Low Dose Extract + Bifenthrin(Group D)	186.00±18.77	192.33±17.61	0.134
High Dose Extract + Bifenthrin(Group E)	176.67 ±11.86	179.67±16.75	0.605

n=6; Values are Mean ± S.E.M
**Significantly different from the control group*

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

	Group A (Control)	Group B (Bifenthrin only)	Group C (Kalanchoe pinnata Extract only	Group D (Bifenthrin + Kalanchoe pinnata Extract-low dose)	Group E (Bifenthrin + Kalanchoe pinnata Extract-High dose)	P- value
White blood cells (10^3/µL)	16.62±2.07	13.66±1.19	13.65±1.74	17.65±1.75	16.67±1.78	0.357
Lymphocytes (10^3/µL)	15.06±2.01	10.18±1.04*	15.23±1.79	14.98±1.73	14.28±1.29	0.004
Monocytes (10^3/µL)	1.14±0.14	1.06±0.17	1.12±0.07	1.15±0.13	1.75±0.64	0.550
Granulocytes (10^3/µL)	0.38±0.07	0.44±0.11	0.33±0.07	0.53±0.20	0.45±0.12	0.766
Red blood cells (10^6/µL)	6.22±0.20	4.51±0.14*	6.24±0.24	6.68±0.20	6.18±0.30	0.002
Haemoglobin (g/L)	14.06±0.29	10.66±0.22*	13.90±0.38	14.35±0.34	13.42±0.52	0.000
Haematocrit (%)	37.86±0.95	40.50±0.49	38.03±0.79	39.45±0.65	38.38±0.95	0.617
Mean corpuscular volume (µm^3)	61.06±1.96	42.32±1.43	61.18±1.35	59.25±1.68	62.68±2.61	0.607
Mean corpuscular haemoglobin (pg)	22.68±0.62	18.54±0.34	22.33±0.40	21.53±0.51	21.80±0.43	0.601
Mean corpuscular haemoglobin concentration (g/dL)	37.14±0.34	30.18±0.53	36.53±0.39	36.35±0.44	34.93±0.77	0.624
Basophils (%)	653.40±55.61	334.40±37.14*	612.00±12.00	602.17±35.35	604.00±85.40	0.008
Reticulocytes (%)	0.12±0.02	0.54±0.0.11*	0.98±0.25	0.92±0.28	0.86±0.28	0.000
Plateletcrit (%)	0.50±0.04	0.51±0.03	0.55±0.02	0.54±0.02	0.64±0.06	0.122

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

As shown in Table 2, haematological analysis for serum levels shows that bifenthrin decreased some haematological parameters such as lymphocytes and basophils, red blood cells and haemoglobin with associated reticulocytosis which agrees with previous studies (Ojewole, 2005).Red blood cells are anucleated cells in the blood involved with the transportation of oxygen. They are called red blood cells because of the red coloring of haemoglobin (Hb) which is measured to detect anaemia and its severity. Haemoglobin and red blood cell count is low in all anaemia (Mark *et al.*, 2006).

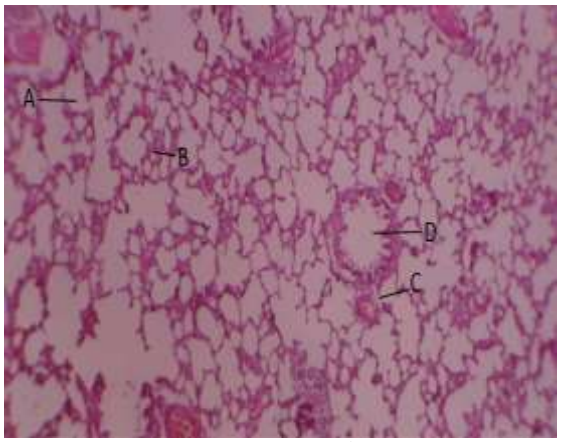

Plate 1: Rat lungs (Control) Composed of normal tissue: **A.** alveoli **B.** interstitial space **C**. bronchial artery **D.** terminal bronchiole (H&E x 40)

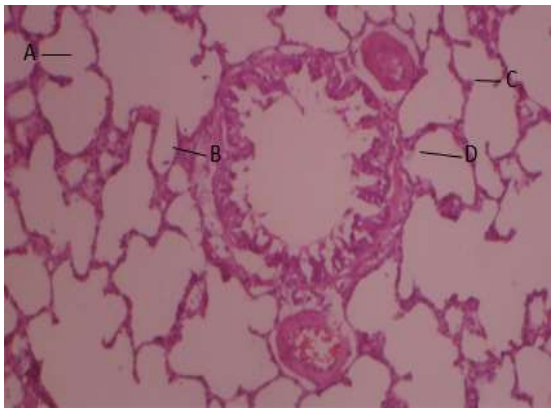

Plate 2: Rat lungs (Control): Composed of normal tissue: **A.** alveoli **B.** interstitial space **C.** bronchial artery **D.** terminal bronchiole (H&E x 100)

Lymphocytes are white blood cells that are uniform in appearance but vary in function and they include T, B and natural killer cells. These cells are responsible for antibody production, direct cell mediated killing of virus-infected and tumor cells and regulation of the immune response. The lymphopenia observed in this study may have been due to the immune response to the inhaled bifenthrin fumes in the lungs. Basophils are a component of the granulocytes. They are fewer in number but the largest in size. They also function to defend the body against allergens, pathogens, parasites and are involved in blood clotting. The basopenia observed may have resulted from the defense against the inhaled bifenthrin fumes (an allergen), which may have resulted in the depletion of their numbers. Therefore, the deranged haematological parameters observed in this study may lead to increased susceptibility to infections and some anaemia in the exposed research animals which are capable of compromising their health and may ultimately lead to mortality.

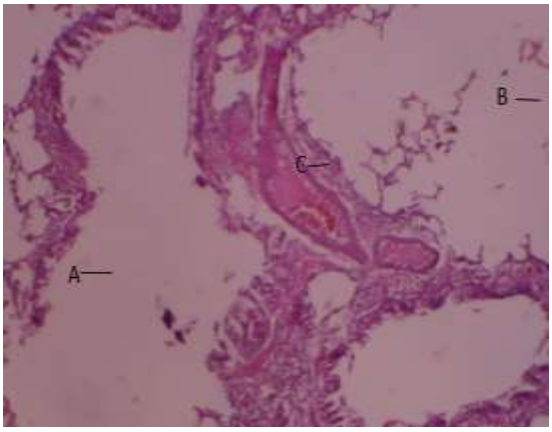

Plate 3: Rat lungs given Bifenthrin only showing: **A**. marked bronchiolar dilation **B.** marked alveolar dilation **C.** vascular ulceration (H&E 40)

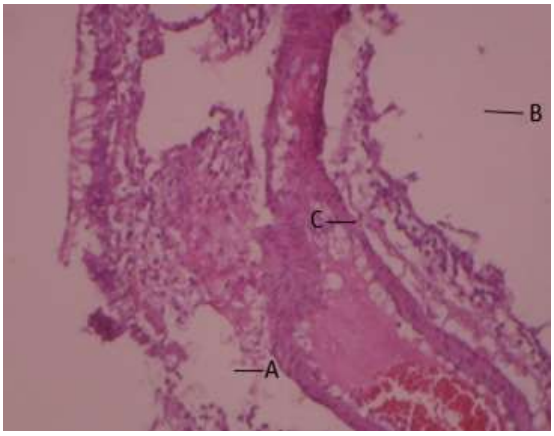

Plate 4: Rat lungs given Bifenthrin only showing: **A.** marked bronchiolar dilation **B.** marked alveolar dilatation **C.** vascular ulceration (H&E x 100)

As shown in Plate 1 and 2 above, the histological sections of the lung of control (Group A) showed normal histoarchitecture of the lung, viz., normal alveol, interstitial space, normal bronchial artery and terminal bronchiole. As shown in Plate 3 and 4, there were observable histological variations in the lung histoarchitecture of the rats exposed to only bifenthrin (GROUP B). These histological variations in the lung histoarchitecture of the rats exposed to only bifenthrin

include marked bronchiolar dilation, marked alveolar dilatation and severe vascular ulceration. These findings are in accordance with previous studies done by Mahamood and Patil, 2002.

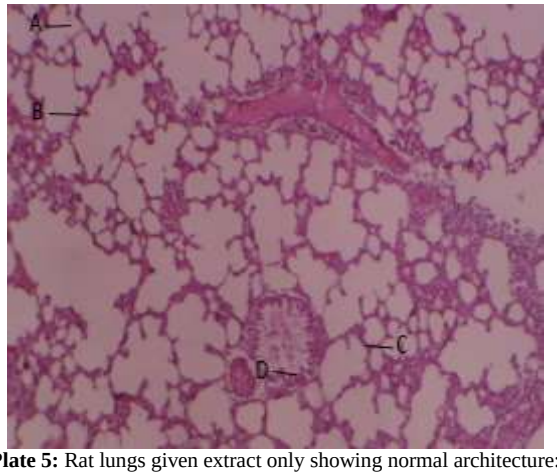

Plate 5: Rat lungs given extract only showing normal architecture: **A.** alveoli, **B**. interstitial space, **C**. bronchiole **D.** bronchial artery (H&E x 40)

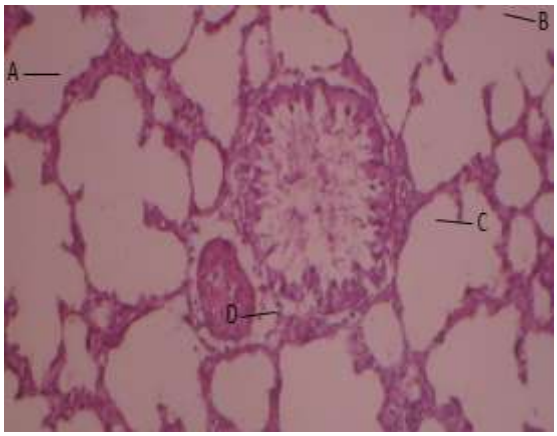

Plate 6: Rat lungs given extract only showing normal architecture: **A.** alveoli **B.** interstitial space **C.** bronchiole **D.** bronchial artery (H&E x 100)

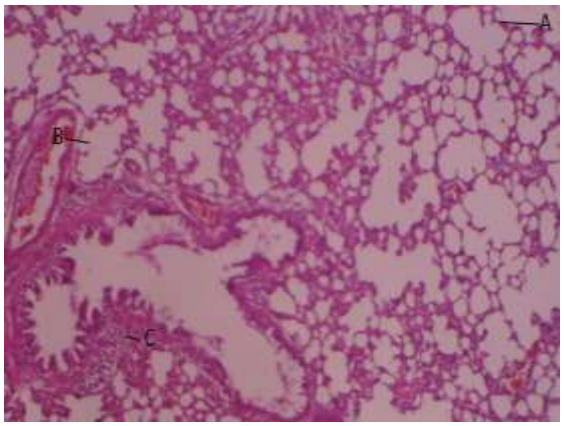

Plate 7: Rat lungs given Bifenthrin + 200mg/kg extract showing normal architecture: **A.** alveoli **B**. bronchial artery **C.** bronchiole (H&E x 40)

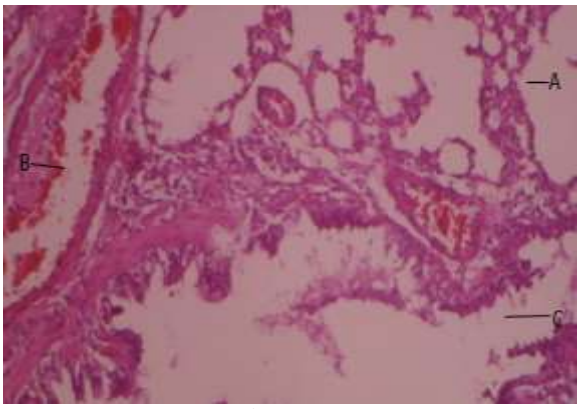

Plate 8: Rat lungs given Bifenthrin + 200mg/kg extract showing normal architecture: **A.** alveoli **B.** bronchial artery **C.** bronchiole (H&E x 100)

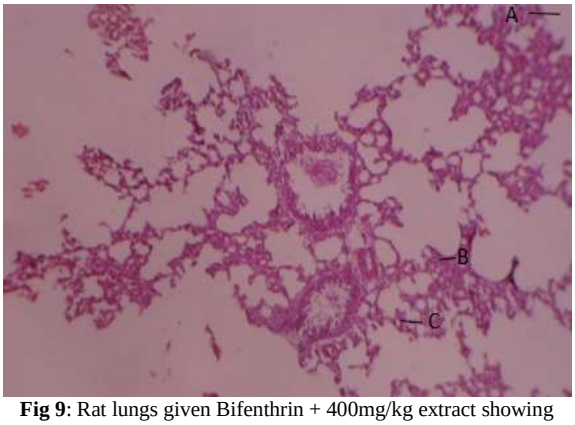

Fig 9: Rat lungs given Bifenthrin + 400mg/kg extract showing normal architecture: **A.** dilated alveoli **B**. bronchial artery **C**. bronchiole (H&E x 40)

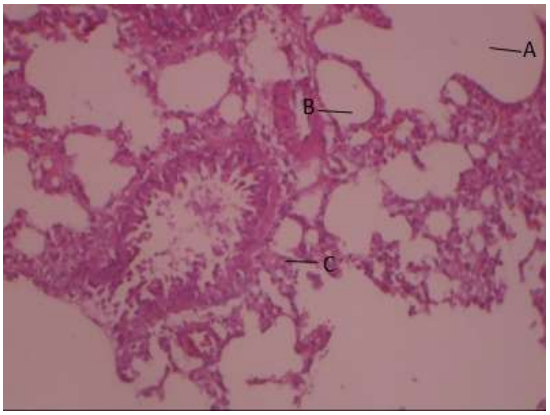

Fig 10: Rat lungs given Bifenthrin + 400mg/kg extract showing normal architecture: **A**. dilated alveoli **B**. bronchial artery **C**. bronchiole (H&E x 100)

As shown in Plate 5 and 6 above, *Kalanchoe pinnata* shows no negative effect on the histology of the lungs as the alveoli, interstitial space, terminal bronchiole and bronchial artery were were found to be histologically normal in the rats that were administered only Kalanchoe *pinnata* leaf extract .As shown in Plate 7 and 8, at low doses (Group D)

Kalanchoe pinnata showed protective effect against bifenthrin-induced alveolar dilatation, bronchiolar dilatation and vascular ulceration. Interstitial pneumonitis was completely prevented and the accumulated particulate matters in alveolar ducts were cleared allowing the flow of oxygen into the alveolar sac and release of carbon dioxide from the blood.

As shown in Plate 9 and 10, at high doses (Group E) *Kalanchoe pinnata* showed some protective effects against bifenthrin-induced lung injuries (alveolar dilatation, bronchiolar dilatation and vascular ulceration). Interstitial pneumonitis was partially prevented which also allowed the exchange of oxygen and carbon dioxide between inspired air and blood.Our histological findings agree with a similar work done by Ojewole *et al.*, 2005 where they used aqueous leaf extract of *Kalanchoe pinnata* to ameliorate formaldehyde lung toxicity in Wistar rats.

Conclusion: In conclusion, *Kalanchoe pinnata* had ameliorative effects against bifenthrin-induced bronchiolar dilatation, alveolar distension and vascular ulceration and its effect is inversely proportional to the doses administered. At low doses, the effects appear to be more potent and can be compared to that of the control group. Therefore, it can be used as a substitute to combat interstitial pneumonitis and other lung injuries caused by bifenthrin inhalation.

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