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Effects of Aqueous Leaf Extract of *Ageratum conyzoides* on Spray-Paint-Induced Injury in the Lungs of Adult Wistar Rats

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ABSTRACT: The aim of this study was to investigate the effects of aqueous leaf extract of *Ageratum conyzoides* on spray-paint-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 six 6 rats per group. The haematological outcome showed that spray paint caused some derangements in haematolodical parameters especially lymphocytes, monocytes, granulocytes and platelets. Histologically, interstitial haemorrhage, pulmonary edema, and activation of lymphoid tissue were observed in the rat treated with spray paint only. *Ageratum conyzoides* shrunk the local activated immune system and the florid-activated broncho-alveolar aggregates of the immune system in the *Ageratum conyzoides treated rats*. In conclusion, *Ageratum conyzoides* has ameliorative effects against spray-paint-induced injury in the lungs of Wistar rats and its effect is inversely proportional to dosage. It is more potent at low doses.

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Ageratum conyzoides is a 30 to 80-cm tall, upright, herbaceous annual plant. Fine white hairs cover the stems; the opposite, pubescent leaves have long petioles and glandular trichomes. The inflorescences are self-incompatible and have 30 to 50 pink flowers grouped in a corymb (Ming, 1999). Humans do not consume *Ageratum conyzoides* unless it is consumed medicinally. However, in some cultures, it is a delicacy for domestic guinea pigs, horses, and cattle. It is also used to feed fish (Okunade, 2002).In polyurethane (PU) goods such as foams, paints, lacquers, inks, insulating materials, varnishes, rubber modifiers, and bonding and vulcanizing agents, isocyanates-industrial chemicals with highly unsaturated N=C=O groups—are utilized. These low molecular weight allergens are one of the most frequently recognized causes of occupational asthma in Western nations, with spray painters at the highest risk (Pronk *et al*., 2005).Other than irritating asthma, hypersensitivity, pneumonitis, and rapid lung function decrease, isocyanate exposure can also cause allergic asthma. The most prevalent causes of occupational asthma are di-isocyanates, which crosslink polyurethane and are very reactive monomers. End users, such as auto-body spray painters, who make up a sizable population at risk, make up a major portion of exposed workers (Pronk *et al.*, 2007). The most popular di-isocyanate monomers include toluene di-isocyanate (TDI), methyl diphenyl di-isocyanate (MDI) (Cullen *et al*., 1996), and hexamethylene di-isocyanate (HDI) (Pronk *et al.*, 2007). Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the world and can be caused by anatomic narrowing of the airways or blocking of

airways with mucus and subsequently interferes with normal breathing (Colter *et al.,* 2009). Respiratory infections are the most frequent complication of immune deficiency (Enarson and Chretien, 1999). Isocyanates are powerful irritants to the mucous membranes of the eyes and gastrointestinal and respiratory tracts. They are extremely toxic to humans. Signs and symptoms of methyl isocyanate poisoning include cough, dyspnoea, chest pain, lacrimation, eye lid oedema and unconsciousness (Mark *et al.,* 2006). Hence, the objective of this paper was to evaluate the effects of aqueous leaf extract of *Ageratum conyzoides* on spray-paint-induced injury in the lungs of adult Wistar rats.

MATERIALS AND METHODS

Sample Collection and Treatment: The leaves of *Ageratum conyzoides* that were used in this research work were collected in front of College of Medicine administrative building, University of Benin, beside the car park and identified as *Ageratum conyzoides* by the Department of Plant Biology and Biotechnology, University of Benin.

The fresh matured leaves were air-dried for two weeks. The air-dried plants were grinded using the British milling machine into fine powder and weighed. 500g of the powdered plant were soaked in 1.5litres of distilled water for 24hours with constant shaking and stirring using a stirring rod. After 24 hours, it was filtered using filter paper. The residues were discarded, and then the filtrate was concentrated to paste level using crucible and water bath at 45°C to actualize the final crude extract. The final crude extract was then stored in a refrigerator at 4^0c.

Experimental animals: 30 adult Wistar rats weighing between 180g and 250g were purchased from the Animal House, Department of Anatomy, University of Benin, Benin City, Edo State, Nigeria and were used for this experimental research. The rats were given a period of 2 weeks to adapt to their new environment before commencement of the experiment. During this period, the animals were allowed access to standard animal feed (Vital grower's feed, manufactured by Bendel Flour Mill, Ewu) and clean water *ad libitum.*

Phytochemical constituents of *Ageratum conyzoides* include alkaloids, flavonoids, tannins, saponins and glycosides (Amadi *et al.,* 2012). Acute oral toxicity of the extract was evaluated. Appropriate doses of the extract were made by diluting with distilled water into 250mg/kg body weight and 500mg/kg body weight which were administered to the rats. 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).

Experimental design: Thirty (30) experimental adult female Wistar rats were randomly assigned into Five (5) groups; group A-E comprising of six (6) rats per group. Animals in control group were designated as **Group A** and they received Grower mash and water *ad libitum* throughout the experimental period. Animals assigned to Group B were given *Ageratum conyzoides* extract orally at 500mg/Kg. Animals assigned to Group C were exposed to 15ml of spray paint only via inhalation. Animals assigned to Group D were exposed to 15ml of spray paint via inhalation and received low dose of *Ageratum conyzoides* plant extract (250mg/kg body weight) and Group E were also exposed to 15ml of spray paint via inhalation and received high dose of *Ageratum conyzoides* plant extract (500mg/kg body weight). The experimental period lasted for 30days. Administration of plant extract was done orally via the use of an orogastric tube for 30days.

Method of sacrifice and sample collection: After the 30th day, the animals were sacrificed under chloroform anesthesia. Blood samples were collected with plain specimen bottles for biochemical analysis and the lungs of each rat were harvested and immediately fixed in 10% formal saline for 24 hours before histological analysis.

Determination of Phytochemical constituents of *Ageratum conyzoides*

Qualitative analysis of each phytochemical of *Ageratum conyzoides* was done using Gas chromatography (Amadi *et a.,* 2012). Phytochemical constituents of *Ageratum conyzoides* include alkaloids, flavonoids, tannins, saponins and glycosides (Amadi *et al.,* 2012). Acute oral toxicity of the extract was evaluated. Appropriate doses of the extract were made by diluting with distilled water into 250mg/kg body weight and 500mg/kg body weight which were administered to the rats.

Determination of histological Parameters: The harvested tissues were histologically assessed using the following methods: fixation, embedding and tissue staining for microscopy (Drury and wallington, 1980). The tissue sections were examined under Leica DM750 research microscope with digital camera (Leica ICC50) attached. Digital photomicrographs of the tissue sections were taken at H & E x40 and x100 magnifications. White blood cells (WBCs), lymphocytes, monocytes, granulocytes, red blood cells (RBCs), haemoglobin, haematocrit, mean

corpuscular volume (MCV), mean corpuscular haemoglobin, mea corpuscular haemoglobin concentration, platelets, mean platelet volume were analyzed using an auto-analyzer (2006 model, manufactured by Hoddlierand Stoughton Group of company, London with a recognized biochemical kit (2010 model, Diagnostical Merek, London).The weights of the experimental animals were taken after 30 days and the differences between them and previous weights were noted.

Statistical Data Analysis: The data were subjected to statistical analysis and P values calculated using the student's t-test

RESULTS AND DISCUSSION

Medicinal plants are widely accepted to be a blessing in the developing countries. The present study was designed to examine the effects of aqueous leaf extract of *Ageratum conyzoides* on spray-paint-induced injury in the lungs of adult Wistar rats. Findings for the study of organ weight (Table2) and body weight (Table 3) show that there was no significant change in the organ and body weight of the treated rats across all the groups when compared with the control and this concur with previous work (Ming, 1999). Findings of this study for haematological analysis for serum levels showed significant decrease in the number of lymphocytes in the blood of rats in Group D (Table 1), a condition known as lymphopenia. This is evident of active immunological response to infection. There was significant increase in the number of monocytes and granulocytes in the blood of rats in group D (Table 1) when compared to the control which is indicative of active continuous immunological response against infection. There was a significant increase in the number of platelets in the group administered spray paint only (Group C), (Table 1). This shows that there were efforts to combat interstitial hemorrhage caused by spray paint in the lungs. There was also a significant increase in the mean platelet volume of rats administered high dose of *Ageratum conyzoides*plant extract only (Group B), (Table 1). This reflects an increase in the production of platelets and is associated with reduced survival rates in infections.

Table 1: Comparison of Haematological Parameters in All Experimental Groups

	Group A	Group B	Group C	Group D	Group E	P- Value
White blood cells (10^3/µL)	8.32±1.08	8.78±0.59	7.36±0.49	7.82±1.17	7.20±0.42	0.627
Lymphocytes (10^3/µL)	94.60±0.37	93.62±0.41	92.56±0.76	90.70±1.58*	92.26±0.90	0.068
Monocytes (10^3/µL)	3.98±0.27	4.40±0.50	5.80±0.64	7.14±1.27*	5.96±0.79	0.058
Granulocytes (10^3/µL)	1.42±0.15	1.58±0.11	1.64±0.13	2.08±0.38*	1.78±0.17	0.273
Red blood cells (10^6/µL)	6.58±0.16	6.77±0.10	6.79±0.24	6.50±0.14	6.98±0.11	0.250
Haemoglobin (g/dL)	9.50±0.22	9.94±0.25	9.36±0.46	9.02±0.24	9.88±0.24	0.200
Haematocrit (%)	37.86±0.41	33.16±5.97	37.56±1.23	36.82±0.70	39.26±0.44	0.608
Mean corpuscular volume (µm^3)	57.68±1.58	57.56±0.70	55.42±0.78	56.76±0.76	56.30±0.69	0.461
Mean corpuscular haemoglobin (pg)	14.38±0.32	14.62±0.28	13.68±0.25	13.84±0.21	14.10±0.24	0.113
Mean corpuscular haemoglobin concentration (g/dL)	25.06±0.58	25.50±0.23	24.84±0.70	24.42±0.21	25.10±0.42	0.596
Platelets (10^3/µL)	510.40±32.02	493.00±42.61	782.00±49.92*	632.40±65.78	489.80±38.58	0.002
Mean platelet volume (µm^3)	6.74±0.11	7.36±0.25*	6.90±0.21	6.78±0.12	6.68±0.10	0.067

** Significantly different from the control group*

Table 2: Changes in Weights of Organs in All the Experimental Groups

	Group A	GROUP B	GROUP C	GROUP D	GROUP E	P- VALUE
Lungs weight (g)	1.46±0.07	1.38±0.08	1.60±0.08	1.52±0.11	1.36±0.10	0.328
Lungs-somatic index (%)	0.76±0.06	0.76±0.04	0.86±0.03	0.86±0.09	0.70±0.05	0.306

Table 3: Changes in Weights of Rats in All the Experimental Groups

Groups	Initial Body Weight	Final Body Weight	P-Value
GROUP A	183.20±7.21	193.60±7.91	0.297
GROUP B	168.40±3.60	181.40±7.66	0.063
GROUP C	187.20±12.26	187.20±10.19	1.000
GROUP D	174.80±10.51	180.20±7.95	0.460
GROUP E	190.00±10.70	195.00±10.84	0.400

*n=6, Mean value ± SEM, **P<0.01: The statistical values obtained were converted into graphical representations in the form of bar charts*

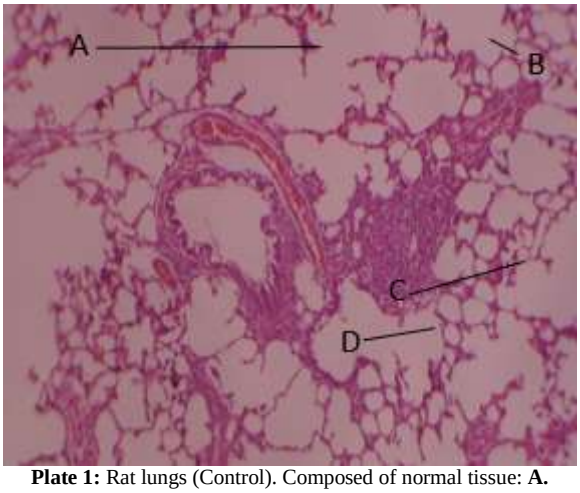

Plate 1: Rat lungs (Control). Composed of normal tissue: **A.** alveolar sacs, **B.** interstitial space, **C.** terminal bronchiole, **D.** bronchial artery **(H&E x 40)**

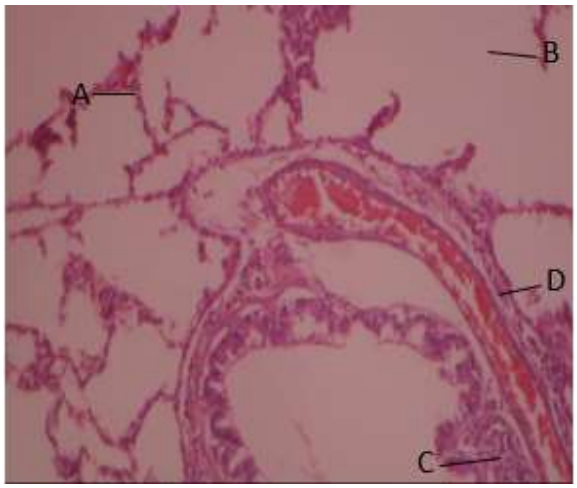

Plate 2: Rat lungs (Control). Composed of normal tissue: **A.** alveolar sacs, **B.** interstitial space, **C.** terminal bronchiole, **D.** bronchial artery **(H&E x100)**

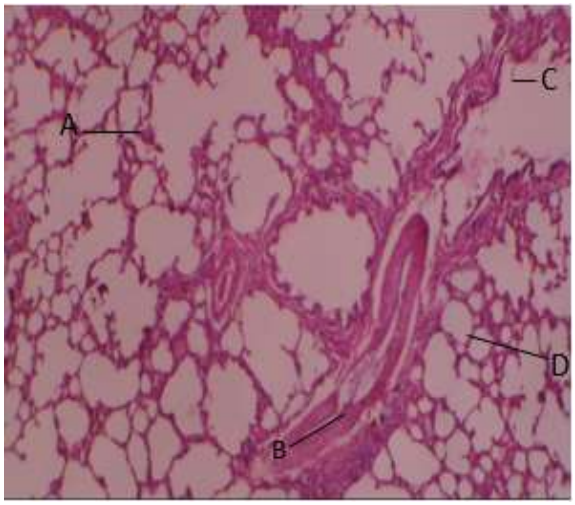

Plate 3: Rat lungs given 500mg/kg Plant extract only showing normal architecture: **A.** alveoli, **B.** interstitial space, **C.**terminal bronchiole, **D.** bronchial artery **(H&E x 40)**

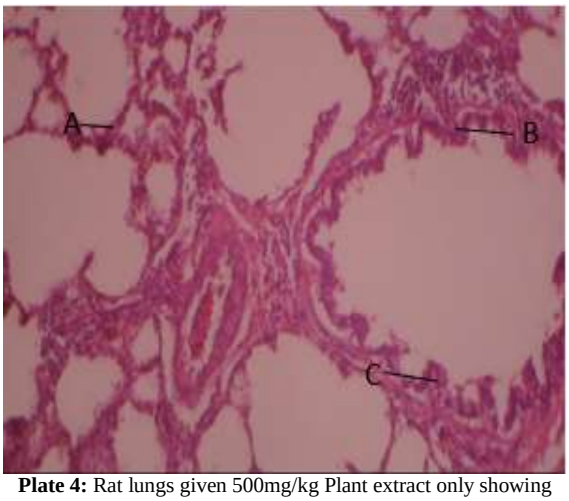

Plate 4: Rat lungs given 500mg/kg Plant extract only showing normal architecture: **A.** alveoli, **B.** terminal bronchiole, **C.** bronchial artery **(H&E x 100)**

Plate 5: Rat lungs given Spray paint only showing: **A.** interstitial haemorrhage, **B.** bronchiolar luminal debris, **C.** infiltrates of inflammatory cells, **D.** interstitial oedema **(H&E x 40)**

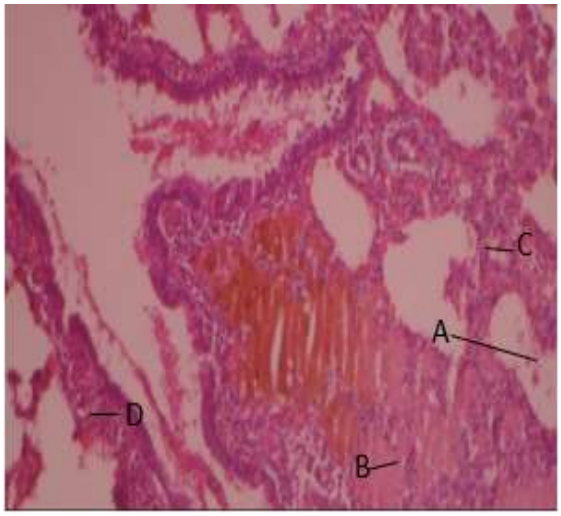

Plate 6: Rat lungs given Spray paint only showing: **A.** interstitial haemorrhage, **B.** bronchiolar luminal debris, **C.** infiltrates of inflammatory cells, **D.** interstitial oedema **(H&E x 100)**

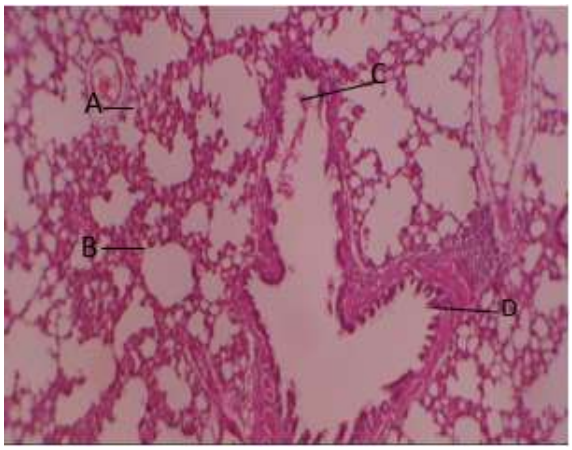

Plate 7: Rat lungs given Spray paint + 250mg/kg extract showing normal architecture of: **A.** alveoli, **B.** terminal bronchiole, **C.** bronchial artery, **D.** Florid activation of bronchio-alveolar lymphoid aggregates **(H&E x 40)**

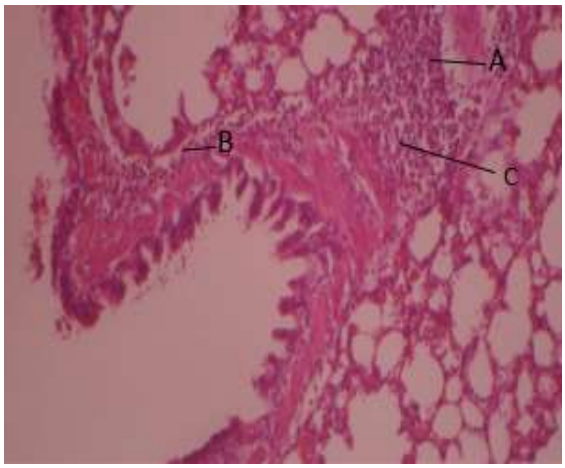

Plate 8: Rat lungs given Spray paint+ 250mg/kg extract showing normal architecture: **A.** alveoli, **B.** terminal bronchiole, and **C.** Florid activation of bronchio-alveolar lymphoid aggregates **(H&E x 100)**

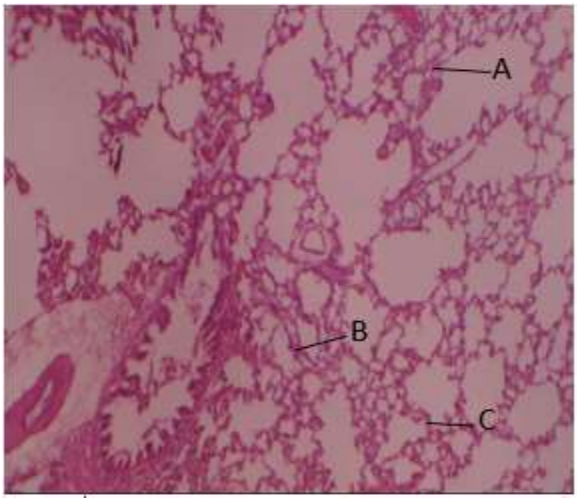

Plate 9: Rat lungs given Spray paint + 500mg/kg extract showing: **A.** normal alveoli, **B.** interstitial oedema, **C.**bronchiolar luminal debris **(H&E x 40)**

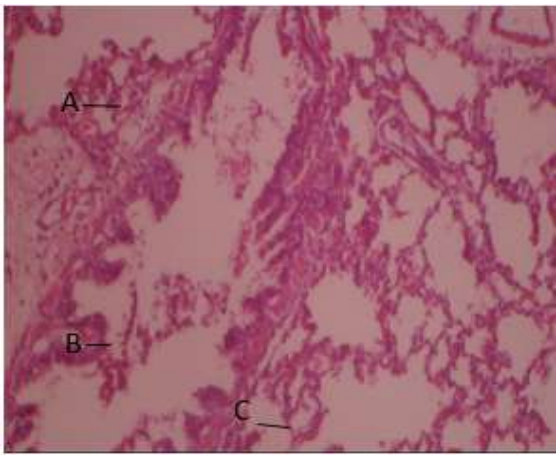

Plate 10: Rat lungs given Spray paint + 500mg/kg extract showing: **A.** normal alveoli, **B.** mild interstitial oedema, **C.** bronchiolar luminal debris **(H&E x 100)**

Observations based on photomicrography show that spray paint caused interstitial haemorrhage, bronchiolar luminal debris, inflammatory cell infiltration and interstitial eodema(FPlate 5 and 6). *Ageratum conyzoides* had no negative effects on the histology of the lungs Low doses of *Ageratum conyzoides*caused an expanded lumen of the bronchioles. (Plate 3 and 4) *Ageratum conyzoides*shrunk the local activated immune system and also the florid activated bronchio-alveolar aggregates of the immune system (Plate 7 and 8). At high doses, *Ageratum conyzoides* showed a more activated immune system and constricted bronchial airway (Plate 9 and 10).which is indicative of having a less ameliorative effect when compared to the low dose.

*Conclusion***:** In conclusion, *Ageratum conyzoides*had ameliorative effects against spray-paint-induced alveolar interstitial haemorrhage, pulmonary eodema and activation of lymphoid tissue 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 lung diseases.

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