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EFFECTS OF AQUEOUS EXTRACT OF *Allium cepa* (Red Onion) ON OVALBUMIN-INDUCED ALLERGIC ASTHMA IN WISTAR RATS

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ABSTRACT
Asthma is a chronic inflammatory disease of airways that involves inflammatory cells and mediators to elicit inflammatory response. Allium cepa is a common plant used as condiment and has anti–inflammatory, antifungal and antibacterial properties. The aim of this study was to investigate the effect of aqueous extract of Allium cepa (EAC) on leucocytes infiltration and lung histology in Ovalbumin-induced allergic asthma in Wistar rats. Allergic asthma was induced in rats by systemic sensitization with ovalbumin (OVA) via intraperitoneal (i.p.) injections followed by intranasal (i.n.) challenge. Extract of Allium cepa at doses of 150mg/kg and 300mg/kg b.w. ; and dexamethasone (1mg/kg) were given to the treated groups. The asthmatic group showed a significant ($p<0.05$) increase in number of eosinophil and lymphocyte in the Blood and bronchoalveolar fluid (BALF), which was significantly ($p<0.05$) decreased in Allium cepa treated groups. Histological assessment of the lung tissue showed various degrees of inflammation in all OVA-induced animals, but a decreased inflammation was observed in those treated with Allium cepa and dexamethasone. It is therefore, concluded that aqueous extract of Allium cepa reduced cellular infiltration and lung inflammation of allergic asthmatic Wistar rats.
Key words: Asthma, Ovalbumin, Allium cepa, Leucocytes, Rat

INTRODUCTION

Asthma belongs to the category of obstructive pulmonary diseases (OPD). These diseases are characterised by difficulty in expiration due to decreased intrapulmonary pressure (Mitchell *et al.,* 2011). A higher prevalence of asthma among Nigerian adolescents and adults compared with regional and global averages (Musa and Aliyu, 2014).

Approximately two-thirds of asthma cases are allergic, characterised by excessive production of IgE and associated with antigen-antibody induced inflammatory cascade, bronchoconstriction, increased mucus production and mucosal edema (Manohar and selvakumar, 2012). The infiltration of leucocytes into the lungs with release of mast cells bronchoconstrictor mediators and elevated level of immunoglobulin E (IgE), stimulate allergic inflammation (Chaudhari *et al.,* 2011).

Allium cepa (Red onion) is one of the oldest cultivated plants used both as food, condiment and for medicinal purposes (Lanzotti, 2006). Onion is cultivated all over the world especially in Europe, Asia, North America and Africa (Nasri *et al.,* 2012). In Nigeria, onion is cultivated widely in the northern part where it is commonly called *Albasa.*

Onions are known to possess medicinal properties from ancient time. Humans have used them for centuries for medicinal purposes. Researches have shown that *Allium cepa* have some therapeutic properties: lowers blood cholesterol levels (Ghalehkhandi *et al.,* 2012); hypoglycemic activity (Ogunmodede *et al.,* 2012); hepatoprotective effect (Ozougwu and Eyo, 2014); analgesic, anti-inflammatory and antioxidant activity (Nasri *et al.,* 2012).

Traditional medicinal practitioners in Nigeria recommend the use of *Allium cepa* for relief of epileptic seizures, asthmatic symptoms etc. However, there is paucity of scientific information on the specific role of onion in allergic asthma.

A simple and sensitive animal model of asthma is using ovalbumin sensitization followed by challenge. Allergic response is observed in lungs 24 hours after OVA challenge (Manoharet *et al.,* 2012). Rats exhibit features of airway allergy and allergic asthma when exposed to allergen that is similar to that of humans (Moffet *et al*., 2003). This study therefore investigates the effects of aqueous extract of *Allium cepa* (EAC) on cellular infiltration in ovalbumin-induced allergic asthma in Wistar rats.

MATERIALS AND METHODS

Drugs and chemicals

Ovalbumin (Mayer and Baker ltd. Bagenham, England), Aluminium hydroxide (BDH chemicals ltd. Poole, England), Dexamethasone (1mg/kg, Hubei Tianyao Pharmaceutical Co. Ltd), Ketamin hydrochloride (50mg/kg).

Animals
Twenty-five Wister strain rats (130 –150g) of both sexes were allowed to acclimatize for two weeks before experiment. They were maintained on a commercial poultry feed and water *ad libitum*.

Collection of Plant Material and Extraction
Fresh red onion bulbs were purchased from Samaru market Zaria, Kaduna state Nigeria in the month of August, 2015. The botanical identification was done at the herbarium unit of Biological Science Department, Ahmadu Bello University, Zaria with voucher number 2196. Onion bulbs were washed, cut into small pieces and homogenized. The resulting mixture was soaked in 1L of distilled water and was allowed to stand for 24 hours with intermittent shaking. The mixture was then filtrated and the filtrate evaporated to dryness. The dried extract was then stored at 4^{o}C before use.

Experimental Design
The twenty-five (25) Wistar rats were randomly divided into five (5) groups as follows:
Group1: normal saline only intraperitoneally
Group2: 200mg ovalbumin (OVA) intranasally only
Group3: 200mg OVA intranasally + 150mg/kg Extract *Allium cepa* intraperitoneally
Group4: 200mg OVA intranasally + 300mg/kg Extract *Allium cepa* intraperitoneally
Group5: 200mg OVA intranasally + 1mg/kg dexamethasone intraperitoneally
The animals in group 1 were given an intraperitoneal injection of normal saline (0.9% NaCl) on days 0, 7 and 14. Groups 2-5 were sensitized with intraperitoneal injection of ovalbumin followed by ovalbumin challenge. An hour before each OVA challenge, the animals in group 3 and 4 were given intraperitoneal injection of extract *Allium cepa* 150mg/kg and 300mg/kg body weight respectively, while group 5 were given intraperitoneal injection of Dexamethasone (1mg/kg body weight).

Ovalbumin Sensitization and Ovalbumin Challenge
Animal in groups 2-5, were sensitized by intraperitoneal injection of ovalbumin (20mg/kg) in aluminium hydroxide (100mg/kg) in normal saline on days 0, 7 and 14 **(i.p)** (Russo *et al*., 2001). Three (3) days after ovalbumin sensitization, all animals in groups 2-5 were challenged with 50µL of ovalbumin at 200mg via intra nasal route for four consecutive days (Days 18, 19, 20 and 21). The control group (non-sensitized) were treated with aluminium hydroxide and instillation of normal saline (Agbonon *et al.,* 2005).

Collection of Broncho Alveolar Fluid (BALF) and differencial cell count.
The rats were anesthetised with Ketamin hydrochloride (50mg/kg) at 0.45ml/rat via intraperitoneal route 24 hours after the last challenge (22nd day). Broncho Alveolar lavage procedure was performed in tracheotomised trachea. The anterior end of the left trachea was tied with a thread and the trachea was cannulated, 2ml of normal saline was injected into the cannulated trachea and was aspirated 10 seconds later as BALF). The procedure was repeated and the collected BALF was centrifuged to separate the cells from the supernatant (Chaudhari *et al.,* 2011) Differential cell counts were performed after staining with Giemsa solution. About 300 cells were counted and were differentiated by general leukocyte morphology to determine the number of cellular infiltration

Collection of Blood sample and differencial cell count
Blood was collected by cardiac puncture and was kept in EDTA tube which was later used for systemic differential white blood cell count. A magnification of times 100 was used to count the differential white blood cells (Lewis *et al.,* 2008).

Histological Examination
Left Lung was removed completely from the chest cavity, and fixed with 10% neutral buffered formalin and the histological preparation and analysis, was carried out by a trained histologist at the histological section in the Department of Human Anatomy, A. B. U. Zaria, Histological sections were viewed with magnification of ×100.

Statistical analysis
All results were expressed as mean ± standard errors of the mean (SEM). Statistical significance of differences was assessed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. The mean difference between cell count in blood and in BALF was assessed by independent-sample T-test. In all cases, $P< 0.05$ was considered statistically significant. Statistical Package for Social Science (SPSS) version 15 was used for the analysis.

Results and Discussion
There was a statistical significant (P<0.05) increase in neutrophil, eosinophil, and lymphocyte counts in the blood of ovalbumin-induced allergic asthma in Wistar rats (Table 1). Administration of 150mg/kg extract *Allium cepa* (EAC) caused a significant (P<0.05) decrease in only the neutrophil count, but treatment with 300mg/kg EAC or Dexamethasone (DEX) 1mg/kg, significantly (P<0.05) decreased both eosinophil and lymphocyte counts. Monocytes count was significantly (P<0.05) decreased in OVA group, while treatment with 150mg EAC reversed the effect but was significantly (P<0.05) decreased in DEX treated group. There was no significant change in basophil count in all the groups (Table 1).
The development of asthma in rats is confirmed by an increase in inflammatory mediators in the blood, bronchoalveolar lavage fluid and change in histology of the lung tissue (Kucharewicz *et al.,* 2008). In this study, the increase in eosinophil and lymphocyte counts in blood of OVA-induced allergic asthma model is indicative of inflammatory response. Sensitization followed by challenge with OVA in OVA-induced asthma model in Wistar rats is known to increase inflammatory mediators like eosinophils, neutrophils, T-lymphocytes, monocytes (Kucharewicz *et al.,* 2008). Study by Zhang *et al.* (2014) showed that the blood eosinophil/lymphocyte ratio and eosinophil/neutrophil ratio are increased in eosinophilic asthma.

The decreases of inflammatory mediators with *Allium cepa* demonstrate an anti-inflammatory property of the plant that was consistent with the effect of Dexamethasone, a standard antiasthma drug.

Result in table 2, showed the effects of aqueous extract of *Allium cepa* on WBC differential count in bronchoalveolar fluid (BALF) of ovalbumin-induced allergic asthma in Wistar rats. The eosinophil and lymphocyte count in OVA (Asthma) group were significantly ($P<0.05$) higher compared with the control (Non-sensitized) group. Whereas treatment with EAC at 150mg/kg and 300mg/kg significantly ($P<0.05$) decreased both eosinophil and lymphocyte count which was similar to the effect of the standard drug Dexamethasone (1mg/kg). There was no significant ($P<0.05$) change in monocyte count in all the groups. The effect of dexamethasone on cell count in BALF and blood is consistent with study reported by Salama *et al.* (2012).

The increase in eosinophil and lymphocyte counts in bronchoalveolar lavage fluid (BALF) of OVA treated group, indicates cellular infiltration into the BALF, which was also reported by Wardlaw *et al.* (2000). To further confirm cellular infiltration into the BALF, we assessed the percentage of Eosinophil count (Fig 1) and lymphocytes count (Fig 2) in the blood as well as BALF of all the groups. The percentage of both eosinophil and lymphocyte count was higher in the blood than that of the BALF of all the groups, in this manner, eosinophil count was significantly ($P<0.05$) decreased with both doses of EAC treatment as well as DEX. While lymphocyte count was significantly ($P<0.05$) decreased with only the dose of 300mg/kg EAC, which appeared to be more effective than 150mg/kg EAC and DEX treated group.

Eosinophil infiltration into BALF is the basic feature of allergic asthma and it is apparent even in very mild asthmatic condition (Wengmann and Renz, 2005). Bronchoalveolar lavage fluid is a diagnostic tool for toxic substances in bronchoalveolar, asthma and chronic obstructive pulmonary diseases (COPD) (Meyer, 2007). Also, the increased lymphocyte in BALF of OVA treated group may orchestrate eosinophil inflammation, which is involved in the pathogenesis of allergic asthma (Kumari and Rana, 2012).

Both monocytes and neutrophils are not the hallmark of allergic asthma, as such their presence in large number in BALF does not confirm asthma (Wardlaw *et al.,* 2000). However, Macrophages and neutrophils are the most numerous cells in the airways, macrophages act as antigen presenting cells and can be activated by allergens through low-affinity IgE receptors to release inflammatory mediators and cytokines that amplify the inflammation (Wenzel, 2003).

The lung histology a normal cytoachitecture of the lungs in the control group (plate 1.), the OVA group showed a marked lung inflammation associated with cellular infiltration, edema and congestion (plate 2). Groups treated with EAC at 150mg/kg showed a mild congestion and cellular infiltration but no edema (plate 3), while 300mg/kg *Allium cepa* treated group showed only a mild cellular infiltration (plate 4) and DEX group showed no cellular infiltration but mild congestion (plate 4).

Therefore, the pathological changes, which appeared in all OVA-induced animals at various degrees have confirmed the presence of allergic asthma induced by ovalbumin, Since inflammation, cellular infiltration, and edema are the hallmark of allergic asthma as stated by Suralkar and Kasture (2012) and Huang *et al.* (2014). The reduced lung inflammation with *Allium cepa* may be attributed to the anti-inflammatory property of the plant as well as structural effect on the airways.

The preliminary phytochemical investigation of *Allium cepa,* showed the presence of steroids, saponins, alkaloids and flavonoids (Lanzotti, 2006). Flavonoids are known to possess anti-inflammatory effects and antioxidant properties that may be responsible for anti-inflammatory and antioxidant activity (Kumar *et al.,* 2010). The flavonoids in *Allium cepa* with potent anti-inflammatory effects are quercetin and kaemferol (Joskova *et al.,* 2011; Nasri *et al.,* 2012)

Studies have reported the anti-inflammatory response of quercetin and its inhibitory effect on muscarinic receptors in the bronchial smooth muscles cells resulting in bronchodilation, It also exerts an inhibitory effect on histamine released during all phases of allergic reactions (Joskova *et al.,* 2011). Reports have shown that kaempferol inhibits the enzymes cyclooxygenases (COX,) Lipoxygenases (LOX) and inducible nitric oxide synthase (iNOX) (Calderón-Montaño *et al.,* 2011). These enzymes are known to play important roles in inflammation by participating in the synthesis of eicosanoids and production of reactive species (Nasri *et al.,* 2012).

It is concluded from this study, that aqueous extract of *Allium cepa* decreases the inflammatory responses of ovalbumin-induced allergic asthma in rats in a similar way compared to that of a standard anti-asthma drug (dexamethasone).

Table 1: Effects of Aqueous Extract of *Allium cepa* on Differential Cell Count In Blood of Ovalbumin-Induced Allergic Asthma In Wistar Rats

	Control	OVA 200mg	OVA 200mg + ACP 150mg/kg	OVA 200mg + ACP 300mg/kg	OVA 200mg + DEX 1mg/kg
LYMPHOCYE CELLS (%)	28.00±0.77	48.60±1.75[a]	40.60±3.16	28.40±0.75[b]	35.40±2.11[b]
NEUTROPHL CELLS (%)	60.60±1.08	68.60±0.51[a]	47.82±2.40[a]	63.40±0.24[b]	62.60±2.75[b]
EOSINOPHIL CELLS (%)	3.00±0.32	4.80±0.58[a]	3.40±0.24[a]	2.20±0.49[b]	2.60±0.57[b]
MONOCYTE CELLS (%)	6.40±0.51	5.60±0.68[a]	6.20±0.37[b]	5.00±0.55[a]	3.20±0.37[b]
BASOPHIL CELLS (%)	0.80±0.37	0.80±0.37	0.60±0.40	0.40±0.24	0.40±0.24

Values with different superscript are significant (P<0.05) along a row.

Table2: Effects of Aqueous Extract of *Allium cepa* on Differential Cell Count In Broncho Alveolar Fluid (Balf) of Ovalbumin-Induced Allergic Asthma In Wistar Rats

	Control	OVA 200mg	OVA 200mg + ACP 150mg/kg	OVA 200mg + ACP 300mg/kg	OVA 200mg + DEX 1mg/kg
LYMPHOCYTE CELLS (%)	18.60±2.38	28.40±1.03[a]	17.00±1.22[b]	17.00±3.18[b]	17.60±1.12[b]
NEUTROPHIL CELLS (%)	6.20±0.66	5.40±0.87[a]	6.80±1.02[b]	6.60±0.51[b]	6.40±0.68[b]
EOSINOPHIL CELLS (%)	0.80±0.37	2.80±0.37[a]	0.80±0.37[b]	0.80±0.37[b]	0.60±0.40[b]
MONOCYTE CELLS (%)	71.20±2.60	74.40±1.83	75.40±1.54	75.40±3.33	72.20±1.46

Values with different superscript are significant (P<0.05) along a row.

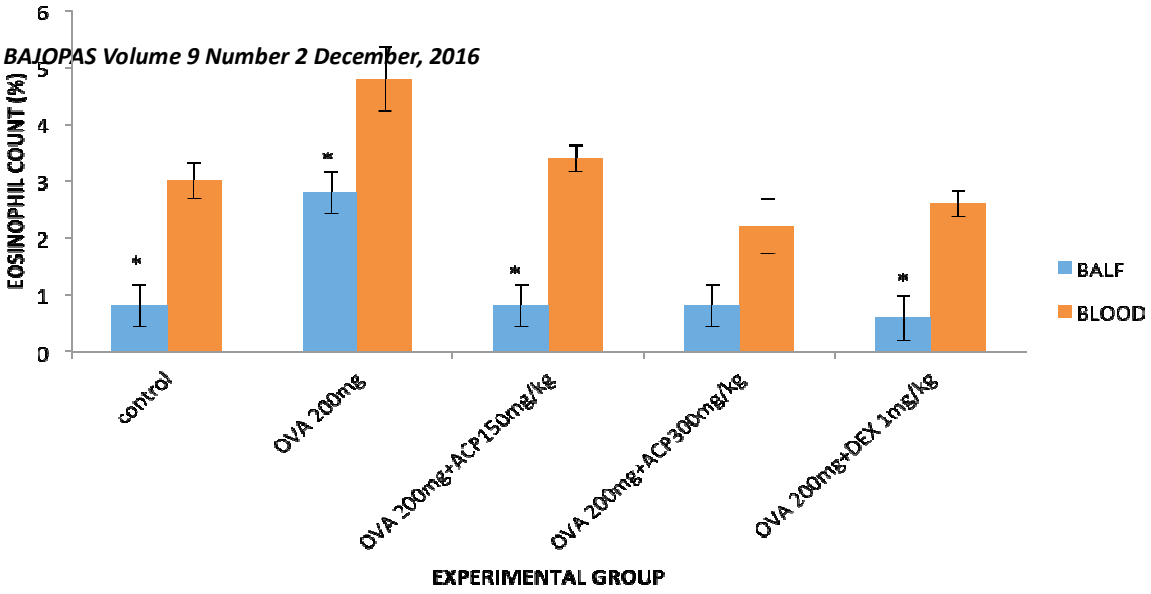

Fig 1: The Effects of Aqueous Extract of *Allium cepa* on percentage of Eosinophil Count In Blood And Balf of Ovalbumin-Induced Allergic Asthma In Wistar Rats

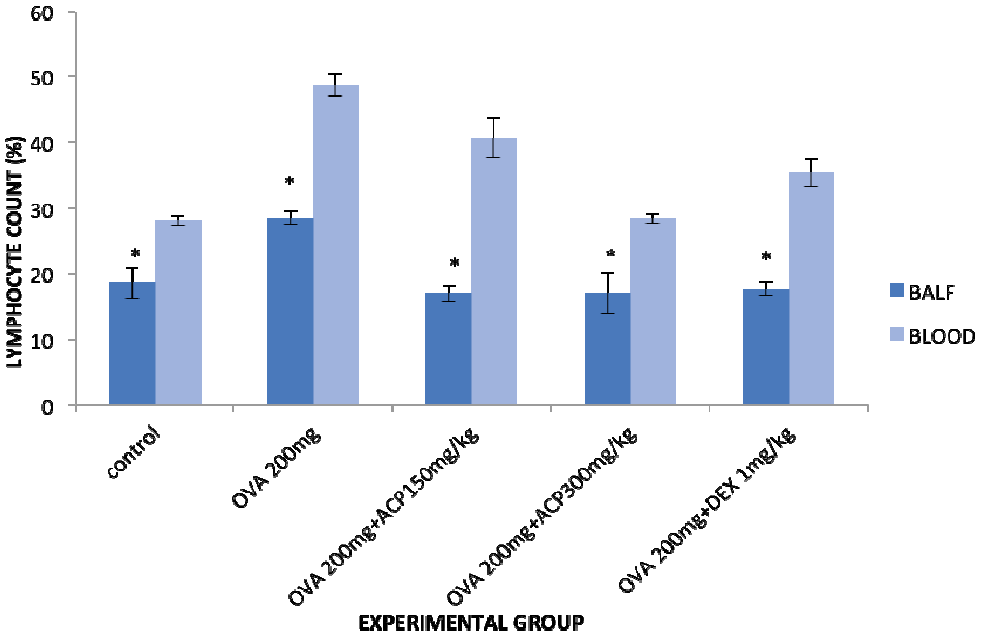

Fig 2: The Effects of Aqueous Extract of *Allium cepa* on percentage of lymphocytes Count In Blood and BALF of Ovalbumin-Induced Allergic Asthma In Wistar Rats

Histological analysis

The slides in plates 1-5 showed the histological changes on the effect of Aqueous Extract of *Allium Cepa* on Lung Histology of Ovalbumin-Induced Allergic Asthma In Wistar Rats.

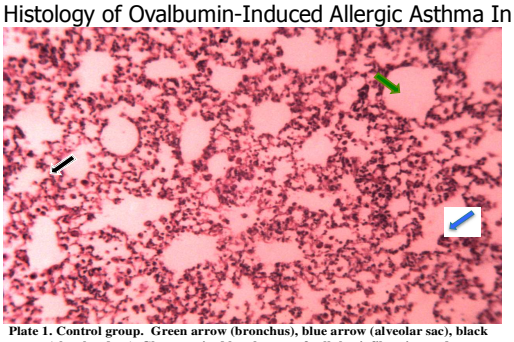

Plate 1. Control group. Green arrow (bronchus), blue arrow (alveolar sac), black arrow (alveolar duct). Characterised by absence of cellular infiltration and pathological changes . (H & E X100)

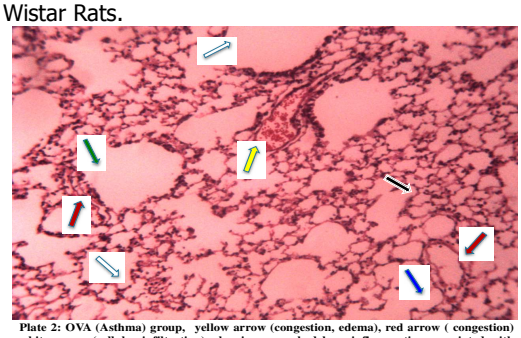

Plate 2: OVA (Asthma) group, yellow arrow (congestion, edema), red arrow (congestion) white arrow (cellular infiltration), showing a marked lung inflammation associated with cellular infiltration, edema and congestion. (H & E x 100)

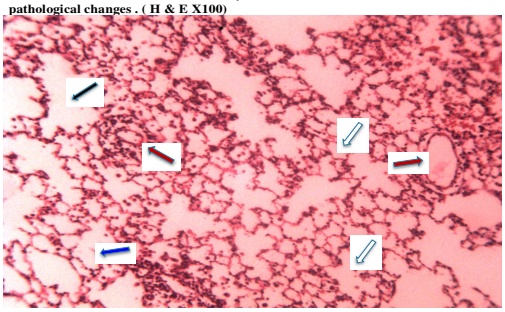

Plate 3: 150mg/kg *Allium cepa* treated group. red arrow (congestion), white arrow (cellular infiltration), showing mild congestion and cellular infiltration but no edema. (H & E X100)

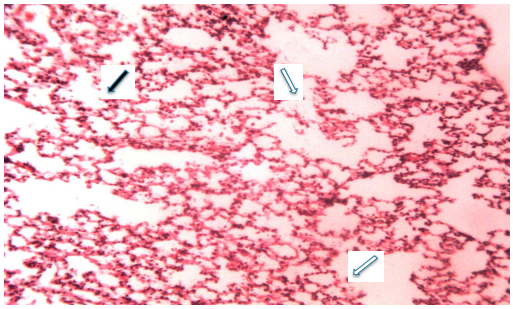

Plate 4: 300mg/kg *Allium cepa* treated group. , white arrow (cellular infiltration). Showing only a mild cellular infiltration. (H & E X100)

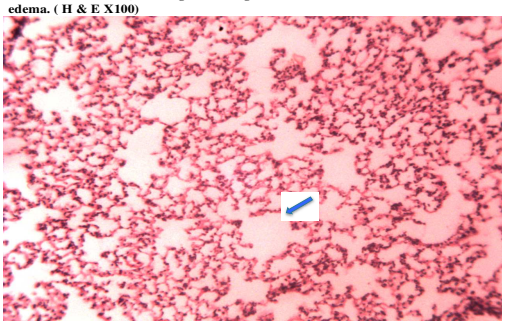

Plate 5: DEXA 1mg/kg treated group. , Showing absence of cellular infiltration but mild congestion. (H & E X100)

Contribution of Authors

F.A Dawud: Experimental design, practical experiment and manuscript preparation

A. B. Dabo: practical experiment, result preparation , photomicroscopy

N. W Yusuf: Statistical analysis and manuscript preparation

I .A. Umar: Idea of the experiment**,** manuscript editing**.**

Conflicts of interest

The authors declare that there is no conflict of interests in the publication of this paper.

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