Effect of mangrove crab (*Sesarma huzardi*) extract on haematology and lung histology in swiss mice infected with *Bordetella pertussis*

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EFFECT OF MANGROVE CRAB (*SESARMA HUZARDI*) EXTRACT ON HAEMATOLOGY AND LUNG HISTOLOGY IN SWISS MICE INFECTED WITH *BORDETELLA PERTUSSIS*

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Abstract
Pertussis or whooping cough is a severe human respiratory tract disease caused by *Bordetella pertussis*, a known worldwide pathogen that affects infants, children and adults. This study is aimed at investigating the changes in haematological parameters and histopathological changes of the lungs associated with *Bordetella pertussis* infection in swiss mice and to evaluate the potential of *Sesarma huzardi* extract to reverse these adverse changes concerning erythromycin treatment. The animals were divided into five groups: group 1 was normal to control, group 2 was infected with *Bordetella pertussis* without treatment (negative control), groups 3 and 4 were *Bordetella pertussis* infected and treated with 300mg/kg and 600mg/kg of the extract respectively and group 5 was infected and treated with 4000mg/70kg of erythromycin in divided doses. The animals were inoculated with a single infectious dose of *Bordetella pertussis* bacteria and were consequently treated with the graded doses of the extract and erythromycin for eighteen days after the animals were confirmed infected. The mice were euthanized using diethyl ether anaesthesia and blood samples were taken for haematological investigation and lung tissue was harvested and processed for histological examination. The result shows that inoculation of mice with an infective dose of *B. pertussis* reveals a gradual decrease in PCV, Hemoglobin, Platelet, RBC and Eosinophil levels when compared to the normal control but these alterations were reversed when treated with *Sesarma huzardi* extract and there was a steady increase in PCV, Hemoglobin, platelet and RBC as the day progresses. The result showed that *Sesarma huzardi* extract reversed the changes in the haematological parameters and pathological changes in the lungs of mice infected with *Bordetella pertussis* in a dose and time-dependent manner which suggests curative potentials of *Sesarma huzardi* extract against *B. pertussis.*

Keywords: *Sesarma huzardi*, *Bordetella pertussis*, erythromycin, Pertussis

Introduction

Ever since the discovery of pertussis as a clinical disease more than 1,600 years ago, *Bordetella pertussis* remains a foremost global pathogen that affects infants, children, and adults (Dorjietal., 2017). *Bordetella pertussis* is a complex bacterium that expresses several bacterial factors with immune-modulating functions and produces diverse bacterial factors accountable for the symptoms seen during the disease (Mattoo and Cherry, 2005). Pertussis is mostly a toxin-mediated disease. The bacteria close to the cilia of the respiratory epithelial cells produce toxins that paralyze the cilia, and cause inflammation of the respiratory tract, which interferes with the clearing of pulmonary secretions (Sheridan et al., 2012). Pertussis antigens allow the organism invades host defences by enhancing lymphocytosis but impairing chemotaxis (NCDC, 2011).The indulgence of this leaves us with the inquiry of why has pertussis remained a key challenge to overcome internationally and what are the looming dangers if nothing is done to restrain the prevalence of this disease. Numerous studies have increasingly proven that pertussis toxin(PT) is the leukocytosis-enhancing factor that is produced by *Bordetella pertussis*. Furthermore, other experimental animals, including rats (Samore and Siber 1992), pigs (Elahi et al., 2005) and macaques (Hinds et al., 1996), develop leukocytosis (an increase in the number of white blood cell especially during infection) when administered purified pertussis toxin(PT). Mice

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and baboons inoculated with *B. pertussis* have lowered levels of leukocytosis when induced with PT-specific monoclonal antibodies (Nguyen et al., 2015).

In a study by Temple et al. (2009) to establish the influence of living *Bordetella pertussis* on the induction and duration of pathophysiological reactions in mice infected intranasally with graded doses of culture, reported that fatally infected mice showed loss of body weight, spleen atrophy, obvious hypothermia and hypoglycemia (low blood sugar), and highly increased levels of leukocytes and serum immune-reactive insulin while non-fatal infected mice showed normal weight gain, almost normal temperature, spleen enlargement, not too pronounced hypoglycemia, lower but obvious increased levels of leukocytes and serum immune-reactive insulin, and histamine sensitization. Leukocytosis (an increase in the number of white blood cells especially during infection) and lymphocytosis (an increase in the number of lymphocytes in the blood) are clear indications of pertussis infection (Beck et al., 2014). Similarly, Momoh et al. (2013) noted that infection with *S. typhi*, similar bacteria to the one under study produced a reduction in pack cell volume (PCV), Red blood cell (RBC) and Hemoglobin (Hb) while there was an increase in monocyte, neutrophil and WBC but there was no significant difference. Crabs are decapod crustaceans which belong to the infra order Brachyura. They are mainly covered with thick exoskeletons. Their lower region is completely hidden under the thoracic cavity. They can be found in most tropical and subtropical regions of the world as reported by Sammy et al. (2009).

Sesarma huzardi is a species of marine crab that is found in tropical and subtropical estuaries and other maritime areas of land along the Atlantic coast of the Americas (Renata et al., 2012). They are known to be good sources of essential macro and micro minerals such as potassium, phosphorus, calcium, magnesium, copper, iron, manganese, and zinc. Park and Kim (2010) and Sujeetha et al. (2015) reported the biomedical and nutritional properties of crabs to include Omega3 (a polyunsaturated acid) contained in crab meat which helps in protecting against heart diseases and inhibits aggressive behaviour. Mahae *et al*. (2011) noted that the selenium contained in crab meat plays an important role in human's antioxidant defence system by preventing cells and tissues from damage and helps in the proper functioning of the immune system and metabolism of thyroid hormone while riboflavin present in them helps in the production of steroids and red blood cells, maintenance of the skin, promote normal growth and iron absorption from the digestive tract and support antioxidant activity. Kong et al. (2010) and Suneeta (2014) in their separate studies reported that crabs lower blood pressure, protect against heart diseases and possess anti-inflammatory properties. Chitosan derived from crab shell have several properties including anti-microbial and antibacterial properties due to its peculiar characteristics (Mahae et al., 2011). Chitosan fights against numerous pathogenic organisms like fungi, spoilage microorganisms, and gram-positive and gram-negative bacteria (Mahae et al., 2011). However, this is the first attempt at establishing the antibacterial effects of crab extract which was opinionated by erratic evidence of the healing potential of crabs in whooping cough among the Bonny people of Rivers State, Nigeria.This study is aimed at determining the effect of *Sesarma huzardi* (land crab) extract on haematology and lungs histology in swiss mice infected with *Bordetella pertussis*

Material and Methods

Sesarma huzardi was caught using a trap in the Buguma Creek Rivers State, Nigeria. The samples collected were transferred into perforated plastic containers to allow for air during transportation and were transported to the Pharmacognosy research laboratory, Department of Pharmacognosy, University of Port Harcourt, Nigeria. The samples were identified by Mr. Otufu Paciya using Food and Agriculture Organization species identification sheets for freshwater and marine crab species.

Using the Shahidi and Synowiecki (1991) extraction method, 60 of the freshly collected crabs were sacrificed and the shell was separated from the meat and washed with tap water to remove all impurities. The crab shells and meat were then transferred to the oven and dried at 70^{o}c until they were completely dry. Using a laboratory mortar and pestle, the dried crab shells and meat were ground and sieved into the size of 500µm. 40g of the sieved crab was measured using WANT precision electric weighing balance into a beaker and 200ml of cord liver oil was added and stirred with magnetic stirrer until it was completely mixed for 20minutes. The beaker was then transferred into a water bath at a temperature of 60^{o}Cand allowed for 30 minutes. The mixture was

then filtered with a white handkerchief to drain off the oil and the residue was transferred into a beaker. The residue was treated with 2% potassium hydroxide (KOH) at a ratio of 1:20 w/v and was stirred continuously for 2 hours at a temperature of 90^{o}C to remove protein from the crab. The sample was filtered under vacuum and the residues were continuously washed until the pH became neutral i.e pH=7. This was done to ensure that all the salt had been removed after removing the protein. The deproteinized crab was transferred into an oven and dried at 60^{o}C until it was completely dry (Shahidi & Synowiecki, 1991). Two-point five percent w/v of hydrochloric acid (Hcl) was used at room temperature (20^{o}C) for 6 hours to remove the mineral content of the deproteinized crab at a ratio of 1:20 w/v. The samples were filtered under a vacuum and washed with tap water until the pH was neutral. The demineralized crab was then transferred to the oven and dried at a temperature 60^{o}C until completely dried. (Shahidi and Synowiecki, 1991).The demineralized crab was treated with 300 ml acetone for 10 minutes and dried for 2 hours at an ambient temperature and the residues were removed to achieve decolourization. The decolourized sample was washed in running water, filtered and dried at 60^{o}C until it was completely dried to obtain crab chitin (Shahidi & Synowiecki, 1991). Deacetylation of chitin was carried out using the method of Yen *et al.* (2009). The obtained chitin was treated with 40% w/v aqueous sodium hydroxide (NaoH) in the ratio of chitin to the solution 1:15 w/v at 105^{o}C in a water bath for 2 hours. Thereafter, the chitin was filtered with a filter pump and washed with deionized water until pH was neutral to obtain the extract. The obtained extract was then dried at 60^{o}C for 2 hours in the oven. The dried extract was preserved in a well-labeled bottle and kept for the experiment.

The test organism *Bordetella pertussis* (ATCC$^{®}$9340^{TM}) was gotten from the American Type Culture Collection (ATCC), USA. The culture media used for isolation according to ATCC is medium 35: Bordet Gengou/Broth medium from a human clinical specimen at a growth temperature of 37^{o}c in aerobic atmosphere. The product was received freeze-dried at 2^{o}C-8^{o}Cand stored at -80^{o}C. The bacterium was reconstituted using Regan-Lowe agar (Charcoal blood Agar) in the Department of Microbiology laboratory, University of Port Harcourt.

A total of hundred and twenty-two (122) animals (swiss mice) was divided into five groups for the curative treatment study. Group 1 (normal) had 10 animals, group 2 (negative control) had 28 animals; groups 3 to 5 consisted of 28 swiss mice each Group 1 served as the normal control without treatment but was fed with the normal animal feed and water. Group 2 (negative control group) consisted of *B. pertussis* inoculated mice without treatment. Group 3 consisted of *B. pertussis*-infected mice exposed to a low dose (300mg/kg) of *Sesarma huzardi* extract while group 4 consisted of *B. pertussis*-infected mice exposed to a high dose (600mg/kg) of *Sesarma huzardi* extract and group 5 consisted of *B. pertussis* infected mice exposed to 4000mg/70kg of erythromycin. On day 0, at day 6days intervals and on day 18, seven animals were sacrificed using diethyl ether anaesthesia; samples of blood were collected and the liver was removed for assessment of liver function status and histopathological examination respectively.

One hundred and twenty-two animals were intraperitoneally challenged with the infective dose of *Bordetella pertussis* which was calculated to be 5 x 10^{5}cfu/ml. After infection had set in (through physical observation of signs like weakness, non-productive cough, anorexia and the isolation of the organism from the blood of the infected animals on day 0) seven animals were sacrificed and blood samples and liver tissue was collected for preliminary investigation and the rest animals from the other treatment groups were given two times daily of the various doses of the extract and the standard antibiotics(erythromycin) for 18days.

The extract solution for the study was prepared by dissolving 0.5g of the extract in 1 ml of di-methyl-sulfoxide (DMSO) solvent to have a stock concentration of 500mg/ml. Since 70kg (70000g) takes 4000mg of erythromycin daily at the severe case of whooping cough, then 25g (average weight of test animal) will take 25g×4000mg/70000g=1.429mg. This means that 25g will take 1.429mg/ml or 2.858mg/0.5ml (1.429×2) or 5.716mg/0.25ml (1.429×4). 5.716mg/0.25ml was prepared from 500mg tablet of erythromycin tablet thus 500mg/Xml=5.716mg/ml. therefore, X=500mg x ml/5.716mg= 87.47ml. Hence, a 500mg tablet of erythromycin was dissolved in 87.47ml distilled water to prepare the erythromycin solution for the study. Each animal was anaesthetized with diethyl ether in a desiccator and blood was collected by cardiac puncture method and transferred into a well labeled sample bottle containing anti-coagulant. The haematological analysis was carried

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out using the method described by Randox Laboratories Limited, United Kingdom (2015). The study animals (swiss mice) were subjected to diethyl ether anaesthesia in a decicator and dissected aseptically to collect the liver for histopathological studies. The collected tissues were kept in 10% chloroform for preservation and were subsequently trimmed to the size of 3-4mm thickness for fixation. These tissues were fixed, dehydrated, cleared, impregnated, embedded, sectioned and stained with hematoxylin and eosin before mounting according to the method described by Baker (1945). The results of the measurements are shown as Mean± Standard Deviation of Mean. The mean differences were obtained by ANOVA and post hoc with the least significant difference (LSD) (Mead & Curnow, 1982).

Results

Effect of *Sesarma huzardi* extract on Post-Inoculation Treatment on Hematological Indices in *B. pertussis* infected mouse

From the result, inoculation of mice with an infective dose of *B. pertussis* reveals a gradual decrease in PCV, Hemoglobin, Platelet, RBC and Eosinophil levels when compared to the normal control but these alterations were reversed when treated with *Sesarma huzardi* extract. Treatment with *Sesarma huzardi* extract reveals a steady increase in PCV (Table 1) Hemoglobin (Table 2) platelet (Table 3) RBC (Table 4) as the day progresses. Contrarily, the negative control shows a constant decrease in all the parameters. Statistical (ANOVA) comparison shows a significant difference ($p<0.05$) between normal control, negative control, standard drug and the treated groups on 6^{th}, 12^{th} and 18^{th} day. Similarly, inoculation with an infective dose of *B. pertussis* shows an increase in WBC, neutrophils, lymphocytes and monocytes when compared with the normal control. However, treatment with *Sesarma huzardi* extract reversed the variations causing a gradual decrease in the haematological parameters (neutrophils, lymphocytes, WBC and monocytes). ANOVA comparison between the normal control, negative control, standard drug and the treatment group shows a significant difference in WBC on days 6, 12 and 18. However, neutrophils showed no significant ($p>0.05$) difference on days 12 and 18 when compared to the standard drug. Similarly, there was a significant difference ($P<0.05$) in lymphocytes on days 6 and 12 but showed no significant ($P>0.05$) on day 18 when compared to the normal control and standard drug. Finally, monocytes count showed no significant difference about the standard drug on days 6 and 12 but a significant difference ($P<0.05$) when compared to negative control on day 18.

Table 1: Effect of Post – IT on *Sesarma huzardi* extract on PCV (g/dl)] in *B. pertussis* infected mice

	DAY 0	DAY 6	DAY 12	DAY 18
Control	37.00±0.000	37.00±0.000	37.00±0.000	37.00±0.000
Negative control	24.67±1.155	22.33±.577	18.67±1.155	15.33±1.155
Erythromycin	24.67±1.155	28.33±.577	34.33±.577	36.67±.577
Low Dose	24.67±1.155	26.00±0.000^{abc}	27.33±.577^{abc}	28.00±1.000^{abc}
High Dose	24.67±1.155	25.67±.577^{abc}	28.67±.577^{abc}	31.67±.577^{abc}

a= Significant ($p<0.05$) between test groups and control
b= Significant ($p<0.05$) between test groups and negative control
c= Significant ($p<0.05$) between test groups and erythromycin
Control = Animal fed with normal feed and water
Negative control = Animal infected with *Bordetella pertussis* without treatment
Low dose = 300mg/kg
High dose =600mg/kg
Erythromycin = standard antibiotics drug

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Table 2: Effect of Post - IT on *Sesarma huzardi* extract on Hgb (g/dl)] in B. pertussis infected mice

	DAY 0	DAY 6	DAY 12	DAY 18
Control	12.30±0.000	12.30±0.000	12.30±0.000	12.30±0.000
Negative control	8.40±.200	7.47±.115	7.00±.173	6.47±.153
Erythromycin	8.40±.200	9.13±.208	11.10±.173	12.20±.100
Low Dose	8.40±.200	8.27±.058^{abc}	8.57±.115^{abc}	9.00±.100^{abc}
High Dose	8.40±.200	8.33±.058^{abc}	9.27±.252^{abc}	9.60±.173^{abc}

Table 3: Effect of Post - IT on *Sesarma huzardi* extract on Platelets count (x10^3/µl)] in *B. pertussis* infected mice

	DAY 0	DAY 6	DAY 12	DAY 18
Control	100.00±0.000	100.00±0.000	100.00±0.000	100.00±0.000
Negative control	56.00±0.000	53.33±1.155	50.33±.577	48.33±.577
Erythromycin	56.00±0.000	73.67±3.512	85.00±2.000	99.33±.577
Low Dose	56.00±0.000	58.33±1.155^{abc}	60.33±.577^{abc}	65.33±1.155^{abc}
High Dose	56.00±0.000	61.00±1.732^{abc}	66.33±2.517^{abc}	72.00±3.000^{abc}

Table 4: Effect of Post - IT on *Sesarma huzardi* extract on RBC (x10^3/µl) in *B. pertussis* infected mice

	DAY 0	DAY 6	DAY 12	DAY 18
Control	9.29±0.000	9.29±0.000	9.29±0.000	9.29±0.000
Negative control	4.90±.300	4.50±.200	3.77±.115	3.07±.153
Erythromycin	4.90±.300	6.33±.058	7.90±.200	9.28±.017
Low Dose	4.90±.300	5.00±.173^{abc}	5.30±.100^{abc}	5.60±.173^{abc}
High Dose	4.90±.300	5.47±.058^{abc}	6.07±.153^{abc}	6.73±.208^{abc}

Table 5: Effect of Post - IT on *Sesarma huzardi* extract on WBC (x10^3/µl) in *B. pertussis* infected mice

	DAY 0	DAY 6	DAY 12	DAY 18
Control	5.10±0.000	5.10±0.000	5.10±0.000	5.10±0.000
Negative control	5.77±.058	6.43±.153	7.70±.173	9.27±.153
Erythromycin	5.77±.058	5.33±.058	5.20±0.000	5.13±.058
Low Dose	5.77±.058	5.80±0.000^{abc}	5.70±0.000^{abc}	5.67±.058^{abc}
High Dose	5.77±.058	5.77±.058^{abc}	5.63±.058^{abc}	5.43±.058^{abc}

Table 6: Effect of Post - IT on *Sesarma huzardi* extract on Neutrophil count (x10^3/µl) in *B. pertussis* infected mice

	DAY 0	DAY 6	DAY 12	DAY 18
Control	20.00±0.000	20.00±0.000	20.00±0.000	20.00±0.000
Negative control	24.67±1.155	27.33±.577	30.00±1.000	42.00±1.732
Erythromycin	24.67±1.155	21.67±.577	21.00±0.000	20.00±0.000
Low Dose	24.67±1.155	24.00±0.000^{abc}	23.67±.577^{abc}	23.33±.577^{abc}
High Dose	24.67±1.155	23.67±.577^{abc}	23.00±0.000^{abc}	22.33±.577^{abc}

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Table 7: Effect of Post - IT on *Sesarma huzardi* extract on Lymphocyte count (x10^3/µl) in *B. pertussis* infected mice

	DAY 0	DAY 6	DAY 12	DAY 18
Control	82.00±0.000	82.00±0.000	82.00±0.000	82.00±0.000
Negative control	88.00±1.000	91.33±2.082	94.00±1.000	101.67±2.517
Erythromycin	88.00±1.000	84.33±.577	83.67±.577	82.00±0.000
Low Dose	88.00±1.000	88.67±.577^{abc}	86.67±.577^{abc}	86.67±.577^{abc}
High Dose	88.00±1.000	88.33±.577^{abc}	85.67±.577^{abc}	84.67±.577^{b}

Table 8: Effect of Post - IT on *Sesarma huzardi* extract on Monocyte count (x10^3/µl) in *B. pertussis* infected mice

	DAY 0	DAY 6	DAY 12	DAY 18
Control	2.00±0.000	2.00±0.000	2.00±0.000	2.00±0.000
Negative control	4.67±.577	6.67±.577	9.00±1.000	11.00±1.000
Erythromycin	4.67±.577	3.33±.577	2.67±.577	2.00±0.000
Low Dose	4.67±.577	4.00±0.000^{ab}	4.00±0.000^{ab}	3.33±.577^{b}
High Dose	4.67±.577	3.67±.577^{ab}	3.67±.577^{ab}	3.33±.577^{b}

Effect of *Sesarma huzardi* extract on lung Histo-architeture in *B. pertussis* infected mice

The lungs histopathological examination of the control animals reveals a normal structure with clear alveolar spaces, epithelial cells and blood vessels. There was no histologic alteration in the lungs. However, mice lung tissues infected with *B. pertussis* with no treatment for four days show various distortions in the lung tissues such as widened interstitial, interstitial inflammation and bullae formation. The lung tissues in mice administered with (300mg/kg) of *Sesarma huzardi* extract for 12 days shows infected tissues with bullae formation while those treated for 18 days shows no interstitial inflammation. The lung tissues not treated (negative control) for 18 days showed widened interstitial, interstitial inflammation, haemorrhage and bullae formation. However, Lungs tissues in mice treated with (600mg/kg) of *Sesarma huzardi* extract for 6 days, and 12 days shows mild interstitial inflammation, bullae formation and widened interstitial while treatment for 18 days shows no obvious histologic change while those treated with erythromycin for 6 days showed interstitial inflammation and bullae formation. However, lung tissues infected with *B. pertussis* and treated with erythromycin for 18 days shows no obvious histologic change and appeared normal as the control. This is shown in plates 1-14

Histological Plates

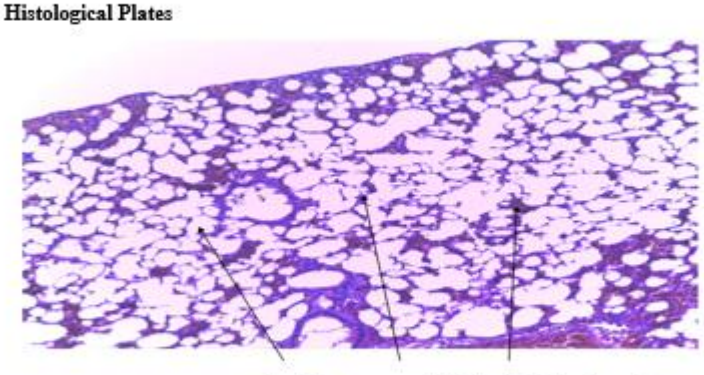

Plate 1: Photomicrograph of lungs tissue of normal mice showing no histologic alteration

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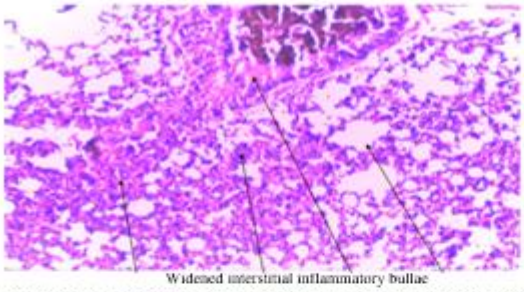

Plate 2: Photomicrograph oflungs of mice infected with ***B.pertussis*** showing interstitial inflammation and bullae formation (Day 0)

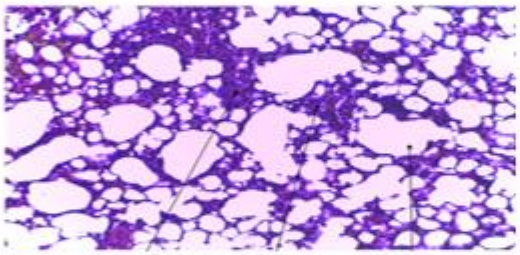

Plate 3: Photomicrograph of lungs of mice infected with ***B.pertussis*** without treatment for 6days showing interstitial inflammation and bullae formation

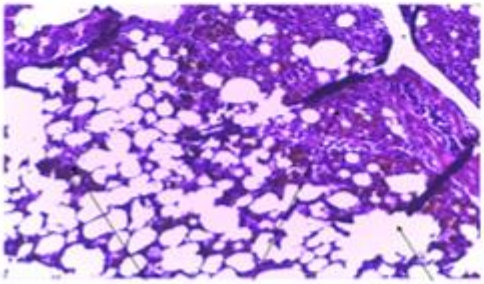

Plate 4: Photomicrograph oflungs of mice infected with ***B.pertussis*** treated with 4000mg/70kg of erythromycin for 6days showing interstitial inflammation and bullae formation

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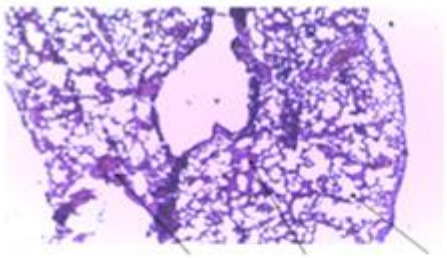

Plate 5: Photomicrograph oflungs of mice infected with *B.pertussis*and treated with 300mg/kg of *Sesarmahuzardi*extract for 6days showing interstitial inflammation and bullae formation

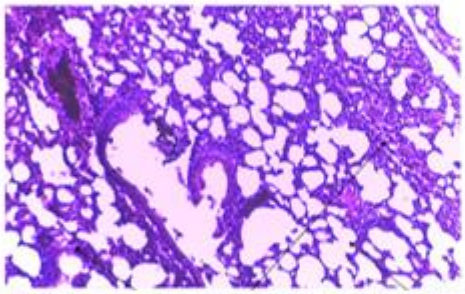

Plate 6: Photomicrograph oflungs of mice infected with *B.pertussis*and treated with 600mg/kg of *Sesarmahuzardi*extract for 6days showing interstitial inflammation and bullae formation

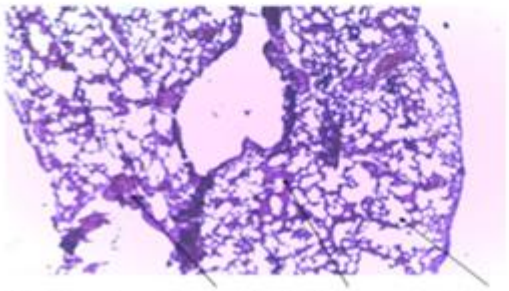

Plate 7: Photomicrograph oflungs of mice infected with *B.pertussis*without treatment for 12days showing interstitial inflammation and bullae formation

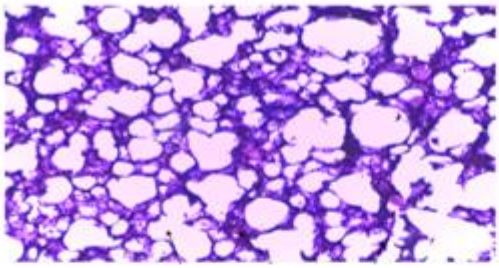

Plate 8: Photomicrograph oflungs of mice infected with ***B.pertussis***treated with 4000mg/70kg of erythromycin for 12days showing no obvious histologic change with clear alveolar spaces and pulmonary vessels

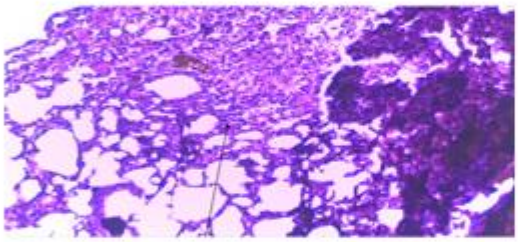

Plate 9: Photomicrograph of lungs of mice infected with ***B. pertussis*** and treated with 300mg/kg of ***Sesarma huzardi*** extract for 12days showing mild interstitial inflammation and bullae formation

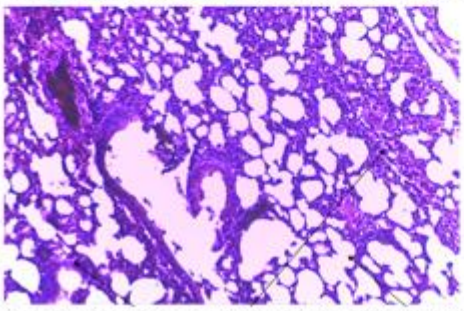

Plate 10: Photomicrograph oflungs of mice infected with ***B. pertussis*** and treated with 600mg/kg of ***Sesarma huzardi*** extract for 12days showing interstitial inflammation and bullae formation

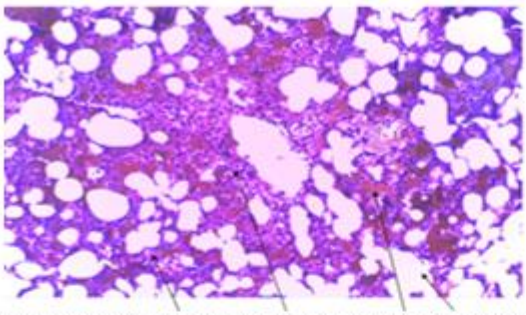

Plate 11: Photomicrograph oflungs of mice infected with *B. pertussis* without treatment for 18days showing interstitial inflammation and bullae formation

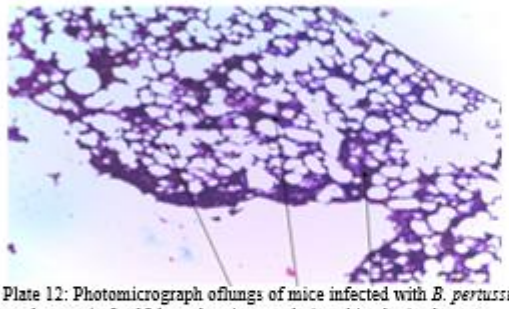

Plate 12: Photomicrograph oflungs of mice infected with *B. pertussis* and treated with 4000mg/70kg of erythromycin for 18days showing no obvious histologic change

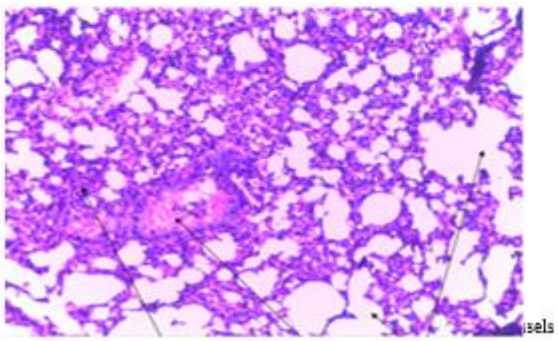

Plate 13: Photomicrograph oflungs of mice infected with *B. pertussis* and treated with 300mg/kg of *Sesarma huzardi* extract for 18days showing no histologic alteration

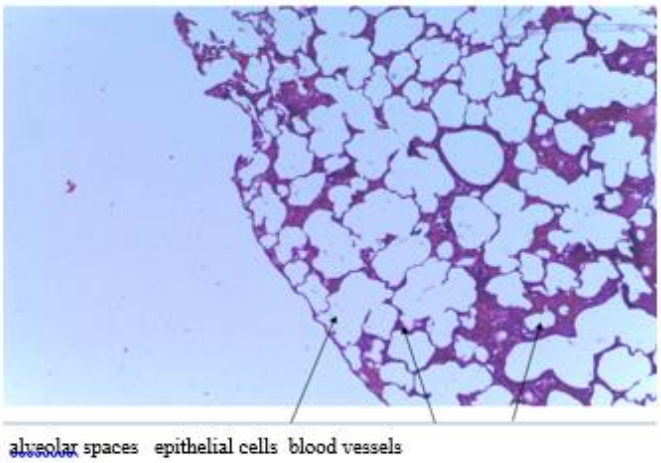

Plate 14: Photomicrograph of lung of mice infected with *B. pertussis* and treated with 600mg/kg of *Sesarma huzardi* extract for 18days showing normal lungs tissue

Discussion

Several studies have shown that during *B. pertussis* infection, the common haematological changes include leukocytosis (an increase in the number of white blood cells (WBC)) lymphocytosis (an increase in the number of lymphocytes in the blood) and monocytosis (an increase in the number of monocytes in the blood (Nguyen et al., 2015, Black et al., 2014; Temple et al., 2009) Significant decrease in mean levels reduced PCV (Pack Cell Volume), Red Blood Cell (RBC) and Hemoglobin while there is an increased neutrophil (Momoh et al, 2013). The result of this study agrees with the reports from other researchers. The increase in neutrophil and white blood cells is associated with the invasion of the hemopoietic organ (bone marrow) by *B. pertussis* while monocytosis and lymphocytosis are attributed to the increased release of these cells from the myeloid/ lymphoid tissues in response to the infection (Das and Mukherjee, 2003). However, the decrease in RBC, PCV and haemoglobin could be a result of the destruction of Red Blood Cell by the infection (Dangana et al., 2010) hemophagocytosis (a potentially fatal disease of normal but overactive histiocytes and lymphocytes that are common in infants) and bone marrow suppression (Khosla et al., 1995). The result of post-inoculation treatment with *Sesarma huzardi* extract further revealed the reversal of the usual trend of significant increase ($p<0.05$) in WBC, lymphocytes, monocytes, neutrophils and decrease in PCV, RBC, platelets and haemoglobin usually associated with *B. pertussis* infection the result of the post-inoculation treatment with *Sesarma huzardi* extract reversed the effect of *B. pertussis* infection which suggests the curative potentials of *Sesarma huzardi* extract against *B. pertussis* when combined with other therapeutic agents. The result of this study agrees with the report of Andreasen and Carbonetti, (2008), Karen et al. (2017) who said infection of *B. pertussis* causes lung tissue inflammation, necrosis and widened interstation. However, treatment with *Sesarma huzardi* slowly reversed the trend with an increase in time and dosage. The result confirms the anti-*Bordetella pertussis* property of the extract.

Conclusion

Inoculation of mice with an infective dose of *B. pertussis* produces haematological changes such as leukocytosis (an increase in the number of white blood cells (WBC)) lymphocytosis (an increase in the number of lymphocytes in the blood) and monocytosis (an increase in the number of monocytes in the blood) Significant decrease in mean levels of PCV (Pack Cell Volume), Red Blood Cell (RBC) and Hemoglobin with an increase in neutrophil but treatment with *Sesarma huzardi* extract reversed the changes in a dose and time-dependent manner. Similarly, infection with B. pertussis caused distortions in the lung tissue such as widened interstitial inflammation and bullae formation but treatment with *Sesarma huzardi* extract reversed the changes in a dose and time-dependent manner.

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