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# Effect of Cov-Pla1 and Cov-Pla3 extract on some pulmonary function parameters in rabbits

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Transmission of COVID-19 is facilitated by uptake of droplets containing coronavirus from the breath, sneeze or cough of infected persons. This represents the commonest mode of coronavirus infection and spread to mucous membranes of the respiratory system. The virus rapidly replicates in alveolar cells, triggering a strong immune response, resulting in cytokine storm syndromes and pulmonary tissue damage. These pathologic processes contribute to a compromised pulmonary function. Thus, evaluation of pulmonary function would give insights into modulatory effect of agents that may be beneficial in ameliorating this pathology. The study evaluated effects of Cov-Pla1 and Cov-Pla3 (polyherbal products of the research team, positioned for treatment of Covid-19) on pulmonary function in bleomycin-induced lung injury in rabbits. Rabbits of both sexes were divided into six groups and treated with the extracts alone or the extract following pre-treatment with bleomycin. Targeted respiratory function parameters were monitored at baseline and on day three. Vital capacity, tidal volume, inspiratory reserve volume and inspiratory capacity in the groups treated with Cov-Pla1 and Cov-Pla3 at 125 and 500 mg/kg respectively were compared with the bleomycin only group. In bleomycin pre-treated groups, the two preparations at 125 mg/kg showed increased vital capacity compared to the bleomyicn only group. This pattern was repeated with the other parameters that were evaluated. These results imply that Cov-Pla1 and Cov-Pla3 at the 125 mg/kg dose have ameliorative effects on bleomycin induced lung injury and could be beneficial in situations such as COVID-19 where there are active insults to the respiratory system.

**Keywords:** COVID-19, Cov-Pla, Herbal Preparation, Respiratory function

## INTRODUCTION

An infection caused by a viral organism that is now referred to as the novel corona virus, also known as the Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) was discovered in China in late 2019.

This infection has continued to ravage the world since the time, in pandemic proportions, and is now known as COVID-19. Many symptoms of the infection have been reported in patients. These range from common symptoms such as fever, cough, myalgia or fatigue and pneumonia, often complicated with dyspnea. Less commonly reported symptoms include headache, diarrhoea, haemoptysis, runny nose, and phlegm producing cough (Huang et al., 2020). COVID-19 has defied all forms of treatment and there are currently no therapeutically effective drugs.

The involvement of the respiratory system in the symptomology of COVID-19 has also been reported in other coronaviruses. This finding has clinical implications. This followed the discovery that in some of these types of viral infections, pneumonia together with renal failure accounted for almost 55% mortality in one cohort of patients (Gralinski et al., 2013). Furthermore, respiratory failure which was a major cause of death in previous viral pandemics has been the leading case of mortality with COVID-19 as well (Saber-Ayad et al., 2020). In COVID-19, acute lung injury (ALI) which precedes, acute respiratory distress syndrome (ARDS) is common (Wu et al., 2020). This pathogenesis is thought to be due to rapid SARS-CoV-2 replication in alveolar cells, triggering a strong immune response, resulting in cytokine storm syndromes and pulmonary tissue damage. Cytokine storm is characterized by the uncontrolled production of pro-inflammatory cytokines and are important causes of acute respiratory distress syndrome (ARDS) as well as multi-organ failure (Li et al., 2020). These pathologic processes ultimately contribute to a compromised respiratory or pulmonary function. Thus evaluation of pulmonary function would give insights into modulatory effect of agents that may be beneficial in ameliorating this pathology. The study evaluated the effect of the poyherbal products Cov-Pla1 and Cov-Pla3 on pulmonary function in the rabbit model of bleomycin induced lung injury.

## METHODOLOGY

### Collection and preparation of plant materials

The collected plants were identified and authenticated by Mr. Joseph Azila of the Federal College of Forestry, Jos, Nigeria. Voucher specimens were deposited at the herbarium of the institute. The plants were dried individually in a drying cabinet maintained at temperatures between 35 °C and 40 °C until constant weights were obtained. The dried plant parts were individually reduced to fine powder using a mortar and pestle then weighed in appropriate percentage proportions (4:2:2:1:1) to produced Cov-Pla 1 and (4:2:2:1.5:0.5) to produce Cov-Pla 3 powders. The powder

was soaked in 70% ethanol and left to stand overnight. It was thereafter decanted, filtered and evaporated to dryness under reduced pressure at 40°C. A dry crisp dark brownish solid extract was obtained. The extract yield was noted and recorded while it was placed in an airtight container then refrigerated until required for the investigations.

### Experimental animals

Rabbits of both sexes weighing between 0.8 and 2.2 kg were used for the experiment. They were sourced from the Animal Facility Unit of the Department of Pharmacology, University of Jos and kept in a pathogen free environment under control conditions (27 ± 2°C; 70-80 % humidity; natural day/night lighting cycle) in the Animal Experimental Unit. Commercial food pellets and water were supplied *ad libitum*. Animal experimental protocols were in accordance with the current guidelines for the care of laboratory animals by the U.S. National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (National Research Council, 2011).

### Ethical considerations

Approval to conduct the study was given by the Animal Ethics Committee of the Department of Pharmacology and Toxicology, University of Jos, Nigeria; under the ethical certificate number F17.00379 dated 5th June, 2020.

### Induction of lung injury in rabbits

Bleomycin was used to induce lung injury in rabbits. As reported in various literature sources, (Matute-Bello et al., 2008; Moeller et al., 2008), it is capable of acute inflammatory injury followed by reversible fibrosis within a short period of time and with good reproducibility. The Oropharyngeal aspiration model of delivery was used in administering bleomycin to the rabbits. In the procedure, rabbits were held vertically and the tongue gently pulled out of the mouth using forceps thereby blocking the swallow reflex. The liquid was dropped onto the back of the tongue using a pipette while simultaneously closing the nose thereby forcing the animal to breathe through the mouth and in the process aspirating the liquid. The nose and tongue were released after at least two breaths had been completed (Lakatos et al., 2008; DeVooght et al, 2009; Bale et al., 2016). The Oropharyngeal administration procedure for bleomycin was performed twice on alternate days with the animal receiving half of the treatment dose on each occasion as described by Bale et al., (2016).

### Treatment groups

The rabbits were randomly divided into six groups of three rabbits each. The groups received various treatments as follows:

Group 1: Normal Saline only daily for 3 days
Group 2: Bleomycin 4 U/kg body weight only (2 U/kg body weight on day 0 and again on the second day)
Group 3: Cov-Pla1 extract 125 mg/kg body weight daily for 3 days

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**Table 1.** Effect of Cov-Pla1 and Cov-Pla3 on vital capacity.

| Treatment | Vital capacity (L) | |
|---|---|---|
| | Baseline | Day 3 |
| Control | 3.06 ± 1.45 | 3.57 ± 0.97 |
| Bleomycin 4 U/kg | 1.27 ± 0.41 | 2.73 ± 0.40 |
| Bleomycin 4 U/kg + Cov Pla1 125 mg/kg | 2.09 ±1.04 | 4.04 ± 2.07 |
| Bleomycin 4 U/kg + Cov Pla1 500 mg/kg | 1.18 ± 0.34 | 3.37 ± 1.62 |
| Cov Pla1 125 mg/kg | 2.19 ± 0.47 | 4.83 ± 2.39 |
| Cov Pla1 500 mg/kg | 2.21 ± 1.13 | 1.93 ± 0.58 |
| Bleomycin 4 U/kg + Cov Pla3 125 mg/kg | 2.09 ± 1.13 | 5.03 ± 1.16 |
| Bleomycin 4 U/kg + Cov Pla3 500 mg/kg | 1.33 ± 0.45 | 4.10 ± 0.09 |
| Cov Pla3 125 mg/kg | 0.74 ± 0.08 | 3.84 ± 0.94 |
| Cov Pla3 500 mg/kg | 1.57 ± 0.47 | 4.13 ± 2.00 |

Values are mean ± SEM; n = 3.

and Bleomycin 4 U/kg body weight (2 U/kg body weight on day 0 and again on the second day)
Group 4: Cov-Pla1 extract 500 mg/kg body weight daily for 3 days and Bleomycin 4 U/kg body weight (2 U/kg body weight on day 0 and again on the second day);
Group 5: Cov-Pla1 extract 125 mg/kg body weight daily for 3 days
Group 6: Cov-Pla1 extract 500 mg/kg body weight daily for 3 days

**Monitoring**

Targeted respiratory function parameters were monitored at baseline (Day 0) and on day three. The Surgifield digital spirometer (Surgifield, England) was used to monitor the targeted respiratory function parameters. The parameters monitored included the vital capacity (VC), Tidal Volume (TV), Inspiratory Reserve Volume (IRV) and the Inspiratory Capacity (IC).  Results were analysed using multivariate analysis on the SPSS statistical software version 25 and expressed as Mean ± Standard error of mean. *P* values less than 0.05 were considered statistically significant.

**RESULTS AND DISCUSSION**

The purpose of the study was to evaluate the effect of the poyherbal products (Cov-Pla1 and Cov-Pla3) on pulmonary function in the rabbit model of bleomycin-induced lung injury. A high dose of bleomycin is reported to cause lethal lung injury as well as pulmonary fibrosis in humans and murine models. This results in acute alveolitis and interstitial inflammation actively characterized by recruitment of neutrophils, lymphocytes, and macrophages in the acute phase (Hoshino et al., 2009). This acute inflammatory injury may occur in a timeframe as short as 24 hours followed by reversible fibrosis (Matute-Bello et al., 2008; Moeller et al., 2008; Jana et al., 2019) and closely resembles acute lung injury (ALI) (Mouratis et al., 2011).

Acute lung injury (ALI) and its severe form, acute respiratory distress syndrome (ARDS) are syndromes of acute diffuse damage to the pulmonary parenchyma as a result of various local or systemic insults and occur within hours to days (Wang et al., 2008). These sequence of events are prevalent in COVID-19.

Evaluation of pulmonary function in animal models of respiratory disease is scientifically and clinically important. In the assessment of the effectiveness of potential therapeutic agents, respiratory physiology and evaluation of improvement in respiratory parameters are fundamental to the identification of improved lung mechanics (Milton et al., 2011). This is more so with Cov-Pla1 and Cov-Pla3 that are being evaluated for the amelioration of lung injury in rabbits.

In the results vital capacity, tidal volume, inspiratory reserve volume and inspiratory capacity in the groups treated with Cov-Pla1 (125 mg/kg; 500 mg/kg) and Cov-Pla3 (125 mg/kg; 500 mg/kg) respectively were compared with the group that received only bleomycin only to evaluate whether there are any ameliorative effects on the parameters. In Table 1, bleomycin plus the two preparations at the 125 mg/kg showed an increase in vital capacity in comparison to the group that was treated with only bleomyicn. Even though these results were found not to be statistically significant. The same pattern was repeated with the tidal volume, inspiratory reserve volume and inspiratory capacity (shown in Tables 2, 3 and 4). However when a higher dose of the preparations were administered with bleomycin, the paramenters appeared to marginally decreased though not in a statistically significant manner. These may mean that the higher dose of the preparations seem to aggravate the injury cause by bleomycin. The implication of these results is that while Cov-Pla1 and Cov-Pla3 at the 125 mg/kg dose have ameliorative effects on bleomycin induced lung injury as evidenced by the favourable effect on the parameters evaluated, caution should be exercised with these agents as the dose increases.

**Source of Funding**

**Table 2.** Effect of Cov-Pla1 and Cov-Pla3 on tidal volume.

| Treatment | Tidal volume (L) | |
|---|---|---|
| | Baseline | Day 3 |
| Control | 1.22 ± 0.51 | 1.12 ± 0.33 |
| Bleomycin 4 U/kg | 0.17 ± 0.04 | 1.22 ± 0.29 |
| Bleomycin 4 U/kg + Cov Pla1 125 mg/kg | 0.51 ± 0.25 | 2.38 ± 0.00 |
| Bleomycin 4 U/kg + Cov Pla1 500 mg/kg | 0.50 ± 0.13 | 2.33 ± 0.00 |
| Cov Pla1 125 mg/kg | 0.78 ± 0.18 | 2.62 ± 1.41 |
| Cov Pla1 500 mg/kg | 0.68 ± 0.38 | 0.00 ± 0.00 |
| Bleomycin 4 U/kg + Cov Pla3 125 mg/kg | 0.47 ± 0.32 | 1.19 ± 0.44 |
| Bleomycin 4 U/kg + Cov Pla3 500 mg/kg | 0.55 ± 0.56 | 1.59 ± 0.37 |
| Cov Pla3 125 mg/kg | 0.21 ± 0.03 | 1.04 ± 0.53 |
| Cov Pla3 500 mg/kg | 0.52 ± 0.16 | 0.00 ± 0.00 |

Values are Mean ± SEM; n = 3.

**Table 3.** Effect of Cov-Pla1 and Cov-Pla3 on inspiratory reserve volume.

| Treatment | Inspiratory Reserve Volume (L) | |
|---|---|---|
| | Baseline | Day 3 |
| Control | 3.70 ± 0.70 | 4.20 ± 0.56 |
| Bleomycin 4 U/kg | 1.94 ± 1.21 | 5.24 ± 1.54 |
| Bleomycin 4 U/kg + Cov Pla1 125 mg/kg | 2.81 ± 1.66 | 7.54 ± 0.00 |
| Bleomycin 4 U/kg + Cov Pla1 500 mg/kg | 2.00 ± 1.53 | 0.00 ± 0.00 |
| Cov Pla1 125 mg/kg | 1.67 ± 0.67 | 4.8 ± 2.21 |
| Cov Pla1 500 mg/kg | 2.33 ± 0.88 | 0.00 ± 0.00 |
| Bleomycin 4 U/kg + Cov Pla3 125 mg/kg | 2.27 ± 1.75 | 4.71 ± 0.62 |
| Bleomycin 4 U/kg + Cov Pla3 500 mg/kg | 2.56 ± 1.19 | 3.49 ± 0.79 |
| Cov Pla3 125 mg/kg | 0.46 ± 0.16 | 4.70 ± 2.36 |
| Cov Pla3 500 mg/kg | 1.27 ± 0.51 | 0.00 ± 0.00 |

Values are Mean ± SEM; n = 3.

**Table 4.** Effect of Cov-Pla1 and Cov-Pla3 on inspiratory capacity.

| Treatment | Inspiratory capacity (L) | |
|---|---|---|
| | Baseline | Day 3 |
| Control | 4.92 ± 1.20 | 5.29 ± 0.78 |
| Bleomycin 4 U/kg | 2.11 ± 1.25 | 6.47 ± 1.79 |
| Bleomycin 4 U/kg + Cov Pla1 125 mg/kg | 3. 32 ± 0.89 | 9.92 ± 0.00 |
| Bleomycin 4 U/kg + Cov Pla1 500 mg/kg | 2.15 ± 1.61 | 0.77 ± 0.00 |
| Cov Pla1 125 mg/kg | 2.52 ± 0.74 | 2.42 ± 1.38 |
| Cov Pla1 500 mg/kg | 3.00 ± 1.29 | 0.00 ± 0.00 |
| Bleomycin 4 U/kg + Cov Pla3 125 mg/kg | 2.74 ± 2.07 | 5.9 ± 0.98 |
| Bleomycin 4 U/kg + Cov Pla3 500 mg/kg | 3.11 ± 1.16 | 5.08 ± 0.42 |
| Cov Pla3 125 mg/kg | 0.67 ± 0.19 | 8.62 ± 0.27 |
| Cov Pla3 500 mg/kg | 1.79 ± 0.66 | 00.00 ± 0.00 |

Values are Mean ± SEM; n = 3.

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other Infectious Diseases.

## CONFLICT OF INTERESTS

The authors declare no conflict of competing interests

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## Addendum

Cov-Pla1 was eventually registered as Pla-tonic while Cov-Pla3 as Immunopla

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