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Attenuation of Bleomycin-induced Toxicity on Selected Respiratory Function Metrics in Rabbits by a Polyherbal Preparation

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ABSTRACT

Introduction: Bleomycin, a commonly used chemotherapeutic agent, is known for its potential to induce respiratory toxicity. Despite its efficacy in treating various cancers, its use is limited by adverse effects, including pulmonary complications. These respiratory disorders and diseases can significantly impact an individual's quality of life, leading to various health complications.
Objective: The study assessed the effect of a polyherbal preparation (*CoV Pla-2* extract) on tidal volume (V_T), vital capacity (VC), inspiratory capacity (IC) and inspiratory reserve volume (IRV) following bleomycin-induced lung injury in rabbits.
Methods: Lung injury was induced in rabbits using bleomycin administered via oropharyngeal aspiration. Animals were randomly divided into six groups of three rabbits each and received treatments as follows: Group 1: Normal Saline (5 ml/kg); Group 2: Bleomycin (4 U/kg); Group 3: *CoV Pla-2* extract (125 mg/kg) and Bleomycin (4 U/kg); Group 4: *CoV Pla-2* extract (500 mg/kg) and Bleomycin (4 U/kg); Group 5: *CoV Pla-2* extract (125 mg/kg); Group 6: *CoV Pla-2* extract (500 mg/kg). Normal saline and the extract were administered daily for 14 days *per oral*, while Bleomycin was administered on days 0 and 2. A digital spirometer was used to monitor some pulmonary function parameters.
Results: Results showed that administration of bleomycin decreased Inspiratory Capacity, Vital Capacity, Inspiratory Reserve Volume and Tidal Volume when compared to control. Co-administration of the extract and bleomycin caused an increase in these pulmonary function parameters, when compared to the animals administered bleomycin alone. **Conclusion:** The findings from this study suggest that *CoV Pla-2* extract can modify bleomycin-induced lung injury, as seen in increase in Tidal Volume, Vital Capacity, Inspiratory capacity and Inspiratory Reserve Volume following injury due to bleomycin.

1. Introduction

Respiratory function is a pivotal element of both human and animal health, exerting a direct influence on overall well-being. Numerous factors, including environmental pollutants, infections, and chronic diseases, have the potential to compromise respiratory function, giving rise to conditions such as chronic obstructive pulmonary disease (COPD), asthma, bronchitis, and even life-threatening situations like acute respiratory distress syndrome (ARDS) and pneumonia[1]. Some Coronavirus disease 2019 (COVID-19) patients have previously experienced shortness of breath, difficulty in breathing, viral pneumonia, respiratory failure, and even multiple organ failure[2]. In response to these challenges, researchers have been delving into the therapeutic potential of natural extracts derived from plants and herbs, aiming to discover their positive effects on respiratory function.

Bleomycin, an antineoplastic antibiotic derived from Streptomyces species, is frequently employed in cancer treatment for its cytotoxic effects. However, one of its well-known adverse effects is pulmonary toxicity, which can manifest as pulmonary fibrosis, a severe and potentially life-threatening condition[3].

Pulmonary toxicity induced by bleomycin is marked by a deterioration in pulmonary function parameters, encompassing diminished lung compliance, decreased vital capacity, and compromised diffusion capacity[4]. The drug induces the production of reactive oxygen species (ROS) and free radicals, causing oxidative stress in lung tissue. This oxidative damage can result in cellular injury and inflammation, creating a conducive environment for the onset of fibrosis[5].

The bleomycin-induced lung fibrosis model stands as the commonly utilized experimental model, recognized as a marker of the inflammatory response within the alveolus[6]. Known for its ease of establishment, robustness, reproducibility, and versatility, the Bleomycin model has been applied across various animal species, including dogs, guinea pigs, rats, hamsters, primates[7,8,9].

For ages, plant extracts have been integral to traditional medicine due to their potential therapeutic properties. Many herbal extracts contain compounds recognized for their anti-inflammatory properties, offering assistance in mitigating inflammation within the airways. This proves especially advantageous for conditions such as asthma and COPD.

An established approach in traditional therapeutic medicine is the amalgamation of various medicinal herbs to achieve the intended therapeutic efficacy[10]. This is essential in treatment because in order to obtain the desired therapeutic effect, different plant phytochemicals often need to be combined, as the active phytochemical constituents of individual plants may be insufficient.

In this present study, several plants were repurposed based on their known pharmacological activities and combined into a formulation and named "*CoV Pla-2*". This formulation comprised parts from five plants (*Vernonia amygdalina*, *Carissa edulis*, *Garcinia kola*, *Zingiber officinale* and *Artemisia annua*) of African ethnopharmacological relevance.

Oxidative stress plays a pivotal role in the initiation and advancement of respiratory diseases. Herbal extracts abundant in antioxidants, like *Vernonia amygdalina*, offer a potential shield against oxidative stress, thereby potentially diminishing the susceptibility to respiratory diseases[11]. Extract from the leaves of the *Vernonia amygdalina* have demonstrated anti-inflammatory effects, offering relief from inflamed airways such as seen in pulmonary toxicity and enhancing airway function[12]. *Garcinia kola* has been noted for its ability to induce dilation in alveolar ducts, alveolar sacs, and alveoli, consequently enhancing respiratory activities in experimental animals[13]. Additionally, extracts of *Carissa edulis* have been identified to boost the immune system's response, potentially aiding the body in more effectively combating respiratory infections[14,15]. This is in addition to its reported anti-oxidant and anti-inflammatory properties[16].

Zingiber officinale (ginger) is a common spice and also a widely used medicinal plant with reported antiviral activity against human respiratory syncytial virus (HRSV)[17]. It has also been reported to relax airway smooth muscles and attenuate airway hyperresponsiveness[18]. *Artemisia annua* has been reported to possess anti-inflammatory and antioxidant properties because of the high content of alkaloids, lactones, flavonoids, phenols, quinines, tannins and terpenoids[19], as well as its antiviral and immunomodulation effects[20].

This study investigated the effect of a polyherbal preparation (*CoV Pla-2*) on pulmonary function following Bleomycin-induced lung injury in rabbits.

2. Methods

Collection and Preparation of Plant Materials

The plant specimens were collected from several locations in Pankshin and Farin gada market in Plateau State and Toro in Bauchi State, Nigeria. They were identified and authenticated by Mr. Joseph Azila of the Federal College of Forestry, Jos, Nigeria. Samples were subsequently deposited at the College herbarium for reference. The plants were separately dried in a cabinet at temperatures of 40°C until constant weights were achieved. The dried components of the five plants within the mixture were finely powdered using a mortar and pestle, weighed according to a ratio of 40:20:20:15:5 (*Vernonia amygdalina leaves*: *Carissa edulis* fruits: *Garcinia kola* fruits: *Zingiber officinale* rhizome: *Artemisia annua* leaves respectively), and combined to form a powder. The resulting mixture was soaked overnight in 70 % ethanol, followed by decanting, filtering, and evaporation under reduced pressure at 40°C. The extract obtained was then stored in an airtight container and refrigerated until ready for use.

Experimental animals

Rabbits of both genders, with a weight range of 1.0 to 2.1 kg, were procured from the Animal Experimental Unit of the Department of Pharmacology and Toxicology, University of Jos, Nigeria. The rabbits were accommodated at the same facility under controlled conditions of 27 ± 2°C temperature, 70-80 % relative humidity, and a 12-hour dark/light cycle. They were provided with a diet comprising commercial food pellets, and access to water was granted *ad libitum*.

Ethical considerations

All animal experimentation procedures followed the "Guidelines for the Care of Laboratory Animals" as stipulated by the U.S. National Institute of Health[21]. The study protocol received approval from the Animal Ethics Committee of the Department of Pharmacology and Toxicology, University of Jos, Nigeria, under the ethical certificate number F17.00379.

Induction of Lung Injury in Rabbits

In this study, Bleomycin was chosen due to its capability to induce rapid acute inflammatory injury and reversible fibrosis with high reproducibility, as documented in previous research[22]. The administration of bleomycin to the rabbits was conducted using the oropharyngeal aspiration model.

During the procedure, rabbits were held in a vertical position, and their tongues were gently extended from the mouth using forceps to temporarily inhibit the swallow reflex. The administration of Bleomycin in liquid form involved depositing it at the back of the tongue using a pipette. Simultaneously, the nose was blocked to encourage the animal to breathe through the mouth, facilitating the ingestion of the liquid. The nose and tongue were released after at least two breaths had been completed, as reported in literature[23,24]. This protocol was repeated twice on alternate days, with the animal receiving half of the treatment dose during each instance as described by Bale and colleagues[23].

Treatment groups

Eighteen rabbits were randomly assigned to six groups, each consisting of three rabbits, and subjected to different treatments as outlined below:

Group 1: Normal Saline only daily for 14 days; Group 2: bleomycin 4 U/kg only (2 U/kg on day 0 and again on the second day); Group 3: *CoV Pla-2* extract 125 mg/kg daily for 14 days and bleomycin 4 U/kg (2 U/kg on day 0 and again on the second day); Group 4: *CoV Pla-2* extract 500 mg/kg daily for 14 days and bleomycin 4 U/kg (2 U/kg on day 0 and again on the second day); Group 5: *CoV Pla-2* extract 125 mg/kg daily for 14 days; Group 6: *CoV Pla-2* extract 500 mg/kg daily for 14 days.

Monitoring

Vital capacity (VC), Tidal Volume (TV), Expiratory Reserve Volume (ERV), Inspiratory Reserve Volume (IRV) and the Inspiratory Capacity (IC) were assessed at baseline (Day 0) and on days 3, 7, 10 and 15, using the digital spirometer.

Statistical Analysis: Statistical analysis of all generated data was performed using SPSS version 23 software IBM. Analysis of variance (ANOVA) and least significance difference (LSD) test were performed at p-values of less than 0.05 ($P<0.05$). Values were reported as Mean ± Standard Deviation.

3. Results

Tidal volume decreased in the group that received bleomycin at a dose of 4 U/kg, compared to the normal saline control at baseline and on day 7 and day 15 (Table 1). Animals that received bleomycin (4 U/kg) and the extract (125 mg/kg) showed a decrease in tidal volume at day 3 only, when compared to animals that received bleomycin

alone, though insignificantly (Table 1). Tidal volume was observed to be lower at baseline and on days 3, 10 and 15 in rabbits that received bleomycin and the extract (500 mg/kg), compared to the the animals that received bleomycin alone (Table 1). Administration of the extract alone (125 mg/kg) showed a decrease in tidal volume at baseline, and on days 3, 10 and 15, when compared to the normal saline group. Administration of the extract alone at 500 mg/kg produced decrease in tidal volume at baseline, and on day 3, when compared to animals administered normal saline (Table 1).

Table 1: *Effect of CoV-Pla2 Extract on Tidal Volume in Rabbits*

Group	Treatment	Tidal Volume (L)				
		Baseline	Day 3	Day 7	Day 10	Day 15
1	Normal Saline (5 ml/kg)	1.22±0.88	1.11±0.56	1.15±0.49	0.97±0.17	1.20±0.86
2	Bleomycin (4 U/kg)	0.17±0.07	1.22±0.51	0.69±0.70	0.99±0.45	0.64±0.15
3	Bleomycin (4 U/kg) + Ext. (125 mg/kg)	0.33±0.17	1.51±0.38	0.86±0.54	0.99±0.53	1.43±0.75
4	Bleomycin (4 U/kg) + Ext (500 mg/kg)	0.13±0.05	0.93±0.99	0.72±0.63	0.84±0.66	0.47±0.23
5	Ext. (125 mg/kg)	0.40±0.18	0.90±1.56	1.30±1.36	0.82±0.15	0.54±0.58
6	Ext. (500 mg/kg)	0.40±0.34	0.56±0.97	1.39±1.59	1.68±0.99	1.27±1.08

Values are mean ± SEM (n=3); Ext. = Extract

Inspiratory capacity increased at day 3 and decreased at days 7, 10 and 15 in the animals that received bleomycin alone at a dose of 4 U/kg when compared to animals that received normal saline (Table 2). A decrease in inspiratory capacity was observed in the group that received bleomycin (4 U/kg) plus Extract (125 mg/kg) at baseline and on day 10 when compared to animals given bleomycin alone (Table 2). Bleomycin (4 U/kg) + Extract (500 mg/kg) showed variability over the 15-day period, with a decrease observed at days 3 and 15 when compared to the bleomycin only group. Animals that were administered only the extract at a dose of 125 mg/kg showed a decrease in the parameter values compared to the normal saline control at baseline and on days 3, 7, 10 and 15 (Table 2). When compared to the normal saline control, administration of a higher dose of the extract (500 mg/kg) showed fluctuations over the 15-day period, with a decrease observed at baseline and on days 3, 7, 10 and 15.

Table 2: *Effect of CoV-Pla2 Extract on Inspiratory Capacity in Rabbits*

			Inspiratory Capacity (L)			
Group	Treatment	Baseline	Day 3	Day 7	Day 10	Day 15
1	Normal Saline (5 ml/kg)	4.93±2.07	5.29±1.35	4.79±2.87	6.83±3.11	5.22±3.19
2	Bleomycin (4 U/kg)	2.11±2.17	6.47±3.09	1.88±1.88	5.46±2.05	5.02±1.71
3	Bleomycin (4 U/kg) + Ext. (125 mg/kg)	0.90±0.82	7.43±1.40	5.04±1.03	4.90±2.17	6.38±1.86
4	Bleomycin (4 U/kg) + Ext. (500 mg/kg)	2.47±1.00	2.30±2.63	3.61±3.25	5.70±2.32	2.62±1.22
5	Ext. (125 mg/kg)	1.56±1.28	0.37±0.63[a,b]	3.11±4.90	4.98±1.63	2.88±3.41
6	Ext. (500 mg/kg)	2.89±3.49	1.37±2.37[#]	2.95±3.53	6.59±0.61	4.71±2.75

[a]$P<0.05$ *vs* normal Saline; [b]$P<0.05$ vs Bleomycin + Extract (500 mg/kg);
Values are mean ± SEM (n=3); Ext. = Extract

When compared to the normal saline control, administration of bleomycin (4 U/kg) alone caused a decrease in Inspiratory Reserve Volume at days 3 and 15. Inspiratory Reserve Volume increased in the group that received bleomycin (4 U/kg) + Extract (125 mg/kg) on days 3, 7 and 15 compared to animals administered bleomycin alone (4 U/kg), though insignificantly (Table 3). Inspiratory Reserve Volume similarly increased in the group that received bleomycin (4 U/kg) + Extract (500 mg/kg) at baseline, days 7 and 10 compared to animals administered bleomycin alone (4 U/kg) (Table 3). Animals in group 5 that received only the extract at a dose of 125 mg/kg showed an increase in Inspiratory Reserve Volume observed at day 7 followed by a decrease at Day 15, when compared to normal saline (Table 3). Inspiratory Reserve Volume was observed to increase in the group administered the extract at 500 mg/kg on day 7 when compared to the animals that received only normal saline, though in an insignificant manner (Table 3).

Table 3: *Effect of CoV-Pla2 Extract on Inspiratory Reserve Volume in Rabbits*

			Inspiratory Reserve Volume (L)			
Group	Treatment	Baseline	Day 3	Day 7	Day 10	Day 15
1	Normal Saline (5 ml/kg)	3.70±1.21	4.18±0.97	3.63±2.85	5.86±2.94	4.02±2.42
2	Bleomycin (4 U/kg)	1.94±2.09	5.25±2.66	1.19±1.18	4.48±1.61	4.38±1.60
3	Bleomycin (4 U/kg) +Ext. (125 mg/kg)	0.58±0.73	5.92±1.10	4.18±0.58	3.73±1.46	4.95±1.13
4	Bleomycin (4 U/kg) + Ext. (500 mg/kg)	2.34±0.98	1.36±1.66	2.89±2.63	4.86±1.98	2.15±1.04
5	Ext. (125 mg/kg)	1.16±1.26	2.80±4.84	5.15±4.46	2.83±1.87	2.26±2.71
6	Ext. (500 mg/kg)	2.43±3.13	0.81±1.40	4.89±4.50	4.90±1.02	3.44±1.78

Values are mean ± SEM (n=3); Ext. = Extract

A decrease in vital capacity values in animals administered bleomycin alone was observed at baseline and on day 3, 7, 10 and 15 compared to the normal saline control (Table 4). The group that received bleomycin and the extract (125 mg/kg) showed an increase in the vital capacity at day 3, 7, 10 and 15, compared to animals administered bleomycin alone that gradually decreased over the subsequent days but remained higher than the baseline value. The animals that received bleomycin along with a higher dose of extract (500 mg/kg) showed an increase in vital capacity values at Day 7 and Day 10, followed by a decrease on Day 15 (Table 4). The animals administered only the extract (125 mg/kg) showed a increase in the parameter values at Day 3, which gradually decreased over the subsequent days but remained higher than baseline. Animals in the group that received only the extract, but at a higher dose of 500 mg/kg, showed fluctuations over the 15-day period, with no clear trend observed (Table 4).

Table 4: *Effect of CoV-Pla2 Extract on Vital Capacit*

			Vital Capacity (L)			
Group	Treatment	Baseline	Day 3	Day 7	Day 10	Day 15
1	Normal Saline (5 ml/kg)	3.07±2.52	3.57±1.67	4.06±1.67	3.87±2.00	2.76±1.84
2	Bleomycin (4 U/kg)	1.27±0.72	2.73±0.70	2.32±1.43	3.03±1.45	2.40±0.58
	Bleomycin (4 U/kg)	1.10±0.63	6.74±2.03	3.77±1.43	3.57±0.37	4.30±0.31
3	+ Ext. (125 mg/kg)					
	Bleomycin (4 U/kg)	1.21±0.98	3.05±0.99	5.59±2.30	5.34±2.04	1.47±0.54
4	+ Ext. (500 mg/kg)					
5	Ext. (125 mg/kg)	1.67±1.13	6.76±3.35	6.92±2.81	3.97±2.13	2.81±2.66
6	Ext. (500 mg/kg)	2.54±2.63	3.70±0.58	2.58±1.19	4.49±2.84	3.80±1.59

Values are mean ± SEM (n=3); Ext. = Extract

4. Discussion

Bleomycin has found extensive use as a chemotherapeutic agent due to its unique feature of not inducing significant myelosuppression or immunosuppression, unlike many other cytotoxic drugs. Nevertheless, its clinical application is constrained by dose-dependent pulmonary toxicity. The instillation of bleomycin into the lungs of experimental animals has been employed as a model for studying human pulmonary fibrosis. This bleomycin model is comparatively straightforward to establish and induces consistent lung injury[25,26].

While the precise mechanism remains unclear, bleomycin-induced cell injury in the lung triggers an inflammatory response characterized by the recruitment of inflammatory cells and an upsurge in cytokine production[27]. In this present study, bleomycin challenge resulted in the reduction of forced vital capacity, one of the parameters evaluated. Vital capacity, a metric indicating the maximum volume of air that can be exhaled from the lungs after a deep inhalation, increased in animals administered the plant extract in the presence of bleomycin. This signifies the capacity of the polyherbal extract to alleviate the effects of bleomycin's injury by harnessing the antioxidant and anti-inflammatory properties present in certain plants within the blend. The ability of extracts to protect against bleomycin-induced lung injury was also previously reported[28].

The current study showed a decrease in tidal volume after bleomycin administration on days 7 and 15. These alterations may be attributed to morphological changes in the lungs induced by bleomycin treatment, consistent with findings reported in previous studies[29,30]. In addition, these changes were more likely to reduce the surface area

available for gas exchange, which is a major feature of the bleomycin model[31].

In this study, we confirmed that administering the highest dose of the extract immediately helped alleviate bleomycin-induced lung injury, evident in a significant reduction ($P<0.05$) in tidal volume compared to the control group. A consistent decrease was observed on days 3, and 15 at the lowest dose of extract when compared to the rabbits administered bleomycin alone. This is in line with the observations of Mcelroy and colleagues[32].

In clinical research, improvements in lung function are primarily used to gauge the success of therapeutics. In The present study, we showed that bleomycin (BLM) decreased inspiratory capacity on days 7, 10 and 15 after challenge in the animal model. A decrease in inspiratory capacity following bleomycin administration was reported by Li and collegues[33]. At the low dose of extract (125 mg/kg) in the presence of bleomycin, inspiratory capacity increased at days 3 and 7 and 15, compared to the bleomycin-treated group. This increase was also observed at the high dose (500 mg/kg), though this increase was not consistent across all the days. Despite this observation, the ability of the extract to mitigate against the noxious effects of bleomycin, was similar to the report of some scientists, who also observed an increase in inspiratory capacity following an intervention with an agent with protective properties[33].

A similar pattern was observed in the animals administered bleomycin alone, as Inspiratory Reserve Volume (IRV) decreased when compared to control, though in an insignificant manner ($P>0.05$). Co-administration of the extract and bleomycin caused an increase in this parameter, when compared to the animals administered bleomycin alone. This also clearly points to the ability of the extract to relieve against lung injury induced by bleomycin.

5. Conclusion

In conclusion, the findings from this study suggest that *CoV Pla-2* preparation can attenuate bleomycin-induced lung injury, as seen in increase in Tidal Volume, Vital Capacity, Inspiratory capacity and Inspiratory Reserve Volume following injury due to bleomycin.

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Conflict of Interest: The authors declare that no conflict of interest exists.

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