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Original Article

Anti-Inflammatory and Antioxidant Effects of *Aframomum Pruinosum* Seeds in Wistar Rats' Bleomycin-Induced Lung Injury

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ABSTRACT

Introduction: *Aframomum pruinosum* seeds have been widely used in traditional medicine to treat lung infections as a cytoprotective plant-derived medicine. The present study investigated the anti-inflammatory and antioxidant effects of *A. pruinosum* in Wistar rats subjected to bleomycin-induced lung injury.

Materials and methods: Bleomycin was administered subcutaneously on the first, second, and third day of the study, and other substances (Indomethacin 2 mg/kg and aqueous extract 100, 200, and 400 mg/kg) by oral route through water. Thirty-five Wistar rats (5-week-old) were divided into seven groups of five rats each, normal rats (Group 1) received distilled water (5 mL/kg), and control group of *A. pruinosum* aqueous extract (Group 2) to observe its adverse effects, received the highest dose of *A. pruinosum* (400 mg/kg), the negative group (Group 3) received bleomycin 0.3 mg/kg and distilled water 5 mL/kg, positive group (Group 4) received bleomycin 0.3 mg/kg and indomethacin 2 mg/kg, Group 5 received bleomycin 0.3 mg/kg and aqueous extract of *A. pruinosum* 100 mg/kg, Group 6 received bleomycin 0.3 mg/kg and aqueous extract of *A. pruinosum* 200mg/kg, and Group 7 received bleomycin 0.3 mg/kg and aqueous extract of *A. pruinosum* 400 mg/kg. GraphPad Prism 8.0.1.244 was used to compare data.

Results: The body weight of rats (group 3) exposed to bleomycin has decreased compared to the normal group. Groups exposed to bleomycin and treated with indomethacin or aqueous extract have increased body weight compared to the negative group. Co-treatment of bleomycin and aqueous extract of *A. pruinosum* (400 mg/kg) significantly increased lung weight compared to the negative control. Administration of aqueous extract of *A. pruinosum* (400 mg/kg) significantly decreased the levels of Interleukin 1 beta, TNF-α, Interleukin 1 beta, and increased Interleukin 10 level compared to the negative control. Co-treatment of bleomycin and aqueous extract significantly alleviated the toxic effects of bleomycin in oxidative parameters, such as superoxide dismutase, catalase, malondialdehyde, and protein, compared to the negative control, and protected the rats against oxidative damage.

Conclusion: The aqueous extract of *Aframomum pruinosum* seeds had anti-inflammatory and antioxidant potential effects, which might be considered an effective therapeutic against lung lesions.

1. Introduction

Acute and chronic lung disorders are known as interstitial lung diseases (ILD)[1]. Acute interstitial pneumonia is characterized clinically by a rapid onset of respiratory failure and has a grave prognosis, with over 70 percent mortality in patients within three months, despite mechanical ventilation[2]. Acute interstitial pneumonia resembles respiratory distress syndrome (ARDS), which is induced by diverse causes such as highly concentrated oxygen, poisonous gases such as ammonia, chlorine, ozone and formaldehyde, severe infections, and shock status[3]. The histologic basis of interstitial pneumonia is the infiltration of leukocytes such as monocytes, neutrophils, and eosinophils into the pulmonary interstitial space and diffuse alveolar destruction. The majority of chronic ILD is referred

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to as idiopathic pulmonary fibrosis[4]. Multiple mediators, including reactive oxygen species (ROS), cytokines, chemokines, eicosanoids, prostaglandin, and apoptosis-related genes, may be involved in the establishment of ILD[4]. However, the pathogenesis of human ILD is still not well investigated[5]. The ILD remains a principal threat to public health and remains a problem worldwide, despite significant developments in modern medicine; no fully effective medications exist to promote lung function, provide total organ protection, or assist in regulating pnemocytes[5]. Therefore, more studies in animals have been conducted, targeting the development of an appropriate drug in the treatment of ILD, which involves the use of medicinal plants[6].

Bleomycin was discovered by Umezawa in 1966 and was originally isolated from the fungus *Streptomyces verticillus.* Bleomycin exerts antitumor effects by inducing tumor cell death and inhibition of tumor angiogenesis[7]. Bleomycin is a prototypical endotoxin derived from *Streptomyces verticillus* and is the initial stimulus leading to septic shock syndrome[7]. Bleomycin can directly activate macrophages, endothelial cells, and the complement-triggering production of inflammatory mediators, such as nitric oxide (NO), tumor necrosis factor-a (TNF-a), interleukins (ILs), and leukotrienes[8]. In particular, a large amount of NO is produced from L-arginine by inducible NOS (iNOS), thereby causing detrimental effects in the lungs[8]. Although physiological NO production, such as nitrogen dioxide and nitrous acid, has beneficial microbicidal, anti-parasite, and anti-tumor effects[9]. Excessive NO produced by iNOS is a mediator for inflammatory diseases and causes cell injury by generating reactive radicals, such as peroxynitrite[9]. Bleomycin, a member of the glycopeptide group of antibiotics, is a chemotherapeutic medicine used clinically for a variety of human malignancies, such as melanoma and malignant neoplasm[9]. It has been reported that administration of a high dose of bleomycin often leads to lung injury and pulmonary fibrosis in bleomycin-treated patients[10]. Bleomycin-induced lung fibrosis is a widely used animal model for human idiopathic pulmonary fibrosis[11]. Several studies have indicated that ROS, such as oxygen radicals, are involved in bleomycin-induced lung injury because the antioxidants superoxide dismutase and N-acetyl-l-cysteine can partly inhibit bleomycin-induced lung injury. Therefore, bleomycin-induced lung fibrosis is mediated by the generation of ROS, in the fact that bleomycin stimulates the production of cytokines[12]. Interferon gamma is an important cytokine in this respect because it can activate the respiratory or oxidative burst products to generate nitric oxide, hydrogen peroxide, hypochlorite, hydroxyl, and superoxide[12]. On the other hand, skin reactions are the most common side effects, including erythema, hyperpigmentation of the skin, striae, vesiculation, skin peeling, thickening of the skin and nail beds, hyperkeratosis, and ulceration may also occur[12].

Although there are many medicines used for treating lung diseases, such as adriamycin, vinblastine, and dacarbazine, most of these medicines produce several adverse reactions, such as diarrhea, depression, anxiety, and difficulty sleeping[11]. In addition, the major limitation of bleomycin therapy is usually pulmonary toxicity, which can be life-threatening and has been described in up to 10 % of patients receiving the drug[13]. One of the potential determinants of bleomycin toxicity is the bleomycin hydrolase enzyme, which is primarily responsible for metabolizing bleomycin to nontoxic molecules[14]. Two organs are the most common sites of bleomycin toxicity, the lungs and the skin, which have the lowest levels of the bleomycin hydrolase enzyme due to the feasibility of cloning the gene that encodes bleomycin hydrolase[15]. Hence, exploring new compounds from plant-based sources presented a viable alternative.

In Cameroon, 20 species of the *Aframomum* genus, such as *Zingiberaceae*, were found and widely used for medicinal, ethno-dietary, cultural, and spiritual purposes[16]. Among different species of the *Aframomum*, *Aframomum pruinosum* is used to cure several afflictions, such as women's sterility and schizophrenia. *Aframomum* species might also have antisympathetic properties and tranquilizing effects because the plant inhibits sympathetic neurons[16]. phytochemical analysis of two extracts (aqueous extract and ethanolic extract of *A. pruinosum*) was performed with both qualitative and quantitative approaches. Qualitative tests for alkaloids, saponins, glycosides, and reducing sugar were performed[17]. Quantitative determination of total phenolic compounds was assessed, and the total flavonoid content was estimated[17]. The gas chromatography/mass spectrometry (TOPTION Gas Chromatography Machine Supplier China; GC/MS) analyses of *A. pruinosum* essential oils allowed the identification of 73 components, including E-R-neolidol, hydrocarbons, β-pinene, and sesquiterpenes. Different phytochemical characteristics of plants, such as alkaloids, triterpenoids, steroids, and flavonoids, are attributed to *A. pruinosum's* prospective curative properties[18]. Anti-inflammatory activity on cytokines of *A. pruinosum* seeds has never been reported before. The present study aimed to evaluate the anti-inflammatory and antioxidant effects of *A. pruinosum* seeds aqueous extract on bleomycin-induced lung injury in the Wistar animal model.

2. Materials and Methods

2.1. Ethical approval

Animals were handled by considering the ethical guidelines of the Cameroon National Veterinary Laboratory as referenced by the approval and head central No 001/17CCS/MINPIA/RD-NW/DD-MELSSV.

2.2. Collection and preparation of plant material

The fresh seeds of *A. pruinosum* were collected from mature plants cultivated in the Western region of Cameroon, more precisely in the subdivisions of Babadjou, in a village known as Bamepa'ah (5° 14' 5" North and 10° 25 57" East) in September 2023. Firstly, the seeds were identified with the help of local herbalists. The gathered information included vernacular name (Tso'oh or seeds of peace), parts used such as root, leaves, fruits, and seeds, quantity used (half-coffee spoon in a cup of water), and the

ailment treated (jaundice, epilepsy, infertility, and respiratory impairment). Secondly, the samples were collected with acceptable bio-conservative methods and were properly sorted out, cleaned, and transported to the University of Bamenda, Cameroon. The plant samples were finally provided to an acknowledged taxonomist for botanical authentication, and the dry plant samples of the *A. pruinosum* were deposited and kept at room temperature at the University of Bamenda, Cameroon, for future reference. The seeds of *A. pruinosum* were collected from fruits and air-dried away from direct sunlight at 22 to 25 ^{o}C for two months until properly dried. Then, 1.5 kg of seeds were ground into fine homogenous powder using an electrical mill (Pascal engineering Co., ltd., Gatwick Road Crawley, Sussex, England), followed by sieving through a mesh sieve to obtain 1.3 kg and stored at room temperature, waiting for extraction.

2.2.1. Extraction of Aframomum pruinosum seeds

The dried powder (800g) was macerated in 7.5 liters of distilled water for 48 hours to extract the solution with active compounds of *A. pruinosum.* The solution was filtered using Whatman's No. 1 filter papers (Z240079, made in England)[19]. The obtained filtrate was evaporated at 40^{o}C to dryness for 72 hours in a thermostat oven (DHG-9101-15APEC) in the biochemistry laboratory, University of Bamenda, Cameroon. A dark brown solid extract was obtained, weighed, and 36.43 g recorded, representing an extraction yield of 4.55 %. This dark brown solid was stored in a tightly labelled bottle in a refrigerator at 4^{o}C until further use. Based on the information from the local herbalists, the doses of 100, 200, and 400mg/kg body weight (bw) were selected. The extract (400mg) was dissolved in 50 mL of distilled water and administered by the oral route (drinking) to rats.

2.3. Animals

Thirty-five healthy male and female Wistar rats (*Rattus norvegicus*, 5-week-old, average weight from 90 to 130 g) were obtained from a breeder at Mile 4, Nkwen Bamenda, North-west region, Cameroon. The experimental rats were raised in the animal house and kept under standard laboratory conditions (12 hours light and dark sequence, temperature 23 ± 3 ^{o}C, and 35 to 60% humidity)[16]. The animals were caged in 20 plastic cages of dimensions (45.5 cm in diameter and 22.5 cm in height), with an appropriate diet (corn flour, soya beans, and wheat brown)[16], and tap water *ad libitum*. All the animals were acclimatized for one week before the beginning of the experiment, and to ensure that the new substances were completely administered to the rats and the desired dose was in their bodies, food was withdrawn 12 hours before the initiation of the experiments.

2.4. Experimental design

After the acclimatization period of one week, 35 male and female Wistar rats were weighed and divided into seven different groups[17]. Bleomycin was administered subcutaneously, and other substances (indomethacin and aqueous extract) by the oral route. The groups were named 1 to 7 and designed as follows. Group 1 was the normal group and received distilled water (5mL/kg bw), Group 2 was the control of extract and received the highest dose of aqueous extract *A. pruinosum* (400 mg/kg) to control possible lung toxicity by extract, Group 3 was the negative group and received bleomycin (0.3 mg/kg) and distilled water (5 mL/kg), Group 4 was the positive group and received bleomycin (0.3 mg/kg) and indomethacin (2 mg/kg), Group 5 received bleomycin (0.3 mg/kg) and aqueous extract of *A. pruinosum* (100 mg/kg), Group 6 received bleomycin (0.3mg/kg) and aqueous extract of *A. pruinosum* (200 mg/kg), and Group 7 received bleomycin (0.3 mg/kg) and aqueous extract of *A. pruinosum* (400 mg/kg). From Groups 3 to 7, bleomycin (0.3 mg/kg) was subcutaneously administered to induce lung injury every Tuesday, Thursday, and Saturday, and treatment was applied every Wednesday, Friday, and Sunday for four weeks. At the end of the experiment on the twenty-ninth day, the animals were sacrificed. Blood samples were collected and centrifuged (Lmc 3000 Biosan, France) at 3000 rpm for 15 minutes to obtain 2 mL of clear serum. The Serum obtained was stored at -20 ^{o}C and used for cytokine tests such as Interleukin 1 beta (IL-1β), Interleukin 6 (IL-6), and Interleukin 10 (IL-10). Then, animals were dissected, and lung tissues were removed and weighed to calculate lung index (lung weight/rat weight). One part of the lung assessed oxidative stress parameters, and the other was trimmed down for histological analysis.

2.4.1. Cytokine tests

Interleukin 1 beta, tumor necrosis factor-alpha, interleukin 6, and interleukin-10 were measured using a specific ELISA kit (Alpha Diagnostic International, U.S) as used, and the experiment was carried out in the biological science laboratory of the University of Buea, Cameroon. Briefly, the plates were sensitized by putting 20 µl of the antibody and incubated for 18 hours at 5 ^{0}C. After the incubation, 20 µl of serum was added to the wells, the wells were washed and discarded after 10 minutes. After washing and drying, 20 µl of the conjugate antibody was added into each well after two hours at room temperature, 100 µl of the substrate was added into each well and the reaction was stopped after 30 minutes by adding 20 µl of the stop solution and the results read using an ELISA machine (Alpha Diagnostic International, U.S) at a wave length of 450 nm.

2.5. Antioxidant activity

Measurement of the biomarkers in oxidative stress included the procedure to estimate malondialdehyde (MDA) activity by the method of Ohkawa et al.[20]. The activity of catalase (CAT) in lung tissues was evaluated by the method of Sinha[21]. The superoxide dismutase (SOD) activity was determined in the supernatant of homogenate by the method of Misra and Fridovich[22]. The protein content in the homogenate was measured using the method described by Gornall et al.[23].

2.6. Histological studies

To observe the lung sections under a microscope, 10% formalin was used for the fixation of the collected lung sample[20]. Later, samples were fixed, sectioned, and stained. In fixation, lung samples of all experimental groups were kept in 10% neutral formalin. After fixation, tissues were dehydrated in different percentages of alcohol (75%, 95%, and 100% absolute), embedded in a paraffin block, and serially sectioned (5 µm) using a microtome (Leica, Germany). Lung sections were stained with Mayer hematoxylin and eosin (H&E)[19]. Bleomycin-induced lung injury was observed using a microscope (Zeiss, Hallherbermoos, Germany)[19].

2.7. Statistical analysis

All data were presented as mean ± SEM. The significant difference among means was assessed by one-way and two-way Analysis of Variance (ANOVA), followed by Bonferroni post-test using GraphPad Prism, version 8.0.1.244. Differences between means were considered significant at $p < 0.05$.

3. Results

3.1. Body weight

There was an increase in the body weight of normal rats during the four weeks at the animal house. Bleomycin in the negative group did not significantly decrease ($p > 0.05$) the body weight of rats compared to the normal rats. Groups 5, 6, and 7, receiving the aqueous extract (100, 200, and 400 mg/kg), did not significantly obtain higher body weight compared to the negative group (Group 3). Figure 1 shows an increasing weight in Group 4.

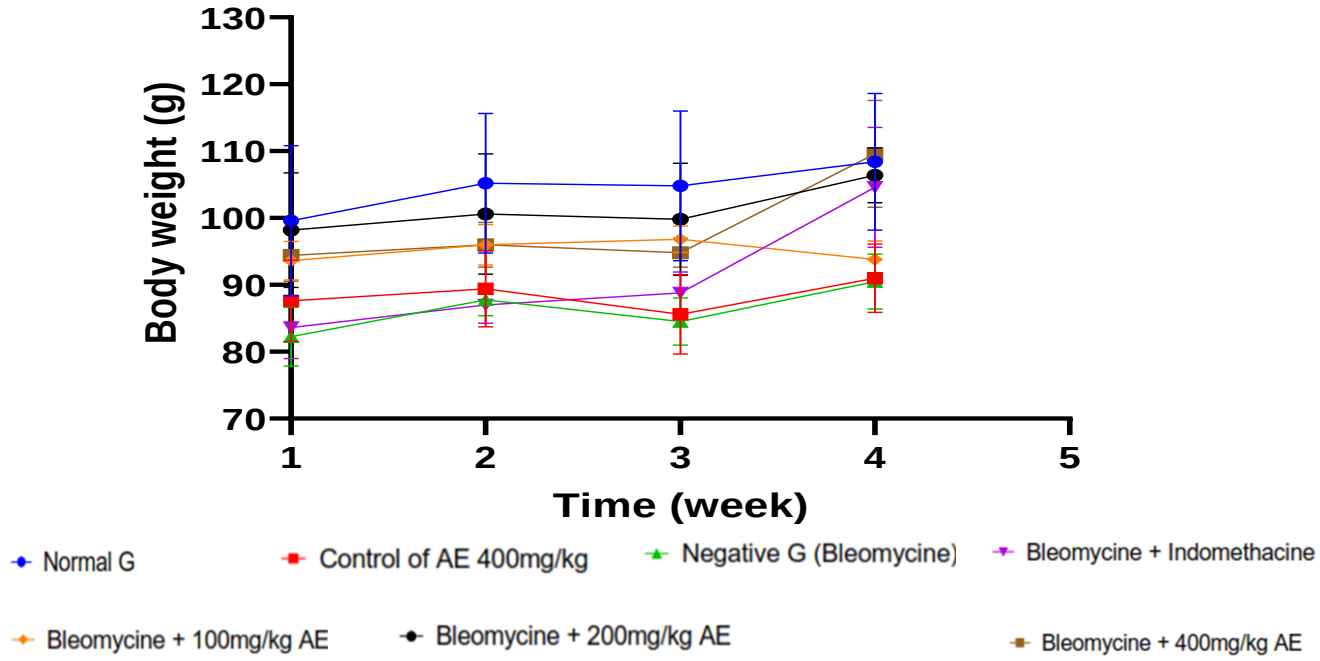

Figure 1. Effect of aqueous extract of *Aframomum pruinosum* on the body weights of bleomycin-induced lung injury in 5-week-old rats. The curves represent means ± SEM, Normal G (blue line): Normal group, Negative G (green line): Negative group, AE: Aqueous extract.

3.2. Lung index

The lung weight of the negative group (Group 3) was significantly decreased ($p < 0.001$; Figure 2) compared to the normal group (Group 1). In groups 5, 6, and 7 (100, 200, and 400 mg/kg of the *A. pruinosum* seeds' aqueous extract), the lung weight significantly increased ($p < 0.01$, $p < 0.05$, $p < 0.001$, respectively) compared to the negative group (Group 3). The lung weight of the rats in Group 4 significantly increased ($p < 0.001$) compared to the negative group (Group 3, Figure 2).

3.3. Interleukin 1 beta

The concentration of IL-1β was also evaluated during the experiment. Figure 3 indicated that the negative group (bleomycin only) had a higher concentration ($p < 0.01$) of IL-1β than the normal group (Group 1). The level of IL-1β significantly reduced ($p < 0.05$) in groups 6 and 7, compared to the negative group (Group 3). Interleukin 1 beta concentration in Group 4 significantly decreased ($p < 0.05$) compared to the negative group (Group 3).

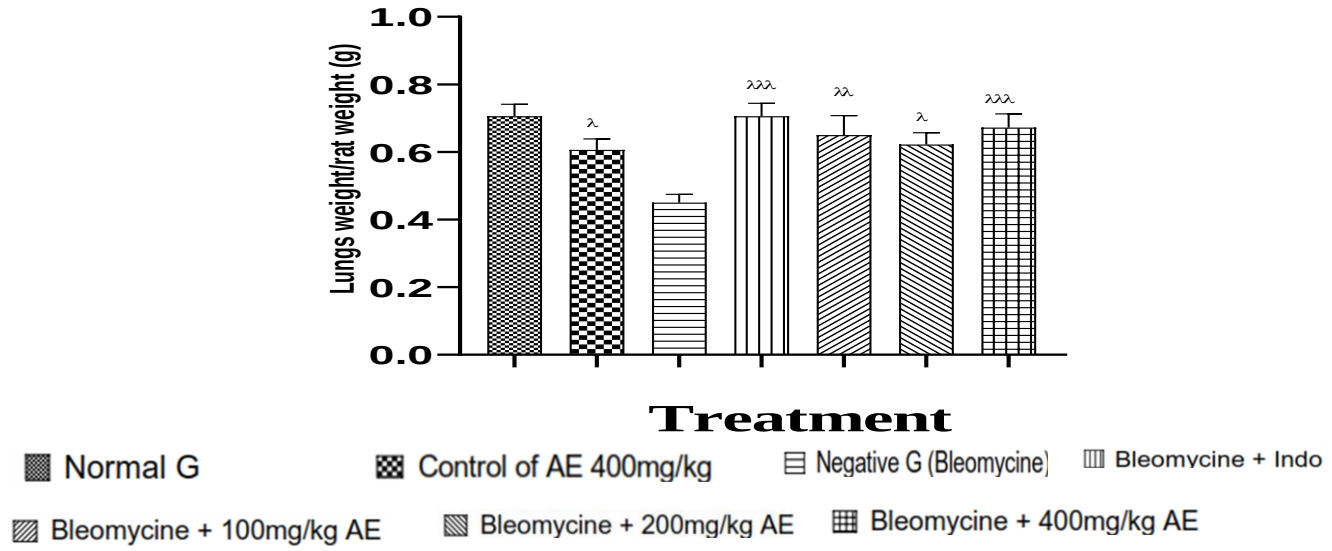

Figure 2. Effects of the aqueous extract of *Aframomum pruinosum* seeds on lung index (lung weight/rat weight) in bleomycin-induced lung injury of 5-week-old rats. The histograms represent means ± SEM. Group 3 is significantly different ($p < 0.001$) compared to the normal group. Groups 4, 5, 6, and 7 are significantly different ($p < 0.05$, $p < 0.01$, and $p< 0.001$) compared to the negative group. Normal G: Normal group, AE: Aqueous extract, Indo: Indomethacin, Negative G: Negative group.

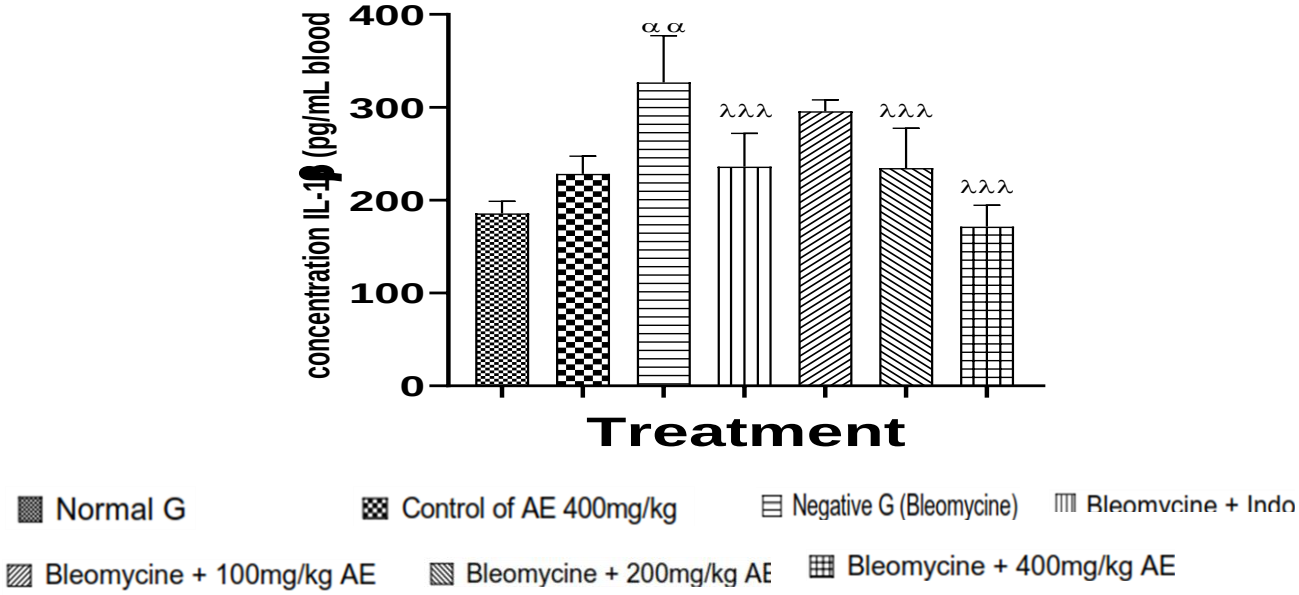

Figure 3. Effect of the aqueous extract of *Aframomum pruinosum* seeds on the concentration of interleukin-1 beta in the serum of bleomycin-exposed rats aged 5 weeks. The histograms represent means ± SEM. Group 3 is significantly different ($p < 0.001$) compared to the normal group. Groups 4, 6, and 7 are significantly different ($p < 0.001$) compared to the negative group. Normal G: Normal group, AE: Aqueous extract, Indo: Indomethacin, Negative G: Negative group.

3.4. Tumor necrosis factor

In the negative group (Group 3), the concentration of TNF-α significantly increased ($p < 0.001$) compared to the normal group (Group 1). Considering groups 5, 6, and 7, the concentration of TNF-α significantly decreased ($p < 0.05$) compared to the negative group (Group 3, Figure 4).

3.5. Interleukin 6

Interleukin-6 significantly increased ($p < 0.01$) in the negative group (Group 3, Figure 5) compared to the normal group (Group 1). In groups 6 and 7, IL-6 significantly decreased ($p < 0.001$) compared to the negative group (Group 3).

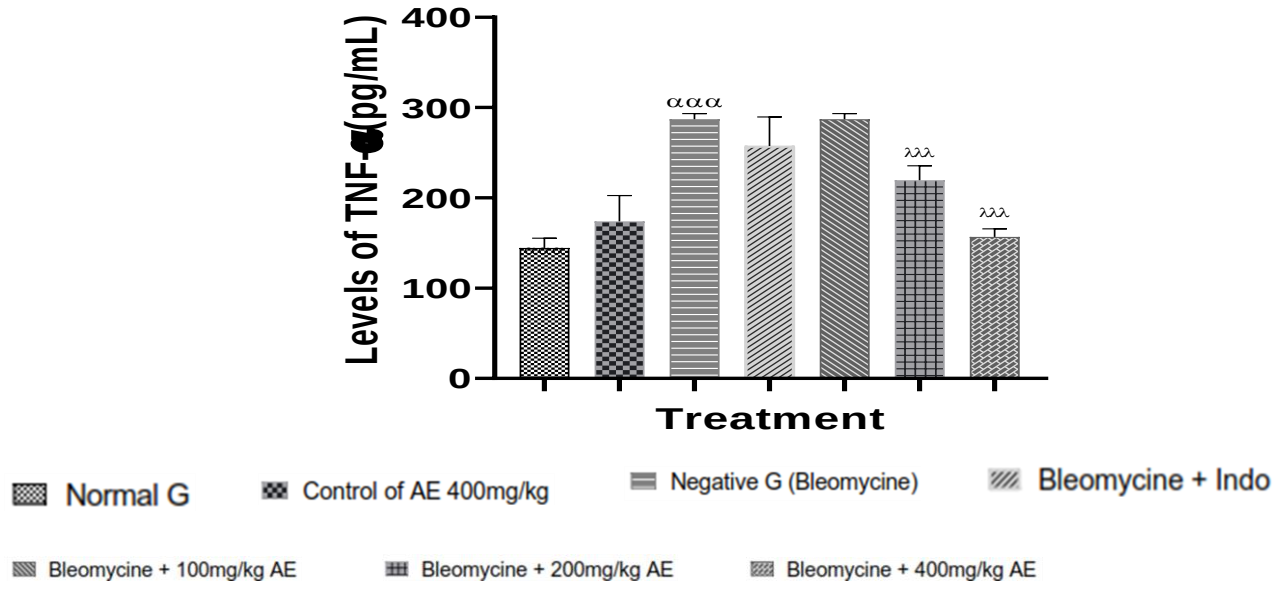

Figure 4. Effect of the aqueous extract of *Aframomum pruinosum* seeds on the concentration of tumor necrosis factor-alpha in the serum of 5-week-old bleomycin-exposed rats. The histograms represent means ± SEM. Group 3 is significantly different ($p < 0.001$) compared to the normal group. Groups 6 and 7 are significantly different ($p < 0.001$) compared to the negative group. Normal G: Normal group, AE: Aqueous extract, Indo: Indomethacin, Negative G: Negative group.

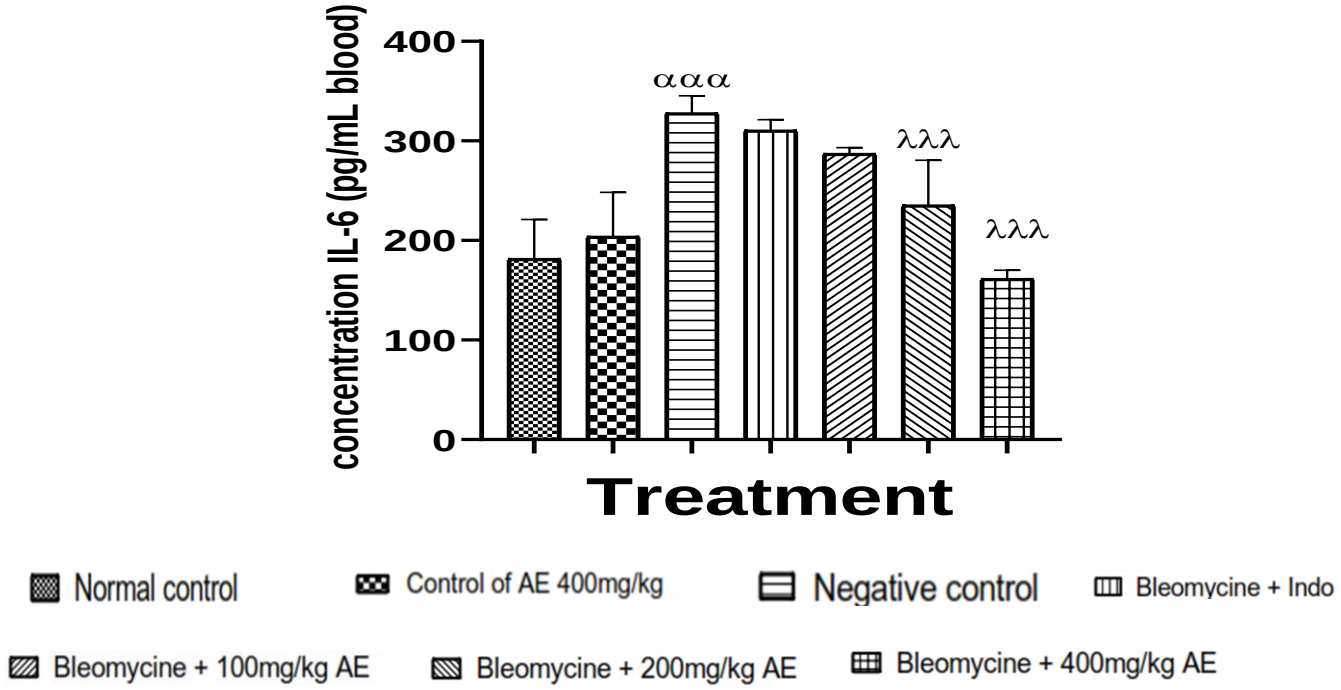

Figure 5. Effects of the aqueous extract of *Aframomum pruinosum* seeds on the concentration of interleukin-6 in the serum of 5-week-old bleomycin-exposed rats. The values represent means ± SEM of five rats per group. Group 3 is significantly different ($p < 0.001$) compared to the normal group. Groups 6 and 7 are significantly different ($p < 0.001$) compared to the negative group. Normal G: Normal group, AE: Aqueous extract, Indo: Indomethacin, Negative G: Negative group.

3.6. Interleukin 10

In Figure 6, IL-10 significantly decreased ($p < 0.001$) in the negative group (Group 3) compared to the normal group (Group 1). Considering groups 6 and 7, IL-10 significantly increased ($p < 0.001$) compared to the negative group (Group 3). In Group 4, IL-10 significantly increased ($p < 0.01$) compared to the negative group (Group 3, Figure 6).

3.7. Oxidative stress and protein level

In Group 3, bleomycin significantly increased ($p < 0.01$) the protein activity in the lungs compared to the normal group 1 (Table 1). In Groups 4 and 7, Co-administration with bleomycin and the plant extract, and/or significantly decreased ($p < 0.01$) the protein activity compared to the negative group (Group 3). In Group 7, treatment with

bleomycin and the highest dose of the plant extract (400 mg/kg) significantly decreased lung catalase activity of the rats compared to the negative group (Group 3; $p < 0.05$). Co-treatment of rats with bleomycin and *A. pruinosum* seeds (Group7, 400 mg/kg) increased slightly the activity of SOD (70 U/mg protein), as compared to the negative group (Group 3; $p < 0.05$). In Group 3, MDA activity significantly increased ($p < 0.01$) compared to the normal group (Group 1). In groups 6 and 7, MDA activity significantly increased ($p < 0.05$) compared to the negative group (Group 3).

3.8. Histology

In Figure 7, the lung sections of the normal group (Group 1, Figure 7A) and the control of aqueous extract group (Group 2, Figure 7B) illustrated normal alveoli, alveolar duct, and alveolar sac with the thin alveolar wall, which demonstrated normal terminal bronchiole and bronchial wall and regular lung histology. Whereas, the negative group (Group 3, Figure 7C) showed lung alterations characterized by alveoli coated by hyperplastic type II pneumocytes, macrophages, and thick alveolar walls. The positive group (Group 4, Figure 7D) presented a few abnormalities, such as disarrangement of alveolar architecture. The rats in Group 5 still presented abnormalities such as interstitial fibrosis and intra-alveolar hemorrhage (Figure 7E). In groups 6 and 7, administration of the bleomycin with aqueous extract of *A. pruinosum* at the doses of 200 and 400 mg/kg normalized the lung architectures (Figure 7F and 7G). The dose of 400 mg/kg of *A. pruinosum* indicated an improvement in lung structures similar to the normal group (Figure 7G). The lung structures in Group 2 (400mg/kg; Figure 7B) were not similar to the normal group (Figure 7A).

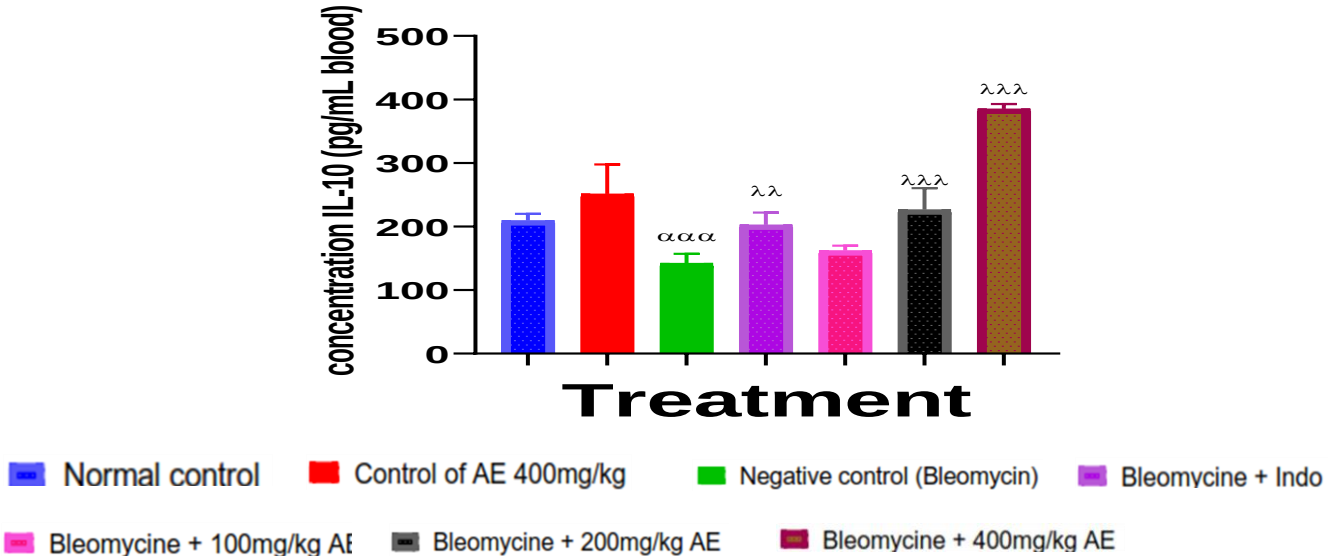

Figure 6. Effect of the aqueous extract of *Aframomum pruinosum* seeds on the concentration of interleukin 10 in the serum of 5-week-old bleomycin-exposed rats. The histograms represent means ± SEM of five rats per group. Group 3 is significantly different ($p < 0.001$) compared to the normal group. Groups 6 and 7 are significantly different ($p < 0.001$) compared to the negative group. Normal G: Normal group; AE: Aqueous extract; Indo: Indomethacin; Negative G: Negative group.

Table 1. Effect of the aqueous extract of *Aframomum pruinosum* seeds on oxidative stress and protein level in 5-week-old bleomycin-exposed rats

	Stress parameters (%)			
Animal groups	**Lung proteins (mg/g)**	**CAT (IU/mg of proteins)**	**SOD (IU/mg of proteins)**	**MDA activity (IU/mg of proteins)**
Group 1 (Distilled water 5 ml/kg)	0,054 ± 0,001	0,805 ± 0,083	89,501 ± 10,202	0,065 ± 0,012
Group 2 (Control of AE 400 mg/kg)	0,053 ± 0,009$^{\lambda}$ (1,48)	0,845 ± 0,058 (4,73)	80,657 ± 12,230 (9,88)	0,066 ± 0,036 (13,50)
Group 3 (Negative control, bleomycin only)	0,073 ± 0,011$^{\alpha}$ (26,82)	0,727 ± 0,072 (9,75)	69,334 ± 9,977$^{\alpha}$ (22,53)	0,096 ± 0,009$^{\alpha\alpha}$ (47,69)
Group 4 (positive group, bleomycin 4 mg/kg + Indomethacin 2 mg/kg)	0,049 ± 0,009$^{\lambda\lambda}$ (33,33)	0,642 ± 0,129 (11,691)	64,933 ± 11,723 (6,34)	0,083 ± 0,010 (11,27)
Group 5 (Bleomycin + 100 mg/kg AE)	0,058 ± 0,009 (21,13)	0,6878 ± 0,039 (5,39)	67,788 ± 8,529 (2,23)	0,083 ± 0,018 (11,27)
Group 6 (Bleomycin + 200 mg/kg AE)	0,062 ± 0,012 (14,90)	0,720 ±0,123 (0,93)	64,480 ± 13,947 (7,00)	0,072 ± 0,010$^{\lambda}$ (23,25)
Group 7 (Bleomycin + 400 mg/kg AE)	0,050 ± 0,010$^{\lambda\lambda}$ (31,43)	0,606 ± 0,135$^{\alpha}$ (16,61)	70,460 ± 11,377 (1,62)	0,057 ± 0,007$^{\lambda\lambda}$ (40,00)

The values ($^{\lambda, \alpha, \lambda\lambda, \alpha\alpha}$) are expressed as mean ± SEM. Group 3 is significantly different ($p < 0.05$) compared to group 1. Groups 4, 6, and 7 are significantly different ($p < 0.05$, $p < 0.01$) compared to the negative group. CAT: Catalase, SOD: Superoxide dismutase, MDA: Malondialdehyde, AE: Aqueous extract.

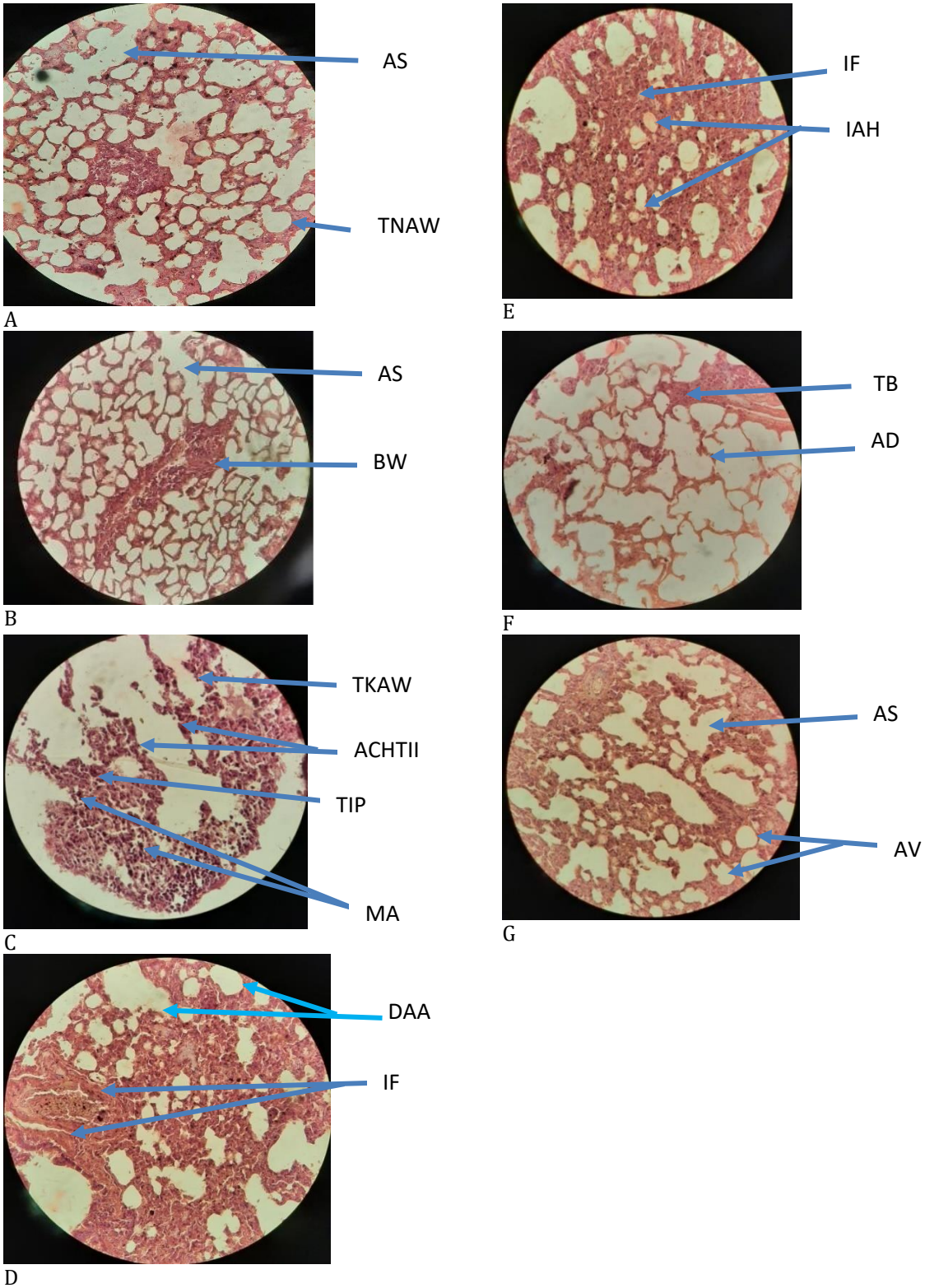

Figure 7. Microphotographs of the lungs of rats in different treatment groups. This figure demonstrates the microphotographs of the lung structure to observe the effect of the aqueous extract of A. pruinosum seeds in intoxicated rats by bleomycin and morphological analysis. Figure 7A: Normal group, Figure 7B: Control of aqueous extract 400 mg/kg, Figure 7C: Negative control, Figure 7D: Bleomycine + indomethacin, Figure 7E: Bleomycine + 100mg/kg aqueous extract, Figure 7F: Bleomycine + 200mg/kg aqueous extract, Figure 7G: Bleomycine + 400mg/kg aqueous extract. DAA: Disarrangement of alveolar architecture, IF: Interstitial fibrosis, IAH: Intra-alveolar haemorrhage, ACHTII: Alveoli coated by hyperplastic type II pneumocytes, TIP: Type I pneumocytes, MA: Macrophages, TKAW: Thick alveolar wall, TNAW: Thin alveolar wall, BW: Bronchial wall, AD: Alveolar duct, AS: Alveolar sac, AV: Alveoli, TB: Terminal bronchiole. H&E, Magnification (x 400).

4. Discussion

Bleomycin exerts its antitumor effect by inducing tumor cell death. Moreover, the effects of bleomycin are cell cycle, with bleomycin main effects occurring during the cell cycle,

namely G2 and M phases[24]. The current results indicated that the body weight of the negative group was lower compared to normal group, suggesting that the cumulative dose of bleomycin has reached certain values in the body and produced hyperkeratosis and ulceration may occur to reduce the body weight and damaged the cell cycle in many organs, including bone tissues, liver, skin and lungs. The results on figure indicated that, administration of aqueous extract increased the body weight of rats compared to negative group, suggesting that aqueous extract of *A. pruinosum* might act on cell cycle and related enzymes Skp-Cullin-F-box complex (SCF) and Antigen presenting Cell (APC) and reduce and or replace ulceration to increase the body weight of rats. Moreover, the lung weight of the negative group was significantly reduced compared to the normal group. The major limitation of bleomycin therapy is usually pulmonary toxicity, which can be considered threatening and has been described in up to 10% of patients receiving the medicine. In addition, one of the potential determinants of bleomycin toxicity is bleomycin hydrolase, the enzyme that is primarily responsible for metabolizing bleomycin to nontoxic molecules[25]. Therefore, in the current study, the lack of hydrolases lung could be responsible for the reduction of lung weight in the negative group. Co-administration of aqueous extract of *A. pruinosum* significantly increased the lung weight compared to the negative group, probably because the extract contains bioactive components such as hydrocarbons, β-pinene, and sesquiterpenes that activated or replaced hydrolase to encode bleomycin hydrolase, which reduced the side effects of bleomycin and then increased the lung weight.

The inflammation is initiated by complex processes triggered by fungi such as bleomycin, which is a prototypical endotoxin derived from *Streptomyces verticillus*[7]. Bleomycin can directly activate macrophages, which trigger the production of inflammatory mediators such as NO, TNF-α, ILs, and leukotrienes, and reduction of proinflammatory mediators such as IL-10, IL-17, and transforming growth factor beta (TGF-β)[5]. The current results indicated that in the negative group, bleomycin significantly increased the level of TNF-α, IL-1β, IL-6, and decreased IL-10 level compared to the normal group, suggesting the adverse effects of bleomycin as a fungus, which activated macrophages to trigger the production of inflammatory mediators such as cytokines.

Treatment with aqueous extract of reduced the side effects of bleomycin and significantly reduced the concentration of TNF-α, IL-1β, and IL-6, and significantly increased the concentration of IL-10. The current results suggested that *A. pruinosum* contains some components that could inhibit Nuclear Factor-kappa B (NF-kB). In fact, among the components contained in *Glycyrrhizae radix*, liquiritigenin effectively blocks the potentiated cytotoxicity induced by cadmium in mice[26]. Kim et al.[27] demonstrated the anti-inflammatory effects of liquiritigenin as a consequence of the inhibition of NF-kB-dependent iNOS and proinflammatory cytokines production. The present results indicated that aqueous extract significantly reduced the production of TNF-α, IL-1β, IL-6, and significantly increased the production of IL-10 compared to the negative group. The current results suggested that *A. pruinosum* might act at the level of NF-kB via iNOS to alleviate inflammatory mediators. Liquiritigenin effectively blocked the induction of both iNOS protein and its mRNA[28], suggesting that components of the aqueous extract of *A. pruinosum* might act by blocking mRNA before inhibiting TNF-α, IL-1β, and IL-6 at the transcriptional level. The iNOS gene promoter includes the binding sites for transcription factors such as NF-kB, AP-1, C/EBP, and CREB[29], NF-kB, in particular, is an essential transcription factor, suggesting that the extract of *A. pruinosum* could act on those transcription factors to regulate cytokines.

Nuclear Factor-kappa B forms a homo or heterodimer complex, and NF-kB heterodimers of p65 and p50 subunits can be activated by exposure cells to bleomycin or inflammatory cytokines. In the nucleus, NF-Kb dimers bind to target DNA elements and activate transcription of genes encoding for proteins involved in immune or inflammatory reactions[30]. In the current study, the significant regulation in cytokine concentration compared to the negative group suggested that components of aqueous extract might act at p65 and p50 to bind NF-Kb to DNA elements and inhibit transcription of genes involved in inflammatory reactions. Chemical investigations on essential oils from Aframomum species revealed that *A. pruinosum* seed oils were rich in (E)-nerolidol, while the leaf essential oil was dominated by sesquiterpenes[27].

It is well recognized that activation of IkBx kinase complex phosphorylates IkBx, and Ikappa B kinase complex may be activated by different upstream kinase family member[28]. Therefore, it remains to be established whether the aqueous extract of *A. pruinosum* acts on these upstream kinases to know the exact pharmacological target of *A. pruinosum*.

In the current result, bleomycin reduced the activities of CAT and SOD also increased protein and MDA levels in negative group compared to normal group. It is known that bleomycin induces the generation of reactive oxygen radicals by forming complex Fe^{3+}, which is subsequently oxidized to Fe^{2+}, resulting in the reduction of oxygen to free radicals[31]. These free radicals affect the enzymes CAT, MDA, and SOD dangerously[31]. Treatment with aqueous extract of *A. pruinosum* increased significantly rat's CAT and SOD, also reduced protein and MDA levels compared to negative group, suggesting that *A. pruinosum* substances might act to combine and reduce free radicals of bleomycin. *A. pruinosum* components might act on the enzymes of the membrane to restore the stability of these oxidative stress biomarkers.

In the current study, the control group did not indicate any significant difference compared to the normal group in all experimental tests, suggesting that the *A. pruinosum* at a dose of 400 mg/kg could be used as a food supplement. phytochemical analysis of the two extracts of *A. pruinosum* (aqueous extract and ethanolic extract) was performed with both qualitative and quantitative approaches. Qualitative tests for alkaloids, saponins, glycosides, and reducing sugars were performed according to the methods described by Ukoha et al.[32]. The

quantitative determination of total phenolic compounds was assessed using the Folin-Ciocalteu method[33], while the total flavonoids content was estimated as described by Sonfack et al.[34].

5. Conclusion

In general, the current results demonstrated that the aqueous extract of *Aframomum pruinosum* seeds possesses anti-inflammatory and antioxidant effects, which result from the inhibition of TNF-α, IL-1β, IL-6, and the activation of IL-10. The antioxidant effects arise from increased activities of CAT and SOD, along with a reduction of protein and MDA levels. The experimental dose of aqueous extract (400 mg/kg) was above the normal dose. The present findings demonstrated the existence of a new class of anti-inflammatory compounds in the aqueous extract of *A. pruinosum* seeds and offered the possibility of treatment for inflammatory diseases and oxidative stress. Isolation of active molecules from *A. pruinosum* seeds and studying the action of *A. pruinosum* molecules on different target cells is suggested for further studies.

Declarations

Competing interests

The authors declared there is no conflict of interests.

Authors' contributions

Oumar Mahamat, Dita Tsomelou Gric, and Youmbie Djanche Duplex Bonheur conceived and designed the study. Dita Tsomelou Gric and Tuekam Kayo Raoul Polycarpe performed the experiments. Dita Tsomelou Gric and Youmbie Djanche Duplex Bonheur analyzed and interpreted the data. Oumar Mahamat and Youmbie Djanche Duplex Bonheur contributed to the writing of the manuscript. All authors have read and approved the final edition of the manuscript.

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Ethical considerations

The article was written originally based on the collected data from the present research, and it is submitted for the first time to this journal. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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