

Bacoside A Averts Oxidative Stress and Inflammation in Pulmonary Disease Induced by Cigarette Smoke in Rats Through NRF2/ATF4 and TLR4/NF- κ B Signaling Pathways

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ABSTRACT

Background: Chronic Obstructive Pulmonary Disease (COPD) has emerged as a foremost cause of worldwide illness. Exposure to Cigarette Smoke (CS) contributes to Oxidative Stress (OS) and inflammation, which is a chief cause of COPD. **Objectives:** Bacoside A (BA) is a triterpenoid saponin extracted from the plant *Bacopa monnieri* (Brahmi), renowned as a potent nerve stimulant, exerting antioxidant, anti-inflammatory, and anticancer activities. **Materials and Methods:** Previously, it has been investigated that BA acts as a neuroprotective agent in CS-induced brain damage in rats. Hence, this current study assessed the protective effect of BA in relieving lung inflammation and Oxidative Stress (OS) caused by CS-triggered COPD. Albino rats were disseminated into 5 sets: sham, CS-induced, CS+BA (5 mg/kg bw), CS+BA (10 mg/kg bw), and CS+BA (15 mg/kg bw). COPD was developed to assess breathing capacity, inflammatory cells in BALF, OS markers, cytokines, histopathology, and western blot. **Results:** BA dose-dependently alleviates the OS, MDA, cytokines, inflammatory cells, and histopathological variations, while enhancing antioxidant status in COPD-stimulated rats. **Conclusion:** Our data exhibited that BA could protect against COPD in CS-stimulated rats by suppressing inflammation and OS in the lungs by modulating the TLR4/NF- κ B and Nrf2/ATF4 signalling pathways.

Keywords: Bacoside A, COPD, Inflammation, Nrf2/ATF4, Oxidative stress, TLR4/NF- κ B.

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INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a chronic respiratory disorder that remodels the airway structure because of enduring inflammation of the lungs and airways.^{1,2} A recent estimation revealed that more than 3 million people are involved in COPD-related deaths, which will be accountable for almost 8 million per year in 2030, making it the third leading cause of mortality globally.^{3,4} It typically exhibits as insistent airflow control and respiratory symptoms.⁵ The main COPD symptoms comprise dyspnea and cough, and the major reasons for airflow blockade comprise Cigarette Smoke (CS), industrial, tuberculosis, and natural exposures.⁶ This will severely interrupt the COPD patient's physical health and quality of life. CS covers numerous destructive constituents and exerts a harmful influence on the respiratory tract. Investigations have exposed that enduring exposure to smoking causes erosion in the airway wall structure

and enhances the mucous glands producing secretions, which eventually leads to obstructive bronchitis formation.⁷ The trouble in breathing was activated by the merger of chronic bronchitis and emphysema. Previously, it has been revealed that COPD-triggered inflammation significantly contributes to comorbidities, including lung carcinoma.⁸ Thus, it has developed as one of the vital causes of COPD progression. Conversely, there is no effective handling that can efficiently accomplish COPD and its correlated impediments. There are treatments comprising long-acting β -2-agonists, corticosteroids, long-acting muscarinic antagonists, and so on. They are accessible only to deliver short-term assistance and do not halt COPD progress. Accordingly, the present therapeutic modalities against COPD offer symptomatic respite only.⁹

CS may excite the lung cells to generate more than Reactive Oxygen Species (ROS), which triggers the formation and discharge of inflammatory mediators, thus producing lung inflammation.¹⁰ CS constantly prompts inflammation in the airway via multifaceted signalling paths and generates Reactive Oxygen Species (ROS), Interleukins (IL), chemokines, and proteases through the stimulus of macrophages and epithelial cells in the airways.^{11,12} The promotion of inflammation indexes marks in chronic airway inflammation and organizational



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variations, affecting lung functional loss.¹³ Furthermore, air pollution as well as exogenous oxidants in CS generates Oxidative Stress (OS) in COPD, which leads to the endogenous production of ROS by structural and inflammatory cells inside the lung.¹⁴ OS may aggravate chronic inflammation, exacerbate emphysema and fibrosis, lead to corticosteroid resistance, hasten lung maturity, prompt DNA mutilation, and incite the development of auto antibodies.¹⁴ Hence, the repression of inflammatory reactions and OS is determined as a vital approach for averting COPD development.

The Nuclear Factor Kappa B (NF- κ B) signaling has been documented as a characteristic pro-inflammatory signaling cascade, established on its pro-inflammatory gene expressions, probably through the stimulation of Interleukin-1 Beta (IL-1 β), Tumor Necrosis Factor Alpha (TNF- α), and Interleukin-6 (IL-6).^{15,16} Besides, NF- κ B signaling is a crucial factor in pulmonary inflammation and acute lung injury. Anti-inflammatory compounds can control inflammatory reactions by restraining the NF- κ B pathway.¹⁷ An early report showed that curcumin can avert CS-triggered inflammation in both *in vitro* and *in vivo* models by controlling NF- κ B signalling.¹⁸ Numerous studies have exposed the vital role of NF- κ B in the inflammation of COPD patients' airways, which controls several inflammatory gene expressions in airway cells.^{19,20} The inflammatory cells in the bronchial biopsies presented anomalous stimulation of NF- κ B in COPD individuals. Activation of NF- κ B is an OS-focused mechanism because of a disparity between oxidants and antioxidants in the airways. This is similarly triggered by many inflammatory intermediaries, including TNF- α and IL-1 β , through the stimulation of Toll-Like Receptors (TLRs) owing to incitement via viral/bacterial pathogens. Primarily, when instigation, NF- κ B translocates from the cytoplasm to the nucleus after separating commencing I κ B α , the negative controller of NF- κ B, which is degraded throughout this progression. It includes I κ B α phosphorylation, which is catalysed by I κ B Kinase (I κ K). This stimulates the cytokines discharge comprising IL-8, IL-6, and TNF, hence boosting immune cell translocation to the airway's inflamed cells of COPD patients.^{21,22}

Nuclear factor erythroid 2-Related Factor 2 (Nrf2) delimits the cell's OS responses and pulmonary inflammation.²³ OS is a vital feature in COPD pathogenesis.²⁴ Thus, treatment with antioxidants has recently been involved in consideration in COPD research. As a transcription factor, Nrf2 standardizes antioxidant capacity.²⁵ Nrf2 adjusts numerous antioxidant as well as detoxification genes, and the Nrf2 signaling is concerned with CS-prompted emphysema.²⁶ A crucial antioxidant enzyme in COPD, namely γ -Glutamylcysteine Synthetase (γ -GCS) catalyses the primary regulatory step in glutathione biosynthesis, which is a vital part in antioxidant defences.²⁷ The regulatory enzyme Heme Oxygenase-1 (HO-1) involves heme biosynthesis and exerts anti-proliferative, anti-inflammatory, antioxidant,

and anti-apoptotic properties.²⁸ COPD-correlated airway gene expression is a significant transcriptional regulator known as Activating Transcription Factor 4 (ATF4), and its hyper expression in airway epithelial cells reiterates COPD-related manifestation of gene variations. Ultimately, Superoxide Dismutase (SOD) damages superoxide radicals.²⁹ It has been investigated that Nrf2-deficient mice are extremely vulnerable to CS-developed lung impairments.³⁰ Furthermore, Nrf2 over-expression was described to defend in contrast to CS-triggered cell apoptosis.³¹ These accounts propose that Nrf2 stimulation acts against the OS perceived in CS-induced emphysema.

Bacoside A (BA) is a triterpenoid saponin extracted from the herbal plant *Bacopa monnieri* (Brahmi), which is renowned as a potent nerve stimulant and cardiogenic, that is used in the indigenous system of medicine.³² BA exhibits several pharmacological properties such as antioxidant,³³ antitoxic,³⁴ anti-inflammatory,³⁵ antibacterial,³⁶ anticancer,³⁷ memory enhancer,³⁸ analgesic,³⁹ anti-hyperglycemic, and hepatoprotective⁴⁰ activities. Hence, this current research was conducted to evaluate the efficacy of BA on CS-stimulated COPD in rats and to assess the underlying antioxidative and anti-inflammatory mechanisms.

MATERIALS AND METHODS

Chemicals

Bacoside A (BA, purity >98%) was acquired from Solarbio (Beijing, China). Dimethyl Sulfoxides (DMSO), ketamine, and Sodium Dodecyl Sulfate (SDS) were obtained from Ruicong Ltd., (Shanghai, China). Biochemical assay kits were delivered by Ese-Bio (Shanghai, China). ELISA assay kits were obtained from Elabscience Biotechnology (Wuhan, China). The antibodies (TLR4, p-I κ B α , I κ B α , p-NF- κ B, NF- κ B, γ -GCS, Nrf2, HO-1, ATF4, SOD, and β -actin) and secondary antibody for western blot assay were acquired from ZSGB-bio (Beijing, China). Biochemicals and solvents of analytical grade were used.

Investigational animals

Adult male albino rats weighing 190-220 g were used for the experiments. The rats were preserved in sterile polypropylene coops under specific research environments. Animals were fed a fixed pellet diet with ad libitum and an unceasing 12 hr light/dark phase. The animals were adapted one week before the tests. All treatment protocols were conducted according to the Institutional Animal Ethics Guidelines. All treatment protocols were conducted as per the Institutional Animal and Ethics Committee, Beidahuang Group General Hospital Ethical No: KY-2025040901.

Experimental plan

Rats (Total=30) were separated into 5 sets containing 6 individually. Group 1 - Normal control (NC); Group 2 - COPD model rats; Group 3 - COPD+BA (5 mg/kg bw, low dose); Group

4 and 5 - COPD+BA (10 mg/kg bw, middle dose), and COPD+BA (15 mg/kg bw, high dose).

Induction of experimental COPD and drug treatment in rats

Experimental COPD was induced by the exposure to Cigarette Smoke (CS) as reported previously.⁴¹ The NC rats were exposed to free air, and other groups were induced to smoke CS two times a day for an incessant 12 weeks with a distinct smoking device. Different concentrations (5, 10, and 15 mg/kg bw) of BA were intragastrically administered for 6 successive days before CS exposure and continued for the treatment. An equivalent amount of normal saline was administered to NC rats. After the experimental period, animals were anesthetized with ketamine (24 mg/kg body weight) and injected intramuscularly, followed by cervical beheading.

Assessment of respiratory function

The tidal volume and peak expiratory flow rate were recorded on the 77th day with a whole-body plethysmograph (Biosystem XA, USA) with a respiratory function analysis system. These were anesthetized on the 85th day, and a cannula was inserted into the trachea to attain functional residual capacity and forced expiratory volume at 100 ms with an involuntary maneuvers system (Bio-System for Maneuvers, Data Sciences International).

Hematological parameters in BALF

The rat's lungs were isolated to Bronchoalveolar Lavage Fluid (BALF) was centrifuged at 800×g for ten min at 4°C. Add 1 mL of saline into the separate supernatants, which were obtained as pellets. BALF pellet suspension was mixed with Turk's solution, and the White Blood (WBC) Cells per 1 µL were counted with a hemocytometer. Furthermore, the sums of monocytes/macrophages, neutrophils, and WBC were counted through staining with Wright-Giemsa solution and detected under a microscope.

Analysis of plasma proteins

The blood samples from the abdominal vein were subsequently centrifuged at 1500×g for 15 min at 4°C, the plasma was extracted and stored at 80°C for auxiliary biochemical estimation. ELISA kit (Elabscience Biotechnology, Wuhan, China) was used to determine leptin and C-Reactive Protein (CRP) according to the kit protocol.

Analysis of OS markers in the lung tissue homogenate

Homogenized the lungs and lysed them with 0.05 M Tris. HCl extraction buffer on ice. Subsequently, the cell lysates were centrifuged at 12,000×g for 10 min at 4°C. Biochemical analysis was performed by using the resulting cell lysates. The

concentration of Malondialdehyde (MDA), Glutathione (GSH), and Superoxide Dismutase (SOD) were determined by using commercial kits (Ese-Bio, Shanghai, China) following their protocols.

Estimation of inflammatory mediators

The intensities of cytokines, chiefly IL-1 β , TNF- α , and IL-6, were estimated by an ELISA kit provided by Elabscience Biotechnology (Wuhan, China), as per their instructions.

Histopathological examination of the lung tissues

The lung cells were removed, fixation with formalin (4%) for one day, and then paraffin-embedded. The lungs were cut into 5 µm slices and were hematoxylin and eosin (H&E) stained for microscopic study. An experienced pathologist carried out the histological examination of all experimental groups using an Olympus BX60 microscope (Olympus, Tokyo, Japan).

Western blotting assay

Lung tissue proteins were isolated with an ice-cold solution of lysis buffer, and the protein quantity was estimated by employing an assay kit (Ese-Bio, Shanghai, China). When SDS-PAGE electrophoresis was performed, the gel was transferred to a PVDF film. The PVDF film was wrapped with skimmed milk (5%) and the diluent (1:1000) of the primary antibody (TLR4, p-I κ B α , I κ B α , NF- κ B, NF- κ B, γ -GCS, Nrf2, HO-1, ATF4, and β -actin) and kept at 4°C for one day. Then, the diluent (1:5000) of the HRP-conjugated secondary antibody with skimmed milk powder (5%) and applied to the PVDF film, and then incubated. Lastly, an ECL chemiluminescence device was employed for the membrane development. Quantified the protein bands through densitometry with the software Image J and homogeneous to β -actin expression.

Statistical study

The statistical examination was conducted by the software version 9.0 GraphPad Prism and expressed as mean $\pm$ SD. It was working to implement an Analysis of Variance (ANOVA) and subsequently Duncan's assessment. Student *t*-test has been conducted, and $p < 0.05$ was considered statistically significant.

RESULTS

BA ameliorates CS-induced lung injury

CS-induced rats exhibited lung functional parameters such as the peak expiratory flow rate, tidal volume, and forced expiratory volume were considerably decreased ($p < 0.05$) versus NC (Figure 1A). Lung emphysema biomarkers, including functional residual volume and lung capacity, were remarkably increased ($p < 0.05$) in the CS-induced rats than NC. Conversely, treatment with BA (5, 10, and 15 mg/kg bw) in the CS-developed rats restored near normal levels of these lung functional parameters in an

amount-related manner. These findings point out that BA diminished the CS-stimulated lung damage in rats.

Effect of BA on BALF cell count and plasma proteins

The level of WBC, macrophages, neutrophils, and CRP was markedly elevated ($p < 0.05$), whereas remarkably moderated

($p < 0.05$) the leptin level of the plasma in the CS-induced group in contrast to NC (Figure 1B). Administration of BA (5, 10, and 15 mg/kg bw) presented a substantial decrease ($p < 0.05$) in WBC, macrophages, neutrophils, and CRP while enhancing ($p < 0.05$) the plasma leptin levels in a concentration-related way against

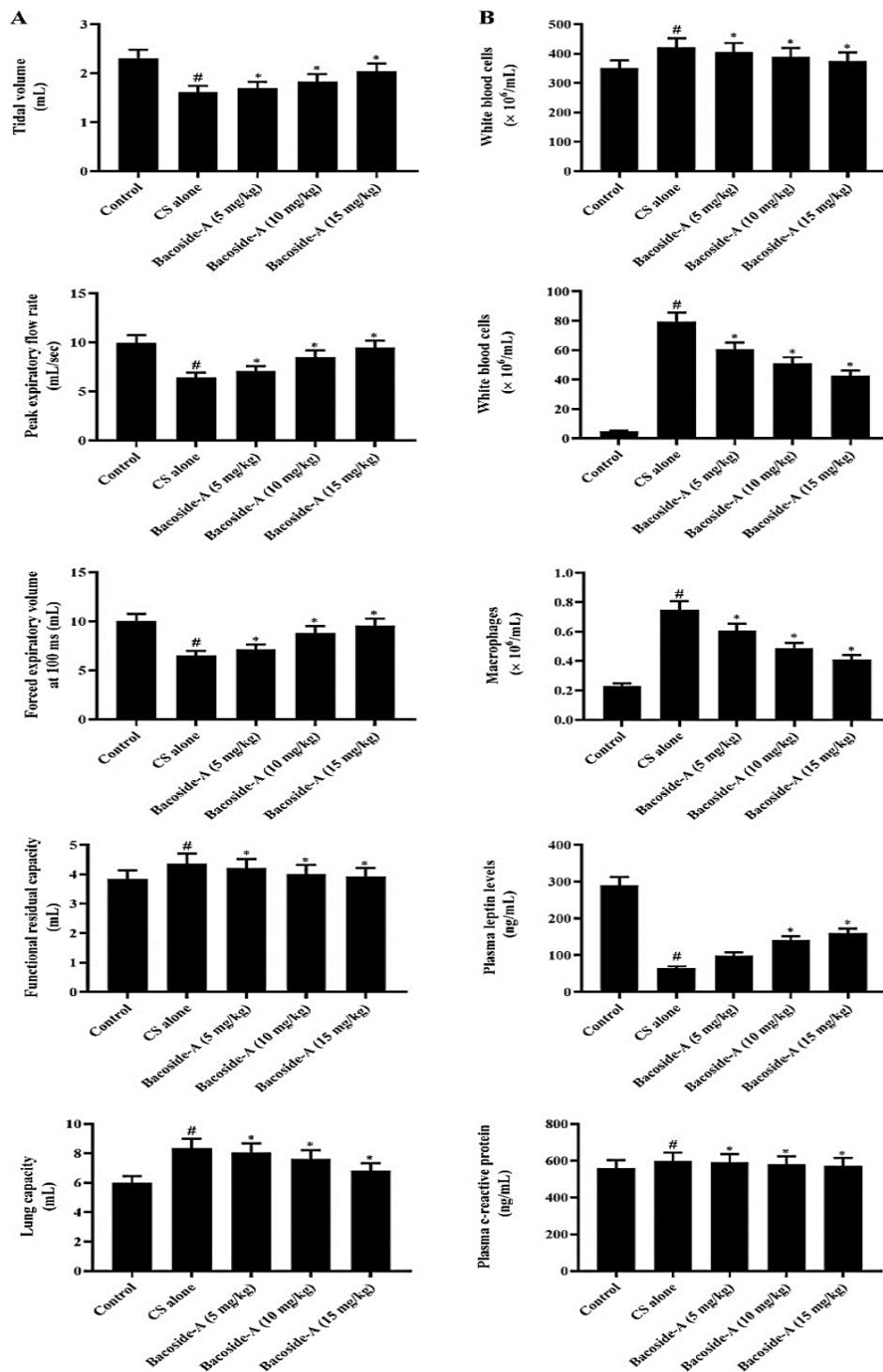


Figure 1: A) BA ameliorates CS-induced lung injury in rats. The levels of tidal volume, peak expiratory flow rate, forced expiratory volume, functional residual capacity, and lung capacity in experimental rats. B) Effect of BA on BALF cell count and plasma proteins. The levels of WBC, neutrophils, macrophages, plasma leptin, and CRP in experimental rats. The findings were exhibited as mean $\pm$ SD of 6 observations, and the statistical significance is considered as # $p < 0.05$ against the NC and * $p < 0.05$ against the CS-induced model.

the CS-induced rats. These data emphasized the pulmonary protective effect of BA on CS-stimulated rats.

BA attenuates CS-stimulated OS in rats

To examine the antioxidant action of BA on CS-induced rats, study the effects of BA on OS markers such as MDA, GSH

and SOD in lung tissue homogenate (Figure 2A). CS-induced rats were exposed to a higher ($p<0.05$) level of MDA, whereas considerably diminished ($p<0.05$) antioxidant status such as SOD activity and GSH level against the NC group. Administration of BA (5, 10, and 15 mg/kg bw) in CS-induced rats uncovered a substantial decrease ($p<0.05$) in MDA levels, while enhanced

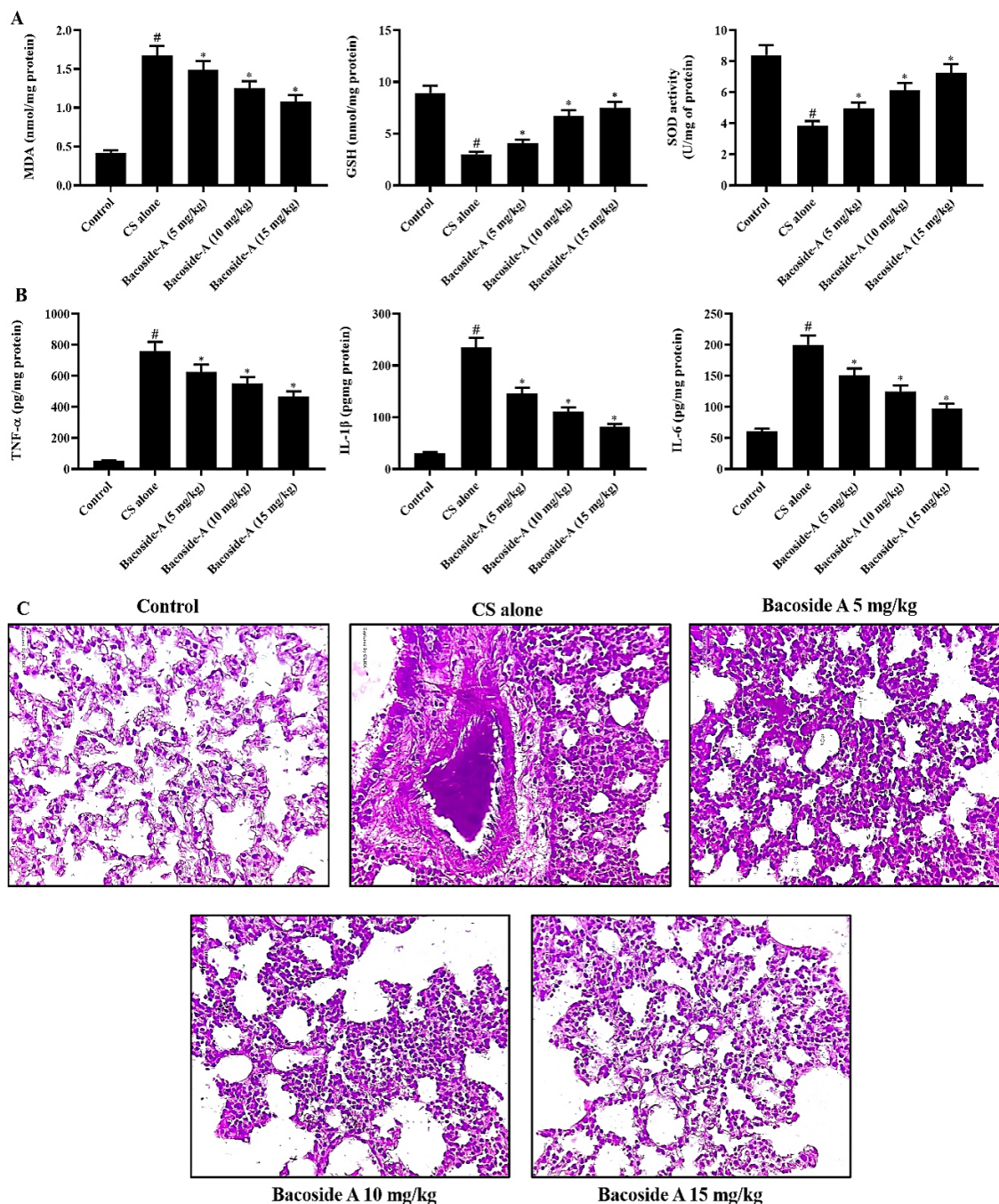


Figure 2: A) BA attenuates CS-stimulated OS in rats. The status of MDA, GSH, and SOD of experimental animals. B) BA diminishes CS-stimulated inflammatory mediators. The cytokines containing TNF- α , IL-1 β , and IL-6 were determined by ELISA kit. The findings were exhibited as Mean $\pm$ SD of 6 observations and the statistical significance is considered as $^{\#}p<0.05$ against the NC and $^*p<0.05$ against the CS-induced model. C) BA inhibits CS-stimulated histopathological changes in the lungs. The histopathological changes of lung tissue of NC and investigational groups (H&E staining, 20X), Scale bar=50 μ m. NC rats showing typical lung cells. CS-induced rat lung tissues exhibiting enlarged alveolar spaces organized with deprivation of alveoli, numerous infiltrating inflammatory cells, signs of emphysema, and inter alveolar edema. Administration of CS+BA (5, 10, and 15 mg/kg bw) reduced histopathological variations in a concentration-related manner.

($p < 0.05$) the SOD and GSH level in an amount-related way. These data suggested that BA could remarkably alleviate CS-induced lung impairments and ameliorate antioxidant function.

BA diminishes CS-stimulated inflammatory mediators

Inflammatory cytokines comprising TNF- α , IL-1 β , and IL-6 were also determined (Figure 2B). CS-induced rats unveiled considerably elevated ($p < 0.05$) cytokines of TNF- α , IL-1 β , and IL-6 concentrations when compared to the NC. In contrast, BA (5, 10, and 15 mg/kg bw) administered to CS-prompted rats markedly reduced ($p < 0.05$) the quantity of TNF- α , IL-1 β , and IL-6 levels in a dosage-related way. These outcomes uncovered the anti-inflammatory activity of BA against the CS-induced rat model.

BA inhibits CS-stimulated histopathological changes in the lungs

Histopathological analysis of CS-induced rats' lung tissues displayed enlarged alveolar spaces organized with deprivation of alveoli, numerous infiltrating inflammatory cells, signs of emphysema, and interalveolar edema in contrast to NC (Figure 2C). These histopathological variations are reduced by BA (5, 10, and 15 mg/kg bw) in a quantity-related manner.

Influence of BA on NF- κ B downstream mediators

To establish the anti-inflammatory potential of BA on CS-induced COPD, we analyzed the intensities of numerous downstream intermediaries of the NF- κ B signalling (Figure 3). The intensity of TLR-4, p-NF- κ B, and I κ B α was remarkably elevated ($p < 0.05$) in the CS-stimulated group than NC. BA (5, 10, and 15 mg/kg bw) diminished substantially the level of these mediators in a quantity-related way.

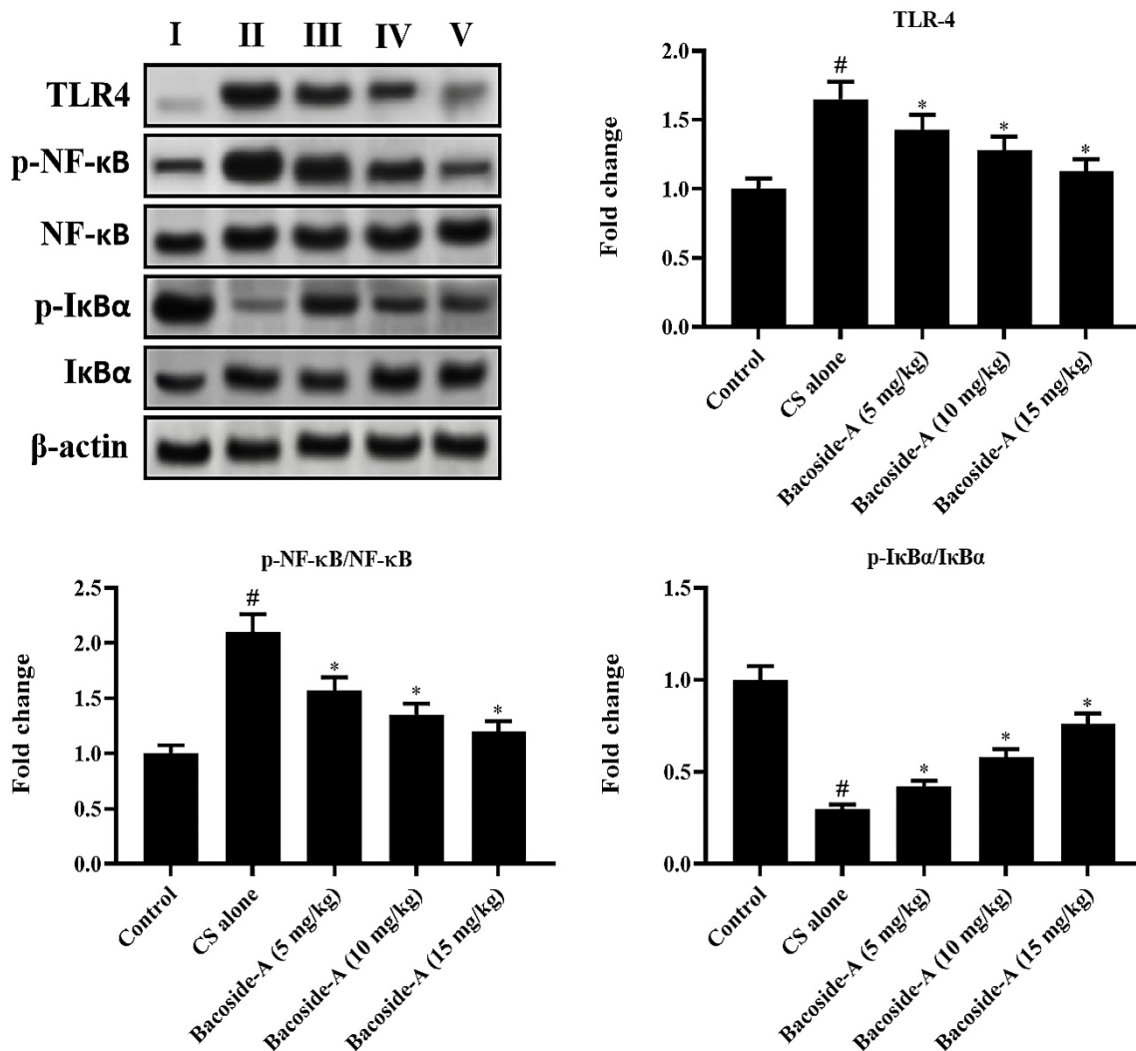


Figure 3: Influence of BA on NF- κ B downstream mediators. The intensities of TLR-4, p-NF- κ B, NF- κ B, p-I κ B α , and I κ B α protein expression levels were evaluated by western blot. The findings were exhibited as mean $\pm$ SD of 6 observations and the statistical significance is considered as [#] $p < 0.05$ against the NC and ^{*} $p < 0.05$ against the CS-induced model.

Effect of BA on Nrf2/ATF4 signaling pathway

The protein levels of Nrf2, γ -GCS, HO-1, and ATF4 were expressively elevated ($p < 0.05$), in CS-stimulated rats versus NC (Figure 4). Conversely, these protein expression levels were reverted in a dosage-reliant way to near-normal levels after supplementation with BA (5, 10, and 15 mg/kg bw).

DISCUSSION

COPD is an overwhelming disorder that affects weakened breathing function owing to emphysema and chronic bronchitis.^{1,2} The key characteristics of COPD involve OS and chronic inflammation in the lungs, which eventually leads to diminished airflow. It has been believed CS is a crucial element that induces OS and the inflammatory reaction, leading to COPD pathogenesis.^{6,7} Precisely, the extra-pulmonary appearance of COPD is classically chronic general inflammation. Furthermore, the signs include extreme phlegm formation and CS-produced airway inflammation, which are the main reasons for COPD.^{7,8} As a triterpenoid saponin, BA exerts antioxidant,³³ anti-inflammatory,³⁵ and anticancer³⁷ activities. Previously, it has been investigated that BA acts as a neuroprotective agent in CS-induced brain damage in rats.⁴² As demonstrated by decreased inflammatory cytokines and OS factors, as well as increased lung function and antioxidant factors in CS-induced COPD in rats, BA (5, 10, and 15 mg/kg bw) dose-dependently inhibited histopathological damage, MDA level, inflammatory cells in BALF, OS, and inflammation in COPD. These results

demonstrated that BA shielded lungs against CS-stimulated COPD by reducing inflammation and OS through the control of Nrf2/ATF4 and TLR4/NF- κ B pathway activation.

The lung dysfunction that affects the capability to breathe is the distinctive symptom of COPD. During this disease, the lung's bronchial tubes become constricted. When the patient exhales, they are inclined to fold and develop a blockage with mucus, which prevents air from flowing through the lungs. Hence, primarily, we propose to explore the activity of BA on lung function and breathing capacity by numerous parameters.^{41,43,44} Our data revealed that BA (5, 10, and 15 mg/kg bw) enhanced peak expiratory flow rate, tidal volume, and forced expiratory volume in a quantity-related manner in rats which designates that BA affects a decrease in lung capacity, functional residual capacity, and airway blockage, thus recovers breathing ability. It has been established that COPD patients have an increased count of WBCs, neutrophils, and macrophages.^{41,45} In the current research, BA triggers an amount-related decrease in these indices. Another characteristic feature of COPD is systemic inflammation, which frequently requires to be occupied in description. As exposed in this report, BA triggers an elevation of plasma leptin and a reduction of plasma CRP, which is measured as a vital manifestation of systemic inflammation.^{41,46} Neutrophils and macrophages are stimulated throughout COPD for the creation of cytokines comprising IL-1 β , TNF- α , and IL-6. We have established that BA diminishes systemic inflammation by decreasing the level of IL-1 β , IL-6, and TNF- α . These

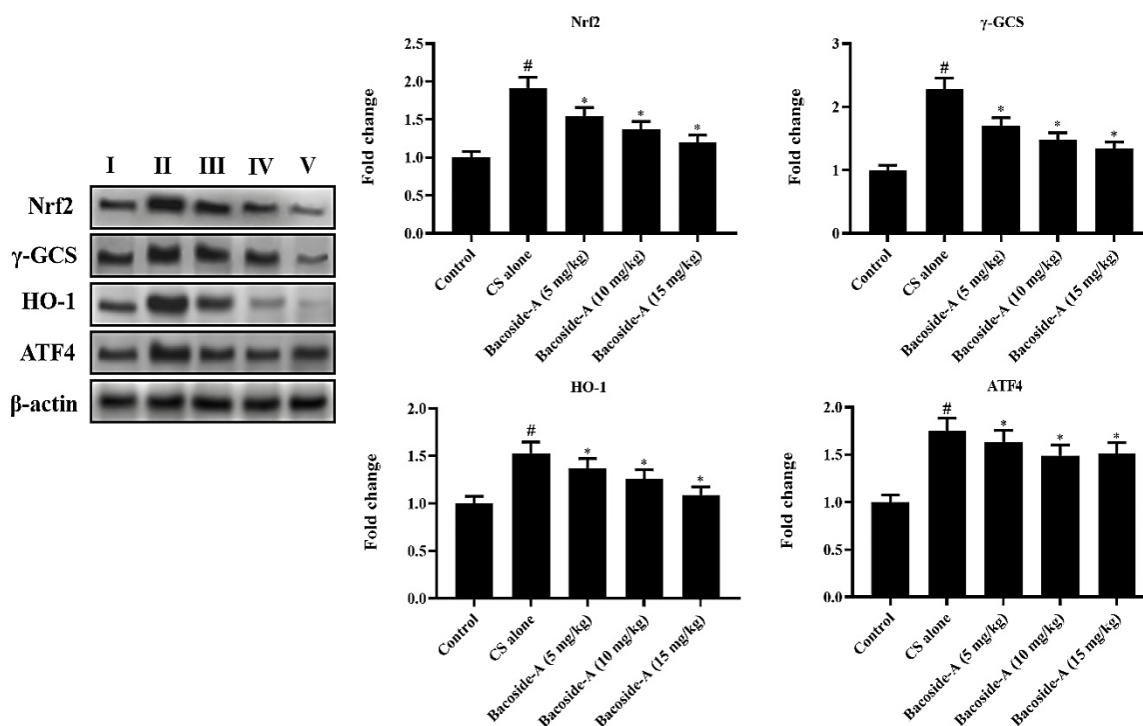


Figure 4: Effect of BA on Nrf2/ATF4 signalling pathway. The protein expression of Nrf2, γ -GCS, HO-1, and ATF4 were evaluated by western blot. The findings were exhibited as mean $\pm$ SD of 6 observations and the statistical significance is considered as # $p < 0.05$ against the NC and * $p < 0.05$ against the CS-induced model.

interpretations were further supported by lung histopathology investigation, where BA was presented to avert injury to the lung alveoli and reinstate the typical lung tissue structure. Herein, we detected that the BA-administered rats were exposed to a decline in BALF inflammatory cell counts, cytokines, and amelioration of histopathology developed by CS exposure. Furthermore, these cytokines can tempt the neutrophil's recruitment to the lung, producing chronic bronchial inflammation and emphysema.^{19,20} Our results showed that BA improved lung function in a concentration-related manner while expressively suppressing inflammatory cell numbers, histopathology, and pro-inflammatory cytokines triggered by CS.

NF- κ B is a crucial transcription factor that controls several inflammatory gene expressions linked with COPD pathogenesis.^{19,20} CS-triggered inflammation is mostly focused on the NF- κ B signaling cascade, and the activation of NF- κ B has been detected in COPD patients.²⁰ The anti-inflammatory regulation of BA was further analyzed on the NF- κ B downstream mediators, which are supposed to be vital in stimulating lung inflammation in COPD patients.^{18,19} Our report has exposed that BA (5, 10, and 15 mg/kg bw) dosage-dependently represses the TLR4 activation along with repression of NF- κ B and I κ B α phosphorylation. We observed that pro-inflammatory cytokines and NF- κ Bp65 phosphorylation were elevated in macrophages following CS exposure, which was supported in the rat model of CS-induced COPD. Similar to the previous publication, our data showed that BA inhibited NF- κ Bp65 nuclear translocation and phosphorylation in the COPD rat model.¹⁸

Macrophages and neutrophils are the chief inflammatory cells that produce ROS and inflammatory intermediaries that hasten lung damage.¹⁰⁻¹² Likewise, OS plays a vital part in COPD progression. CS exposure to COPD in rat models unveiled elevated lipid peroxidation, which stimulates lung impairment.¹⁴ Our results showed that BA (5, 10, and 15 mg/kg bw) remarkably reduced CS-triggered MDA however, augmenting the antioxidant activity of SOD and GSH levels in a quantity-related mode. Gene expression modifications follow in lung tissues as a preliminary, cautious reaction to CS chronic introduction.⁴⁷ The transformed genes are correlated with remodeling, OS, and other significant signaling networks.⁴⁸ As a transcription element, Nrf2 controls the phase-II antioxidant gene expressions and converses defence against OS.⁴⁹ ATF4 was upregulated in OS through communication with Nrf2. It has been well established that ATF4 plays a crucial role in regulating the expression of genes that participate in oxidative stress in COPD. Stimulation of Nrf2/ATF4 gene expression upregulates the inflammatory reaction to oxidants.⁴⁷⁻⁴⁹ BA attenuated the ROS level and elevated the protein expression of Nrf2 and ATF4, prominent in enhanced antioxidant enzyme levels. CS exposure elevates the protein intensities of antioxidant enzymes for instance HO-1, SOD, and γ -GCS. Herein, BA augmented the proteins of these antioxidant

enzymes. Thus, improving antioxidants might be favourable for CS-induced COPD management.

CONCLUSION

In summary, BA (5, 10, and 15 mg/kg bw) dose-dependently ameliorates lung function, while alleviating histopathological alterations, inflammatory cells in BALF, OS, and inflammation in CS-induced COPD in rats. Our data exhibited that exposure to CS triggered NF- κ B and Nrf2 pathways in the lungs, and these variations were repressed by BA administration. It also triggers the suppression of NF- κ B signalling downstream mediators as a probable mechanism for the improvement of airway and systemic inflammation. BA has therapeutic potential for CS-stimulated COPD in rats. We studied only Protein expression of TLR-4, NF- κ B, I κ B α , Nrf2, γ -GCS, SOD, HO-1, and ATF4 this marker needs to be study using RT-PCR in future.

ABBREVIATIONS

COPD: Chronic obstructive pulmonary disease; **CS:** Cigarette smoke; **OS:** Oxidative stress; **BA:** Bacoside A; **TLR4:** Toll-like receptor 4; **NF- κ B:** Nuclear Factor Kappa B; **Nrf2:** Nuclear factor erythroid 2-related factor 2; **ATF4:** Activating Transcription Factor 4; **IL:** interleukins; **IL-1 β :** Interleukin-1 beta; **I κ B α :** Inhibitor of nuclear factor kappa B; **IL-8:** Interleukin-8; **DMSO:** Dimethyl sulfoxides; **ELISA:** Enzyme-linked immunosorbent assay; **SOD:** Superoxide dismutase; **HO-1:** Heme Oxygenase-1; **WBC:** White blood cell; **BALF:** bronchoalveolar lavage fluid; **CRP:** C-reactive protein; **MDA:** Malondialdehyde; **GSH:** Glutathione; **SOD:** Superoxide dismutase; **H&E:** Hematoxylin and eosin (H&E); **ANOVA:** Analysis of variance.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

All treatment protocols were conducted as per the Institutional Animal and Ethics Committee, Beidahuang Group General Hospital Ethical No: KY-2025040901.

SUMMARY

The purpose of the current study was to determine the effectiveness of BA on CS-stimulated COPD in rats as well as the underlying anti-inflammatory and anti-oxidative mechanisms. Sham, CS-induced, CS + BA (5 mg/kg bw), CS + BA (10 mg/kg bw), and CS + BA (15 mg/kg bw) were the five sets of albino rats that were distributed. COPD was created to evaluate histology, western blot, OS markers, cytokines, inflammatory cells in BALF, and breathing capacity. BA improves antioxidant status in COPD-stimulated rats while dose-dependently reducing OS, MDA, cytokines, inflammatory cells, and histopathological abnormalities. Our findings shown that BA may prevent COPD

in CS-stimulated rats by reducing lung inflammation and OS through the modulation of the Nrf2/ATF4 and TLR4/NF- κ B signaling pathways. Additionally, it initiates the regulation of downstream mediators of NF- κ B signaling, which is a likely mechanism for reducing systemic and airway inflammation. BA may be used to treat rats' CS-stimulated COPD.

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