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ORIGINAL ARTICLE

Betanin ameliorates Lipopolysaccharide-induced acute lung injury in mice via inhibition of inflammatory response and oxidative stress

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Abstract *Background:* Acute lung injury (ALI) is the most common form of inflammatory disease, which has higher morbidity and mortality rates worldwide. ALI is characterized by alterations in the lungs such as epithelial dysfunction, excessive inflammation, and lung edema.

Objective: The current work was aimed to unveil the abrogative properties of betanin on the LPS-induced ALI in mice.

Methodology: In an *in vitro* assay, betanin-treated RAW 264.7 cells viability was evaluated by MTT assay. The TNF-α, IL-1β, and IL-6 levels in the cell lysates were estimated using assay kits. In *in vivo* studies, the ALI was initiated in the BALB/c mice by exposing them to a 3-day intra-tracheal challenge of 5 mg/kg of LPS and then treated with betanin (25 and 50 mg/kg) for three days. Later, the BALF samples were obtained from the mice and used to estimate the inflammatory biomarkers using assay kits. The levels of MDA and the antioxidants SOD and GSH were also examined using commerical kits. The iNOS, COX-2, and PGE-2 expressions were estimated using kits, and finally the histopathological study was conducted on the lung tissues.

Results: The outcomes of *in vitro* studies revealed that betanin appreciably improved the viability of LPS-exposed RAW 264.7 cells. The betanin effectively diminished the levels of IL-6, IL-1β,

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and TNF-α in LPS-induced RAW 264.7 cells. In *in vivo* studies, the findings demonstrated that betanin treatment diminished the lung/bodyweight, lung W/D weight, and protein level in the ALI mice. The inflammatory cell counts and inflammatory biomarkers such as COX-2, PGE2, and iNOS were substantially reduced by the betanin treatment in the ALI mice. Betanin treatment also increased the GSH and SOD and reduced the MDA in the ALI mice. The histopathological alterations in the lungs tissues of ALI mice were effectively ameliorated by the betanin.

Conclusion: In summary, the findings apparently suggest that betanin effectively ameliorated the LPS-triggered ALI in mice through its beneficial properties. Therefore, in the future, it can be used as a new salutary candidate to manage the ALI in medical settings.

1. Introduction

Acute lung injury (ALI) is a form of acute inflammatory lung disease that has a significant morbidity and fatality rate worldwide (Patel et al., 2019). Many factors can contribute to the progression of ALI, including pneumonia, trauma, and burns (Wang et al., 2020). ALI is defined by alterations in lung tissue such as epithelial dysfunction, excessive inflammation, and lung edema (Butt et al., 2016). The pathophysiology of ALI is unknown, but injury to the epithelium and endothelium of the lungs, the production of pro-inflammatory regulators, excessive oxidative stress, and a large influx of neutrophils into the lungs are all believed to be involved. Oxidative stress and severe inflammation are two major causes to ALI development. ALI can result in a variety of direct and indirect consequences, comprising pneumonia and other inflammation-related disorders, including acute cardiac failure, renal disease, liver function impairments, and life-threatening complications. At its core, ALI causes redox instability, oxidative injury, and DNA damage, which leads to the death of lung cells and a change in how the lungs work (Tan et al., 2017).

The discrepancy between the body's inflammatory and anti-inflammatory reactions is one of the probable pathogenesis of ALI, which contributes to the onset of numerous diseases (Wan et al., 2021). ALI is distinguished by the release of inflammatory mediators from the epithelium, which promotes neutrophil and macrophage influx into the injured sites, followed by a rise in inflammatory cytokines (Voiriot et al., 2017). The NF-kB pathway is linked to inflammatory mechanisms and promotes transcription of inflammatory proteins such as pro-inflammatory regulators released by activated macrophages. TNF-α and IL-1β trigger other inflammatory cells to produce more inflammatory cytokines, which are well-known chemoattractant factors for neutrophils. It has been shown that IL-6 is important for increasing the neutrophil numbers and lowering the bacterial load in the lungs (Zhao et al., 2016).

Lipopolysacharide (LPS) is a fundamental element of gram-negative bacteria's cell membrane, which is made up of polysaccharides and lipids, which causes numerous organ failures, especially ALI and its more serious version, ARDS (Wang et al., 2021). LPS can cause inflammatory cell infiltrations that results in powerful immune responses. As a result, LPS has been routinely employed to trigger inflammation in animal models such as ALI (de Souza Xavier Costa N, Ribeiro Júnior G, Dos Santos Alemany AA, Belotti L, Zati DH, Frota Cavalcante M, , 2017). LPS has been proven in studies to cause lung damage via a variety of inflammatory pathways. When animals were administered with LPS, the immune mechanisms were stimulated and substantial pro-inflammatory mediators were generated, disrupting the inflammatory and anti-inflammatory homeostasis and resulting in lung inflammation (Mokhtari-Zaer et al., 2020). In innate and adaptive immune responses, lymphocytes, dendritic cells, granulocytes, and macrophages play important roles. Furthermore, research has shown that macrophages, RAW 264.7 cells are a useful *in vitro* inflammatory macrophage model (Lee et al., 2020). So, the LPS-stimulated inflammatory model can tell if a drug has properties that make it anti-inflammatory.

There are currently no efficient medications for the treatment of ALI (Sahu et al., 2020). The major medications utilized to treat ALI are NSAIDs and glucocorticoids, which have substantial side effects such as upper gastrointestinal responses, renal dysfunction, and arterial thrombotic complications, as well as poor outcomes (Tomazini et al., 2020; Cumhur Cure et al., 2020). As a result, new and potential medications that relieve the pathological signs of ALI should be discovered. Betanin, a nitrogenated heterocyclic compound that is the main bioactive substance of beetroot and confers its red-violet color (Silva et al., 2016). Additionally, betanin is characterized as a bioactive substance that can avert lipid peroxidation and boost the functions of antioxidant systems (Esatbeyoglu et al., 2015). Betanin has the potential to be employed as a complementary therapy to lessen the pathophysiological consequences of oxidative stress and inflammatory processes that result in CVD disorders (Fiordelisi et al., 2019; Satta et al., 2017). Several recent studies have also suggested that betanin has the potential to ameliorate oxidative stress in diabetic rats (Mousavi et al., 2022 Oct), allergic airway inflammation (Li et al., 2022 Mar 30), renal fibrosis in diabetic rats (Sutariya and Saraf, 2017 Feb), and showed cardioprotective effects (Da Silva et al., 2022). Nonetheless, the salutary role of betanin against LPS-induced ALI has not been systematically analyzed yet. Consequently, this work was undertaken to unveil the abrogative roles of betanin on the LPS-induced ALI in mice.

2. Materials and methods

2.1. Reagents

The following chemicals were acquired from Sigma-Aldrich, USA: betanin, LPS, 3-MTT, buffer solution, and other reagents. Thermofisher and Biocompare, USA, provided the ELISA test kits for evaluating biochemical parameters.

2.2. In vitro assays

2.2.1. Collection of cell line

The RAW 264.7 cells, which resemble macrophages, were purchased from the ATCC in the USA and kept on DMEM media supplemented with 10% FBS and 1% antimycotic cocktail at 37 °C in a dampened and CO_2 (5%) supplied chamber. Later the cells reached 80% confluency, they were trypsinized, separated, and used in further experiments.

2.2.2. Cell viability assay

Betanin-treated RAW 264.7 cells' viability was determined by MTT test. Cells were cultivated in a 96-wellplate with DMEM

media at 5×10^3 cells/well for 24 h at 37 °C. Later, different concentrations of betanin, ranging from 10, 15, 20, and 25 µM, were given to the cells at 37 °C for 24 hrs. Later, 20 µl of MTT and 100 µl of DMEM were mixed to each well. Then, 100 µl of DMSO was utilized to liquefy the formed formazan stones in each well, and using a microplate reader, finally the absorbance were taken at 570 nm.

2.3. Measurement of pro-inflammatory cytokines

The IL-6, IL-1β, and TNF-α levels in the cell lysates of control and betanin-administered RAW 264.7 cells were measured using assay kits from a commercial company while following the manufacturer's instructions (Thermofisher Scientific, USA).

2.4. Experimental animals

The mice used in this experiment were 3–4 weeks aged BALB/c mice weighing 27.5 g acquired from institutional animal care. All mice were kept in well-hygienic polypropylene containers with access to the typical rodent food and clean drinking water. Mice were kept in carefully planned laboratory settings with a 24 °C temperature, 60–70% air humidity, and a 12-hour cycle of darkness and light. Before to the start of the studies, the experimental mice were acclimated for a week in a lab setting.

2.5. Experimental design

The four groups of acclimatized animals, each with six animals, were randomly chosen. Animals in group I were designated as usual controls and were not given any pharmacological treatments. ALI was induced in Group-II mice by exposing them to a 3-day intra-tracheal challenge of 5 mg/kg of LPS. The animals in groups III and IV were ALI-initiated mice administered with betanin (25 and 50 mg/kg) for three days, respectively, before receiving an injection of LPS. After anesthesia and cervical dislocation death, the mice's lung tissues were gathered and precisely weighed to estimate the moist weight. Once the tissues had been dried out in an oven set to 80 °C, the dry weight was determined.

2.6. Collection of broncho alveolar lavage fluid (BALF) and total cell count

By injecting aliquots of 30 ml of saline solution into the right middle lobe of the mice—both control and experimental—the BALF was obtained. The BALF was then immediately centrifuged for 5 min at 6000 rpm. The centrifuged BALF sample was then relocated to a new tube and used for the biochemical calculations without any cell remnants. Differential cell labeling was used to figure out how many cells were in the BALF fluid pellets as a whole.

2.7. Quantification of oxidative stress markers

By using assay kits, the level of MDA, SOD, and GSH were estimated in the BALF fluids of both control and treated mice

using the recommended protocols by the manufacturer (Biocompare, USA).

2.8. Determination of inflammatory biomarker levels

Using assay kits and in accordance to the suggested guidelines of the manufacturer, the status of inflammatory regulators like iNOS, COX-2, and prostaglandin-E2 (PGE-2) in the BALF of control and experimental mice were quantified (Biocompare, USA).

2.9. Assessment of lung histopathological score

The lung tissues from both experimental and control mice were taken out and treated with 10% neutral formalin in order to perform the histological analysis and calculate the lung histology scores. The lung tissues were then cut into 5 micro m-diameter and preserved in paraffin wax. The stain of hematoxylin and eosin were utilized to stain the deparaffinized tissues. The stained tissues were examined beneath the light microscope at a magnification of 100x, and the histopathological scores were determined based on the degrees of histological changes, such as neutrophil infiltration of the lungs, edema in the alveolar air sacs, and injuries to the alveolar epithelial cells.

2.10. Statistical analysis

One-way ANOVA and DMRT were performed to evaluate the data, which were statistically evaluated using SPSS software and given as the mean ± SD of three discrete assays. Significant was fixed as a "p" value < 0.05.

3. Results

3.1. Effect of betanin on the viability of LPS-induced RAW 264.7 cells

The effects of betanin on the LPS-exposed RAW 264.7 cell viability are shown in Fig. 1. The outcomes proved that the survival of RAW 264.7 cells was not impacted by betanin alone. The 15 and 20 µM of betanin concentrations were chosen for further testing because the 20 µM of betanin concentration only slightly decreased cell viability. Nevertheless, the cells that had received LPS demonstrated a significant decline in survival. Surprisingly, when cells were administered with 15 and 20 µM of betanin and then exposed to LPS, the viability of the cells increased noticeably (Fig. 1). In light of this, it was evident that the betanin therapy increased the survival of LPS-exposed RAW 264.7 cells.

3.2. Effect of betanin on the pro-inflammatory cytokine levels in the LPS-exposed RAW 264.7 cells

Fig. 2 reveals the results of measuring the status of IL-6, IL-1β, and TNF-α in control and LPS-exposed RAW 264.7 cells using assay kits. As contrasted with the control cells, the LPS-exposed cells displayed elevated IL-6, IL-1β, and TNF-α. Nevertheless, when cells were given 15 and 20 µM of betanin
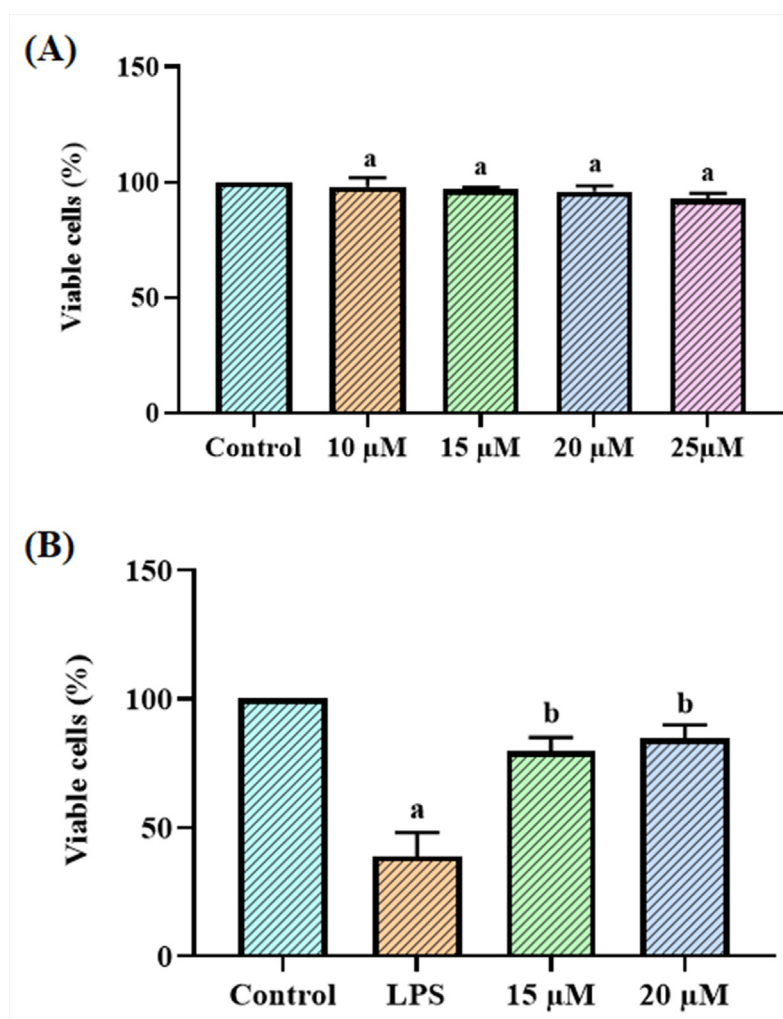
Fig. 1   Effect of betanin on the viability of LPS-induced RAW 264.7 cells The viability of RAW 264.7 cells was unaffected by the betanin treatment alone at various dosages (10–25 µM) **(A)**. Additionally, the treatments with 15 and 20 µM of betanin significantly increased the viability of LPS-exposed RAW 264.7 cells **(B)**. Results presented as the mean ± SD of three replicated tests. The SPSS program was used to analyze the results using one-way ANOVA and DMRT. Note: "a" denotes $p < 0.01$ when compared to control, while "b" denotes $p < 0.05$ when compared to cells that had been treated to LPS.

and then exposed with LPS, the IL-6, IL-1β, and TNF-α were considerably depleted (Fig. 2). These results show the anti-inflammatory property of betanin.

3.3. Effect of betanin on the lung weight, lung/bodyweight ratio, and total protein levels in the LPS-induced ALI mice

The changes in the W/D weight of the lungs, lung/bodyweight ratio, and protein status in the control and LPS-exposed ALI mice are revealed in Fig. 3. It was discovered that the LPS-exposed ALI mice had higher levels of total protein, lung W/D weight, and lung/bodyweight. Nonetheless, the use of betanin substantially reduced these alterations (Fig. 3). When betanin at the dose of 25 and 50 mg/kg were given to ALI mice that had been exposed to LPS, the lung W/D weight, the lung/-bodyweight ratio, and protein content in the BALF all went down in a noticeable way.

3.4. Effect of betanin on the inflammatory cell counts in the BALF of LPS-induced ALI mice

Fig. 4 shows data from the assessment of the levels of inflammatory cells, i.e., macrophages, neutrophils, lymphocytes, and total cell counts. The total cells in the BALF of LPS-exposed ALI mice were augmented. Intriguingly, the LPS-exposed ALI mice were given betanin at 25 and 50 mg/kg, and the outcomes revealed a striking reduction in these increments in the BALF (Fig. 4).

3.5. Effect of betanin on the oxidative and antioxidative biomarker levels in the LPS-induced ALI mice

The levels of oxidative and antioxidative indicators including MDA, SOD, and GSH in control and LPS-exposed ALI mice are presented in Fig. 5. The LPS-exposed ALI mice displayed
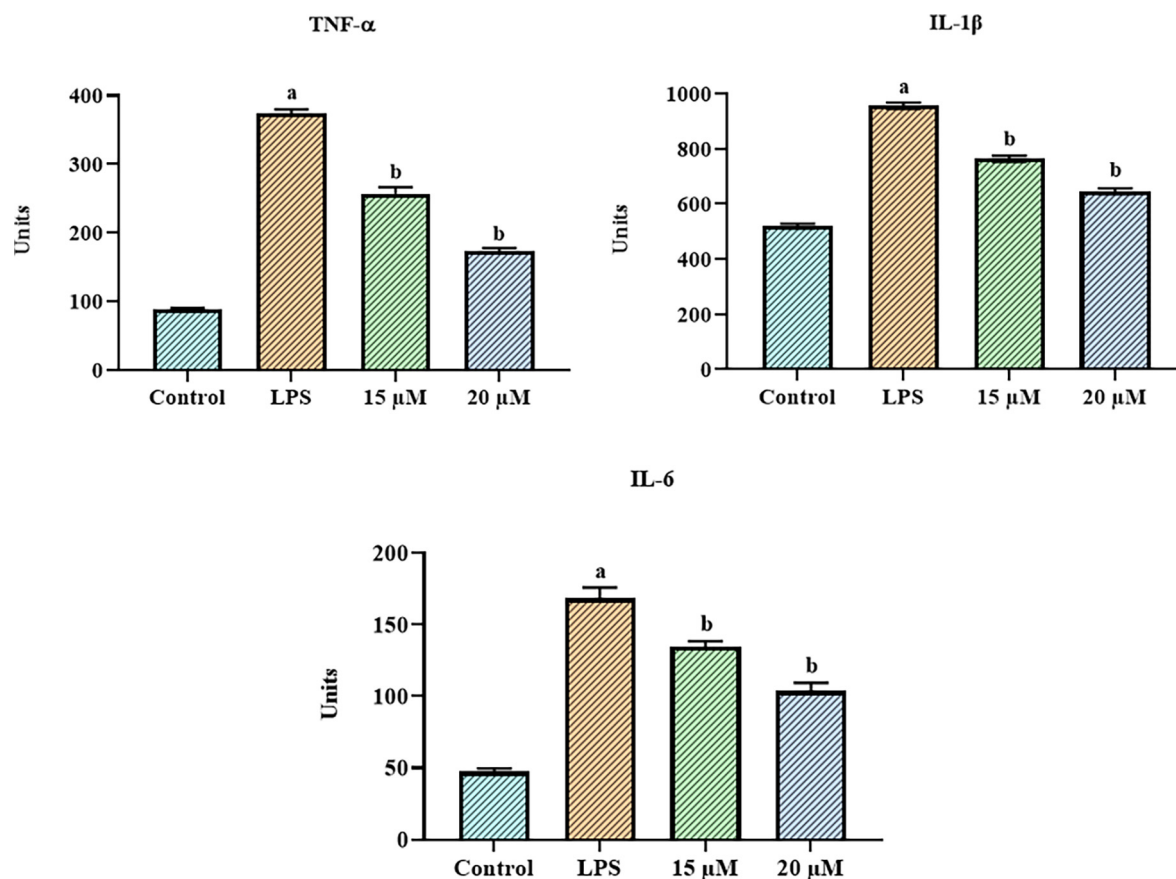
Fig. 2   Effect of betanin on the pro-inflammatory cytokine levels in the LPS-exposed RAW 264.7 cells Betanin treatments at concentrations of 15 and 20 μM reduced the levels of inflammatory cytokines such IL-6, IL-1β, and TNF-α in LPS-exposed RAW 264.7 cells. Results presented as the mean ± SD of three replicated tests. The SPSS program was used to analyze the results using one-way ANOVA and DMRT. Note: "a" denotes $p < 0.01$ when compared to control, while "b" denotes $p < 0.05$ when compared to cells that had been treated to LPS.

an upsurge in MDA and a diminution in GSH and SOD levels. It's interesting to note that the betanin treatment successfully reversed these alterations. Fig. 5 shows that when betanin (25 and 50 mg/kg) was given to animals with ALI caused by LPS, the amount of MDA was greatly reduced and SOD and GSH were raised.

3.6. Effect of betanin on the inflammatory biomarker levels in the LPS-induced ALI mice

Fig. 6 shows the status of inflammatory biomarkers i.e., iNOS, COX-2, and PGE2 in the control and LPS-exposed ALI who were given betanin. When compared to control, the ALI mice demonstrated a substantial elevation in iNOS, COX-2, and PGE2. It's interesting to note that the betanin treatment successfully reversed these alterations. In the LPS-exposed ALI mice, administering with betanin (25 and 50 mg/kg) considerably depleted the iNOS, COX-2, and PGE2 levels (Fig. 6).

3.7. Effect of betanin on the lung histopathology in the LPS-induced ALI mice

The influence of betanin on the lung histopathology in the LPS-exposed ALI mice is depicted in Fig. 7. The lung injury score was raised in the LPS-exposed ALI mice when compared to control due to the enhanced neutrophil penetration, lung edema, and alveolar epithelial cell destruction, as shown in Fig. 7. It's interesting to note that ALI animals treated with betanin (25 and 50 mg/kg) indicated a noticeable diminution in inflammatory signs, lung edema, alveolar damage, and lung injury scores (Fig. 7). These results indicate that betanin therapy reduced the lung tissues' histological changes brought on by LPS.

4. Discussion

The primary clinical manifestations of ALI include breathing problems, hypoxemia, significant capillary extravasation, and noncardiogenic pulmonary embolism (Kotas and Matthay, 2018; Li et al., 2020). Unrestrained and severe lung inflammation is an underlying fundamental immunological process and contributes to its high fatality rate (Fan and Fan, 2018). The buildup of neutrophils in the interstitial, bronchoalveolar, and lung microvasculature is believed as a pivotal causative factor to ALI/ARDS (Blazquez-Prieto and I. Ĺopez-Alonso, C. Huidobro, and G. M. Albaiceta, , 2018). Macrophages are diverse cell types that perform a pivotal function in the ALI pathophysiology. This macrophage aggregation is often
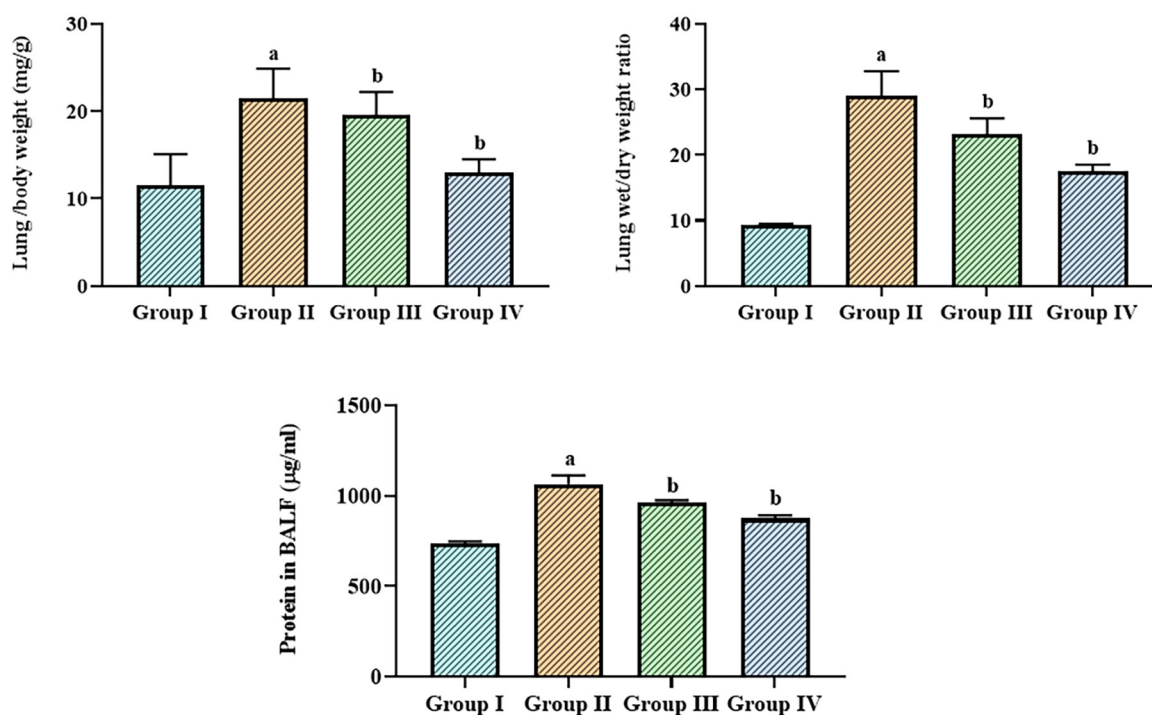
Fig. 3 Effect of betanin on the lung weight, lung/bodyweight ratio, and total protein levels in the LPS-induced ALI mice The lung weight, lung/bodyweight ratio, and protein content in the LPS-exposed ALI mice were successfully decreased by the treatment of betanin (25 and 50 mg/kg). Results presented as the mean ± SD of three replicated tests. The SPSS program was used to analyze the results using one-way ANOVA and DMRT. Note: "a" denotes $p < 0.01$ when compared to control, while "b" denotes $p < 0.05$ when compared to cells that had been treated to LPS.
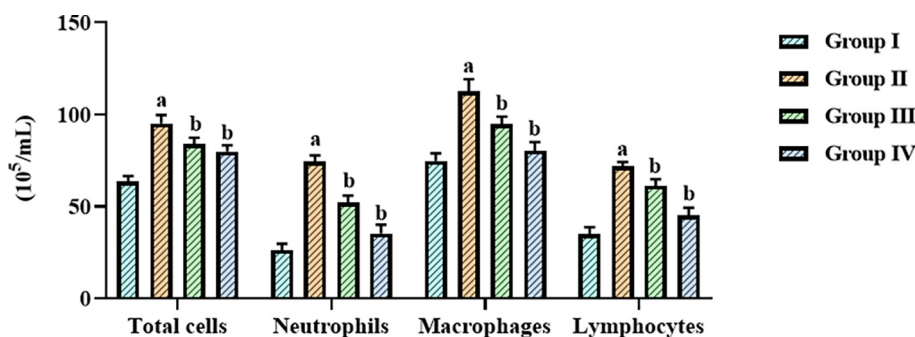

Fig. 4 Effect of betanin on the inflammatory cell counts in the BALF of LPS-induced ALI mice Betanin treatment at doses of 25 and 50 mg/kg significantly reduced the number of lymphocytes, neutrophils, macrophages, and total cells in the BALF of LPS-exposed ALI mice. Results presented as the mean ± SD of three replicated tests. The SPSS program was used to analyze the results using one-way ANOVA and DMRT. Note: "a" denotes $p < 0.01$ when compared to control, while "b" denotes $p < 0.05$ when compared to cells that had been treated to LPS.

seen at the locations of tissue damage resulting from exposure to pulmonary toxicants (Laskin et al., 2019). Following LPS activation, macrophages release a variety of proinflammatory mediators to further worsen the inflammatory response, thereby attracting neutrophils (Hollingsworth et al., 2005). Neutrophils release powerful antibacterial chemicals, such as protease, cationic compounds, and reactive oxidants (Lakshmi et al., 2012). Both neutrophil accumulation in lung capillaries and changes in neutrophil deformability are caused by LPS. Vascular permeability and pulmonary embolism can result from activated neutrophils damaging endothelial cells and destroying basement membranes (Kral-Pointner et al.,

2019). In this work, our findings demonstrated a drastic elevation in the inflammatory cell accumulations such as macrophages and lymphocytes in the BALF of the LPS-activated ALI mice. However, these increments were effectively ameliorated by the betanin treatment, which is evidenced by the reduced inflammatory cell accumulations.

Lung edema is a primary feature of ALI (Hu et al., 2017). The excessive inflammatory response, oxidative stress, and endothelial injury brought on by an inflammatory process that causes microvascular permeability are the main factors contributing to pulmonary edema (Elicker et al., 2016). An essential marker of lung edema is the lung W/D ratio. An
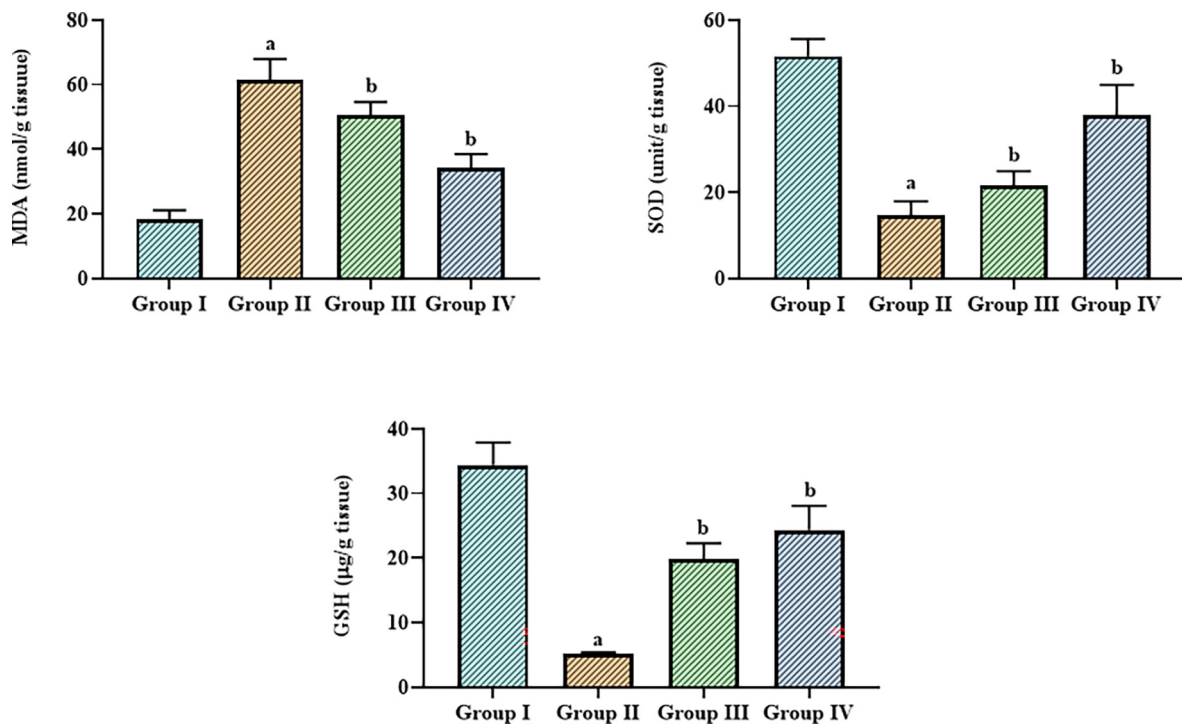
Fig. 5   Effect of betanin on the levels of oxidative and antioxidative biomarkers in the LPS-induced ALI mice The GSH level was decreased and SOD and GSH was elevated by the 25 and 50 mg/kg of betanin treatement in the LPS-induced ALI mice. Results presented as the mean $\pm$ SD of three replicated tests. The SPSS program was used to analyze the results using one-way ANOVA and DMRT. Note: "a" denotes $p < 0.01$ when compared to control, while "b" denotes $p < 0.05$ when compared to cells that had been treated to LPS.
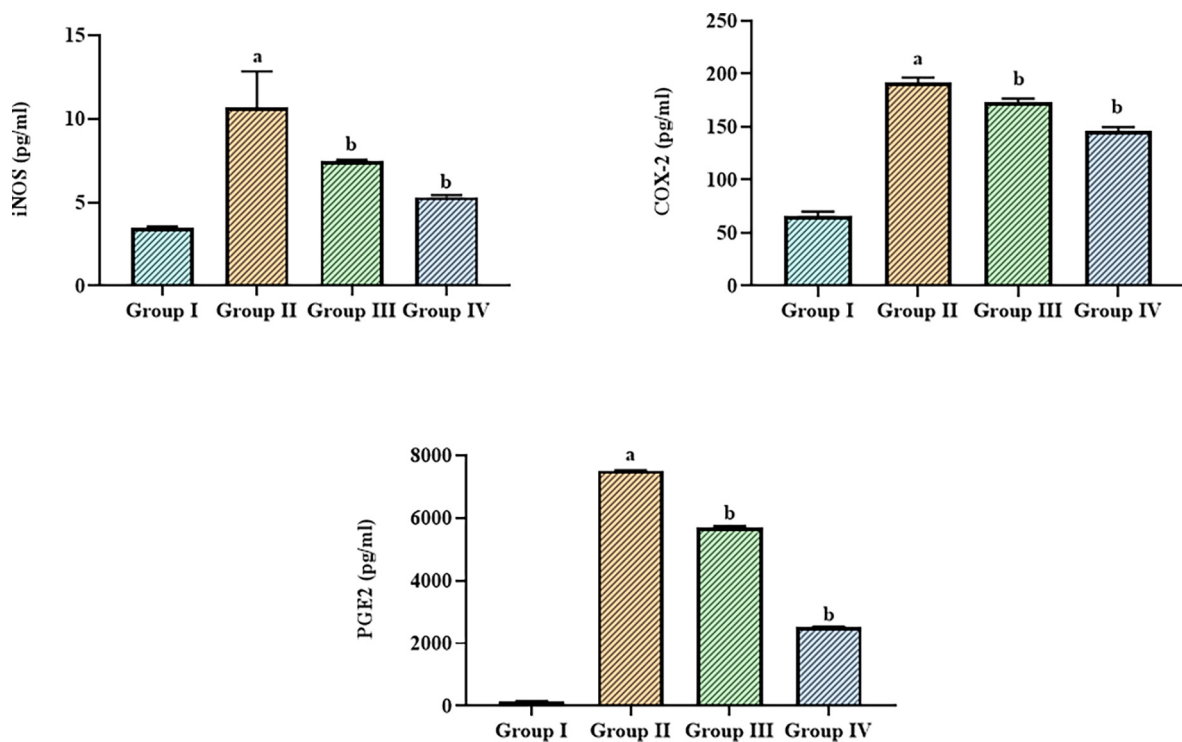

Fig. 6   Effect of betanin on the levels of inflammatory biomarkers in the LPS-induced ALI mice The treatment with 25 and 50 mg/kg of betanin effectively decreased the levels of COX-2, iNOS, and PGE-2 in the LPS-induced ALI mice. Results presented as the mean $\pm$ SD of three replicated tests. The SPSS program was used to analyze the results using one-way ANOVA and DMRT. Note: "a" denotes $p < 0.01$ when compared to control, while "b" denotes $p < 0.05$ when compared to cells that had been treated to LPS.
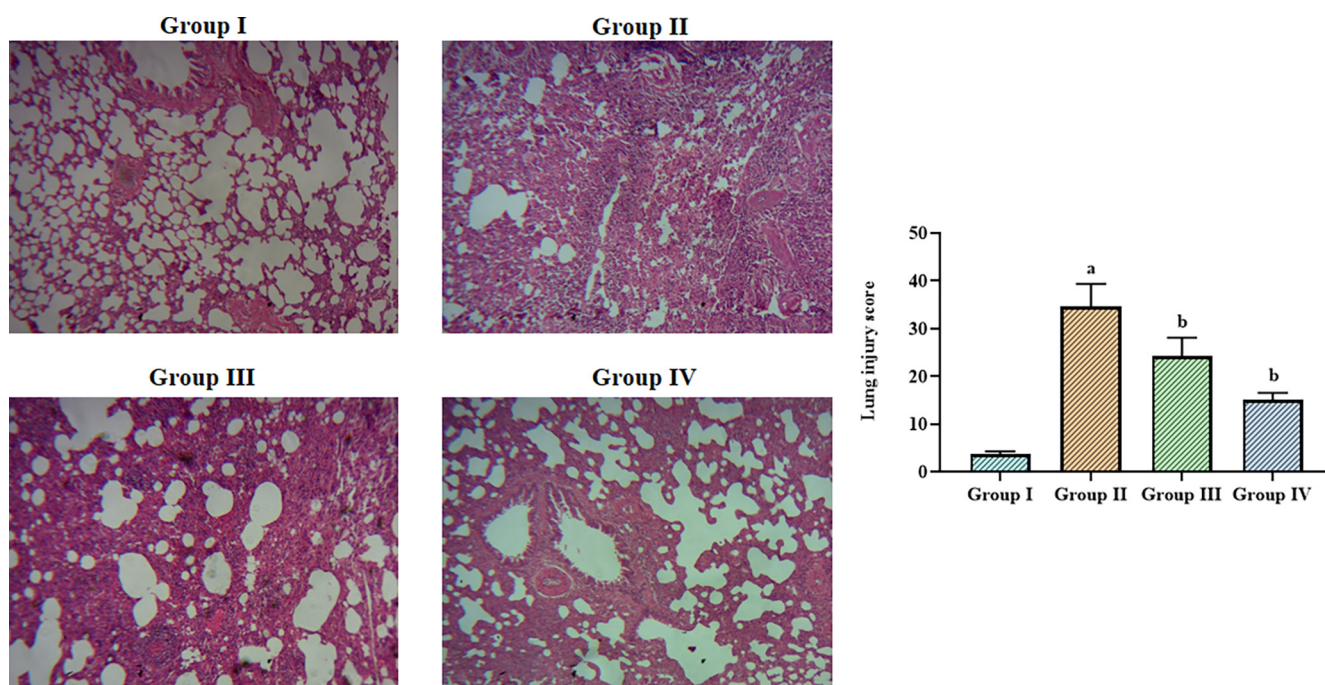
Fig. 7   Effect of betanin on the lung histopathology in the LPS-induced ALI mice The lung tissues of control mice showed normal cellular arrangements **(Group I)**. The LPS-induced ALI mice demonstrated increased inflammatory cell infiltration, lung edema, and alveolar epithelial disruption in the lung tissues **(Group II)**. The treatment with 25 and 50 mg/kg of betanin substantially reduced the histological changes in the lung tissues of ALI mice **(Group III & IV)**. Results presented as the mean ± SD of three replicated tests. The SPSS program was used to analyze the results using one-way ANOVA and DMRT. Note: "a" indicates $p < 0.01$ when compared to control, while "b" indicates $p < 0.05$ when compared to LPS-induced ALI mice.

assessment of the level of water in the lungs is provided by the W/D weight level. Another crucial sign of lung edema is a rise in BALF protein content brought on by protein vasodilatation (Muller-Redetzky et al., 2014). Our findings revealed that the LPS-exposed ALI mice demonstrated increased lung W/D weight and protein levels in the BALF than the control. Interestingly, the treatment with betanin appreciably reduced the lung W/D weight and protein level in the BALF of the LPS-exposed ALI mice. These outcomes demonstrated that the betanin effectively reduced the lung edema in ALI mice.

Inflammation may lead to oxidative stress, and oxidative stress may worsen inflammation. These two processes can interact in a variety of ways (Mittal et al., 2014; Biswas, 2016). According to a previous report, l oxidative stress and excessive inflammation causes lung injury (Zhou et al., 2019). Moreover, MDA is a byproduct of lipid peroxidation catalyzed by ROS that harms cells. Overproduction of ROS also leads to oxidative injury that modifies the body's natural antioxidant defenses by reducing SOD activity (Arunachalam et al., 2019). As a significant antioxidant in the body, SOD protects cells from free radical damage and keeps the body's metabolism in balance (Barrera et al., 2018).

SOD is found in lung tissue in large amounts and defends it against ALI by scavenging ROS (Zhang et al., 2017). GSH is crucial to the antioxidant mechanism because of its potential free radicals scavenging properties.. GSH level fluctuations reflect fluctuations in the body's oxidative state, and when oxidation stimulates the body, GSH biosynthesis rises (Khazri et al., 2016). An earlier finding highlighted that LPS exposure increases the MDA level while decreasing the antioxidant capacity in the LPS-induced ALI model (Dikmen et al.,

2021). In line with this statement, the current findings also demonstrated a significant increase in MDA and a diminution in SOD and GSH in the LPS-exposed ALI mice. However, the treatment with betanin appreciably depleted the MDA content and heightened both SOD and GSH in the ALI mice. These observations reveal the antioxidant properties of betanin.

Several inflammatory-related cytokines and chemokines are released in response to LPS exposure (Tirunavalli et al., 2021). The inflammation in ALI is not only triggered but also amplified and prolonged by activated key cytokines. The augmented cytokines are thought to be directly linked to lung damage (Yang et al., 2020). TNF-α is the first and most significant regulator of an inflammatory reaction. It is mostly generated by monocytes or macrophages that have been stimulated, and it triggers an inflammatory reaction that harms the vascular system's endothelial cells even while causing the pulmonary epithelial network to secrete other cytokines, like IL-6 (Chen et al., 2021; Guo et al., 2022). TNF-α, in along with IL-1β, commences the inflammation by increasing the synthesis of other cytokines (Thompson et al., 2017). TNF-α and IL-1β exacerbate inflammation throughout the body. TNF-α and Il-1β activity was linked to the severity of lung damage. The rate of survival of the ALI mice is also improved by inhibiting TNF-α or IL-6. In order to effectively treat ALI in clinical settings, it is necessary to decrease the abnormal secretion of inflammatory regulators (Hu et al., 2019). When RAW 264.7 cells are administered with LPS, the TLR-4 pathway was activated to identify and bind to LPS, which further led to the stimulation of intracellular signaling cascades comprising NF-κB. This, in turn, encourages the discharge of significant inflammatory signaling molecules, resulting in a cytokine flood (Byun et al., 2013). The current

study found significantly higher levels of IL-6, IL-1, and TNF- in LPS-exposed RAW 264.7 cells than in control. However, the exposure of LPS along with the betanin considerably reduced these cytokines. These findings witnessed the *in vitro* anti-inflammatory effects of betanin.

In LPS-evoked inflammation, a pro-inflammatory mediator secretated by stimulated macrophages performs a pivotal function in commencing and fueling inflammation (Lee et al., 2016). Numerous biological mechanisms, such as neurotransmission, wound repair, apoptosis, and inflammation, are influenced by iNOS. Massive amounts of NO are produced by the iNOS enzymes in living things (Lee et al., 2018). The process mediated by COX-2 results in the production of PGE2, a key lipid that may induce the induction of inflammation (Jiang et al., 2020). Therefore, to research the anti-inflammatory property of betanin, we assessed the status of iNOS, PGE2, and COX-2. The current study found that LPS-exposed ALI mice had significantly higher status of iNOS, PGE2, and COX-2. Interestingly, the betanin treatment exhibited a substantial reduction of these key inflammatory markers in the ALI mice. These results also evidenced the anti-inflammatory roles of betanin.

Furthermore, the role of epithelial damage in the course of ALI has now become increasingly clear. This epithelial damage impairs cell fluid transport and decreases surfactant synthesis (Manicone, 2009). Usually, lung damage reduces oxygenation and exacerbates the systemic inflammatory reaction. Endothelial damage appears minutes to hours following ALI stimulation and leads to endothelial intercellular gaps. Improved microvascular permeability can be attributed to the formation of intercellular gaps (Mao et al., 2009). In this work, the findings of the histopathological study demonstrated neutrophil infiltration, lung edema, and alveolar epithelial membrane destruction. It's interesting to note that ALI mice treated with betanin effectively ameliorated the LPS-triggered histological injuries in ALI mice.

5. Conclusion

The current results first show that betanin effectively ameliorated LPS-induced ALI in mice through its potential anti-inflammatory and antioxidant properties. The betanin substantially inhibited the inflammatory response and augmented the antioxidants in the ALI mice. It also improved the proliferation and abridged the inflammatory cytokines in the RAW 264.7 cells *in vitro*. This study suggested that betanin could effectively ameliorate the LPS-exposed ALI in mice and could be utilized as an anti-inflammatory and anti-oxidant candidate to deal with ALI in medical settings.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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