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Original Article

Uvaol Inhibits Inflammatory Response and Prevents Lipopolysaccharide-Induced Acute Lung Injury in Mice

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ABSTRACT

Background: Sepsis is a clinical disease characterized by systemic inflammatory reactions following infection that can lead to multiorgan failure. Acute Lung Injury (ALI) is a common ailment with increased morbidity and fatality rates worldwide. **Objectives:** The present investigation was planned to investigate the salutary activities of uvaol against Lipopolysaccharide (LPS)-exposed ALI in mice. **Materials and Methods:** The 5 mg/kg of LPS was exposed to the mice for 3 days through the intra-tracheal route to initiate the ALI, and 5 and 10 mg/kg, respectively, of uvaol were administered orally for 3 days the LPS challenge. The mice were scarified under anesthesia, lungs were excised, and wet and dry weights were weighed accurately. All the biochemical parameters, including apoptosis, inflammation, and oxidative stress marker levels, were assessed using the respective assay kits. **Results:** The uvaol (5 and 10 mg/kg) treatment effectively diminished pulmonary edema, total protein, and LDH activity in the ALI mice. The MDA was reduced, and the GSH and SOD levels were increased by the uvaol. The uvaol effectively reduced the inflammatory cytokines and infiltrations in the ALI mice. The PGE-2, iNOS, and COX-2 levels in the BALF of ALI mice were effectively reduced by the uvaol. The Bax and caspase-3 expression was reduced, and the Bcl-2 was elevated by the uvaol. The outcomes of histopathological analysis also supported the therapeutic potential of uvaol against ALI. **Conclusion:** In conclusion, the outcomes of the current exploration highlighted that uvaol considerably prevented the LPS-exposed ALI in mice via its antioxidant and anti-inflammatory effects. Hence, it can be concluded that uvaol can be employed in the treatment of ALI in the future.

Keywords: Lipopolysaccharide, Cytokines, Apoptosis, Uvaol, Lung edema, Sepsis.

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INTRODUCTION

Sepsis is the primary cause of mortality in critical care medicine, with an increasing prevalence each year. Sepsis is characterized by systemic inflammatory reactions following infection that can result in multiorgan failure. Sepsis has recently been identified as a pivotal cause of mortality.[1] Acute Lung Injury (ALI), a disease condition with increased morbidity and fatality rates, is a substantial and diffuse lung injury due to a number of factors. The lung edema and poor gas exchange are hallmarks of this condition, which is caused by alveolar epithelium damage, inflammatory cell infiltrations, and an increase in lung vascular permeability.[2] It is regarded as the main reason of fatalities in patients receiving critical care. ALI is classified as severe clinical illnesses with a death rate of 26-58%, posing a significant public health concern each year.[3]

Organ failure induced by sepsis is exceedingly complex, comprising dysregulated systemic inflammation generated by invading bacteria or their byproducts. Lipopolysaccharide (LPS) is extensively occur in the cell wall of gram-negative bacteria that causes inflammatory reactions and oxidative stress.[4] It was highlighted that LPS is a primary initiator of the inflammatory and pathological reactions found in septic shock patients.[5] ALI occurs when a serious infection or other factors produce endothelial barrier disruption, which leads to the migration of inflammatory cells into the pulmonary system. Multiple immune cascades can be initiated by LPS since it stimulates a wide variety of pro-inflammatory mediators. Animal models of ALI triggered by LPS have been extensively employed in recent years for research into both ALI therapeutics and the mode of action of these medications.[6] In prior investigations, LPS was utilized to establish bacterial infection-caused ALI. Since LPS causes inflammation, encourages leukocyte penetration, and severely impairs normal lung functioning, as previously documented, it promotes secondary injury to the lungs and accelerates the progression of ALI.[7,8]

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LPS causes inflammation in the lungs after being inhaled through the nose because it damages lung parenchyma, produces proteases and ROS, and activates lung inflammatory cells in the interstitial regions. Vascular damage, including diffuse pulmonary injury, bleeding, swelling, and fibrin deposition, occurs at the end.[9] The LPS-exposed ALI model is a well-established model to assess the impacts of medications on ALI; furthermore, LPS treatment has been shown to cause several pathological alterations in the lungs.[10] It has been well stated that inflammation and oxidative stress play a significant role in the ALI progression.[11] Dysregulated pulmonary functioning and cell death in the lungs are the internal outcomes of ALI due to oxidative damage.[12] The significant increase in inflammation is the most consistent trait in animal models exposed to LPS. After being triggered, neutrophils accumulate at the site of injury and release inflammatory mediators.[13]

There is a lack of efficient treatments and specialized medications for treating ALI because its mechanisms have not been adequately explained up to this point. At present, mechanical ventilation and corticosteroid medication remain the mainstays of treatment, along with treating the underlying cause. Unfortunately, pharmacological approaches have not been successful in lowering mortality rates. Therefore, novel medicines that are both safe and effective are required to improve the clinical outcomes of ALI patients.[14] Uvaol, a pentacyclic triterpene, is a major bioactive compound that occurs abundantly in olives (Olea europaea).[15] Uvaol is highlighted to demonstrate numerous biological effects, such as antioxidant,[16] anti-inflammatory,[17] vasodilator effects,[18] antitumor,[19] and wound healing[20] properties. In addition, uvaol is highlighted as interfering with allergic inflammatory reactions via disturbing leucocyte recruitment and cytokine production at the inflammatory sites.[21] Therefore, we hypothesized that uvaol could be beneficial in ameliorating the allergen-induced sepsis. Henceforth, the present exploration is planned to investigate the salutary activities of uvaol against LPS-induced ALI in mice.

MATERIALS AND METHODS

Reagents

The following chemicals, including uvaol, LPS, etc., were acquired from Sigma-Aldrich, USA. All the assays for the estimation biochemical assays were procured from Thermofisher, USA; Biocompare, USA; and Mybiosource, USA, respectively.

Experimental mice

The 3-4-week-old healthy BALB/c mice were employed in this exploration. The mice were housed in clean polypropylene cabins with free access to the pellet food. Mice were caged in a proper laboratory setting with a temperature of 24°C, air moisture of 60-70%, and 12 hr light and dark cycles. Each mouse underwent acclimation to the laboratory settings before the initiation of experiments. All the animals' experiments were approved by the institutional animal ethics committee.

Experimental design

The acclimated mice were alienated into four groups. Group I mice were control. Group II mice were subjected to a 3-day exposure of 5 mg/kg of LPS through the intra-tracheal route to initiate the ALI. The mice from groups III and IV were treated with 5 and 10 mg/kg, respectively, for 3 days before exposure to the LPS. After the completion of the treatments, the mice were anesthetized, sacrificed, and their lung tissues excised. After collection, the tissues were weighed accurately to get a wet weight. After that, the lungs were dehydrated in an oven at 80°C, then weighed properly to get a lung dry weight.

Broncho Alveolar Lavage Fluid (BALF) preparation

The BALF was collected by injecting 30 mL of saline aliquots into the middle right lobe of control and treated mice. After collecting the BALF, it was centrifuged at 6000 rpm for 5 min. The upper aqueous phase was utilized for the biochemical calculations, which ensured that no cell debris was present. Cells in the BALF fluid pellets was determined using differential cell counting. The total protein was assessed by Lowry's method, and the LDH activity was analyzed using assay kits (Biocompare, USA).

Assay of oxidative stress biomarkers

The corresponding assay kits were employed to assess the status of oxidative and antioxidant biomarkers like MDA, GSH, and SOD in the lung tissue samples from control and treated mice. Each assay was conducted thrice using prescribed guidelines of the manufacturer (Mybiosource, USA).

Assay of pro-inflammatory cytokines

The cytokine levels including IL-6 and TNF-α in the BALF of the experimental mice were quantified using corresponding assay kits. Each assay was conducted thrice using prescribed guidelines of the manufacturer (Mybiosource, USA).

Assay of inflammatory markers

The status of inflammatory mediators like PGE-2, iNOS, and COX-2 in the BALF of the mice were estimated using corresponding assay kits. Each assay was conducted thrice using prescribed manual of the manufacturer (Thermofisher, USA).

Analysis of apoptotic marker levels

The Bax, Bcl-2, and caspase-3 expressions in the BALF of the mice was investigated using the corresponding assay kits. Each assay was conducted thrice using prescribed guidelines by manufacturer (Mybiosource, USA).

Histopathological analysis

The lungs were processed with 10% formalin and paraffinized using paraffin wax. The paraffinized lung tissues were then cut into slices of 5 µm diameter using a rotary microtome. Then slides were deparaffinized and stained using eosin and hematoxylin. Finally, an optical microscope was employed to investigate the histological alterations in the lung tissue sections at 40× magnification.

Statistical analysis

One-way ANOVA and Tukey's *post hoc* assay was performed to assess the values obtained from each assay. Values are validated statistically using SPSS and given as a mean±SD of triplicates with $p<0.05$ as significant.

RESULTS

Uvaol decreases the lung W/D weight ratio, LDH activity, and total protein level in the BALF of the experimental mice

Figure 1 shows the total protein, LDH activity, and lung W/D weight ratio in the control and treated mice. As indicated in Figure 1, the ALI mice revealed a severe increase in the lung W/D weight, LDH activity, and total protein in the BALF when compared with control. However, the uvaol (5 and 10 mg/kg) treatment considerably diminished the total protein, LDH activity, and W/D weights in the ALI mice, which proves its beneficial properties against the ALI in mice.

Uvaol inhibits the inflammatory cell infiltration and MPO activity in the experimental mice

The inflammatory cell counts and MPO activity in the BALF were assessed, and the outcomes were revealed in Figure 2. The neutrophils, macrophages, lymphocytes, eosinophils, and total cell counts were severely augmented in the BALF. The ALI mice also exhibited a remarkable elevation in MPO activity. Nonetheless, the 5 and 10 mg/kg of uvaol treatment considerably diminished the inflammatory cell counts and decreased the MPO activity in the BALF. These findings witness the anti-inflammatory properties of uvaol against the ALI condition.

Uvaol treatment increases the antioxidants in the experimental mice

As demonstrated in Figure 3, the MDA level increased drastically while the SOD and GSH levels diminished in the lung tissues of the ALI mice when compared with the control. Interestingly, the 5 and 10 mg/kg of uvaol effectively diminished the MDA while boosted the SOD and GSH levels in the lung tissues of the ALI mice. These outcomes supported the antioxidant properties of uvaol against the ALI.

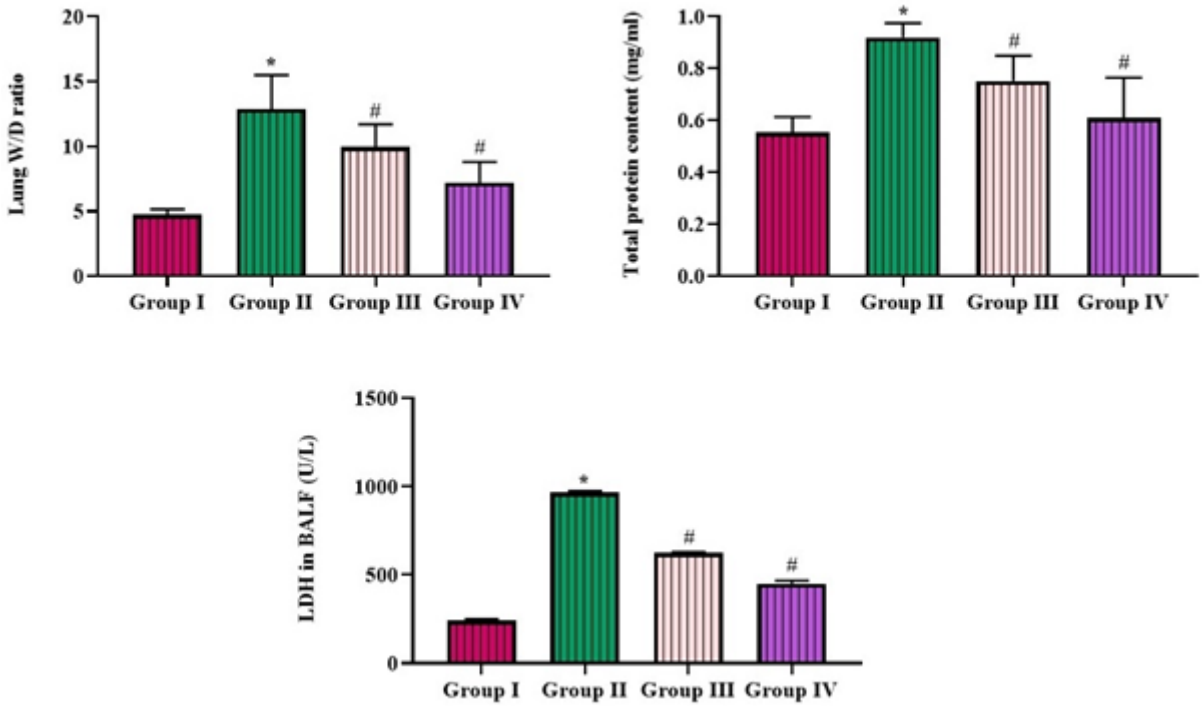

Figure 1: Effect of uvaol on the lung W/D weight ratio, LDH activity, and total protein level in the BALF of the experimental mice.

The values are analyzed by one-way ANOVA and Tukey's *post hoc* assay by using the SPSS software. The data are represented by mean±SD of triplicate assays. '*' represents $p<0.05$ when compared with control and '#' represents $p<0.01$ when compared with LPS-induced ALI mice.

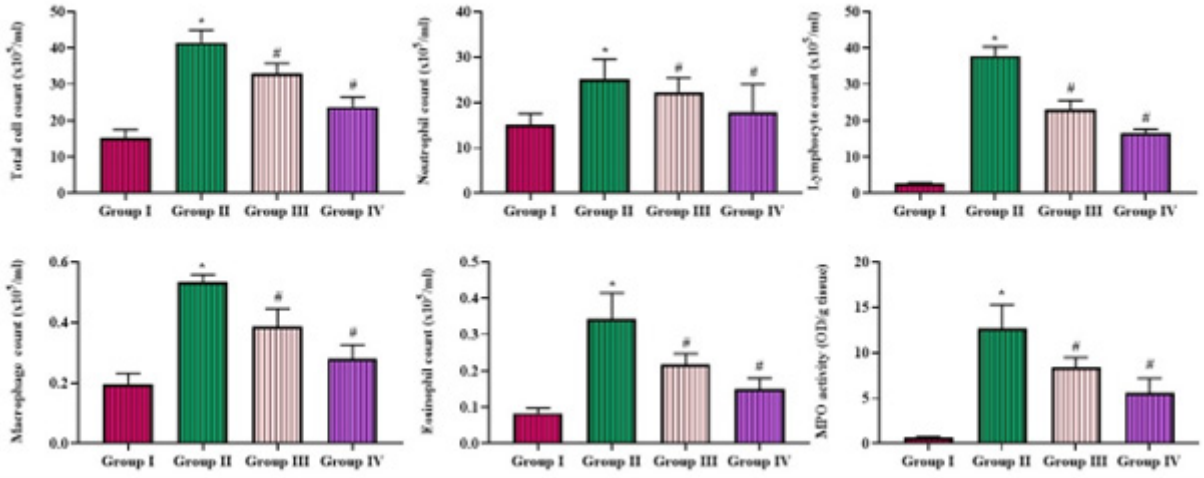

Figure 2: Effect of uvaol on the inflammatory cell infiltration and MPO activity in the BALF of the experimental mice.

The values are analyzed by one-way ANOVA and Tukey's *post hoc* assay by using the SPSS software. The data are represented by mean±SD of triplicate assays. '*' represents $p<0.05$ when compared with control and '#' represents $p<0.01$ when compared with LPS-induced ALI mice.

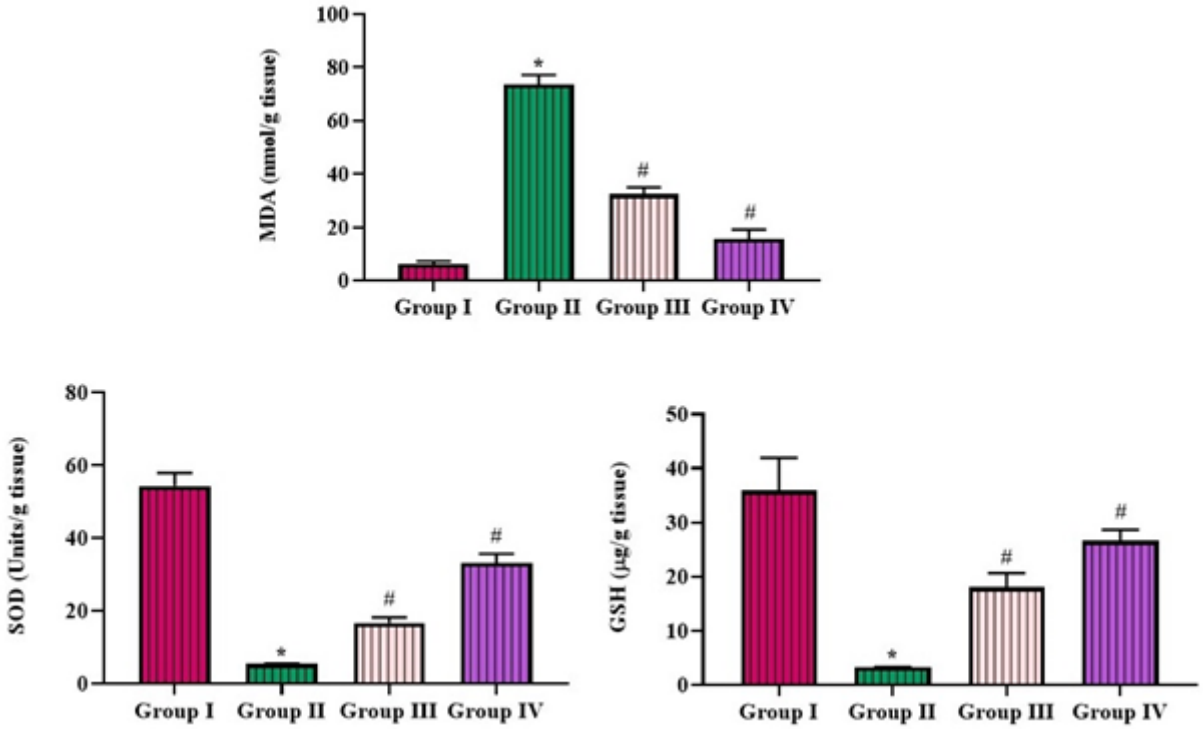

Figure 3: Effect of uvaol on the oxidative stress markers in the experimental mice.

The values are analyzed by one-way ANOVA and Tukey's *post hoc* assay by using the SPSS software. The data are represented by mean±SD of triplicate assays. '*' represents $p<0.05$ when compared with control and '#' represents $p<0.01$ when compared with LPS-induced ALI mice.

Uvaol decreases pro-inflammatory cytokines in the experimental mice

The IL-6 and TNF-α contents in the BALF of control and treated mice were examined, and the findings are illustrated in Figure 4. The increased IL-6 and TNF-α status were noted in the BALF of LPS-induced ALI mice when compared to control. Whereas, the 5 and 10 mg/kg of uvaol treatment considerably diminished these cytokine levels in the BALF. These findings highlight the anti-inflammatory properties of uvaol.

Uvaol treatment decreases the iNOS, COX-2, and PGE-2 levels in the experimental mice

Figure 5 demonstrates the levels iNOS, PGE-2, and COX-2 in the BALF of control and treated mice. The increased iNOS, COX-2,

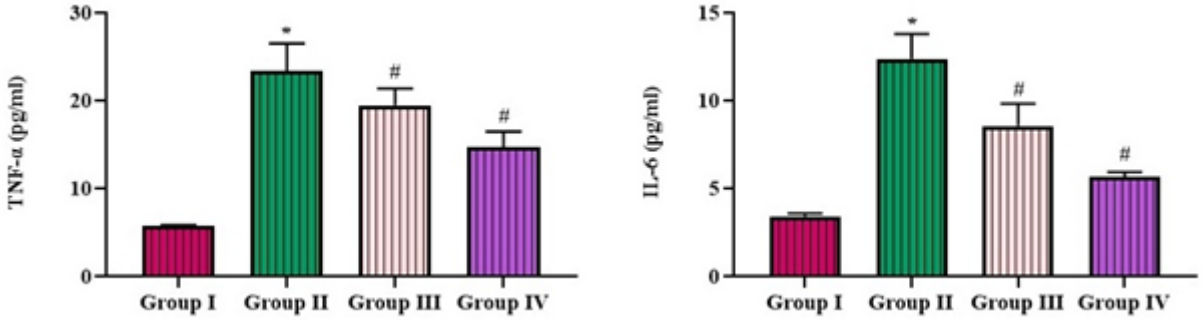

Figure 4: Effect of uvaol on the pro-inflammatory cytokine levels in the BALF of experimental mice.

The values are analyzed by one-way ANOVA and Tukey's *post hoc* assay by using the SPSS software. The data are represented by mean±SD of triplicate assays. '*' represents $p<0.05$ when compared with control and '#' represents $p<0.01$ when compared with LPS-induced ALI mice.

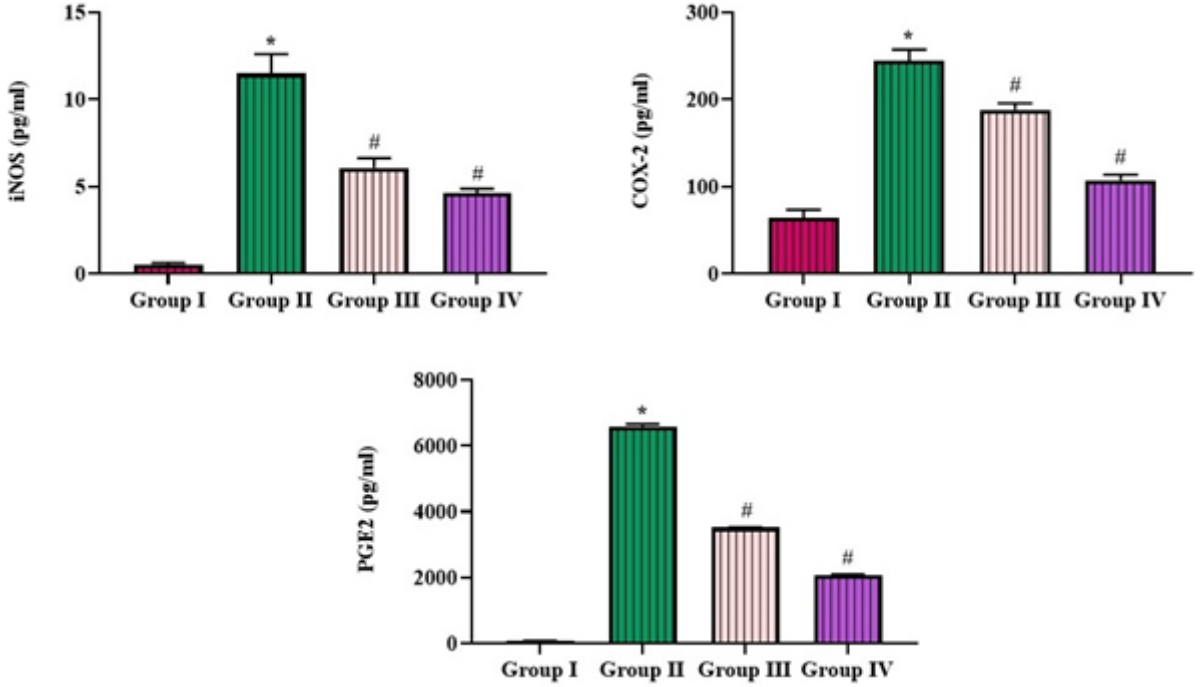

Figure 5: Effect of uvaol on the iNOS, COX-2, and PGE-2 levels in the BALF of the experimental mice.

The values are analyzed by one-way ANOVA and Tukey's *post hoc* assay by using the SPSS software. The data are represented by mean±SD of triplicate assays. '*' represents $p<0.05$ when compared with control and '#' represents $p<0.01$ when compared with LPS-induced ALI mice.

and PGE-2 levels were observed in the BALF of ALI mice. Fascinatingly, 5 and 10 mg/kg of uvaol treatment remarkably reduced the iNOS, COX-2, and PGE-2 levels in the BALF of ALI mice. These outcomes witness the salutary properties of the uvaol treatment on the ALI in mice.

Uvaol treatment decreases apoptotic protein expressions in the BALF of the experimental mice

The expressions of apoptotic proteins like Bax, caspase-3, and Bcl-2 were examined, and the results are given in Figure 6. The ALI mice revealed a significant increase in the apoptotic proteins Bax and caspase-3 expression and diminished the Bcl-2 expression in the BALF when compared with control. Nonetheless, reduced Bax and caspase-3 expression and augmented Bcl-2 expression were observed in the 5 and 10 mg/kg uvaol-treated ALI mice. These findings proved that the uvaol treatment decreased the apoptosis of lung cells in the ALI mice.

Uvaol decreases lung histopathological changes in the experimental mice

The impacts of uvaol on the lung histology of the experimental mice were examined, and the findings are revealed in Figure 7. The ALI mice demonstrated higher inflammatory cell infiltrations, alveolar epithelial damage, pulmonary edema, and

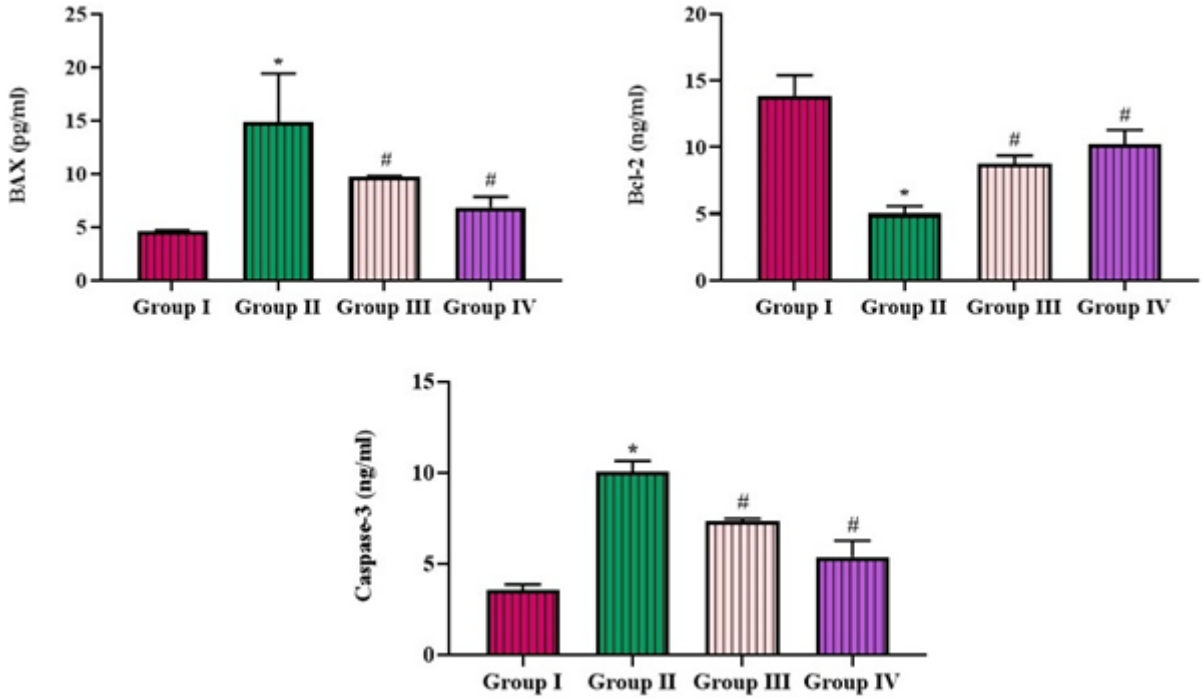

Figure 6: Effect of uvaol on the apoptotic protein expressions in the BALF of the experimental mice.

The values are analyzed by one-way ANOVA and Tukey's *post hoc* assay by using the SPSS software. The data are represented by mean±SD of triplicate assays. '*' represents $p<0.05$ when compared with control and '#' represents $p<0.01$ when compared with LPS-induced ALI mice.

alveolar cell death in the lung tissues. Whereas, the 5 and 10 mg/kg of uvaol treatment exhibited a considerable decrease in lung histological changes, as seen by decreased inflammatory cell infiltration, alveolar epithelial damage, and alveolar cell death. These outcomes highlight that the uvaol treatment prevented the LPS-triggered histological damage in the lung tissues.

DISCUSSION

ALI is characterized by various changes in the lung tissue, such as diffuse alveolar injury, inflammation caused by neutrophils, lung edema development, and surfactant failure. According to clinical studies, ALI commonly causes bilateral lung infiltrates, serious hypoxemia, and lung compliance, which have an impact on morbidity and frequently result in death.[22] ALI leads to inflammation in the lungs, which manifests as inflammatory cell invasion, the release of several proinflammatory cytokines, and alveolar interstitial edema. When the inflammatory cells are stimulated, inflammatory cytokines are released, disrupting the integrity of the pulmonary epithelium and endothelium and causing lung edema. According to Giebelen *et al.*,[23] edema of pulmonary tissues is a sign of both local and systemic inflammation. Additionally, some of the bronchioles in the LPS-treated group exhibited deteriorated lining epithelium and a thick alveolar wall. The extend of pulmonary edema was quantitatively investigated by assessing the W/D ratio. The severity of pulmonary edema increases as the W/D ratio rises. Sun *et al.*[24] employed the lung W/D weight ratio to assess the degree of lung edema. We discovered that uvaol can minimize pulmonary edema by decreasing the lung W/D weight ratio. This outcome signifies that uvaol can successfully prevent LPS-triggered lung edema in ALI mice.

Oxidative stress is now understood to serve a critical function in the pathophysiology of lung injury. Consistent with previous research,[25] we found that LPS-treated mice exhibit aberrant oxidative stress conditions, as witnessed by the augmented MDA levels and reduced SOD and GSH levels in the lungs. MDA, a byproduct of lipid peroxidation,[26] can serve as an indicator of oxidative stress. We also measured the antioxidant SOD and GSH levels in the experimental mice. Antioxidants, including SOD and GSH, are downregulated in response to LPS treatment, contributing to lung injury. SOD can convert the superoxide radicals to hydrogen peroxide, which can then be broken down by GPx into water and oxygen.[27] Therefore, boosting SOD and GSH levels is crucial for protecting cells from oxidative stress. During ALI, the body secretes an excessive amount of ROS, which surpasses its antioxidant ability and leads to oxidative stress and harm to lung tissue. Inhibition of oxidative stress has been shown to alleviate the symptoms of ALI.[28] Similarly, the outcomes of this work showed that uvaol treatment significantly mitigated the oxidative stress in the ALI mice. These findings evidencing the antioxidant potentials of the uvaol treatment on the ALI condition

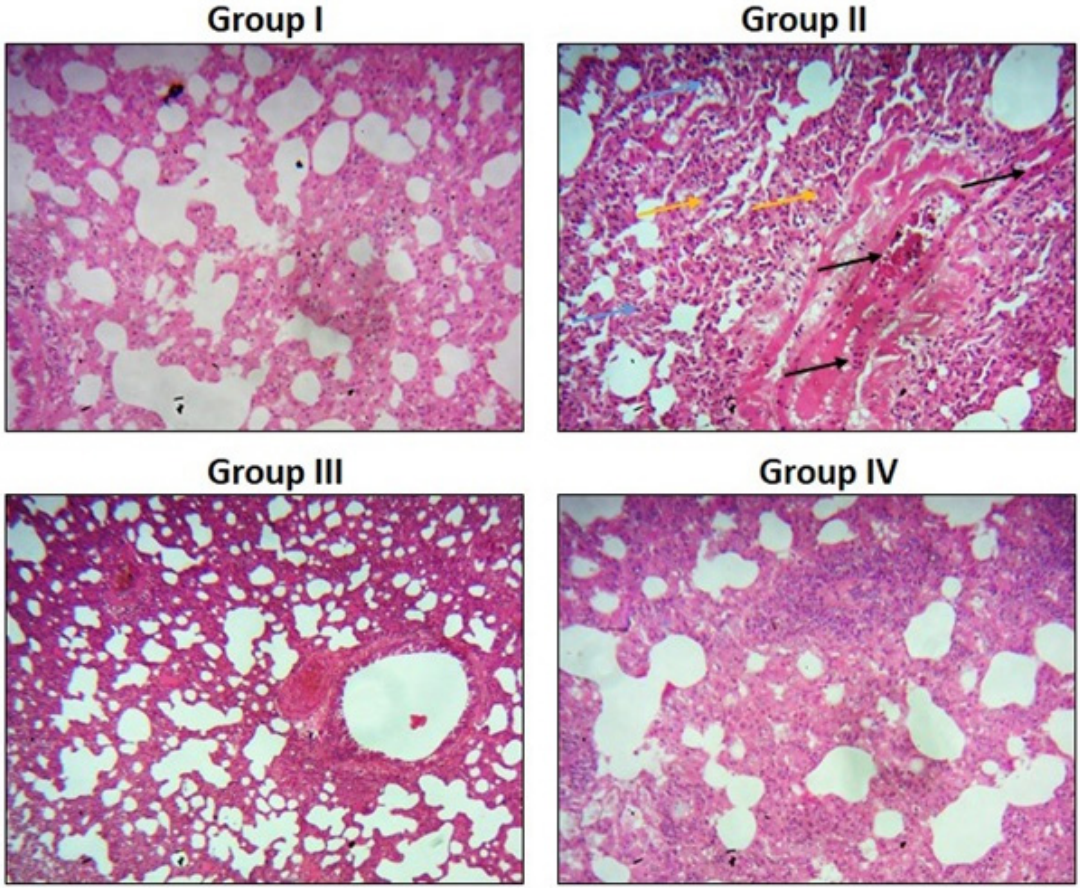

Figure 7: Effect of uvaol on the lung histopathological changes in the experimental mice.

The lung tissues of control mice showed a normal alveolar structures (Group I). The lung tissues of the ALI mice demonstrated increased inflammatory cell infiltrations (black arrows), alveolar epithelial damages, lung edema (yellow arrows), and alveolar cell death (blue arrows) (Group II). The 5 and 10 mg/kg of uvaol treatment exhibited the considerable decrease in the lung histological changes, as seen by decreased inflammatory cell infiltration, alveolar epithelial damages, and alveolar cell death (Group III and IV).

The lung inflammation, which is responsible for the high death rate, is connected to the fundamental immunological mechanism of ALI. Macrophages play a significant function in the pathophysiology of ALI.[29] Macrophages are crucial for the onset of inflammation following lung injury by secreting a variety of cytokines and chemokines that stimulate the induction and migration of neutrophils and other inflammatory cells.[30] Neutrophils are the earliest immune cells to induce an immunological reaction and travel to inflammatory sites.[31] Activation of neutrophils improves their endothelial adhesion, migration, and emigration capabilities. The inflammatory cascade initiated by LPS-stimulated monocytes can worsen lung damage.[32] The outcomes of the current exploration showed that the ALI mice resulted in increased levels of inflammatory cell infiltration into the pulmonary tissues. However, the uvaol treatment considerably mitigated the inflammatory cell infiltrations, which proves its anti-inflammatory properties.

Pulmonary inflammation contributes significantly to the development of ALI. After an ALI, the lungs undergo a significant inflammatory response, and inflammatory cells infiltrate into the alveolar space, where they produce proinflammatory cytokines and chemokines like TNF-α and IL-6.[33] High levels of these cytokines are thought to be intimately related to lung injury. It has been shown that TNF-α has a crucial role in many inflammatory ailments.[13] TNF-α is produced at a high level within lung tissue, where it initiates several inflammatory processes that contribute to the formation of inflammatory responses. IL-6 is a critical mediator in LPS-induced ALI, in addition to its essential role in inflammation and cell-mediated immunity.[34] IL-6 stands out as a very active cytokine that contributes significantly to inflammation. Several clinical problems, including lung damage leading to acute respiratory distress syndrome, often accompany LPS exposure and an increase in IL-6 levels.[35] It has already been highlighted that the exposure to the LPS triggers the generation of TNF-α and IL-6.[36] The present outcomes revealed a remarkable elevation in TNF-α and IL-6 status in the ALI mice. Interestingly, the uvaol treatment appreciably reduced the IL-6 and TNF-α status in the ALI mice, which highlights its strong anti-inflammatory properties.

Lung cell apoptosis is a crucial cause of the pathogenesis of ALI. Apoptosis is an important mechanism of ALI. For instance, the mitochondrial cascade serves a pivotal function in apoptosis.[37] The Bax/Bcl-2 genes enter mitochondria and promote mitochondrial membrane permeability, which results in a reduction in membrane potential. This, in turn, triggers caspase-9/-3, which results in cell apoptosis.[38] In this work, uvaol was found to reduce apoptosis, which is evidenced by elevated Bcl-2 expression and reduced Bax and caspase-3 expressions in the pulmonary tissues of ALI mice, indicating that uvaol may ameliorate ALI in mice.

MPO is a biomarker of neutrophils since its levels are positively correlated with neutrophil counts in different tissues. It is also a crucial indicator for assessing the degree of inflammation. As elevated MPO levels are the most reliable indicator of neutrophil aggregation, inhibiting them significantly reduces the inflammatory response.[39] NO is produced by the enzyme iNOS, which is one of the key components of inflammation in the lung and alveoli. iNOS is a critical player of development of inflammatory ailments. Therefore, any drug that suppresses iNOS may reduce the incidence of lung disorders, as excessive iNOS production is linked to the development of numerous lung ailments.[40,41] It has been suggested that PGE2, a key lipid generated by the COX-2-catalyzed process, may contribute to the activation of inflammation.[42] Inflammation is intimately linked to the activity of COX-2, which participates in the processing of polyunsaturated fatty acids. During the inflammatory process, when PGE2 synthesis is boosted and cell damage is induced, COX-2 expression is dramatically upregulated. Hashemi Goradel *et al.*[43] found that lowering COX-2 levels is effective in treating ALI. In this work, we noted that LPS challenge resulted in a drastic elevation in MPO, iNOS, PGE-2, and COX-2 levels in the ALI mice. Whereas, the uvaol treatment significantly suppressed these levels, which proves the beneficial properties of the uvaol on the ALI.

CONCLUSION

The findings of the current exploration highlighted that uvaol effectively prevented LPS-induced ALI in mice. The results revealed that the uvaol treatment considerably reduced lung edema, inflammation, oxidative stress, apoptosis, and lung injury in the ALI mice. Therefore, it can be concluded that uvaol can be employed in the treatment of ALI in the future. Furthermore, we strongly recommend further molecular-level studies in the future to clearly understand the precise therapeutic roles of uvaol against the ALI condition.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ALI: Acute lung injury; **LPS:** Lipopolysaccharide; **BALF:** Broncho alveolar lavage fluid; **LDH:** Lactate dehydrogenase.

SUMMARY

Sepsis is characterized by systemic inflammatory reactions following infection that can result in multiorgan failure. Sepsis has recently been identified as a pivotal cause of mortality. The uvaol treatment considerably reduced lung edema, inflammation, oxidative stress, apoptosis, and lung injury in the ALI mice. Uvaol treatment significantly suppressed MPO, iNOS, PGE-2, and COX-2 levels, which proves the beneficial properties of the uvaol on the ALI

REFERENCES

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, *et al.* The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016;315(8):801-10. doi: 10.1001/jama.2016.0287, PMID 26903338.
2. An X, Sun X, Hou Y, Yang X, Chen H, Zhang P, *et al.* Protective effect of oxytocin on LPS-induced acute lung injury in mice. Sci Rep. 2019;9(1):2836. doi: 10.1038/s41598-019-39349-1, PMID 30808956.
3. Patel B, Chatterjee S, Davignon S, Herlihy JP. Extracorporeal membrane oxygenation as rescue therapy for severe hypoxemic respiratory failure. J Thorac Dis. 2019;11(Suppl 14):S1688-97. doi: 10.21037/jtd.2019.05.73, PMID 31632746.
4. Kheiry, *et al.* 2019. Kheiry M, Dianat M, Badavi M, Mard SA, Bayati V. p-coumaric acid attenuates lipopolysaccharide-induced lung inflammation in rats by scavenging ROS production: an *in vivo* and *in vitro* study. Inflammation. 2019;42(6):1939-50. doi: 10.1007/s10753-019-01054-6, PMID 31267276.
5. Wang Y, Zhang J, Bo J, Wang X, Zhu J. Hydrogen-rich saline ameliorated LPS-induced acute lung injury via autophagy inhibition through the ROS/AMPK/mTOR pathway in mice. Exp Biol Med (Maywood). 2019;244(9):721-7. doi: 10.1177/1535370219847941, PMID 31042074.
6. Zhou J, Peng Z, Wang J. Trelagliptin alleviates lipopolysaccharide (LPS)-induced inflammation and oxidative stress in acute lung injury mice. Inflammation. 2021;44(4):1507-17. doi: 10.1007/s10753-021-01435-w, PMID 33751359.
7. Tasaka S, Amaya F, Hashimoto S, Ishizaka A. Roles of oxidants and redox signaling in the pathogenesis of acute respiratory distress syndrome. Antioxid Redox Signal. 2008;10(4):739-53. doi: 10.1089/ars.2007.1940, PMID 18179359.
8. Goodman RB, Pugin J, Lee JS, Matthay MA. Cytokine-mediated inflammation in acute lung injury. Cytokine Growth Factor Rev. 2003;14(6):523-35. doi: 10.1016/s1359-6101(03)00059-5, PMID 14563354.
9. Shokry AA, El-Shiekh RA, Kamel G, Bakr AF, Ramadan A. Bioactive phenolics fraction of *Hedera helix* L.(Common Ivy Leaf) standardized extract ameliorates LPS-induced acute lung injury in the mouse model through the inhibition of proinflammatory cytokines and oxidative stress. Heliyon. 2022;8(5):e09477. doi: 10.1016/j.heliyon.2022.e09477, PMID 35647334.
10. Kosutova P, Mikolka P, Kolomaznik M, Balentova S, Adamkov M, Calkovska A, *et al.* Reduction of lung inflammation, oxidative stress and apoptosis by the PDE4 inhibitor Roflumilast in experimental model of acute lung injury. Physiol Res. 2018;67;Suppl 4:S645-54. doi: 10.33549/physiolres.934047, PMID 30607971.
11. Dias-Freitas F, Metelo-Coimbra C, Roncon-Albuquerque Jr. R. Molecular mechanisms underlying hyperoxia acute lung injury. Respir Med. 2016;119:23-8. doi: 10.1016/j.rmed.2016.08.010, PMID 27692143.
12. Tan YB, Mulekar S, Gorodnya O, Weyant MJ, Zamora MR, Simmons JD, *et al.* Pharmacologic protection of mitochondrial DNA integrity may afford a new strategy for suppressing lung ischemia-reperfusion injury. Ann Am Thorac Soc. 2017;14;Suppl 3:S210-5. doi: 10.1513/AnnalsATS.201706-438MG, PMID 28945469.
13. Guo L, Wu X, Zhao S, Zhang X, Qian G, Li S. Autophagy inhibition protects from alveolar barrier dysfunction in LPS-induced ALI mice by targeting alveolar epithelial

cells. Respir Physiol Neurobiol. 2021;283:103532. doi: 10.1016/j.resp.2020.103532, PMID 32950660.

14. Wang F, Fang B, Qiang X, Shao J, Zhou L. The efficacy of mesenchymal stromal cell-derived therapies for acute respiratory distress syndrome—a meta-analysis of preclinical trials. Respir Res. 2020;21:1-10.
15. Sánchez-Quesada C, López-Biedma A, Warleta F, Campos M, Beltrán G, Gaforio JJ. Bioactive properties of the main triterpenes found in olives, virgin olive oil, and leaves of *Olea europaea*. J Agric Food Chem. 2013;61(50):12173-82. doi: 10.1021/jf403154e, PMID 24279741.
16. Allouche Y, Warleta F, Campos M, Sánchez-Quesada C, Uceda M, Beltrán G, *et al.* Antioxidant, antiproliferative, and pro-apoptotic capacities of pentacyclic triterpenes found in the skin of olives on MCF-7 human breast cancer cells and their effects on DNA damage. J Agric Food Chem. 2011;59(1):121-30. doi: 10.1021/jf102319y, PMID 21142067.
17. Ferro JN, Barbosa FT, Barreto EAgra L.C. Triterpenes with healing activity: A systematic review. J Dermatol Treat. 2015;26:465-70.
18. Luna-Vázquez FJ, Ibarra-Alvarado C, Rojas-Molina A, Romo-Mancillas A, López-Vallejo FH, Solís-Gutiérrez M, *et al.* Role of nitric oxide and hydrogen sulfide in the vasodilator effect of ursolic acid and uvaol from black cherry *Prunus serotina* fruits. Molecules. 2016;21(1):78. doi: 10.3390/molecules21010078, PMID 26771591.
19. Martín R, Ibeas E, Carvalho-Tavares J, Hernández M, Ruiz-Gutierrez V, Nieto ML. Natural triterpenic diols promote apoptosis in astrocytoma cells through ROS-mediated mitochondrial depolarization and JNK activation. PLOS ONE. 2009;4(6):e5975. doi: 10.1371/journal.pone.0005975, PMID 19543395.
20. Carmo J, Cavalcante-Araújo P, Silva J, Ferro J, Correia AC, Lagente V, *et al.* Uvaol improves the functioning of fibroblasts and endothelial cells and accelerates the healing of cutaneous wounds in mice. Molecules. 2020 Oct 28;25(21):4982. doi: 10.3390/molecules25214982, PMID 33126422.
21. Lins MP, da Silva Marques P, Smaniotto S, Bandeira de Melo C, Lagente V, Barreto E Uvaol attenuates pleuritis and eosinophilic inflammation in ovalbumin-induced allergy in mice. Agra L.C. Eur J Pharmacol. 2016;780:232-42.
22. Butt Y, Kurdowska A, Allen TC. Acute lung injury: a clinical and molecular review. Arch Pathol Lab Med. 2016;140(4):345-50. doi: 10.5858/arpa.2015-0519-RA, PMID 27028393.
23. Giebelen IA, van Westerloo DJ, Larosa GJ, de Vos AF, van der Poll T. Local stimulation of α7 cholinergic receptors inhibits LPS-induced TNF-α release in the mouse lung. Shock. 2007;28(6):700-3. doi: 10.1097/shk.0b013e318054dd89, PMID 17621262.
24. Sun CY, Xu LQ, Zhang ZB, Chen CH, Huang YZ, Su ZQ, *et al.* Protective effects of pogostone against LPS-induced acute lung injury in mice via regulation of Keap1-Nrf2/NF-κB signaling pathways. Int Immunopharmacol. 2016;32:55-61. doi: 10.1016/j.intimp.2016.01.007, PMID 26800098.
25. Kim JH, Lee S, Cho EJ. Acer okamotoanum and isoquercitrin improve cognitive function via attenuation of oxidative stress in high fat diet- and amyloid beta-induced mice. Food Funct. 2019;10(10):6803-14. doi: 10.1039/c9fo01694e, PMID 31577306.
26. Liu S, Yue Y, Pan P, Zhang L, Su X, Li H, *et al.* IRF-1 intervention in the classical ROS-dependent release of NETs during LPS-induced acute lung injury in mice. Inflammation. 2019;42(1):387-403. doi: 10.1007/s10753-018-0903-7, PMID 30315525.
27. Song X, Ren Z, Wang X, Jia L, Zhang C. Antioxidant, anti-inflammatory and renoprotective effects of acidic-hydrolytic polysaccharides by spent mushroom compost (Lentinula edodes) on LPS-induced kidney injury. Int J Biol Macromol. 2020;151:1267-76. doi: 10.1016/j.ijbiomac.2019.10.173, PMID 31751686.
28. Bi XG, Li ML, Xu W, You JY, Xie D, Yuan XF, *et al.* Helix B surface peptide protects against acute lung injury through reducing oxidative stress and endoplasmic reticulum stress via activation of Nrf2/HO-1 signaling pathway. Eur Rev Med Pharmacol Sci. 2020;24(12):6919-30. doi: 10.26355/eurrev_202006_21683, PMID 32633385.
29. Laskin DL, Malaviya R, Laskin JD. Role of macrophages in acute lung injury and chronic fibrosis induced by pulmonary toxicants. Toxicol Sci. 2019;168(2):287-301. doi: 10.1093/toxsci/kfy309, PMID 30590802.
30. Hou W, Hu SY, Su ZZ, Wang Q, Meng G, Guo T, *et al.* Myricetin attenuates LPS-induced inflammation in RAW 264. 7 macrophages and mouse models. Future Med Chem. 2018;10(19):2253-64. doi: 10.4155/fmc-2018-0172, PMID 30095283.
31. Lei J, Wei Y, Song P, Li Y, Zhang T, Feng Q, *et al.* Cordycepin inhibits LPS-induced acute lung injury by inhibiting inflammation and oxidative stress. Eur J Pharmacol. 2018;818:110-4. doi: 10.1016/j.ejphar.2017.10.029, PMID 29054740.
32. Malleo G, Mazzon E, Siriwardena AK, Cuzzocrea S. TNF-α as a therapeutic target in acute pancreatitis — lessons from experimental models. The Scientific World Journal. 2007;7:431-48. doi: 10.1100/tsw.2007.98, PMID 17450307.
33. Sun XQ, Wu C, Qiu YB, Wu YX, Chen JL, Huang JF, *et al.* Heme oxygenase-1 attenuates seawater drowning-induced acute lung injury through a reduction in inflammation and oxidative stress. Int Immunopharmacol. 2019;74:105634. doi: 10.1016/j.intimp.2019.05.019, PMID 31254959.
34. Guo W, Mao B, Tang X, Zhang Q, Zhao J, Cui S, *et al.* Lactobacillus paracasei CCFM1223 protects against lipopolysaccharide-induced acute liver injury in mice by regulating the "gut-liver" axis. Microorganisms. 2022;10(7):1321. doi: 10.3390/microorganisms10071321, PMID 35889040.
35. Jiang X, Chen L, Zhang Z, Sun Y, Wang X, Wei J. Protective and therapeutic effects of engeletin on LPS-induced acute lung injury. Inflammation. 2018;41(4):1259-65. doi: 10.1007/s10753-018-0773-z, PMID 29704150.
36. Mauer J, Denson JL, Brüning JC. Versatile functions for IL-6 in metabolism and cancer. Trends Immunol. 2015;36(2):92-101. doi: 10.1016/j.it.2014.12.008, PMID 25616716.
37. Yang K, Zou Z, Wu Y, Hu G. MiR-195 suppression alleviates apoptosis and oxidative stress in CCl4-induced ALI in mice by targeting Pim-1. Exp Mol Pathol. 2020;115:104438. doi: 10.1016/j.yexmp.2020.104438, PMID 32277959.
38. Fragoso IT, Ribeiro EL, Gomes FO, Donato MA, Silva AK, Oliveira AC, *et al.* Diethylcarbamazine attenuates LPS-induced acute lung injury in mice by apoptosis of inflammatory cells. Pharmacol Rep. 2017;69(1):81-9. doi: 10.1016/j.pharep.2016.09.021, PMID 27914293.
39. Kan X, Liu B, Guo W, Wei L, Lin Y, Guo Y, *et al.* Myricetin relieves LPS-induced mastitis by inhibiting inflammatory response and repairing the blood-milk barrier. J Cell Physiol. 2019;234(9):16252-62. doi: 10.1002/jcp.28288, PMID 30746687.
40. Song YD, Wu YX, Li XZ, Shen Y, Ding Y, Zhu H, *et al.* Protostemonine attenuates alternatively activated macrophage and DRA-induced asthmatic inflammation. Biochem Pharmacol. 2018;155:198-206. doi: 10.1016/j.bcp.2018.07.003, PMID 29991449.
41. Zheng H, Liang W, He W, Huang C, Chen Q, Yi H, *et al.* Ghrelin attenuates sepsis-induced acute lung injury by inhibiting the NF-Kb, iNOS, and Akt signaling in alveolar macrophages. Am J Physiol Lung Cell Mol Physiol. 2019; 317(3):L381-91. doi: 10.1152/ajplung.00253.2018, PMID 31242025.
42. Jiang F, Guan HN, Liu DY, Wu X, Fan MC, Han JC. Flavonoids from sea buckthorn inhibit the lipopolysaccharide-induced inflammatory response in RAW264.7 macrophages through the MAPK and NF-κB pathways. Food Funct. 2017;8(3):1313-22. doi: 10.1039/c6fo01873d, PMID 28256654.
43. Hashemi Goradel N, Najafi M, Salehi E, Farhood B, Mortezaee K. Cyclooxygenase-2 in cancer: a review. J Cell Physiol. 2019;234(5):5683-99. doi: 10.1002/jcp.27411, PMID 30341914.

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