Research Article Open Access

Anti-inflammatory effect of Phytomarine R-L compound against lipopolysaccharide- induced pulmonary injury in BALB/c mice

Janani Gopi[1], Francesco Marotta[2*], Sushmitha Sriramulu[1], Antara Banerjee[1], N.R. Rajesh Kanna[3], Vignesh Palani[4], Antonio Ayala[5], Fang He[6], R. Vijayashree[3], Surajit Pathak[1*]

[1]Department of medical biotechnology, Faculty of Allied Health Sciences, Chettinad Academy of Research, and Education, Chettinad Hospital and Research Institute (CHRI), Chennai , India; [2]ReGenera R&D International for Aging Intervention and Vitality and Longevity Medical Science Commission, FEMTEC World Federation, Milan, Italy; [3]Department of Pathology, Faculty of Medicine, Chettinad Academy of Research and Education, Kelambakkam, Tamil Nadu, India; [4]Faculty of Medicine, Chettinad Hospital and Research Institute (CHRI), Chennai, India; [5]Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, University of Seville, Spain; [6]Department of Nutrition and Food hygiene, West China School of Public Health and West China Fourth Hospital, Sichuan University, Sichuan China.

***Corresponding Author**: Surajit Pathak, PhD, Professor, Department of medical biotechnology, Faculty of Allied Health Sciences, Chettinad Academy of Research, and Education, Chettinad Hospital and Research Institute (CHRI), Chennai - 603 103, India

Submission Date: September 20th, 2022; **Acceptance Date:** November 14th, 2022; **Publication Date:** November 22nd, 2022

Please cite this article as: Gopi, J., Marotta F., Sriramulu S., Banerjee A., Kanna N. R., Palani V., Ayala A., He F., Vijayashree R., Pathak S. Anti-inflammatory effect of Phytomarine R-L compound against lipopolysaccharide- induced pulmonary injury in BALB/c mice. *Functional Foods in Health and Disease* 2022; 12(11):666-679. DOI: https://www.doi.org/10.31989/ffhd.v12i11.1014

ABSTRACT

Background: This study deals with the induction of lipopolysaccharide induced inflammation in animal model and treating this condition using phytomarine R-L compound (Bloomin'Age, Science of Living, Milan Italy) that is known to possess the anti-inflammatory properties and eventually determine their characteristics through the in-vivo experimentation.

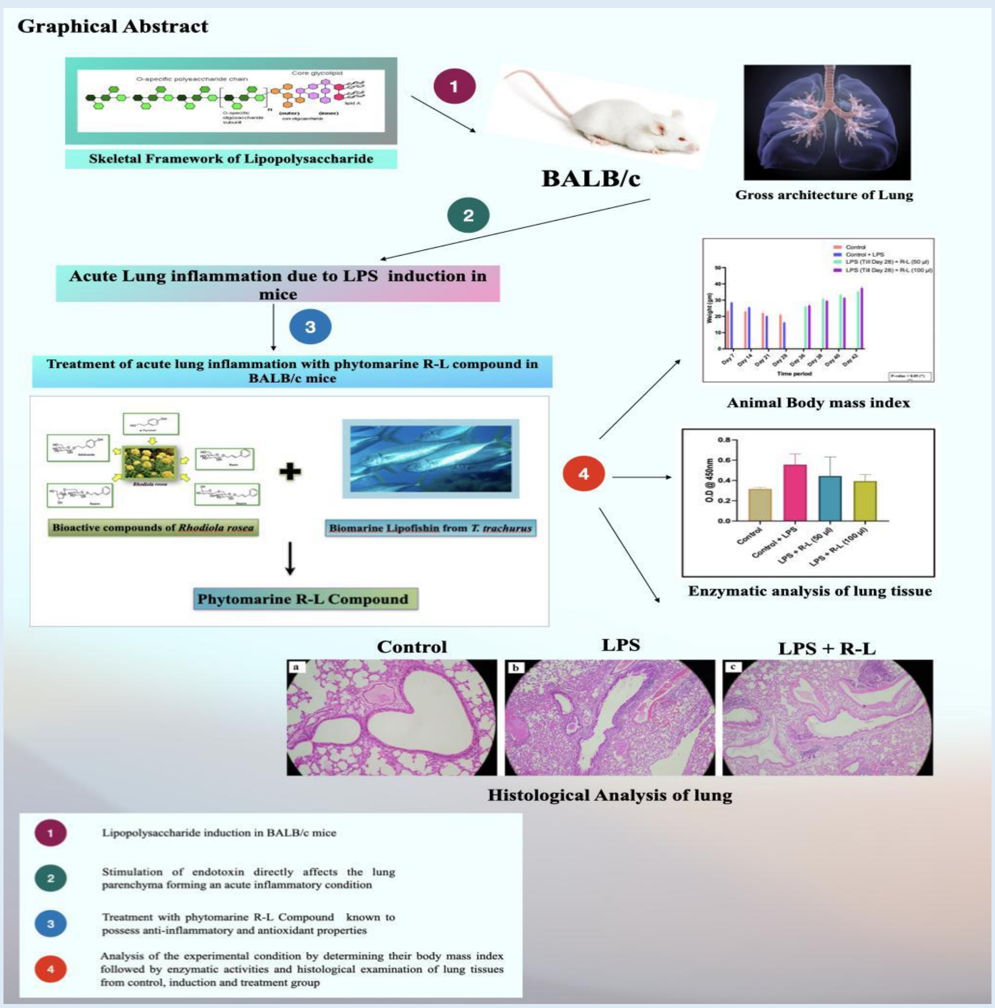
Objective of the study: The present study was subjected to test the therapeutic anti-inflammatory/anti-oxidative and regenerative property of this phytomarine (SBF-LF) in an LPS-induced lung injury in mice models.

Methods: Mice received an intra-nasal administration of LPS at a concentration of 1 mg/ml weekly for four consecutive weeks followed by the oral administration of R-L compound at a concentration of 50 mg/kg body weight intermittently. Broncho-alveolar lavage fluids (BALF) as well as the lung tissues were periodically collected from the control and experimental groups for histological examination. Likewise, the lung tissue homogenate obtained from induction and treatment groups was assayed for myeloperoxidase (MPO) assay, superoxide dismutase (SOD) activity and elastase assay.

Results: The results illustrated that LPS- induced mice group demonstrated an increased inflammatory cell infiltration causing an acute and persisting neutrophilic accumulation in the lung parenchyma with alveolar congestion. Furthermore, the influx of macrophages, lymphocytes as well as inflammatory mediators was observed during the BALF analysis. The treatment with R-L compound resulted in a significant decline in the permeation of inflammatory cells as observed by lung histology and BALF analysis.

Conclusion: Thus, the exploitation of R-L compound with the experimental groups along with other assay methods confirmed its robust anti-inflammatory and antioxidant properties and suggested epithelial regenerative capacity. Further analyzing the efficacy of the R-L compound might help understanding its potential as a therapeutic option in clinical settings.

Keywords: Lungs, BALB/c mice, Lipopolysaccharide, anti-inflammatory, R-L compound



INTRODUCTION

Inflammation is an adaptive response implicated in the aging process and underlying a broad spectrum of pathological as well as physiological processes affecting protective cellular mechanisms whenever tissue injury and infections occur. Acute inflammation mainly encompasses the migration of neutrophils from the vasculature into the inflammatory region that results in an interim matrix with the production of chemoattractant factors through platelets at the site of inflammation which inlay for a period of two to six weeks and gradually shifts to stage of chronic inflammation [1]. Our research work predominantly concentrates on the inflammatory condition in lungs causing asthma, chronic obstructive pulmonary disease involving the chronic bronchitis, as well as emphysema, pneumonia, and acute respiratory distress syndrome. Conscription of leukocytes at the site of inflammation mainly involves the functional response of a significant chemotactic action on cytokines [2]. Thus, the delivery of pathogens is known to be a potent method of inspecting the host's inflammatory reaction [3]. Toxic substances such as LPS, an endotoxin from cell wall of gram-negative bacteria and present in tobacco smoke as well as in a range of environmental and occupational dusts exposure, can ultimately lead to acute and chronic pulmonary inflammation through the infiltration of neutrophils and monocytes into the alveoli.

Thus, LPS is a recognized trigger model for investigating the acute inflammatory response. This is associated to the release of pro-inflammatory cytokines encompassing the tumor necrosis factor alpha (TNF-α), interleukin-1β and interleukin-6. LPS-induced inflammation model in mice reiterates the facets of inflammatory cascade that are interconnected with the pulmonary inflammation and lung ailments in humans [4]. Sequentially, LPS provokes the immunological reactions by interrelating with the membrane receptor CD14 which is a 55 kDa glycosyl phosphatidylinositol connected membrane protein that stimulates the production of multiple cytokines while the protein kinases particularly Fgr, Lyn and Hck engage mainly in the signalling pathway of LPS. Within minutes of LPS stimulation, there is a copious proteins efflux which are tyrosine phosphorylated where the MAP kinases p42, p44 and p38 are enzymatically active and trigger the release of macrophages [5]. Concomitantly, the signalling mechanism of Type I integral protein involves the Toll like receptor 4 (TLR-4) engrosses the recruitment of intracellular adaptor proteins that transmit signals from the TIR domain, initiating the protein kinases as well as transcription factors that provoke the release of inflammatory mediators [6] whose involvement is known to be linked with acute and chronic inflammation in human. Therefore, using intranasal delivery for delivering substances in the bronchus mirrors a clinically compatible and interventional methodology [7, 8].

Over the last decades, the prominence of natural compounds such as the plants and marine species naturally containing many active chemicals have been studied for their potentially health benefits, either preventative or therapeutic. To refrain from the utilization of classic medicine, the prominence of functional foods along with the phytomedicine might sustain the ministrations of the prevailing medical conditions. Functional foods are not like dietary supplements and are rather traceable through natural compounds that can be taken through normal diet. The promising features of functional food can be employed against viral infections, heart disease, respiratory disease, inflammation and cancer. Moreover, the plant-based components of functional foods can furnish both taste as well as food aroma that can be employed as expectorant, antitussive, antimicrobial and irritation soothers in pulmonary medicine [9]. For instance, flavonoids display a broad array of pharmacological activities dealing with the modulation as well as progression of several pulmonary disease conditions [10 - 13]. Some, albeit still extremely limited, experimental studies have investigated the therapeutic potential of marine extracts in LPS-induced lung injury [14, 15].

Rhodiola rosea, which is mostly found in Europe and Southeast Asia, has a rich polyphenol content (41.4 ± 3.41%) [16] and its salidroside moieties are known to possess various pharmacological activities, such as antioxidant, anti-inflammation, anti-apoptotic, anti-depressant, anti-aging, neuroprotective, cardioprotective effects while alleviating cigarette-smoking experimental COPD [17]. Another interesting compound which attracted our research interested was a lipoprotein fraction obtained without chemicals from *T. trachurus* fish from the Galician coast of the Atlantic Ocean. Lipofishin, i.e., a subclass under the proteolipids, which forms a new group of lipoproteins extracted from the muscle of *Trachurus* fish have been demonstrated to possess anti-inflammatory properties [18]. After years of research, in 2021, we devised a phytomarine compound (Bloomin'Age, Science of Living, Milan Italy) by accurately mixing of high-salidroside specific bioactive fractions (SBF) isolated from the Rhodiola rosea and the lipoprotein extracted from the muscle of *Trachurus trachurus* fish (*Rhodiola rosea* plant extract and Lipoproteins from *Trachurus* sp). Our previous results showed the significant anti-inflammatory, antioxidant, potential of R-L compound [19].

Thus, the aim of the present study was to test the therapeutic anti-inflammatory/anti-oxidative and regenerative property of this phytomarine (SBF-LF) in an LPS-induced lung injury in mice model.

METHODS

Reagents: LPS, phosphate buffered saline (PBS), sodium phosphate buffer, Ethylene diamine tetra-acetic acid (EDTA), Sodium hydroxide (NaOH), Concentrated sulphuric acid (H_2SO_4), Glycerin, Giemsa stain, ethanol, methanol, saline, DNase free water and R-L compound, 10% formaldehyde, Xylene, Tris, Ethylenedi-aminetetra-acetic acid disodium salt dihydrate (Na_2EDTA-$2H_2O$), pyrogallol, ammonium chloride, potassium bicarbonate, 3, 3', 5, 5'-Tetramethylbenzidine (TMB), dimethyl sulphoxide (DMSO). *Rhodiola* SBF and LF-*Trachurus sp* (R-L compound) were a kind gift from ReGenera R&D International for Aging Intervention, Italy.

Animals: Healthy female BALB/c mice (*Mus musculus)* aged 8 to 12 weeks old weighing about 20-25 grams approximately were housed in animal house, Chettinad Hospital and Research Institute (CHRI) with the approval number: **IAEC 4/ Proposal 29/ A. Lr: 11/ Dt: 20.12.18**. They were placed in plastic cages with adsorbent bedding matter with 12h light/dark cycle under standard conditions. Further, ad-libitum feeding of water and food pellet was provided during the *in vivo* experimentation.

LPS-induced lung inflammation: For induction of acute phase of inflammation in lungs, 1 mg of lipopolysaccharide was dissolved in 1ml of sterile saline. Then, each mouse was administered 10µl of LPS (1 mg/ml dose concentration) intra-nasally (IN) to the nares of the mice through micropipette by lightly anaesthetizing with isoflurane and was given once in every week for four consecutive weeks [20]. Treatment of R-L compound was subjected to oral administration with a specific concentration 50 mg/kg body weight was predetermined for the drug treatment. Thus, the R-L compound was given orally to the BALB/c mice at a specific dose concentration of 15 mg/ml (i.e., each mice received 1.5 mg for approximately ~ 30g body weight) at regular time intervals (1^{st}, 3^{rd}, 5^{th}, 7^{th} day of week 5) according to protocol [21].

Experimental groups: Mice were randomized and divided into three groups. Group 1 (*n*=6) received oral administration of phosphate buffered saline (PBS) which was allotted as negative control. Group 2 (*n=6*) received LPS (1 mg/ml) which was administered intra-nasally once a day in a week for 4 consecutive weeks and they were sacrificed at the end of four weeks which was allotted as induction/positive control group. Group 3 (*n=6*) received a selected oral administration of 50µl of R-L compound from week 5 continuously at regular intervals after LPS induction to evaluate its effect on the biochemical parameters (Fig.1).

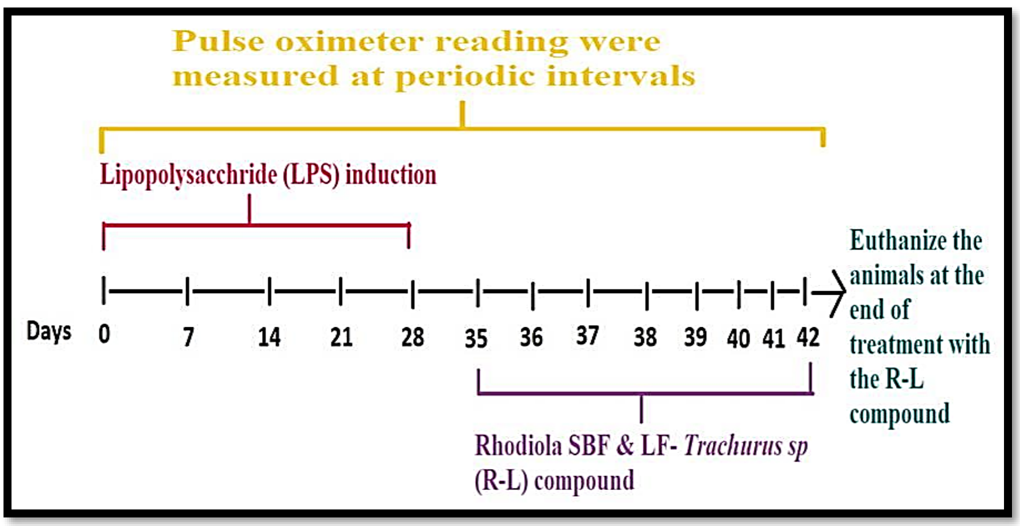


Figure 1: Experimental Design of the study

Tissue Collection and Processing: After instillation of deep anesthesia, the animals were euthanized, and the lungs were collected appropriately from the experimental groups. Mice lungs were obtained for light microscopy without induction or perfusion with fixatives to avoid any translocation of leukocytes within the lung parenchyma or vessels. A portion of lung was used for histology by fixing it in 10% formaldehyde and the remaining portion of lung was stored at -20°C till homogenization and processed for further experimental studies.

Histology assessment of lung tissue: Lung tissues were fixed and embedded in paraffin. Tissue sections were cut at 4–5 µm from paraffin embedded lung tissues and mounted on glass slides for hematoxylin and eosin stain and examined by an optical microscope [22].

Analysis of BALF: The experimental protocol of BALF collection involves euthanizing the mouse through inhalation of carbon-di-oxide gas and taking them back to the laminar airflow chamber. Then, mice were moistened with ethanol and positioned in supine position by pinning the legs with needles on Styrofoam boards. Dissection of the mice was performed, the organs were exposed, and the fluid was accurately collected about ten times in an Eppendorf tube and stored in ice. The processing of the BALF involved centrifuging the fluids for 10 minutes at 4° C and resuspending the cell pellet in 200 µl of PBS. This was followed by centrifuging the fluids for 10 minutes at 4° C and resuspending the cell pellet in 100 to 200 µl of ACK lysis buffer and placing it on ice for 10 minutes to lyse the RBCs. 1 ml of PBS was added to stop the lysis. Finally, the fluids were centrifuged again at 800G for 10 minutes at 4° C and the cell pellet was resuspended in PBS and used for the experimental procedures and assay methods [23].

Estimation of BALF by Giemsa stain: Bronchoalveolar lavage fluid was centrifuged at 1500 revolutions per minute for 10 minutes and red blood cells were extracted by hypotonic lysis. After a second centrifugation the pellet was dissolved in 500 µl saline. For differential cell count analysis, the pulmonary fluids were diluted with saline to retrieve 1 x 10^5 cells/100 µl of saline. Polymorphonuclear cells and macrophages were identified by morphological examination of smears and stained with Wright-Giemsa solution**.**

Elastase assay: The elastase assay was carried out for the lung tissues. Elastase stock has a concentration of 3.33 mg/ml which is to be dissolved in sterile H_2O and the substrate (N-succinyl- ala-ala-ala-p-nitroanilide) in 1.6mM of buffer. 50 µl of tissue supernatant were combined with the elastase enzyme followed by incubation for 15 minutes. Then, substrate was added to the above reaction mixture and absorbance was read at 402nm.

Myeloperoxidase (MPO) assay: The MPO assay was carried out for the lung tissues. The peroxidase activity with 3, 3', 5, 5'-Tetramethylbenzidine (TMB) was measured suitably in the spectrophotometer. Lung tissue supernatant was combined with 0.75 mM hydrogen peroxide (H_2O_2) and TMB solution and the plate was incubated at 37°C for 5 min. The reaction was stopped by adding 2M sulphuric acid (H_2SO_4) and absorption was measured at 450 nm to estimate MPO activity [24].

Superoxide dismutase (SOD) activity: SOD activity in the cell extract was estimated by measuring its ability to inhibit the auto-oxidation of pyrogallol according to the method of Markland [25]. 1ml of assay mixture containing 0.05M sodium phosphate buffer (pH8.0), 0.01M EDTA and 0.27mM pyrogallol (Solution of pyrogallol was made in100mM HCl). The absorbance was measured for 5min at 420nm.The enzyme activity was expressed as U/mg protein, where1U is the amount of enzyme required to bring about 50% inhibition of the auto-oxidation of pyrogallol.

Statistical Analysis: Data was obtained from independent experiments performed in triplicate manner and were presented as mean ± SEM. The unpaired student t test was used to determine significant differences between two groups of data. The p values of <0.05, <0.01 and <0.001 were considered as statistically significant and are indicated by asterisks (*, ** and ***) respectively. All the data was analyzed with Graph Pad Prism 5.0 software.

RESULTS

Animal disease activity index of the mice was analyzed by interpreting the change in the body weight, input of water, and food consumption. Thus, changes in body mass were assessed during the *in vivo* experiment. Initially, in LPSfed mice, there was a gradual loss of body weight and water and food intake when compared to normal mice, whereas, in LPS + R-L compound fed mice there was a marginal gain in the body mass, water and food consumption (**Fig. 2-a, 2-b and 2-c**). During animal experiments, the veterinary pulse oximeter was used to measure the saturated peripheral oxygen (SpO_2) level in all groups at regular time intervals during LPS induction as well as under R-L compound treatment. From graphical representation, **Fig. 2-d** depicts a gradual SpO_2 decline in the LPS induced mice in contrast to control mice, whereas a slight increment in the LPS + R-L compound treatment to the lung injury mice model.

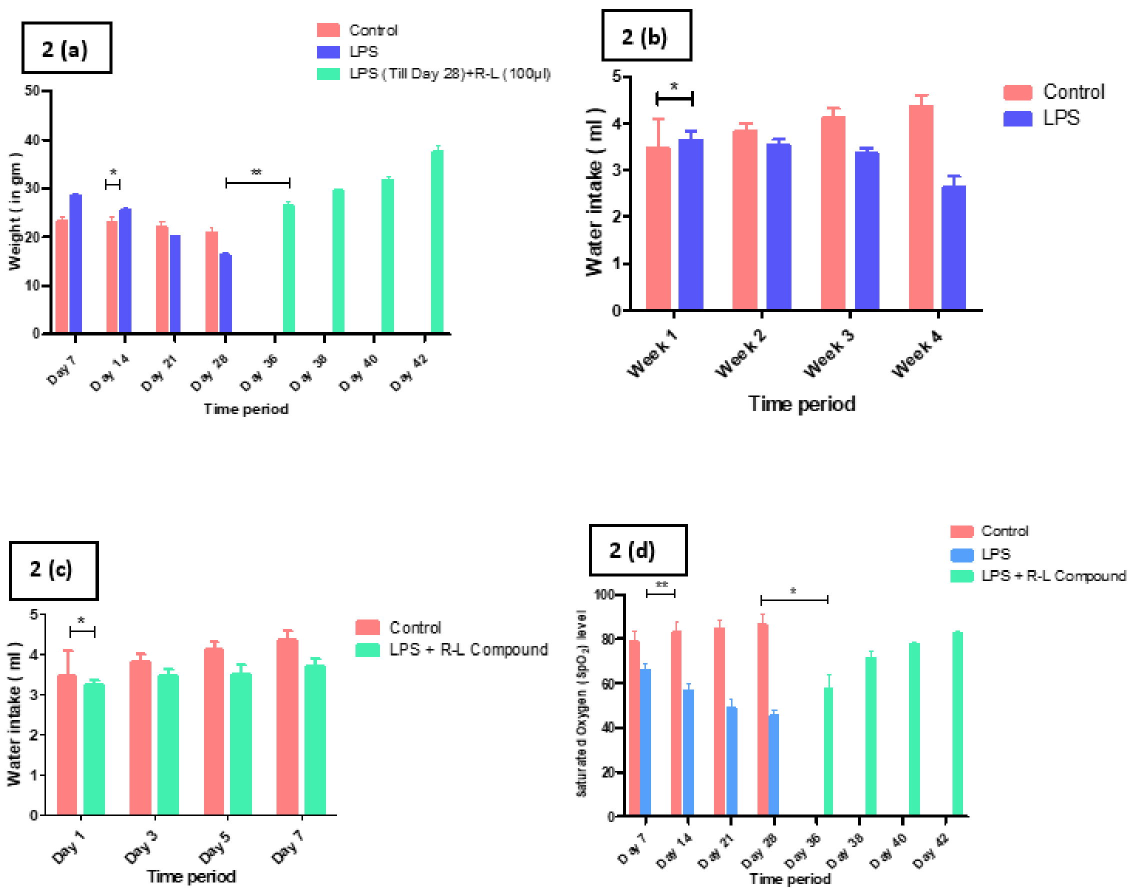


Figure 2: Effect of body mass changes in mice **(2-a)** was observed during the LPS induction as well as during R-L treatment; **(2-b)** Water and food consumption was observed during LPS induction; **(2-c)** Water and food consumption was monitored during R-L treatment; Pulse oximeter reading **(2-d)** during LPS induction and R-L treatment.

Histology assessment of lung tissues: Control mice showed the normal histological structure of the lung sections stained with hematoxylin and eosin. Microscopically, the control tissue section was seen with its lung parenchyma exposed to normoxic condition with alveolar septa and pulmonary capillaries which are found to be preserved **(Fig. 3-a)**. In LPS-induced mice group, the lung tissue sections showed alveolar congestion and enlargement, hemorrhage, infiltration, or aggregation of inflammatory cells such as macrophages, neutrophils and even lymphocytes in airspaces, engorgement of the blood vessel walls and thickening of the alveolar walls as the significant signs of the progression of the disease condition **(Fig. 3-b)**. Thus, the treatment of inflammatory lung changes with the drug (R-L compound) were found to impede the inflammatory cell infiltration in the lung parenchyma as well as in the alveolar and bronchial walls suggesting the recovery of lung tissue to the normoxic condition **(Fig. 3-c)**.

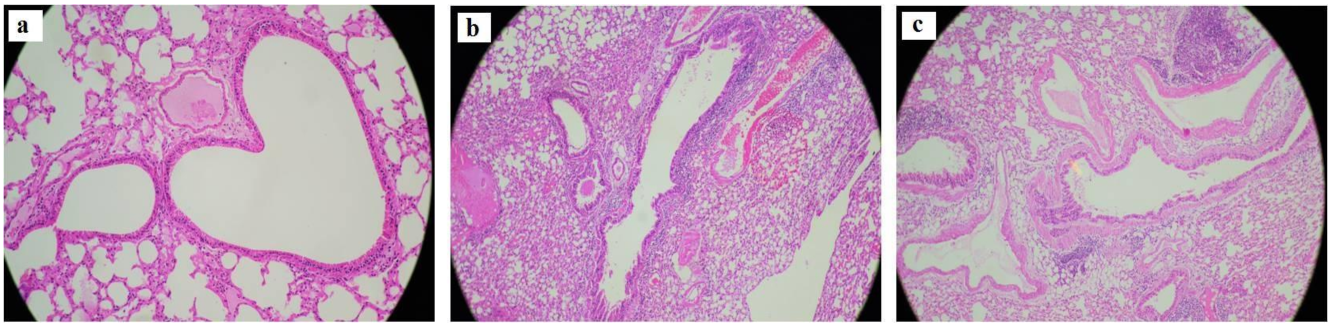


Figure 3: Effect of R-L on lung inflammation. Lung sections were obtained from control mice (**a)** group; LPS treated mice **(b)** group, LPS + R-L compound mice group **(c)**. After hematoxylin and eosin staining, histological examination of lung tissue sections was observed under light microscopy at 20X magnifications.

BALF smear stained using Giemsa stain: The processed BALF was smeared in a glass slide and fixed with methanol followed by Giemsa stain. The smeared glass slide was observed with a compound microscope at 10X magnification. Therefore, in LPS treated group, the BALF cytological analysis revealed that three distinct types of alveolitis were observed namely the lymphocytes, neutrophils, and eosinophils. Especially, in **Fig. 4-b,** the presence of alveolar casts of amphophilic, amorphous material in honeycomb pattern of cells were observed, the infiltration of inflammatory cells in BALF and in the lung, parenchyma was observed following exposure to LPS in mice. Alveolar macrophages are found which is associated with the acute phase of inflammation respectively. Then, in **Fig. 4-c** the R-L compound treated group was observed with a minimal level of inflammatory cells accumulation in comparison with LPS treated group.

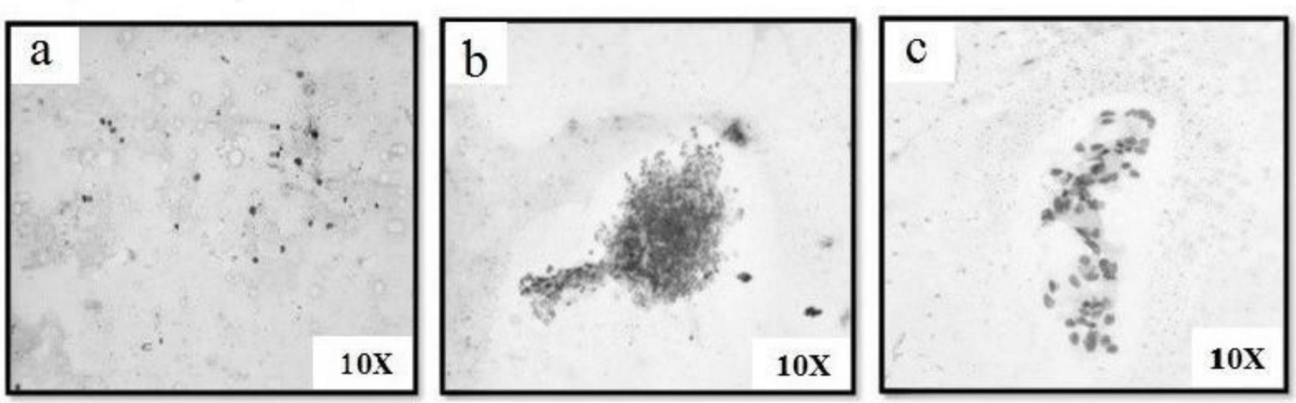


Figure 4: Microscopic images of bronchoalveolar lavage fluid (BALF) stained with Giemsa. Control group **4(a)**, LPS treated mice group **4(b)**; LPS + R-L compound treatment group **4(c)** were observed at 10X magnification. BAL cells depict eosinophils, neutrophils, macrophages, and lymphocytes in LPS challenged mice.

Colorimetric Assay Methods

I. Elastase Assay: The enzymatic activities of elastase in control and treatment groups are summarized in **Fig. 5-a**. The elastase activity significantly increased in the LPS treated group because the alveoli lost their elasticity and secreted the enzymes which are known to be at higher levels in comparison with LPS + R-L treated mice group which exhibit a substantial decrease in their enzymatic reaction.

II. Myeloperoxidase Assay: In myeloperoxidase assay, the absorbance is inversely proportional to the level of MPO enzyme. The enzymatic activities of myeloperoxidase in control and treatment groups are summarized in **Fig. 5-b**. The tissue lysate depicts an increased level of MPO activity in the LPS treated mice group due to enhanced infiltration of enzymatic levels of myeloperoxidase from the azurophilic granules from the neutrophils due to necrosis caused by inflammation. In contrast with the LPS + R-L treated mice group showing a subsequent decrease in MPO enzyme reaction.

III. Superoxide dismutase (SOD) Assay: During LPS induction, the free radical accumulation will be more which is counteracted by the scavenging activity of the SOD enzyme. The specific activities of SOD in control and treatment groups are summarized in **Fig. 5-c**. The SOD tissue lysate was known to significantly increase in the LPS treated in contrast to LPS + R-L treated mice group, showing a significant decrease in SOD appropriately.

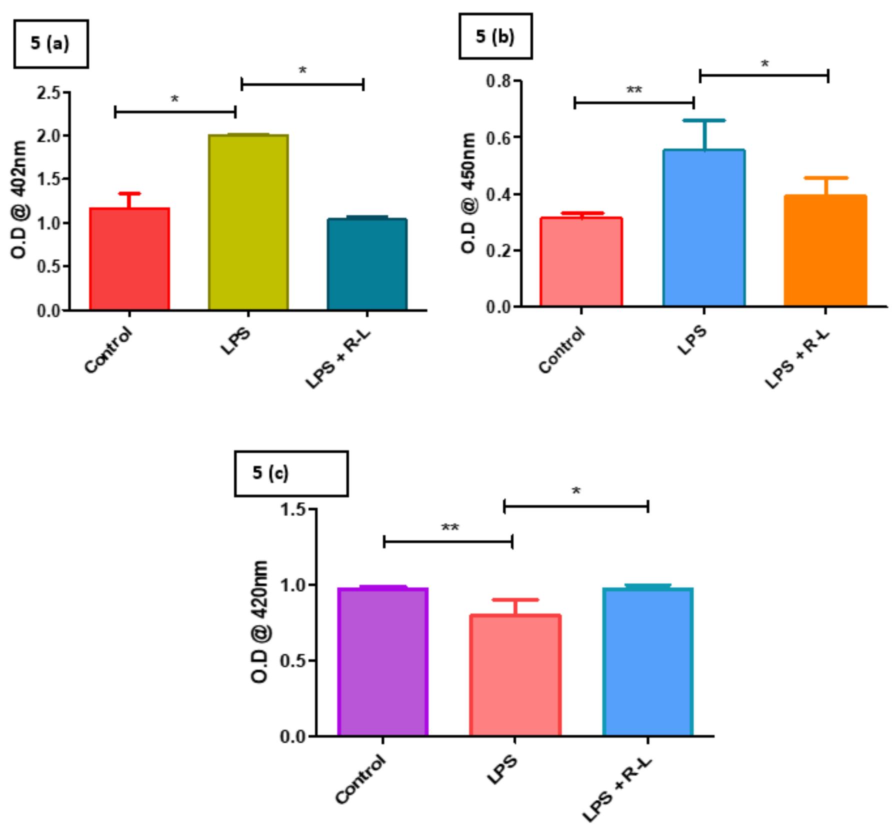


Figure 5: Effect of R-L compound on different assay methods in LPS induced mice. The assay methods such as **(5-a)** Elastase assay; **(5-b)** Myeloperoxidase Assay; **(5-c)** Superoxide dismutase Assay were performed from the lung tissue homogenate extracted from different experimental grouping of mice. Data are represented as the mean ± SD.

DISCUSSION

Generally, infections, as well as their correlated inflammatory mechanisms, lead to a rapid infiltration of neutrophils at the site of inflammation through the peripheral blood [26]. Thus, the neutrophils are necessitated in healthy lungs where they play a significant role as constituent of innate immunity shielding against infection. However, they could also cause substantial damage together with macrophages

Treatment with salidroside has also been shown to alleviate the pro-inflammatory cytokines, chemokines as well as nitric oxide via NF-κB signaling pathway during a variety of in vitro and in vivo inflammatory conditions such as ethanol-induced acute gastric ulcer, murine endotoxemia, murine asthma and on RAW264.7 and J774.1 cell tests [30 - 35]. In the above experimental settings and others, salidroside has acted by setting down the nuclear factor kappa- Initiation of DNA-binding as well as mitogen-activated protein kinases signal or extracellular signal-related kinase transduction pathway production [36] is related with inflammation.

In this study, we evaluated the biological activity of R-L compound after 4-weeks induction of LPS lung damage model in mice in order to test the potential therapeutic intervention, during the acute phase, with the R-L compound which was orally administrated during the fifth week (i.e., Day 35). After the treatment with R-L compound, the lung tissue sections showed significantly beneficial effects in comparison with the control. Thus, R-L compound proved to significantly affect the acute inflammatory burst while also preventing its progression with minimal damage. Concomitantly, the enhanced MPO concentration observed in untreated mice, in RL-treated mice decreased in parallel with an overt reduction in the inflammatory cells' infiltration into the lungs, in agreement with Guan et al. [31]. A great deal of lung injury research studies has been focused on the early phase acute events leading to the destruction of and cytokines at the inflammatory site, as observed in the BALF and in the lung parenchyma of mice after exposure to LPS [27, 28]. Indeed, after 3 days of LPS at 0.5 mg/kg lung injury induction, a remarkable neutrophil infiltration was found [28]. Rhodiola rosea L. extract contains p-tyrosol, salidroside, rosavin, pyridine, rhodiosin and rhodionin which over the past two decades, has been shown to exhibit an anti-inflammatory and anti-oxidative property [29]. epithelial cells in the alveoli and the endothelial cells in the capillary walls, infiltration of inflammation cells and edema of alveoli [7]. This sequence of events at a different degree and duration has also been advocated as one of the multifactorial detrimental factors involved in COPD and smoking-related COPD as we also previously highlighted in a book chapter [37]. No matter the severity and the stage of exotoxin/endotoxin-induced lung injury, tissue and BALF inflammatory changes and alveolar-capillary barrier disruption are common superimposing features. In this respect, it was of interest to note that R-L treated mice could significantly, albeit partially, improve their falling SpO_2 from ≤65% to 80%, as accurately measured by pulse oximeter.

Our prior studies had shown the upregulating property of R-L compound on SIRT-1 and Khloto-1, among others [19]. Although in the present research we did not measure SIRT-1, this seems to support the finding that in a mouse model of asthma, lung SIRT1 expression decreased [38]. Authors suggested that elevating lung SIRT1 levels may be a new strategy for asthma [38] and this is likely to play a role also in tight junctions' integrity as was more recently demonstrated [39]. Klotho-1 a further vitagenes enhanced by R-L compound in our vitro work seems to be in line with the very recent report that its overexpression protects the peritoneal membrane [40].

CONCLUSIONS

Our current SARS-COVID-19 pandemic has identified that counteracting and possibly inhibiting the inflammatory cascade represents the most significant target of pharmaceutics in curbing the lung inflammation at the early acute phase. The relevant findings observed with respect to the R-L compound which helped sparing the inflammation-induced alveolar tissues degradation with a possible remodeling of the alveolar air sacs associated to reduction of elastase was a very noteworthy finding. These latter biochemical and histological effect were notably mirrored by a functional counterpart, i.e., a partial but significant variation of pulse oximeter readings. Indeed, this clinically applicable parameter clearly differentiate among normal control mice against mice induced with LPS as well as the R-L treated mice. These data might hold some interest in the present scenario of the SARS-COVID-19 pandemic which brought respiratory distress and drastic fluctuation in saturated oxygen levels of COVID-19 positive patients, as well in re-infected vaccinated patients, with severe supervening infections. Finally, the potential adjuvant intervention using phytomarine R-L compounds under appropriate dosing and formulation may yield a potential application in clinical protocols treating from asthma and COPD in future while being devoid of adverse effects.

Limitations of the Study: Although, more *in vivo* studies are warranted to unveil deeper imaging and molecular mechanisms in the pathological conditions of the pulmonary inflammation, the present pilot study shows the potentialities of R-L compound which might be beneficial to the human health strategies dealing with the respiratory disease conditions.

List of Abbreviations Used: AHR: Airway hyper-responsiveness, BALF: Bronchoalveolar Lavage Fluid, COPD: Chronic obstructive pulmonary disease, CCL-2: Chemokines (chemokines c-c motif ligand), CRP: C-reactive protein, CXCL: Chemokine C-X-C motif ligand, DMSO: Dimethyl sulphoxide, H_2SO_4 : Sulphuric acid, IFN-γ: Interferon gamma, IL-8: Interleukin-8, IN: Intra-nasal, LAR: Lymphocyte antigen receptors, LPS: Lipopolysaccharide, MIP: Macrophage inflammatory proteins, MPO: Myeloperoxidase, NaOH: Sodium hydroxide, NF-B: Nuclear transcription factor-B, Na_2EDTA: Ethylenediaminetetraacetic acid disodium salt dihydrate, PBS: Phosphate buffered saline, ROS: Reactive oxygen species, STATs - Signal transducers and activators of transcription, SOD: Superoxide dismutase, SBF: Specific bioactive fractions, SPB: Sodium phosphate buffer, SpO_2 : Saturated peripheral oxygen, SARS-COVID: Severe Acute Respiratory Syndrome – Coronavirus, TNF-α :Tumor necrosis factor alpha, TLR-4: Toll like receptor 4, TGF-ß: Transforming growth factor beta, NF-kB: Transcription factor nuclear factor, TLR: Toll like receptor, TMB: 3, 3’, 5, 5’-Tetramethylbenzidine, κBa Inhibitory protein.

Author Contributions: Conceptualization–SP and FM; Supervision–SP, AB, JG and FM; Writing (Original draft preparation) – JG; Experiment, Data analysis and Statistical part – JG. SS, RK, (Review and Editing) – FM, SP, AB, VP, AA, MM, FH and VR; All authors revised and approved the content of the manuscript.

Acknowledgements and Funding: The authors are grateful to Chettinad Academy of Research and Education (CARE), Chennai, India for providing the infrastructural support to carry out the research work. This study was supported by Chettinad Academy of Research and Education (CARE) through their financial support in the completion of the project.

Availability of Data and Materials: Readers can access the data upon request to Corresponding author.

Ethics Approval: The Animal Ethics Committee, Chettinad University approved the experimental methodologies under the approval number: IAEC 4/ Proposal 29/ A. Lr: 11/ Dt: 20.12.18 and the experimentation were carried out under the supervision of the Animal Welfare Committee, CPCSEA.

Conflicts of Interest: Each author declares that he or she has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

REFERENCES

1. Brown, Bryan N., and Stephen F. Badylak. "The role of the host immune response in tissue engineering and regenerative medicine." *Principles of tissue engineering*. Academic Press, 2014. 497-509. DOI: https://doi.org/10.1016/B978-0-12-398358-9.00025
2. Takano, K., Nakagawa, H. Contribution of cytokine-induced neutrophil chemoattractant CINC-2 and CINC-3 to neutrophil recruitment in lipopolysaccharide-induced inflammation in rats. *Inflamm. res.* **50**, 503–508 (2001). DOI: https://doi.org/10.1007/PL00000226
3. Liang, Yongchao, Wanchun Sun, Yong-Guan Zhu, and Peter Christie. "Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review." *Environmental pollution* 147, no. 2 (2007): 422-428. DOI: https://doi.org/10.1016/j.envpol.2006.06.008
4. Van Linthout, S., Spillmann, F., Graiani, G. *et al.* Down-regulation of endothelial TLR4 signalling after apo A-I gene transfer contributes to improved survival in an experimental model of lipopolysaccharide-induced inflammation. *J Mol Med* **89**, 151–160 (2011). DOI: https://doi.org/10.1007/s00109-010-0690-6
5. Mitchell, Jonathon, Su Jin Kim, Alexandra Seelmann, Brendan Veit, Brooke Shepard, Eunok Im, and Sang Hoon Rhee. "Src family kinase tyrosine phosphorylates Toll-like receptor 4 to dissociate MyD88 and Mal/Tirap, suppressing LPS-induced inflammatory responses." *Biochemical pharmacology* 147 (2018): 119-127. DOI: https://doi.org/10.1016/j.bcp.2017.11.015
6. Nair, Syam, Kristina S. Sobotka, Pooja Joshi, Pierre Gressens, Bobbi Fleiss, Claire Thornton, Carina Mallard, and Henrik Hagberg. "Lipopolysaccharide-induced alteration of mitochondrial morphology induces a metabolic shift in microglia modulating the inflammatory response in vitro and in vivo." *Glia* 67, no. 6 (2019): 1047-1061. DOI: https://doi.org/10.1002/glia.23587
7. de Souza Xavier Costa N, Ribeiro Júnior G, dos Santos Alemany AA, Belotti L, Zati DH, Frota Cavalcante M, et al. (2017) Early and late pulmonary effects of nebulized LPS in mice: An acute lung injury model. PLoS ONE 12(9): e0185474. DOI: https://doi.org/10.1371/journal.pone.0185474
8. Di, Marissa E., Beth Kahkonen, Chia-Hsin Liu, and Yuanpu Peter Di. "Lung carcinomas induced by NNK and LPS." In *Methods in cell biology*, vol. 163, pp. 175-185. Academic Press, 2021. DOI: https://doi.org/10.1016/bs.mcb.2021.01.002
9. Widelska, Gabriela, Kamila Kasprzak-Drozd, Karolina Wojtunik-Kulesza, Anna Krajewska, and Anna Oniszczuk. "The impact of functional food on the prevention and treatment of respiratory diseases." Curr. Issues Pharm. Med. Sci., Vol. 33, No. 4, Pages 228-232. DOI: https://doi.org/10.2478/cipms-2020-0037
10. Lv, Xiang, Xuhui Zhou, Jia Yan, Jue Jiang, and Hong Jiang. "Propofol inhibits LPS-induced apoptosis in lung epithelial cell line, BEAS-2B." *Biomedicine & Pharmacotherapy* 87 (2017): 180-187. DOI: https://doi.org/10.1016/j.biopha.2016.12.074
11. Lv, Hongming, Qinmei Liu, Zhongmei Wen, Haihua Feng, Xuming Deng, and Xinxin Ci. "Xanthohumol ameliorates lipopolysaccharide (LPS)-induced acute lung injury via induction of AMPK/GSK3β-Nrf2 signal axis." *Redox biology* 12 (2017): 311-324. DOI: https://doi.org/10.1016/j.redox.2017.03.001
12. Zhang, C., Wang, X., Wang, C., He, C., Ma, Q., Li, J., Wang, W., Xu, Y. T., & Wang, T. (2021). Qingwenzhike Prescription Alleviates Acute Lung Injury Induced by LPS *via* Inhibiting TLR4/NF-kB Pathway and NLRP3 Inflammasome Activation. *Front Pharmacol*. 2021; 12:790072. Published 2021 Dec 23. DOI:https://doi.org/10.3389/fphar.2021.790072
13. Wu, Peiliang, Hanhan Yan, Jiayu Qi, Wenjing Jia, Wentao Zhang, Dan Yao, Cheng Ding, Yali Zhang, Mayun Chen, and Xueding Cai. "L6H9 attenuates LPS-induced acute lung injury in rats through targeting MD2." *Drug Development Research* 81, no. 1 (2020): 85-92. DOI: https://doi.org/10.1002/ddr.21607
14. Li, Xiaoling, Riming Huang, Kaifeng Liu, Mingyue Li, Hui Luo, Liao Cui, Lei Huang, and Lianxiang Luo. "Fucoxanthin attenuates LPS-induced acute lung injury via inhibition of the TLR4/MyD88 signaling axis." Aging (Albany NY) 2021 Jan 31; 13(2): 2655–2667. Published online 2020 Dec 11. DOI: https://doi.org/10.18632/aging.202309.

15. Ahmad TB, Rudd D, Benkendorff K, Mahdi LK, Pratt K-A, Dooley L, et al. (2017) Brominated indoles from a marine mollusc inhibit inflammation in a murine model of acute lung injury. PLoS ONE 12(10): e0186904. DOI: https://doi.org/10.1371/journal.pone.0186904
16. Chiang, Hsiu-Mei, Hsin-Chun Chen, Chin-Sheng Wu, Po-Yuan Wu, and Kuo-Ching Wen. "Rhodiola plants: Chemistry and biological activity." *Journal of Food and Drug Analysis* 23, no. 3 (2015): 359-369. DOI: https://doi.org/10.1016/j.jfda.2015.04.007
17. Luo, Fen, Jingyan Liu, Tianhua Yan, and Mingxing Miao. "Salidroside alleviates cigarette smoke-induced COPD in mice." *Biomedicine & Pharmacotherapy* 86 (2017): 155-161. DOI: https://doi.org/10.1016/j.biopha.2016.12.032
18. Lombardi, Valter RM, Lola Corzo, Iván Carrera, and Ramón Cacabelos. "The search for biomarine-derived compounds with immunomodulatory activity." *Journal of Exploratory Research in Pharmacology* 3, no. 1 (2018): 30-41. DOI: https://doi.org/10.14218/JERP.2018.00006
19. Marotta, Francesco, Sathya P. Thandavan, Surajit Pathak, Sushmitha Sriramulu, Ganesan Jothimani, Dharanivasan Gunasekaran, Devaprasad Markandeyan, and Antara Banerjee. "Vitagenic Effect of Specific Bioactive Fractions of Rhodiola with Trachurus sp. Extract Against Oxidative Stress-Induced Aging in Human Amnion Derived Epithelial Cell Line: In View of a Novel Senolytic." *Current Aging Science* 14, no. 2 (2021): 139-153. DOI: https://doi.org/10.2174/1874609814666210114094030
20. Liu, Chia-Hsin, Zhong Chen, Kong Chen, Fu-Tien Liao, Chia-En Chung, Xiaoping Liu, Yu-Chun Lin, Phouthone Keohavong, George D. Leikauf, and Yuanpu Peter Di. "Lipopolysaccharide-Mediated Chronic Inflammation Promotes Tobacco Carcinogen–Induced Lung Cancer and Determines the Efficacy of ImmunotherapyInflammation-Associated Lung Cancer and Immunotherapy." *Cancer Res* (2021) 81 (1): 144–157. DOI: https://doi.org/10.1158/0008-5472.CAN-20-1994
21. Gahring LC, Myers EJ, Dunn DM, Weiss RB, Rogers SW (2017) Nicotinic alpha 7 receptor expression and modulation of the lung epithelial response to lipopolysaccharide. PLoS ONE 12(4): e0175367.
22. Zhou, X. and Moore, B. B. (2017). Lung Section Staining and Microscopy. Bio-protocol 7(10): e2286. DOI: https://doi.org/10.21769/BioProtoc.2286.
23. Sun, F., Xiao, G. and Qu, Z. (2017). Murine Bronchoalveolar Lavage. Bio-protocol 7(10): e2287. DOI: https://doi.org/10.21769/BioProtoc.2287.
24. Pulli B, Ali M, Forghani R, Schob S, Hsieh KLC, Wojtkiewicz G, et al. (2013) Measuring Myeloperoxidase Activity in Biological Samples. PLoS ONE 8(7): e67976. DOI: https://doi.org/10.1371/journal.pone.0067976
25. Marklund, Stefan, and Gudrun Marklund. "Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase." Eur. J. Biochem. 47,469-474 (1974)
26. Mortaz, E., S. D. Alipoor, I. M. Adcock, S. Mumby, and L. Koenderman. "Update on neutrophil function in severe inflammation. Front Immunol. 2018 Oct 2;9:2171. DOI: https://doi.org/10.3389/fimmu.2018.02171
27. Voynow, Judith A., and Meagan Shinbashi. 2021. "Neutrophil Elastase and Chronic Lung Disease" *Biomolecules* 11, no. 8: 1065. DOI: https://doi.org/10.3390/biom11081065
28. Amlan Chakraborty, Jennifer C. Boer, Cordelia Selomulya, Magdalena Plebanski & Simon G. Royce (2018) Insights into endotoxin-mediated lung inflammation and future treatment strategies, Expert Review of Respiratory Medicine, 12:11, 941-955, DOI: https://doi.org/10.1080/17476348.2018.1523009
29. Ardjomand-Woelkart, Karin, and Rudolf Bauer. "Review and assessment of medicinal safety data of orally used Echinacea preparations." *Planta Med* 2016; 82(01/02): 17-31. DOI: https://doi.org/10.1055/s-0035-1558096
30. Shi, Jia, Tianxi Yu, Kai Song, Shihan Du, Simeng He, Xinxin Hu, Xiangyun Li et al. "Dexmedetomidine ameliorates endotoxin-induced acute lung injury in vivo and in vitro by preserving mitochondrial dynamic equilibrium through the HIF-1a/HO-1 signaling pathway." *Redox biology* 41 (2021): 101954. DOI: https://doi.org/10.1016/j.redox.2021.101954
31. Guan, Shuang, Haihua Feng, Bocui Song, Weixiao Guo, Ying Xiong, Guoren Huang, Weiting Zhong et al. "Salidroside attenuates LPS-induced pro-inflammatory cytokine responses and improves survival in murine endotoxemia." *International immunopharmacology* 11, no. 12 (2011): 2194-2199. https://doi.org/10.1016/j.intimp.2011.09.018
32. Song D, Zhao M, Feng L, Wang P, Li Y, Li W. Salidroside attenuates acute lung injury via inhibition of inflammatory cytokine production. Biomed Pharmacother. 2021 Oct; 142:111949. Epub 2021 Jul 26. DOI: https://doi.org/10.1016/j.biopha.2021.111949.
33. Chang, Xiayun, Fen Luo, Wenjiao Jiang, Lingpeng Zhu, Jin Gao, He He, Tingting Wei, Shilin Gong, and Tianhua Yan. "Protective activity of salidroside against ethanol-induced gastric ulcer via the MAPK/NF-κB pathway in vivo and in vitro." *International*

Immunopharmacology 28, no. 1 (2015): 604-615. DOI: https://doi.org/10.1016/j.intimp.2015.07.031

34. Jing, W. A. N. G., J. I. N. Rong-Guang, X. I. A. O. Lu, W. A. N. G. Qiu-Juan, and Y. A. N. Tian-Hua. "Anti-asthma effects of synthetic salidroside through regulation of Th1/Th2 balance." *Chinese journal of natural medicines* 12, no. 7 (2014): 500-504. DOI: https://doi.org/10.1016/S1875-5364(14)60078-9
35. Huang, Qian, and Xiao-Lan Hu. "Effects of salidroside on the secretion of inflammatory mediators induced by lipopolysaccharide in murine macrophage cell line J774. 1." *Sheng li xue bao:[Acta Physiologica Sinica]* 69, no. 1 (2017): 41-46. PMID: 28217806.
36. Zhang W, Zhang W, Huo L, Chai Y, Liu Z, Ren Z, Yu C. Rosavin suppresses osteoclastogenesis *in vivo* and *in vitro* by blocking the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways. Ann Transl Med. 2021 Mar;9(5):383. DOI: https://doi.org/10.21037/atm-20-4255
37. Marotta, Francesco, Jaganath Arunachalam, Antara Banerjee, Roberto Catanzaro, Sudhir Adalti, Aparimita Das, Alexander Kolyada, and Surajit Pathak. (2019). Oxidative Stress and Smoke-Related Lung Diseases: A Tentative Approach Through the Blood, Lungs, and Gut. In: Chakraborti, S., Chakraborti, T., Das, S., Chattopadhyay, D. (eds) Oxidative Stress in Lung Diseases. Springer, Singapore. DOI: https://doi.org/10.1007/978-981-13-8413-4_2
38. Wang, Yajun, Dongming Li, Guoda Ma, Wen Li, Jun Wu, Tianwen Lai, Dan Huang et al. "Increases in peripheral SIRT 1: A new biological characteristic of asthma." *Respirology* 20, no. 7 (2015): 1066-1072. DOI: https://doi.org/10.1111/resp.12558
39. Fu, C., Hao, S., Xu, X. *et al.* Activation of SIRT1 ameliorates LPS-induced lung injury in mice via decreasing endothelial tight junction permeability. *Acta Pharmacol Sin* **40**, 630–641 (2019). DOI: https://doi.org/10.1038/s41401-018-0045-3
40. Hiroyuki Kadoya, Minoru Satoh, Yuko Nishi, Megumi Kondo, Yoshihisa Wada, Yuji Sogawa, Kengo Kidokoro, Hajime Nagasu, Tamaki Sasaki, Naoki Kashihara, Klotho is a novel therapeutic target in peritoneal fibrosis via Wnt signaling inhibition, *Nephrology Dialysis Transplantation*, Volume 35, Issue 5, May 2020, Pages 773–781, DOI: https://doi.org/10.1093/ndt/gfz298