RESEARCH PAPER

MOLECULAR EVALUATION OF TERMINALIA CHEBULA AND VIRGIN COCONUT OIL ON ACUTE LUNG INJURY: AN IN VIVO STUDY

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ABSTRACT

Background: A significant amount of evidence indicates that Terminalia chebula (TCE) and Virgin Coconut Oil (VCO) are beneficial for their capacity to regulate inflammation and eradicate free radicals. Through polymerase chain reaction (PCR) analysis of the surfactant protein genes SFTPA1 and SFTPA2, the purpose of this work was to evaluate the individual and synergistic effects on acute lung injury that was generated by lipopolysaccharide (LPS) in BALB/c mice. Methods: The BALB/c mice were divided into five groups, each consisting of six animals: a disease control group, a TCE-treated group, a VCO-treated group, a combination post-treatment group (TCE+VCO), and a combined pre- and post-treatment group. Each of these groups contained six mice. The delivery of LPS through the intratracheal route occurred, which led to ALI. For a period of seven days, the therapies were administered orally. In order to investigate the expression of genes, we first extracted total RNA from lung samples, then derived cDNA from it, and last utilized quantitative real-time PCR. Results: Unique amplification peaks were identified for the genes SFTPA1, SFTPA2, and the housekeeping gene GAPDH. In all treatment groups, SFTPA1 expression levels went up. There was a significant increase in SFTPA2 expression in groups that only got VCO and groups that got both VCO and TCE. Conclusion: Based on the findings of this study, it was discovered that the simultaneous administration of Terminalia chebula extract and Virgin Coconut Oil protects mice against acute lung injury caused by LPS.

Keywords: Virgin coconut oil, Terminalia chebula, Acute lung injury

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INTRODUCTION

Acute lung injury (ALI) is characterized by the dysfunction of alveolar epithelial and capillary endothelial cells, arising from multiple direct and indirect causes [1]. This damage leads to extensive lung interstitial and alveolar edema, ultimately resulting in sudden hypoxic respiratory failure. ALI is a common problem in patients who are very sick. Despite extensive research on ALI by both national and international investigators in recent years, the mortality rate associated with ALI in the last few years, the death rate linked to ALI is still between 30%-60%.[2]. Despite this, ALI continues to be a major factor in critical care medicine.

Lipopolysaccharide (LPS) is considered the primary pathogen responsible for the aggravation of ALI [3]. It exerts its effects by regulating neutrophil aggregation, modulating the production of pro-inflammatory mediators, altering platelet aggregation, reducing myeloperoxidase activity and suppressing the release of neutrophil extracellular traps (NETs) [4].

For centuries, the mature, dried fruit of Terminalia chebula Retz. (TC), known as "Haritaki" in Ayurvedic medicine and "Arura" in Tibetan and Mongolian medicinal traditions, has been esteemed as an essential herbal remedy for respiratory disorders. In Ayurvedic medicine, TC is regarded as a valuable medicinal plant with broad therapeutic applications. This is a well-known natural cure. The Jingzhu Materia Medica, an important Tibetan pharmacopoeia, describes the diverse pharmacological properties of TC. It highlights the six flavors, eight characteristics, three post-digestive effects and seventeen therapeutic actions of TC, which contribute to itsclinical its clinical utility and its recognition as “King of Tibetan-Mongolian Medicinals” [5]. The ripe seed of the coconut, or copra (dried coconut pulp), is used to make coconut oil. Copra is mostly used to make oil, which makes up about 65% to 75% of the total. Coconut oil is widely used in both the food and industrial sectors [6].

This oil is rich in medium-chain saturated fatty acids, which have been shown to exert cardioprotective and anti inflammatoryanti-inflammatory effects. It also has strong antioxidants, like carotenoids and tocopherols, as well as vitamins. Furthermore, published research demonstrates that virgin coconut oil contains unsaponifiable compounds, primarily polyphenols and tocotrienols, which possess excess antioxidant properties. This oil exerts several pharmacological effects, such as lowering blood pressure, preventing coronary disease, and protecting the heart and blood vessels. Coconut oil may be a possible candidate for adjuvant therapy in ALI because it affects the inflammatory process [7].

While both Terminalia chebula and virgin coconut oil are acknowledged for their anti-inflammatory, antioxidant, and immunomodulatory effects, there is scant scientific evidence investigating their combined or synergistic therapeutic potential, especially regarding ALI. A thorough examination of the chemical composition of T. chebula and virgin coconut oil may enable additional investigation into the pharmacological potential of these substances. The combination of T. chebula extract and VCO may produce synergistic effects. This study aimed to evaluate the synergistic effects of TCE and VCO in LPS induced animal models of ALI using PCR.

MATERIALS & METHODS

This study was planned, executed, and reported in compliance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines (ARRIVE 2.0) [8].

Study Design: This was an experimental study comparing the impacts of the Terminalia chebula extract (TCE) and virgin coconut oil (VCO), separately and in combination with each other, in a model of lipopolysaccharide (LPS)-induced acute lung injury (ALI) in mice.

Five experimental groups were involved. LPS control group 2. TCE-treated group (post-LPS) 3. VCO-treated group (post-LPS) 4. TCE VCO combination group (after LPS) 5. TCE + VCO combination group (pre- and post-LPS). A mouse was selected as an experimental unit. Animals were considered as independent experimental units.

Sample Size: The study involved the use of 30 BALB/c mice which were then divided into 6 mice per group (n = 6). The sample was chosen according to the previous pilot observations and as the Institutional Animal Ethics Committee (IAEC) suggests.

Inclusion and Exclusion Criteria: Healthy male and female BALB/c mice aged 6 to 8 weeks were included. Mice aged beyond 8 weeks or less than 6 weeks and unhealthy mice were excluded.

Randomization: Randomization of animals to the experimental groups was used to reduce the selection bias. The body weight change was taken into consideration to minimize the confounding variables. There were no animals lost during the experimentation process and they were all incorporated in the final analysis.

Blinding: Analyses of gene expression and histopathological analysis were conducted by the blinded investigators with regards regard to the treatment groups, so that observation bias is reduced.

Outcome Measures: Primary ouctomes outcomes were the histopathology examination of the lung tissue structure and the analysis of the expression level of the surfactant proteins (SFTPA1 and SFTPA2) in the lung tissue. Secondary The secondary outcome was the level of inflammatory cytokines (IL-1β).

Experimental animals, housing and care: The study used BALB/c mice that were 6 to 8 weeks old and weighed 20 to 25 grams (both males and females). The animals were housed in a controlled environment (22±2°C and 50±10 RH) with 12-hour light/dark cycles in polypropylene cages, allowing unrestricted access to food and water. Daily monitoring of animal welfare was done in the course of the study. The animal study complied with IACUC standards and commenced subsequent to obtaining Ethical Clearance from IAEC.

Ethical statement: The Institutional Animal Ethics Committee (IAEC) approved all the experiments with animals. Accent number: IAEC/SSSMC&RI/2023-05. The research was done in accordance with the CPCSEA regulations and ARRIVE 2.0 requirements.

Experimental Procedures

1. TERMINALIA CHEBULA EXTRACT (TCE)

Terminalia chebula (Haritaki) fruits were obtained, washed and sun dried, then grounded. The powder was shifted to remove any large particles, and after that, it was combined with ethanol (0.1% by weight) at a concentration of 98% for a period of three days at room temperature, with consistent stirring. After that, the extract was filtered once more with Whatman No. 1 filter paper, and then it was concentrated with a rotary evaporator (Buchi R-114, Switzerland) at a lower pressure. In order to preserve the semi-solid residue until it was required once again, it was freeze-dried at a weight-to-weight ratio of 11.78%.

2. THE PRODUCTION OF VIRGIN COCONUT OIL (VCO) FROM COCONUTS

When combining newly collected coconut pulps, an electric blender was utilized for the mixing process. Then proceeded to gather the coconut milk after filtering it. During the process of making coconut milk, the pulp was combined with water and then cooked for sixty minutes at a temperature ranging from one hundred to one hundred and twenty degrees Celsius. A double-jacketed coconut oil cooker was used to compress the mix. The oil was filtered and got out of the plant through muslin fabric and then heated it up more until it became clear. The oil was kept at 4°C until it was time to analyze and use it.

3. ADMINISTRATION OF LPS

The mice were sedated with 50 mg/kg of ketamine and 10 mg/kg of xylazine, and then we put Lipopolysaccharide (LPS) (10 µg/ml) into their tracheas to cause lung inflammation. The incision was stitched up, antiseptics (Povidone iodine ointment) were given,—Mice were closely observed for any signs of pain or troubled breathing. Meloxicam (22–25 µl) was administered for postoperative analgesia.

4. DESIGN OF THE EXPERIMENT

The mice were randomly divided into five groups, with six mice in each group. Group I, referred to as the disease control, did not receive any pharmacological intervention after LPS induction and was only given normal saline. After LPS was given, group II got haritaki extract (400 mg/kg, I.O.), group III got VCO (400 µl/kg, I.O.), and group IV got a mix of haritaki and VCO (400 mg/kg + 400 µl/kg). Before and after LPS induction, group V got haritaki and VCO (400 mg/kg and 400 µl/kg, respectively). All pharmacological interventions were given once a day for seven days after LPS was given by oral gavage. At the end of the study, the animals were killed by CO2 asphyxiation.

5. POLYMERASE CHAIN REACTION ANALYSIS RNA SEPARATION

After centrifugation, the homogenate was re-suspended and washed twice with ice-cold PBS before being collected. A High Pure miRNA Isolation Kit was used (Sigma-Aldrich, St. Louis, MO, USA) to extract total RNA, following the standard method DNA strands were made by reverse transcribing 5 µl of total RNA using a SuperScript™ II Reverse Transcriptase Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) and oligo-(dT) primers (Sigma-Aldrich, St. Louis, MO, USA) according to the instructions that came with the kit. To make complementary DNA (cDNA) for SFTPA1, a forward primer that targeted nucleotides 15 to 32 and a reverse primer that targeted

nucleotides 715 to 698 were used. The forward and reverse primer sequences used to make cDNA for SPA-1 are TGGTCGCTGATTTCTTGGA and CGCTGCTCTCACTGACTCA, which are 82 base pairs long. For SPA-2, the sequences are TGAAAAGAAGGAGCAGCGACT and ACCAGGGCTTCCAACACAA, which are 126 base pairs long (Sotiriadis et al., 2015). For each experiment, 100 ng of cDNA was used, and a human GAPDH endogenous control (Applied Biosystems, Foster City, CA, USA) was used as the housekeeping gene (Liu et al., 2005).

The PCR was performed in a 96-well plate utilizing a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), commencing at 50°C for 2 minutes, followed by denaturation at 95°C for 15 seconds, and finishing with an extension at 60°C for 1 minute. Throughout 40 cycles, data on exponential amplification was collected without fail. During the melt stage, the temperature went up by 0.15°C per second to break down the amplified DNA. The procedure was stopped when the temperature reached 95°C, and the amount of amplified DNA was recorded.

Statistical Analysis: The results were in the form of data which were expressed in mean and standard deviation (SD). One-way analysis of variance (ANOVA) followed by post hoc test of Tukey were used to perform statistical comparisons between groups. The p value of 0.05 was taken as significant.

RESULTS

The preventive effectiveness of TCE and VCO was evaluated against LPS-induced pulmonary injury in BALB/c mice. There were no deaths of any animals in the course of experiments. Histopathological observations revealed: - Intense inflammatory infiltration / damage in the alveoli in the control group of LPS - Partial recovery in the TCE or VCO treatment groups on their own - Approaching normal lung architecture and significant diminution of inflammation in the combined TCE + VCO treatment groups, specifically, with pre- and post-treatment

Levels of the SFTPA1 and SFTPA2 genes

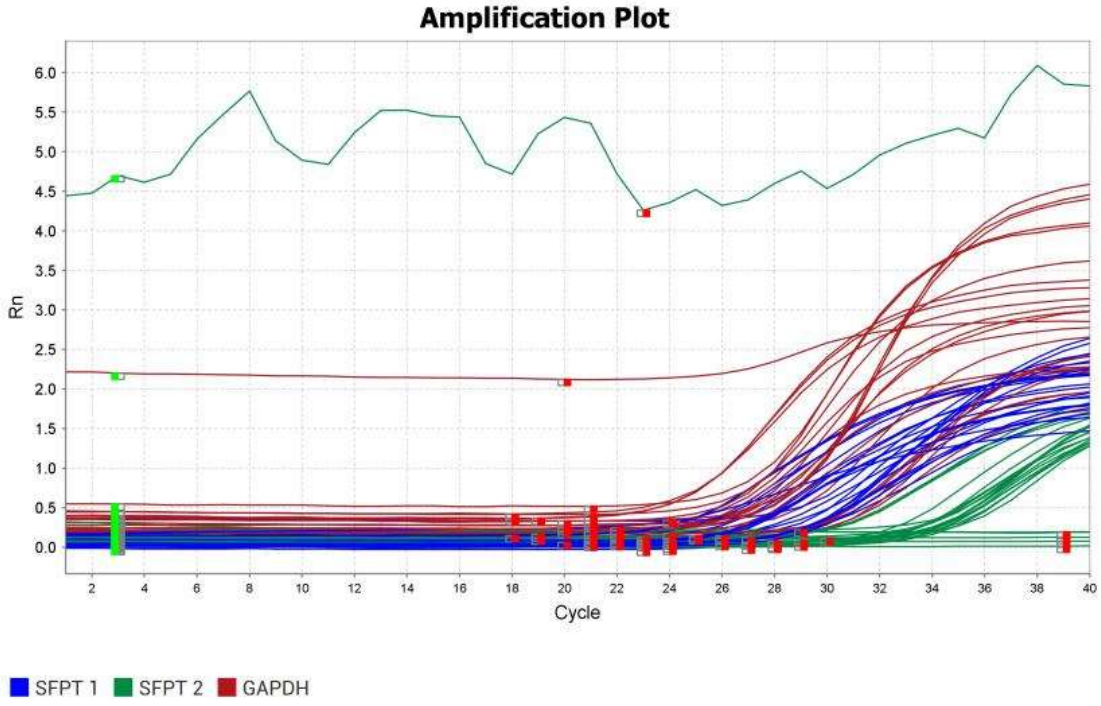

Figure 1: Amplification plot of the quantitative PCR study of SFTPA1 and SFTPA2 The amplification of SFTPA1 and SFTPA2 is shown by blue and green lines respectively and their amplification curves show successful amplification. The red lines are relative to GAPDH, which was used as the reference gene. Each line is indicative of triplicate measurements of each group.

Fig. 1 illustrates the amplification profiles of SFTPA1 and SFTPA2 together with the housekeeping gene GAPDH. During the initial 10 cycles, amplification signals remained below the threshold level for all genes. SFTPA1 exhibited earlier amplification than SFTPA2, indicating relatively higher transcript abundance. Distinct amplification curves and sharp peaks were observed for all genes, demonstrating efficient and specific PCR amplification.

Melting curves

To examine the melting characteristics of the PCR products from the genes under investigation, melt curve analysis was employed. The melting plot in Fig. 2a showed that the degree at where the two genes melted down was the same. It was apparent that the arcs were tightly packed within a narrow temperature range showed this. This showed that the procedure was very specific and that the primers used didn't make any products that weren't specific. Moreover, the appearance of discrete peaks at particular temperatures is an indication of the purity of the genes that are now being expressed. As shown in Figures 2b, 2c, and 2d, the maximum temperature at which GADPH reached its peak was 83.5 degrees Celsius, whereas SFTPA1 reached its peak at 85 degrees Celsius and SFTPA2 reached its peak at 81.5 degrees Celsius. On the other hand, the NTC (No Template Control) curve did not contain a peak that had the potential to interfere with the peaks of the genes that were chosen when the temperature was at the appropriate level. This shows that there was no contamination or amplification in the control. The curves for each group showed clear and distinct patterns across all genes, which showed that primer melting was consistent. Nonetheless, multiple minor peaks were observed in the melt curves of SFTPA2, indicating the presence of amplification products originating from outside the targeted gene.

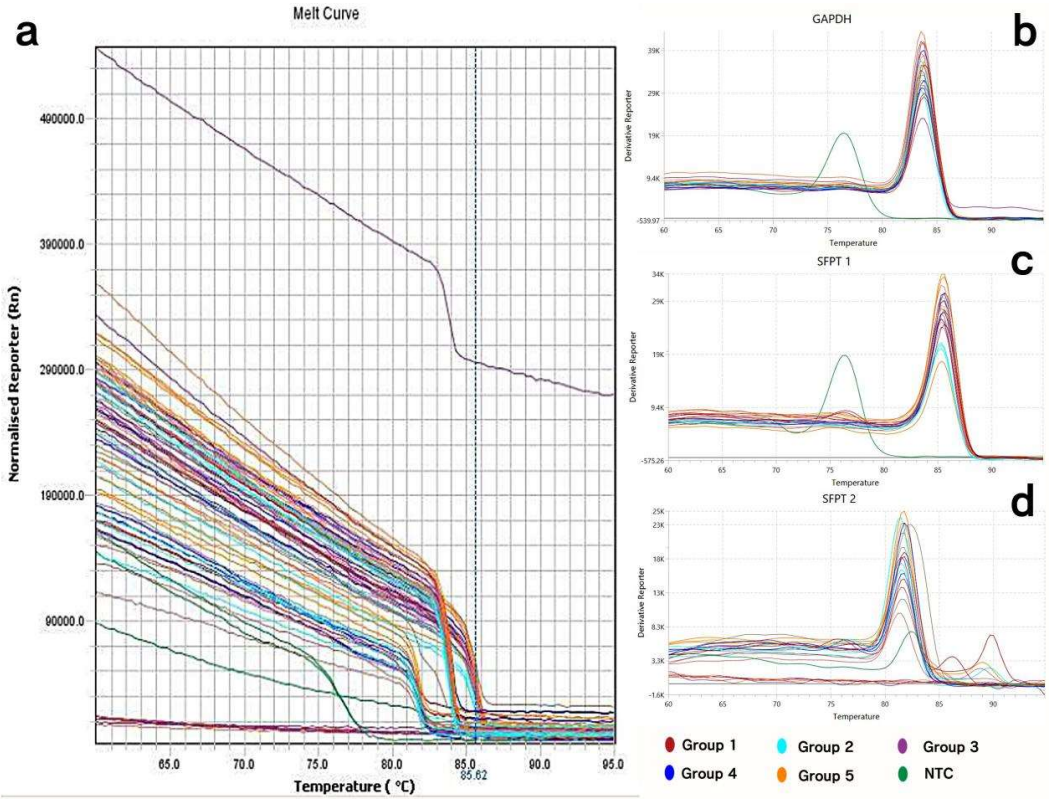

Figure 2: Melt curve analysis SFTPA1 and SFTPA2Figure. 2a displays the normalized reporter signals of the melt curve for different groups across a range of temperatures (65°C to 95°C). The data from Group 1 (red), Group 2 (blue), Group 3 (purple), Group 4 (indigo), and Group 5 (orange) are compared, with the non-template control (NTC) shown in green color. Figure. 2b, 2c, and 2d show the derivative melt curves for GADPH, SFTPA1 and SFTPA2 among various groups, respectively, demonstrating distinct melting profiles.

The expression of the SFTPA1 and SFTPA2 genes

The relative expression of SFTPA1 and SFTPA2 was determined using the 2^-ΔΔCt method with GAPDH as the endogenous control (Table 1). Compared with the disease control group (Group 1), SFTPA1 expression increased by 5.13-fold, 10.43-fold, 10.20-fold, and 14.94-fold in Groups 2, 3, 4, and 5, respectively. The highest SFTPA1 expression was observed in Group 5, indicating enhanced transcriptional activation following combined administration of TCE and VCO.

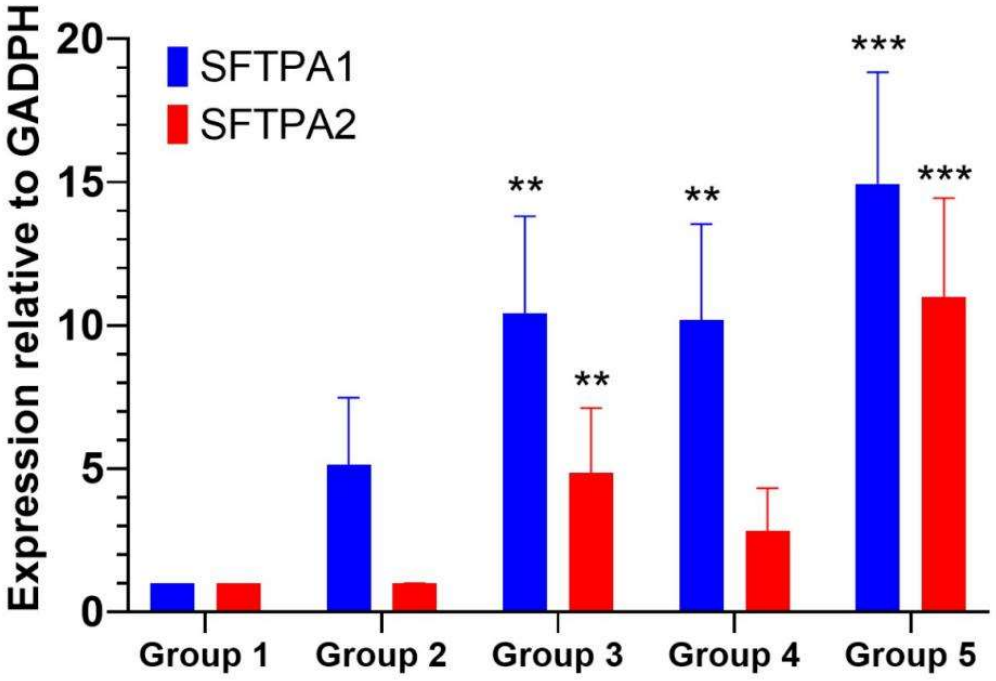

Figure 3: Effect of TCE and VCO on the expression of SFTPA1 and SFTPA2

SFTPA2 expression exhibited a different pattern. Group 2 showed minimal change in expression (0.99-fold) compared with Group 1, whereas Groups 3, 4, and 5 demonstrated 4.85-fold, 2.82-fold, and 10.99-fold increases, respectively. The highest SFTPA2 expression was observed in Group 5, suggesting that combined pre- and post-treatment with TCE and VCO produced the strongest stimulatory effect on surfactant-associated gene expression. These findings support a synergistic role of TCE and VCO in enhancing pulmonary protective mechanisms following LPS-induced lung injury. Gene expression was quantified using GAPDH as the endogenous control. Relative expression levels were calculated using the 2^-ΔΔCt method with Group 1 serving as the calibrator group. ΔCt values are presented as mean ± standard deviation (SD), and fold-change values represent relative gene expression compared with the disease control group (Fig. 3).

The relative expression of SFTPA1 (blue bars) and SFTPA2 (red bars) in various experimental groups (Group 1 to Group 5) was shown. Expression levels are normalized to GAPDH.

Table1: Relative expression of SFTPA1 and SFTPA2 genes in different experimental groups determined by real time PCR.

Group	SFTPA1 ΔCt (Mean ± SD)	SFTPA1 Fold Change	SFTPA2 ΔCt (Mean ± SD)	SFTPA2 Fold Change
Group 1	4.043 ± 2.295	1.00	-8.556 ± 3.442	1.00
Group 2	1.684 ± 2.880	5.13	-8.555 ± 2.883	0.99
Group 3	0.660 ± 1.799	10.43	-10.834 ± 1.876	4.85
Group 4	0.694 ± 0.672	10.20	-10.051 ± 1.882	2.82
Group 5	0.142 ± 2.642	14.94	-12.015 ± 1.168	10.99

Interpretation and Scientific Significance

Terminalia chebulaCombined chebula. Combined administration of TCE and VCO resulted in the highest expression of SFPTA1 and SFTPA2 which was associated with improved histopathological findings compared with the individual treatments.

DISCUSSION

In this study, the surfactant-related protein genes SFTPA1 and SFTPA2 were subjected to PCR analysis in order to investigate the complimentary complementary impact of TCE and VCO on ALI caused by LPS in BALB/c mice. Both before and after the therapy, the simultaneous injection of TCE and VCO resulted in a considerable increase in the expression of SFTPA2, as demonstrated by the unique gene expression patterns that were observed in the treatment groups. This shows that all components responsible for lung protection and regeneration operated in an exceptionally effective manner.

There is a connection between the antioxidant and anti-inflammatory properties that these medications are known to have and the rise in SFTPA1 and SFTPA2 that was discovered among participants that were given either one or both of the treatments. Additionally, the SFTPA gene family is responsible for the production of surfactant proteins, which play a significant role in maintaining the stability of the alveoli and warding off infections in the lungs. These proteins are responsible for controlling the activity of alveolar macrophages and preventing the production of pro-inflammatory cytokines in situations when inflammation is brought on by the activation of endotoxins, as discovered by Wright et al. [9]. There was an increase in the amount of production of SFTPA2 in Group V, which received both TCE and VCO both before and after therapy respectively. This suggests that the treatment had a safeguarding and restoring action at its transcriptional level. This suggests that the combination therapy increases the lung's ability to endure inflammatory assaults.

When compared to the disease control, the expression of SFTPA2 was not significantly increased by administration of TCE alone. On the other hand, when it was blended with VCO, it demonstrated a significant improvement in transcription. This finding lends credence to the hypothesis of a synergistic interaction, in which the polyphenolic antioxidants derived from T. chebula (more specifically, chebulagic and gallic acids) and the medium-chain fatty acids derived from VCO work together to improve antioxidative defense and influence gene expression pathways that are associated with the regulation of inflammation [5, 10].

The observed synergistic effect may be mediated through coordinated modulation of the NF-kB and Nrf2 signalling pathways. NF-kB is a key transcription factor involved in the regulation of pro inflammatory mediators including TNF-α, IL-1β and IL-6 which are markedly evaluated during LPS induced lung injury [11]. Polyphenolic constituents of Terminalia chebula, particularly chebulagic acid, chebulinic acid and gallic acid have been shown to suppress NF-kB activation, thereby reducing inflammatory gene transcription [12]. In contrast, the medium chainmedium-chain fatty acids and phenolic compounds present in virgin coconut oil activate the Nrf2 pathway, enhancing endogenous antioxidant defenses and reducing oxidative stress [13]. Excessive reactive oxygen species generated during ALI contribute to alveolar epithelial injury and impaired surfactant production. Therefore, simultaneous inhibition of inflammatory signaling and enhancement of antioxidant responses may preserve type II alveolar pneumocyte function, promote surfactant homeostasis and contribute to the increased expression of SFTPA2 observed in the combination treatment group.

The enhanced expression of SFTPA2 in the combination-treatment group may indicate enhanced alveolar epithelial recovery and restoration of surfactant homeostasis. Previous studies have demonstrated that T. chebula extract reduces oxidative stress-induced cellular injury, while VCO exhibits significant anti-inflammatory activity through modulation of inflammatory pathways [13,15,16]. The present findings extend these observations by suggesting that concurrent administration of both agents may provide complementary molecular benefits in ALI. Based on the findings, it appears that the combination of T.chebula and VCO could potentially offer a novel phytotherapeutic approach for the treatment of ALI and other pulmonary inflammatory disorders that are connected with it. At the moment, the majority of treatments for ARDS and ALI concentrate on providing supportive care rather than focusing on molecular intervention [11]. As a result, the incorporation of natural compounds that possess antioxidant and anti-inflammatory qualities presents a promising therapeutic strategy [17-20]. Because of this synergy, oxidative damage may be reduced, surfactant equilibrium may be restored, and alveolar repair may be facilitated. All of these things are essential for reversing the pathogenic cascade that occurs in ALI.

However, this work was limited to the mRNA expression profiling of only two surfactant protein genes. Despite the fact that the PCR analysis produced substantial insights into transcriptional regulation, the study was nevertheless limited. It is required to do additional validation by protein quantification tests, such as Western blotting or ELISA, in order to determine the translational significance of the gene expression changes that have been discovered. An analysis of histology and the measurement of inflammatory cytokines, such as TNF-α, IL-1β and IL-6, may provide additional evidence of protection at the tissue level. In addition, research that investigate investigates

dose-response relationships and the investigation of different injection regimens have has the potential to improve the therapeutic synergy between TCE and VCO.

In subsequent research, it is necessary to define the pharmacokinetics and bioavailability of active drugs that are provided concurrently, while also testing the safety and effectiveness of these compounds in clinical settings or sophisticated animal models. This mechanistic knowledge of these findings can be obtained by the use of molecular docking and in silico modeling, which have the potential to shed light on the interactions that exist between active phytoconstituents and inflammatory signaling proteins.

CONCLUSION

Based on the findings of this study, it was discovered that the simultaneous administration of *Terminalia chebula* extract and Virgin coconut oil protects mice against acute lung injury caused by LPS. This is accomplished by boosting the expression of the SFTPA1 and SFTPA2 genes. It would appear that the interaction of many molecular systems that combat inflammation and free radicals is the source of this synergy. The results provide a solid foundation for further research into phytochemical adjunct therapy for inflammatory lung disorders and highlight the potential for unique therapeutic improvements that can be achieved via the combination of traditional medicine and genetic science.

Data Availability

The data are accessible on a reasonable request by the relevant author.

CONFLICT OF INTEREST

The authors state that there is no conflict of interest.

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REFERENCES

1. Johnson ER, Matthay MA. Acute lung injury: epidemiology, pathogenesis, and treatment. J Aerosol Med Pulm Drug Deliv. 2010;23(4):243-252. doi:10.1089/jamp.2009.0775.

2. Singh G, Gladdy G, Chandy TT, Sen N. Incidence and outcome of acute lung injury and acute respiratory distress syndrome in the surgical intensive care unit. Indian J Crit Care Med. 2014;18(10):659-665. doi:10.4103/0972-5229.142175.

3. Rittirsch D, Flierl MA, Day DE, Nadeau BA, McGuire SR, Hoesel LM, et al. Acute lung injury induced by lipopolysaccharide is independent of complement activation. J Immunol. 2008;180(11):7664-7672. doi:10.4049/jimmunol.180.11.7664.

4. Ansari J, Gavins FN. Neutrophils and platelets: immune soldiers fighting together in stroke pathophysiology. Biomedicines. 2021;9(12):1945. doi:10.3390/biomedicines9121945.

5. Bag A, Bhattacharyya SK, Chattopadhyay RR. The development of **Terminalia chebula** Retz. (Combretaceae) in clinical research. Asian Pac J Trop Biomed. 2013;3(3):244-252.

6. Punzalan JK, Rosentrater KA. Copra meal: a review of its production, properties, and prospects. Animals (Basel). 2024;14(11):1689. doi:10.3390/ani14111689.

7. Schwingshackl L, Schlesinger S. Coconut oil and cardiovascular disease risk. Curr Atheroscler Rep. 2023;25(5):231-236. doi:10.1007/s11883-023-01098-y.

8. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. J Cereb Blood Flow Metab. 2020;40(9):1769-1777. doi:10.1177/0271678X20943823.

9. Wright JR. Immunoregulatory functions of surfactant proteins. Nat Rev Immunol. 2001;1(1):58-68.

10. Nevin KG, Rajamohan T. Effect of topical application of virgin coconut oil on skin components and antioxidant status during dermal wound healing in young rats. Skin Pharmacol Physiol. 2010;23(6):290-297.

11. Ware LB, Matthay MA. The acute respiratory distress syndrome. N Engl J Med. 2000;342(18):1334-1349.

12. Karunaweera N, Raju R, Gyengesi E, Münch G. Plant polyphenols as inhibitors of NF-κB induced cytokine production—a potential anti-inflammatory treatment for Alzheimer's disease? Front Mol Neurosci. 2015;8:24. doi:10.3389/fnmol.2015.00024.

13. Intahphuak S, Khonsung P, Panthong A. Anti-inflammatory, analgesic, and antipyretic activities of virgin coconut oil. Pharm Biol. 2010;48(2):151-157.

14. Matalon S, Bartoszewski R, Collawn JF. Role of epithelial sodium channels in the regulation of lung fluid homeostasis. Am J Physiol Lung Cell Mol Physiol. 2019;316(2):L205-L213.

15. Jung HL, Yang SY, Pyo MC, Hong CO, Nam MH, Lee JW, et al. Protective effects of chebulic acid from *Terminalia chebula* Retz. against t-BHP-induced oxidative stress by modulations of Nrf2 and its related enzymes in HepG2 cells. Food Sci Biotechnol. 2019;28(2):555-562. doi:10.1007/s10068-018-0477-z.

16. Peedikayil FC, Sreenivasan P, Narayanan A. Effect of coconut oil in plaque-related gingivitis—a preliminary report. Niger Med J. 2016;57(2):143-147.

17. Wang C, Zhang H, Wang X, Wang X, Li X, Li C, et al. Comprehensive review on fruit of *Terminalia chebula*: traditional uses, phytochemistry, pharmacology, toxicity, and pharmacokinetics. Molecules. 2024;29(23):5547. doi:10.3390/molecules2923554.

18. Chen X, Wang W, Zhang H, Liang N, et al. Plant-derived natural compounds for the treatment of acute lung injury: a systematic review of their anti-inflammatory effects in animal models. Int Immunopharmacol. 2025;146:113807.

18. Kim JH, Kim SH, Chung KH, et al. Medicinal herbal extracts: therapeutic potential in acute lung injury. Future Pharmacol. 2024;4(4):700-715.

19. Fan C, Zhang Z, Lai Z, Yang Y, Li J, Liu L, et al. Chemical evolution and biological evaluation of natural products for efficient therapy of acute lung injury. Adv Sci (Weinh). 2024;11(7):e2305432.