

The protective effect of xanthenone against LPS-induced COVID-19 acute respiratory distress syndrome (ARDS) by modulating the ACE2/Ang-1-7 signaling pathway

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Abstract. – OBJECTIVE: Acute respiratory distress syndrome (ARDS) is an inflammatory lung disease that has a high rate of morbidity and mortality. It's an acute diffusive lung injury caused by the release of pro-inflammatory cytokines into the lungs. Specific microRNAs have been identified to play a crucial role in the renin-angiotensin system signaling pathways the main pathophysiological pathway responsible for ARDS. Since the ARDS life-threatening complication associated with COVID-19 is an ongoing challenge, this current study aimed to investigate the potential efficacy of xanthenone in the treatment of ARDS induced with LPS in mice through ACE2 activation and modulation of miR-200 and ACE2/Ang 1-7 pathways.

MATERIALS AND METHODS: Mice were categorized into three groups randomly. The first set of

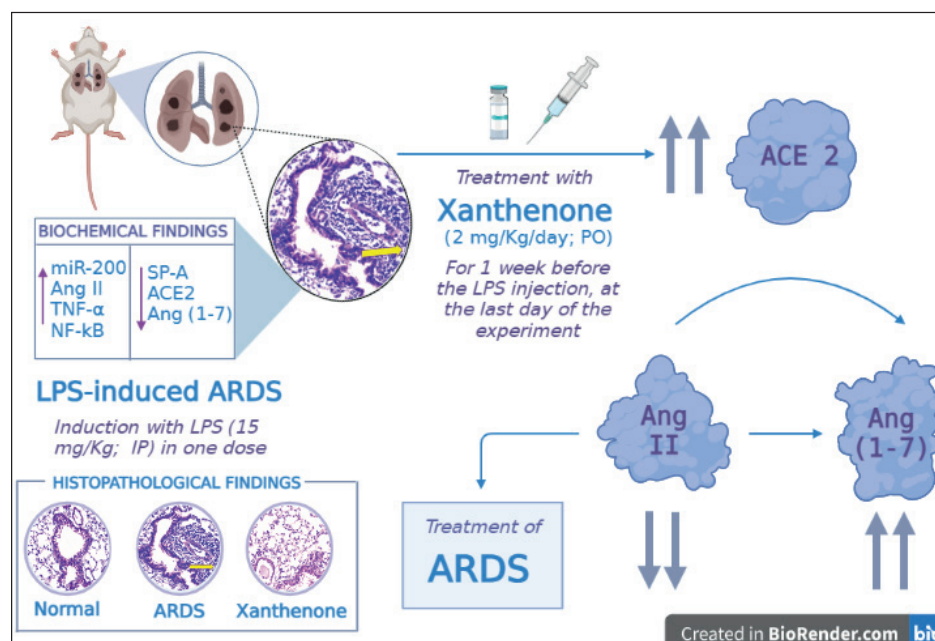
mice served as the normal control group. The ARDS group was injected with LPS (15 mg/kg; i.p.). The last group was treated with xanthenone (2 mg/kg/day; p.o.) for one week before the LPS injection.

RESULTS: Xanthenone treatment resulted in a significant down-regulation of miRNA-200 expression, leading to the activation of ACE2 accompanied with marked inhibition of Angiotensin II as well as increases the levels of Ang 1-7 and SP-A.

CONCLUSIONS: Xanthenone has the potential to be a promising therapeutic drug for the treatment of ARDS COVID-19 complication through activation of ACE2/Ang 1-7 pathways.

Key Words:

Xanthenone, ARDS, COVID-19, ACE2.



Graphical Abstract. Xanthenone downregulates the expression levels of miR-200, TNF-α, and NF-κB, and increases the expression levels of ACE2, Ang 1-7, and SP-A. Xanthenone has the potential to be a promising therapeutic drug for the treatment of ARDS COVID-19 complication through activation of ACE2/Ang 1-7 pathways.

Introduction

Acute respiratory distress syndrome (ARDS) is an acute inflammatory lung disease that eventually causes protein-rich non-hydrostatic pulmonary edema. It can lead to refractory hypoxemia and makes the lungs more stiff with a decreased ability to excrete carbon dioxide¹. Although there has been advances in treatments, mortality rates remain at 34.9% for patients with mild ARDS, 40% for patients having moderate ARDS and 46.1% for patients with severe ARDS². ARDS is a major complication of COVID-19 that seriously threatens patients' lives³. 42% of patients with COVID-19 pneumonia develop ARDS. ARDS also develops in 61-81% of patients requiring admission to the Intensive Care Unit⁴ (ICU). The mortality rates of COVID-19 patients with ARDS range between 26% and 61.5%. These rates can increase in patients who have received mechanical ventilation to be between 65.7% and 94%⁵. It was reported that the alveolar damage in ARDS can result from neutrophil-related epithelial necrosis followed by interstitial flooding and endothelial injury. This leads to a ventilation-to-perfusion mismatch, which aggravates dead space ventilation and decreases lung compliance^{2,6}. Previous research suggested that the renin-angiotensin system (RAS) may play a significant role in the pathogenesis of coronavirus-induced acute respiratory distress syndrome⁷. While angiotensin-converting enzyme 1 (ACE) cleaves angiotensin-1 into angiotensin-2, angiotensin-converting enzyme 2 (ACE2) further converts it into angiotensin 1-7. Angiotensin 1-7 is reported to have potential anti-inflammatory effects and thus its downregulation can cause ARDS⁸. The ACE2/Angiotensin 1-7 axis plays a series of roles in the improvement of endothelial dysfunction. In addition, it has anti-inflammatory, anti-hypertensive, anti-thrombotic, and anti-fibrotic activities. The protective effect of ACE2 is associated with attenuating Angiotensin-2 levels and increasing Angiotensin 1-7 levels in lungs⁹. Emerging evidence has shown that RAS signaling and ACE2 play crucial roles in SARS-CoV-induced ARDS and lethal avian influenza A induced acute lung injury (ALI)¹⁰. According to pathological findings, SARS-CoV-2 is also associated with lung failure and ARDS¹¹, and the majority of severely ill patients with SARS-CoV-2 infection have underlying comorbidities, such as cardiovascular disease, diabetes, and cerebrovascular disease¹².

MicroRNAs (miRNAs) are a unique class of short non-coding RNAs of around 22 nucleotides^{13,14}. They regulate gene expression and adjust the levels of expressed proteins¹⁵. Primary miRNAs are transcribed from DNA sequences and processed into

precursor miRNAs and eventually mature miRNA¹⁶. Furthermore, they bind to the 3' untranslated region (3' UTR) of target messenger RNA (mRNA)¹⁷ to degrade mRNA and repress its translation¹⁸. MiRNAs can also be used as biomarkers for ARDS as the upregulation and downregulation of these miRNAs is significant in the development of ARDS¹⁹. MiR-155 has a big role in immune regulation and it is reported to be upregulated in ARDS and can be considered a promising biomarker in diagnosis of ARDS²⁰. Additionally, miR-146 and miR-223 are also upregulated in ARDS as they are important for immune responses regulation and myeloid differentiation^{21,22}. Moreover, the downregulation of the *miR-let-7* family such as, *miR-let-7f*, *miR-let-7a*, and *miR-let-7b* may play major roles in the inflammatory process, progression and pathogenesis of ARDS¹⁹. Recently, the upregulation miRNA-200 was demonstrated to take a huge part in ARDS development. MiRNA-200 binds to the 3'UTR of the mRNA that is responsible for the transcription of ACE2, resulting in suppression of its expression with a subsequent increase in angiotensin-2 (ACE2 substrate) and a decrease of angiotensin 1-7 that ends with the incidence of ARDS²³.

Xanthenone is a newly discovered activator of the ACE2/Angiotensin 1-7 axis²⁴. Therefore, it may be promising in the management of ARD. Past studies were done on xanthenone as an ACE2 activator²⁵. Xanthenone was also reported to have a neuroprotective effect against cerebral ischemia/reperfusion injury through the exertion of anti-apoptotic, antioxidant, and anti-inflammatory effects²⁶. Moreover, it has been reported that xanthenone also protects experimental pregnant rats from hypertension and proteinuria induced by leptin via the activation of ACE2²⁷. Xanthenone, as an ACE2 activator, can be able to prevent pulmonary fibrosis. It is an antiparasitic drug mainly used in cattle to treat trypanosomiasis and promotes the migration of CD34 cells, inducing vascular repair through an effect mediated by Angiotensin-2. In addition, Xanthenone could improve vascular and pulmonary hypertension, alveolar remodeling, and other pathologies²⁸. Hence, the current study aims to evaluate the potential efficacy of xanthenone in the treatment of ARDS induced with LPS in mice through ACE2 activation and modulation of miR-200 and ACE2/Ang 1-7 pathways.

Materials and Methods

Animals

C57BL/6 male mice (15-20 g) were used in the current study. They were purchased from the Teodor

Bilharis Institute (TBI), Cairo, Egypt. Mice were kept at the animal house of October University for Modern Sciences and Arts (MSA) in plastic cages under constant conditions of humidity 50% and temperature $25 \pm 3^\circ\text{C}$. Free access to water and standard pellet chow were allowed.

Drugs and Chemicals

9-Xanthenone (xanthone) and endotoxin lipopolysaccharide (LPS) from gram-negative bacteria *E. coli* were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Induction of Acute Respiratory Distress Syndrome (ARDS)

LPS (15 mg/kg; i.p) was used for induction of ARDS. This induction method was chosen based on previous literature²⁹⁻³¹ and a pilot study for three doses of LPS (10, 15 and 20 mg).

Experimental Design

Mice were categorized into three groups (n=6) at random. The first set of mice served as the normal control group. The ARDS control group was injected with LPS (15 mg/kg; i.p.). The last group was treated with xanthenone (2 mg/kg/day; p.o.) for one week before the LPS injection on the last day of the experiment. The dose of xanthenone was determined based on previous literature²⁴.

After 6 hours from the last injection of test agents, the animals were anesthetized and sacrificed by cervical dislocation. The lungs were rapidly isolated and washed in ice-cold saline. The isolated lungs were used for the investigation of surfactant protein (SP), miRNA-200, angiotensin-converting enzyme 2 (ACE2), angiotensin II, angiotensin 1-7, nuclear factor- κB (NF- κB) and tumor necrosis factor- α (TNF- α) as well as the histological examination.

Biochemical Parameters

Determination of ACE2 activity in lungs

PromoCell's ACE2 activity assay kit was used for investigation of ACE2 activity in lung tissue according to the manufacturer instructions (PromoCell, Heidelberg, Germany) using fluorescence microplate reader (Thermo Fisher Scientific Oy FI-01621 Vantaa, Finland).

ELISA assay of pulmonary SP-A, Angiotensin II and Angiotensin 1-7

The pulmonary contents of SP-A were measured by enzyme linked immunoassay (ELISA) tech-

nique using a standard kit (Cloud-clone corp; TX., USA). Pulmonary angiotensin 1-7 and angiotensin II contents were investigated using standard ELISA kits (Abbexa Ltd, Cambridge, UK and LifeSpan Biosciences, Inc.). Absorbance was read (OD rang: 490-630) using ELISA plate reader (Stat Fax 2200, Awareness Technologies, Miami, FL, USA).

Quantitative RT-PCR analysis of miR-200 and ACE2

Total RNA was isolated from lungs using TRIzol (Invitrogen; Auckland, New Zealand), according to the manufacturer's instructions and reverse-transcribed into cDNA with the Reverse Transcriptase M-MLV (Promega, Madison, WI, USA). The following primer sequences was used in the current experiment: for ACE2; forward primer sequence: 5'- TGGGCAAACCTCTATGCTG-3', reverse primer sequence: 5'-TTCATTGGCTCCGTTTCTTA-3'; for β -actin; forward primer sequence: 5'-CTGAGAGGGAAATCGTGCGT-3', reverse primer sequence: 5'-TTGTTGGCATAGAGGTCTTTA-3'.

Small RNA species-enriched RNA was isolated for miRNA quantitative reverse transcriptase PCR according to the manufacturer's instructions (mirVana miRNA isolation kit; Ambion, Austin, TX, USA). miRNA was reverse-transcribed by using Ncode miRNA first-strand complementary DNA synthesis kits (Invitrogen; Auckland, New Zealand). Forward primer sequence was designed as the corresponding mature miRNA sequences and U6 snRNA (forward primer sequence: 5'-CTCGCTTCGGCAGCATATACT-3' and reverse primer sequence: 5'-ACGCTTCACGAATTTGCGTGTC-3') were used as normalizing control. The miR-200 specific primers; miR-200 forward primer sequence: 5'-TAACACTGTCTGGTAACGATGT-3' and reverse primer was Qiagen's miScript Universal Primer.

Quantitative reverse transcriptase PCR was performed by using a Power SYBR Green PCR Master Mix on the CFX96 Instrument (Bio-Rad, Hercules, CA, USA). Data analysis was determined by using the relative standard curve method.

Western Blot Analysis of TNF- α

Part of the lung was homogenized using radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris HCl pH 8, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% SDS) provided with phosphatase inhibitor cocktail. After protein quantification according to the manufacturer's instruction (Thermo Fisher Scientific, Waltham, MA, USA), 7.5 μg protein from each sample was

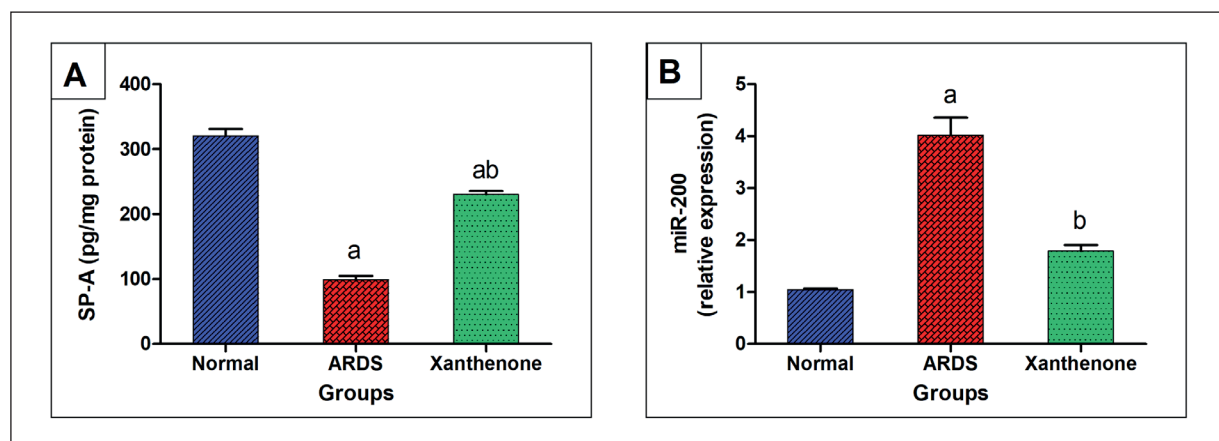


Figure 1. Effect of xanthenone on pulmonary surfactant protein content (SP-A) and miR-200 expression in mice with ARDS. The data are presented as mean \pm SEM (n = 6). **A**, significant difference from normal group and **B**, significant difference from ARDS control group (at $p < 0.05$).

loaded on gel electrophoresis (8% sodium dodecyl sulphate-polyacrylamide) and transferred to PVDF membrane. The membrane was blocked with bovine serum albumin (BSA; 5%) then incubated (1:1000) overnight at 4°C on a roller shaker with either anti-TNF- α (cat #53-7321-82), or anti- β -actin (1:1000; cat#: PA5-16914) antibody (Thermo Fisher Scientific, Waltham, MA, USA). Chemiluminescence detection was performed with an Amersham detection kit according to the manufacturer's protocols and exposed to X-ray film. Protein was quantified by densitometric analysis of the autoradiograms using a scanning laser densitometer (GS-800 system, Bio-Rad, Hercules, CA, USA). Results were expressed as arbitrary units after normalization for β -actin protein expression³².

Histopathologic assessment of lung tissue damage

Autopsy samples of lungs were taken from the different groups and fixed in 10% formalin prepared in saline. Washing was done in tap water then serial dilutions of alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slide microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin (H&E) stains for histopathological examination using the electric light microscope. This is according to the method previously described by Bancroft and Gamble³³.

Immunohistochemical Reaction of NF- κ B

Sections from lung tissue of around 3 μ m thickness embedded in paraffin were used for detection of NF- κ B through the immunostaining with primary antibody polyclonal immunoglobulin-G of mice NF- κ B according the method previously described³⁴. Finally, grading of immunohistochemical reactivity was measured from 4 randomly chosen fields in each section and averaged using image analysis software (Image J, Fiji version; MD, USA).

Statistical Analysis

Data are presented in the form of mean \pm SEM. The comparisons among means of different groups were done via one-way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons posttest³⁵. Kruskal-Wallis test was used for analyzing the histopathological scores and followed by Dunn's multiple comparisons test. The level of significance was taken as $p < 0.05$. All the statistical tests carried out using GraphPad Prism software package, version 5 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Effect of Xanthenone on Pulmonary Surfactant Protein Content (SP-A) and MiR-200 Expression

The results revealed a significant decrease in SP-A by 69.2% upon LPS injection. On the other hand, treatment with xanthenone significantly increased pulmonary SP-A level by 2.3 folds compared to the ARDS control group (Figure 1).

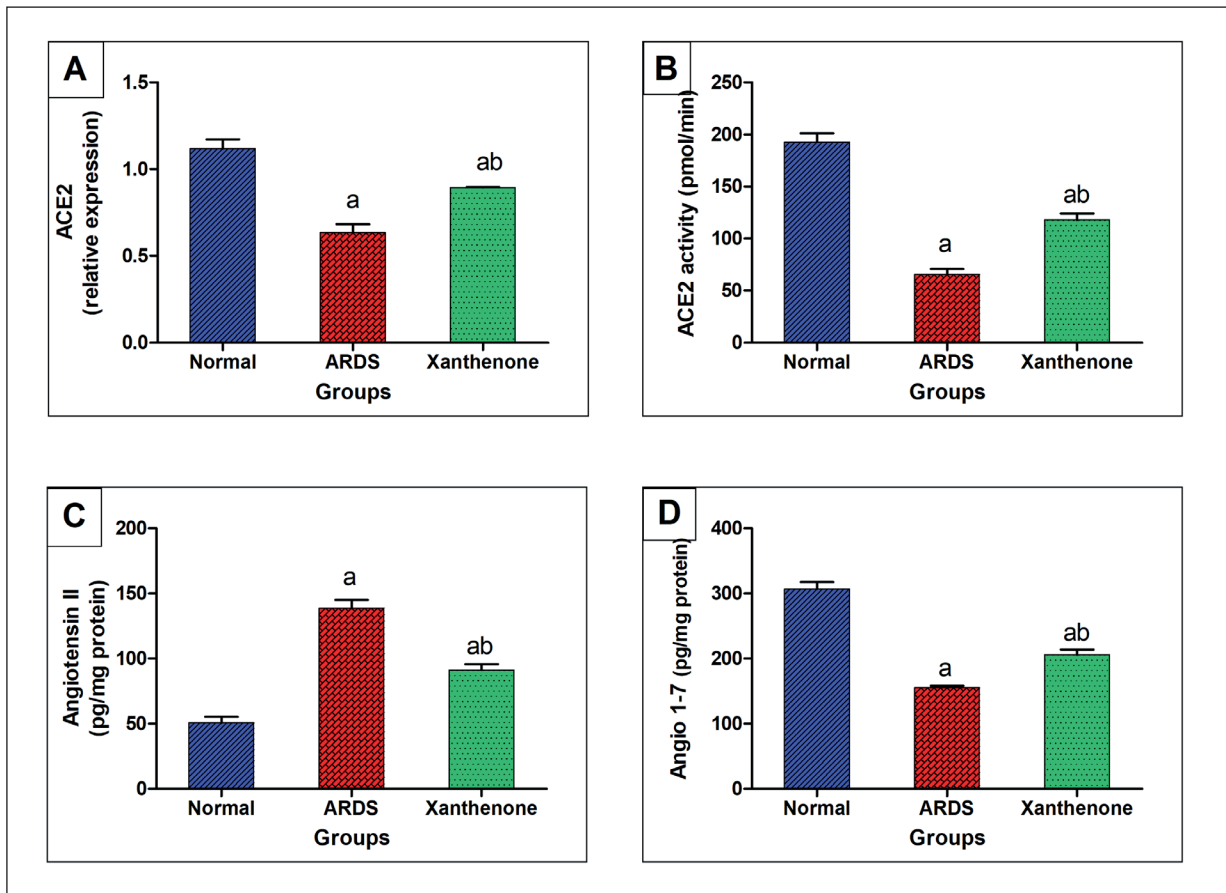


Figure 2. Effect of xanthenone on the relative expression and enzyme activity of angiotensin converting enzyme 2 (ACE2) as well as the pulmonary contents of angiotensin II and angiotensin (1-7) in mice with ARDS. The data are presented as mean \pm SEM (n = 6). **A**, significant difference from normal group and **B**, significant difference from ARDS control group (at $p < 0.05$).

Lungs from ARDS control showed a significant elevation in miR-200 expression by 3.8 folds compared to normal lungs, while xanthenone treatment significantly decreased miR-200 expression by 55.47% compared to ARDS control group.

Effect of Xanthenone on Angiotensin converting Enzyme 2 (ACE2), Angiotensin II and Angiotensin (1-7)

A significant decrease in the pulmonary expression level of ACE2 by 43.2% was observed in the ARDS control group compared to the normal group. Conversely, treatment with xanthenone significantly elevated the pulmonary expression level of ACE2 by 40.9% compared to the ARDS control mice. Moreover, the mice with ARDS exhibited a significantly suppressed ACE2 activity by 66.1% compared to the normal group. On the other hand, ACE2 activity

significantly increased in the xanthenone treated mice by 1.8-folds compared to ARDS control group (Figure 2). This effect was accompanied by a significant elevation in the pulmonary content of angiotensin II by 2.7-folds and a marked suppression the pulmonary content of angiotensin (1-7) by 49.4% compared to normal group. Treatment with xanthenone abolished these effects successfully resulted in suppression of the pulmonary content of angiotensin II by 34.3% and significantly elevated pulmonary level of angiotensin (1-7) with 1.3-folds compared to ARDS control group (Figure 2).

Effect of Xanthenone on the Pulmonary Content of Tumor Necrosis Factor- α (TNF- α)

Results of the current study revealed that the pulmonary TNF- α content was significantly raised by 3.6-folds in the ARDS mice compared

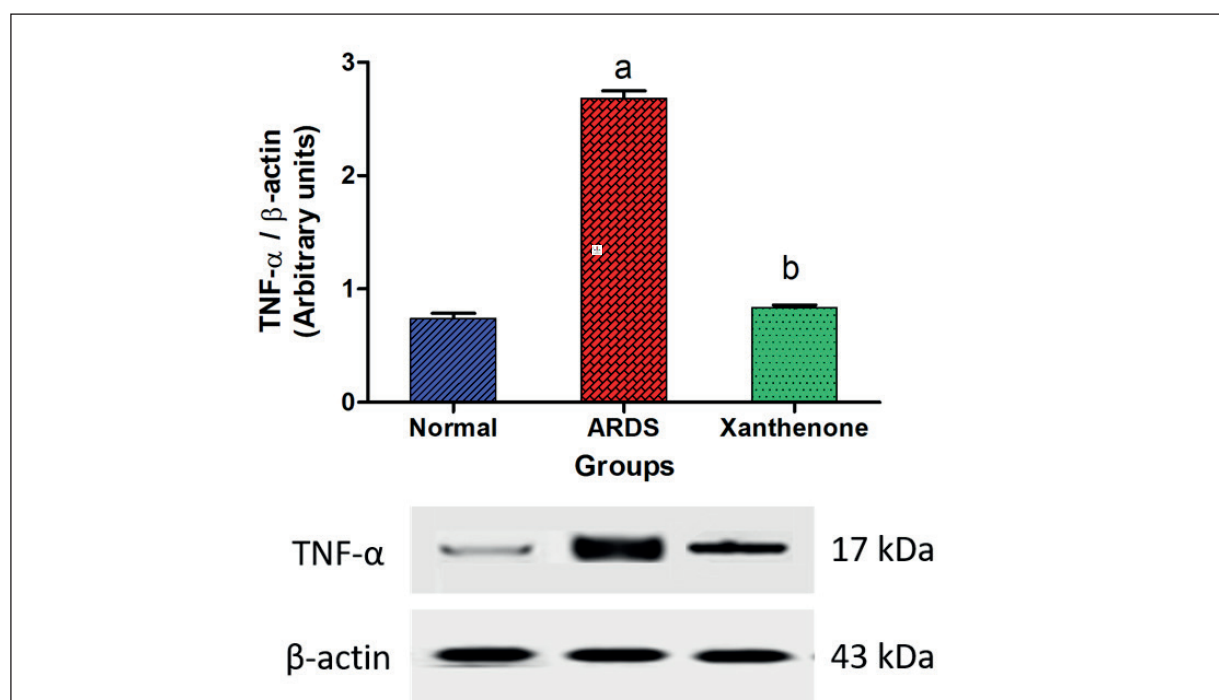


Figure 3. Effect of xanthenone on the pulmonary content of tumor necrosis factor- α (TNF- α) in mice with ARDS. The data are presented as mean \pm SEM (n = 6). **A**, significant difference from normal group and **B**, significant difference from ARDS control group (at $p < 0.05$).

to the normal mice. On the other hand, xanthenone treatment resulted in significant decline in pulmonary TNF- α content by 69.1% compared to the ARDS group (Figure 3).

Effect of Xanthenone on Immunohistochemical Reactivity of Nuclear Factor- κ B (NF- κ B)

The Immuno-staining for NF- κ B showed weak expression in the lung tissue of the normal mice (Figure 4A). The expression of NF- κ B noticeably increased in the lung tissue upon induction of ARDS (Figure 4B). Xanthenone treatment resulted in markedly decreased expression of NF- κ B in lung tissue (Figure 4C) with significant improvement from the induced group. Comparative quantification of the immunohistochemical expression for NF- κ B in lung tissue of mice from all groups is presented in Figure 4D.

Effect of Xanthenone Treatment on Histopathological Alterations of Lungs

Lungs of the normal mice showed the normal histological structure of the bronchioles and the surrounding air alveoli with no histopathological

alteration (Figure 5A). Lung sections from mice injected with LPS showed focal heavy aggregation of inflammatory cells detected in the peribronchiolar tissue with collapsed air alveoli (Figure 5B). Lung sections from the xanthenone-treated mice showed few inflammatory cells infiltration in focal manner with normal histological structure of the bronchioles and surrounding air alveoli as well as blood vessels (Figure 5-C). Scoring histological alterations of lung tissue is presented in Figure 5-D.

Discussion

ARDS is an inflammatory lung disease that has a high rate of morbidity and mortality in hospitals². It is an acute diffusive lung injury caused by the release of pro-inflammatory cytokines into the lungs. As a result, pulmonary vascular permeability increases, and aerated alveoli are lost³. Since the ARDS life-threatening complication associated with COVID-19 is an ongoing challenge, this current study aims to investigate the potential efficacy of xanthenone in the treatment of ARDS induced with LPS in mice through ACE2 activation and

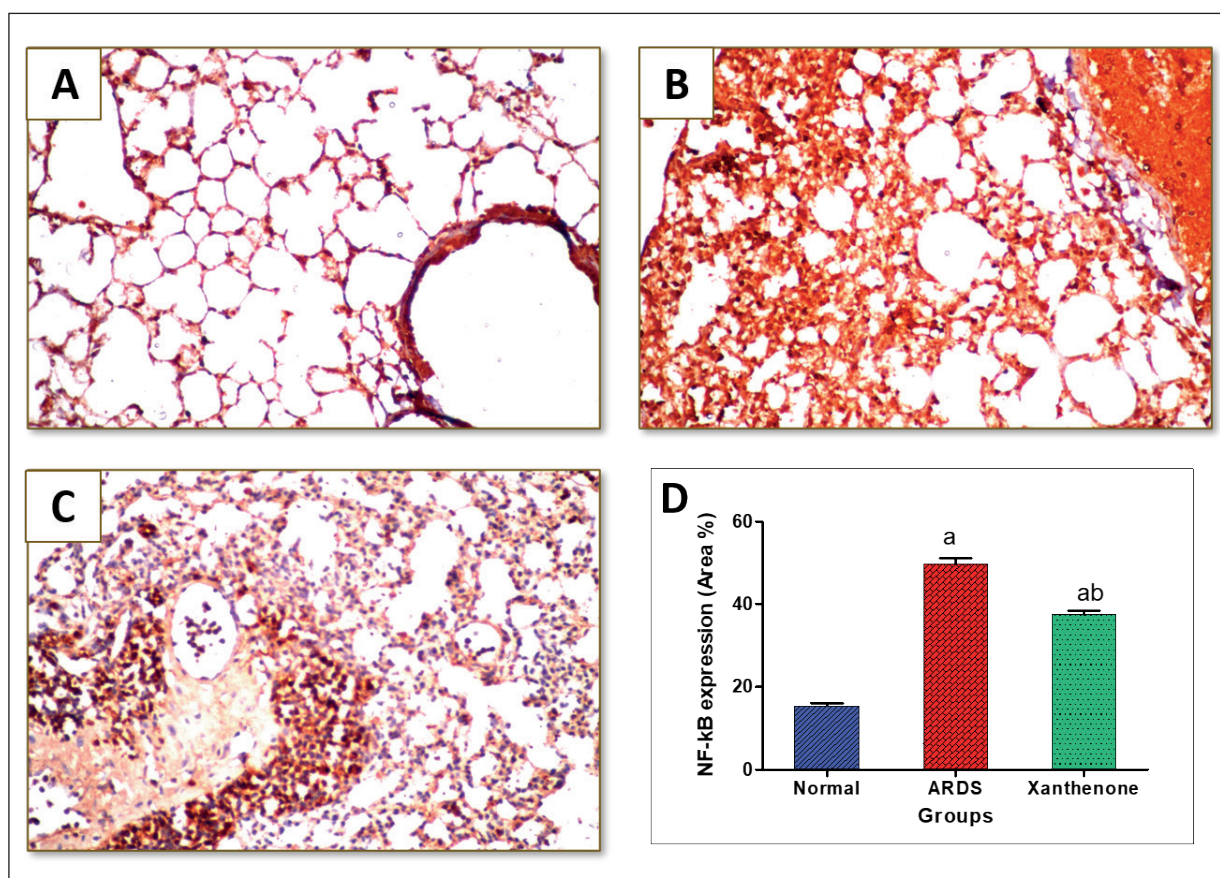


Figure 4. Immunostaining of nuclear factor-kB (NF-kB) in lung tissue of mice with ARDS ($\times 40$). **A**, Normal group, **B** ARDS control group, **C** Xanthenone treated group, **D** represents a comparative quantification of the immunohistochemical expression for NF-kB in lung tissue of mice from all groups. The severity of the immunoactivity is depending on the intensity and distribution of the brown color calculated as area % using image J software. a: represents a significant difference from the normal group, b: represents a significant difference from ARDS control group (at $p < 0.05$).

modulation of miR-200 and ACE2/Angiotensin 1-7 pathways.

In the current study, the LPS induction model has been prioritized as LPS is a part of Gram-negative bacteria outer membrane, and it can cause direct pulmonary injury or extra-pulmonary injury to the airways³⁶. The LPS animal model of ARDS has the ability to reproduce most pathophysiological pathways of human ARDS³⁷. It's well established that LPS mimics human ARDS in rodents and it's become a common method to induce ARDS³⁸. The molecular mechanism by which LPS induces lung injury is as follows, the cellular response to LPS starts when LPS binds to LPS-binding protein, MD-2 and CD14, which makes it easier for LPS to bind to its main receptor, toll-like receptor 4 (TLR-4)³⁹. Intracellular adaptor proteins, such as MyD88 bind to TLR-4 and different intracellular signaling cascades are then activat-

ed⁴⁰. They involve extracellular signal-regulated kinase (ERK1/2) and p38 which are very important in the regulation of the expression of cytokines with pro-inflammatory properties, such as tumor necrosis factor alpha (TNF- α) and Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF- κ B)^{37,39}. The loss of ACE2-mediated effects and RAS homeostasis could be responsible for most of the clinical symptoms reported in patients with COVID 19. Moreover, lung alveoli cells such as type 2 pneumocytes and macrophages express ACE2 and are particularly vulnerable to SARS-CoV-2, that together with the lost ACE2 activity seem to be responsible for the most worrying COVID 19 effects in the respiratory system, causing pneumonia and lung fibrosis²⁸. In the present study xanthenone significantly increased the expression levels of ACE2 in the treated group compared to the induction group, and that results

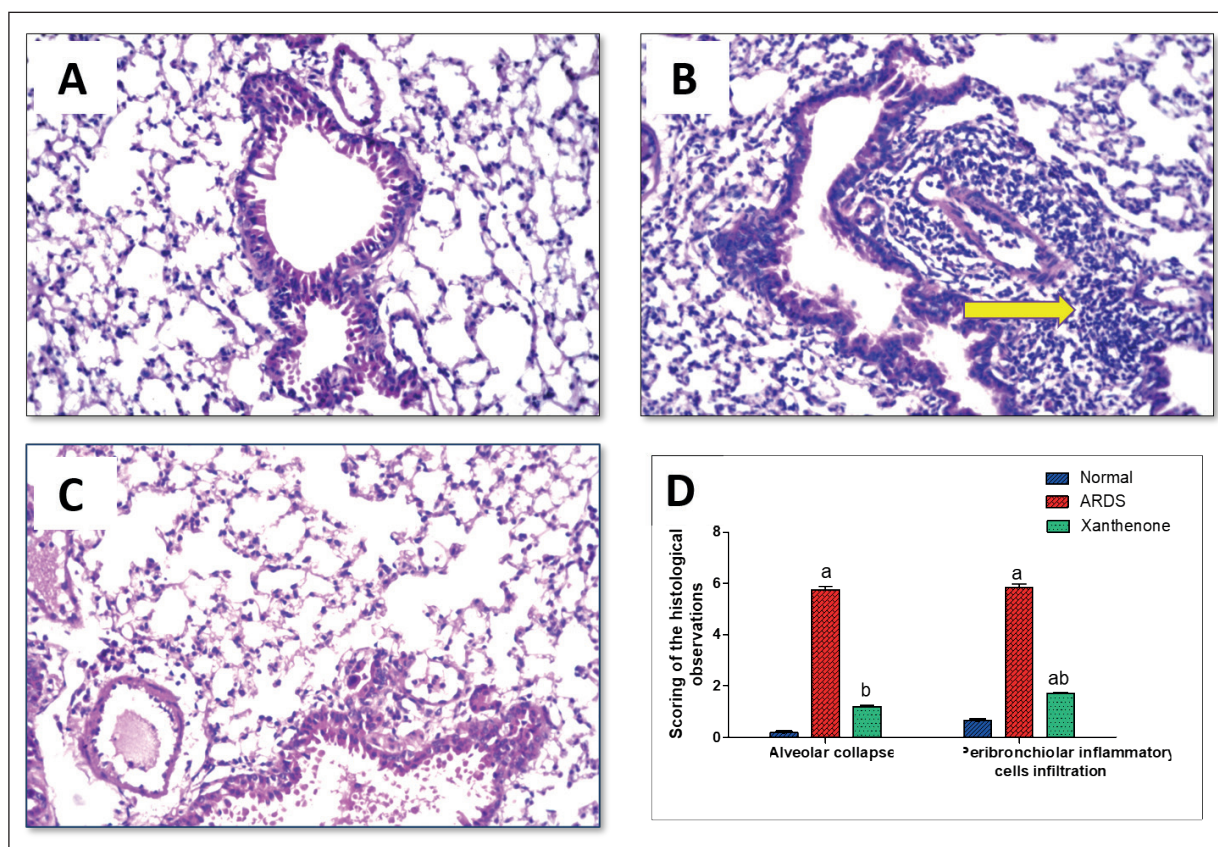


Figure 5. Effect of xanthenone on the histopathological alterations in the lung tissue of mice with ARDS (H&E \times 40). **A**, normal group, **B** ARDS control group, **C** xanthenone-treated group, **D** scoring of the histological observations in the lung tissue from all groups. Data are presented as mean \pm SEM of 6 random non-overlapping fields/section. **a**: significant difference from the normal group, **b**: significant difference from ARDS control group (at $p < 0.05$).

in protection of the lungs against ARDS which agrees with the results of Ibrahim et al²⁷ who reported that xanthenone can be used to prevent leptin-induced hypertension and proteinuria in pregnant Sprague-Dawley rats through ACE2 activation. ACE2 deactivates Ang II by cleaving Ang II into Ang 1-7⁴¹. This cleavage occurs by binding with great affinity to the Ang II type 1 (AT1) and type 2 (AT2) receptor, which mediates blood pressure regulation, fibrosis, cell proliferation and body fluid balance⁴²⁻⁴⁴. More prominently, ACE2 is suggested to have a protective role in respiratory lung injury⁴⁵. Moreover, results of the current study revealed that xanthenone has significantly decreased the expression levels of Ang II in the treatment group compared to the induction group, which results in an improve to the lung injury. Via AT1 and AT2 receptors, Ang II has some biological effects such as, promotion of lung proliferation, speeding up apoptosis induced by fas in alveolar epithelial cells and induction of

both vascular permeability and vascular permeability which eventually results in ARDS⁴⁶⁻⁴⁸. The result of the current study is broadly in line with Abdel-Fattah et al²⁴ who reported that xanthenone counteracted nephrotoxicity induced by gentamicin in rats through the Ang II/ACE2/Ang 1-7 axis. Ang 1-7 basically antagonizes the actions of Ang II particularly proliferation, inflammation and vasoconstriction^{49,50}. Furthermore, it inhibits pro-inflammatory functions by the activation of its Mas receptor⁵¹. In addition, other studies have showed that Ang 1-7 can work on the development of fibrosis and lung remodeling, it also improves the apoptosis of fibrocytes and decreases collagen deposition and the expression levels of transforming growth factors- β (TGF- β)⁵²⁻⁵⁶. The current results showed that xanthenone significantly increased the expression level of Ang 1-7 in the treatment group compared to the induction group. These findings are consistent with the study that revealed the antihypertensive properties of xan-

thenone through ACE2 activation⁵⁷. MiR-200c-3p is a member of the miR-200 family that has major roles in oxidative stress, cancer progression and drug resistance^{58,59}. Furthermore, miR-200c-3p has a critical role in the development of ARDS as it regulates the expression of the mRNA that transcribes ACE2⁶⁰. The results of the present study demonstrated that xanthenone has decreased the expression levels of miR-200 significantly in the treatment group in comparison to the induction group and as a result the mRNA of ACE2 was upregulated and, hence, Ang 1-7 was increased as well, which eventually ameliorates ARDS through ACE2. This result agrees with another study which approved that the inhibition of miR-200c-3p treats ALI/ARDS induced by H5N1 virus infection or LPS *in vivo*²³. Additionally, xanthenone showed a significant increase in SP-A levels in the treated group compared to the induction group which results in lung protection and reduction in the alveolar surface tension. Previous study showed that during the course of ARDS, SP-A is significantly decreased because of the lung injury, which sheds a light on the importance of SP-A as a potential marker for ARDS⁶¹. The necessity of surfactant proteins in general and SP-A in specific in lung physiology is very important as they are responsible for maintaining the functional integrity of the distal airways and decreasing surface tension within the alveoli. So, they reflect alveolar damage when they are downregulated⁶². TNF- α is widely known cytokine that belongs to the TNF super family^{63,64}. It is secreted by macrophages stimulated by LPS⁶⁵. Furthermore, TNF- α downregulates the gene expression of SP-A via the p38 MAPK signal transduction pathway in the lung epithelial cell⁶⁶. One of the earliest manifestations of ARDS is the activation of alveolar and intestinal macrophages which are antigen presenting cells, increase cell surface adhesion molecules and then production of chemokines and cytokines which triggers neutrophils sequestration within the pulmonary microvasculature⁶⁷. These cells then start migrating across the epithelium and endothelium into the alveolar space and subsequently release proinflammatory and cytotoxic compounds such as reactive oxygen species (ROS) and some inflammatory cytokines⁶⁸. This eventually prolongs a vicious cycle by the recruitment of more inflammatory cells which sequentially produce additional cytotoxic mediators that eventually lead to severe injury to the alveoli-capillary membrane and respiratory failure⁶⁷. TNF- α starts appearing in the circulation during the onset of lung injury induced by sepsis or

LPS which implicates these cytokines as potential inflammatory mediators for ARDS⁶⁹. The present results showed that xanthenone significantly decreased the level of TNF- α in the treatment group compared to the induction group which caused reduction in the inflammation in the lungs. The effect of TNF- α in the clinical treatment of ARDS was previously reported; where it was upregulated during the course of ARDS and downregulated upon treatment. This proves the importance of suppressing TNF- α in ARDS treatment and the treatment duration⁷⁰. NF- κ B is an important transcription factor. It was named for its ability to bind with the enhancer of the immunoglobulin kappa light-chain of B cells⁷¹. It's an inducible and inflammatory factor that mediates the inflammatory response by the regulation of the transcription of chemokines, cytokines and adhesion molecules⁷². Moreover, a recent study has showed that the released inflammatory chemokines and cytokines like IL-6, IL-1 β and TNF- α would have a major effect on ARDS progression when the signaling pathway of NF- κ B is activated⁷³. Another study also demonstrates that the activation of NF- κ B initiated by nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor- α (I κ B- α) will accelerate the transcription of like IL-6, IL-1 β and TNF- α ⁷⁴. Results of the current study showed that xanthenone significantly decreased the level of NF- κ B expression in the treatment group compared to the induction group which reduces inflammation and protects the lungs. A previous research has reported that xanthenone downregulates NF- κ B and has an impact on oxidative stress and ACE2/Ang 1-7 signaling through ACE2 upregulation²⁴.

Conclusions

Our investigations have shed new light into the molecular mechanism regarding the potential therapeutic effect of xanthenone against LPS-induced ARDS COVID-19 complication. The current study demonstrates that xanthenone downregulates the expression levels of miR-200, TNF- α , and NF- κ B, and increases the expression levels of ACE2, Ang 1-7, and SP-A. Xanthenone has the potential to be a promising therapeutic drug for the treatment of ARDS COVID-19 complication through activation of ACE2/Ang 1-7 pathways.

Conflict of interest

The authors declare no conflict of interest

Ethical Committee Approval

The study was approved by the Ethical Committee of our Institution of October University for Modern Science and Arts.

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