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Research Article

Sildenafil Blunts Lung Inflammation and Oxidative Stress in a Rat Model of Cholestasis

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Abstract

Background: Cholestasis is a multifaceted disease that influences not only the function of the liver but also affects many other organs. In this context, cholestasis-induced lung injury is a significant clinical complication. Unfortunately, there is no precise therapeutic option against cholestasis-associated lung injury. It has been revealed that oxidative stress and inflammatory response play a role in cholestasis-induced pulmonary damage. Sildenafil is a phosphodiesterase enzyme inhibitor used in the management of erectile dysfunction. Meanwhile, several experiments revealed the effects of sildenafil on oxidative stress and inflammation. This study aimed to evaluate the effect of sildenafil on cholestasis-induced oxidative stress and inflammation in cholestasis-induced lung injury.

Methods: Rats underwent bile duct ligation (BDL) to induce cholestasis. Bronchoalveolar lavage fluid (BALF) levels of inflammatory cells, cytokine, and immunoglobulin were monitored at (3, 7, and 14 days after BDL surgery). Moreover, lung tissue histopathological alterations and biomarkers of oxidative stress were evaluated.

Results: A significant increase in BALF inflammatory cells, TNF-α, and immunoglobulin G (IgG) was evident in BDL animals. Moreover, the infiltration of inflammatory cells, vascular congestion, and hemorrhage were detected in the lung of BDL rats. Increased markers of oxidative stress were also evident in the lung of BDL animals. Sildenafil (10 and 20 mg/kg) significantly blunted inflammatory response, oxidative stress, and histopathological alterations in the lung of cholestatic animals.

Conclusion: The effects of sildenafil on inflammatory response and oxidative stress biomarkers seems to play a crucial role in its protective properties in the lung of cholestatic animals.

Introduction

Cholestasis, the stoppage of bile flow, is a severe clinical complication that primarily influences the liver.[1,2] However, it has been found that other organs, rather the liver, could also be affected by cholestasis.[3-7] Cholestasis-induced lung injury is a severe complication that could lead to lung tissue fibrosis, respiratory complications, and profound hypoxemia.[8-10] Cholestasis-induced lung injury and pulmonary complications could also induce severe damage in newborn infants.[11] Unfortunately, there is no specific pharmacological intervention against cholestasis-induced pulmonary complications so far. Hence, finding novel therapeutic options against this complication could have tremendous clinical value.

The precise mechanism of cholestasis-induced lung injury is not clear. However, some studies mentioned the pivotal role of oxidative stress and its linked events in the pathogenesis of cholestasis-induced lung injury.[8,9,12] Our research team also recently revealed that significant histopathological changes and oxidative stress occur in cholestatic/cirrhotic animals.[12] It has been mentioned that the accumulation of cytotoxic molecules such as hydrophobic bile acids is involved in the mechanism of oxidative stress and lung injury during cholestasis.[11] On

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the other hand, the inflammatory response also seems to play a pivotal role in the mechanism of cholestasis-induced lung injury.[11,13-15] Accumulation of inflammatory cells and releasing cytokines could play a pathogenic role in the mechanism of cholestasis-associated lung damage.[13,14] It is also well-known that inflammation and oxidative stress are two related events.[16,17] Inflammatory cells such as neutrophils are significant reactive oxygen species (ROS).[18]

Sildenafil is a phosphodiesterase type 5 (PDE-5) inhibitor administered to manage erectile dysfunction and pulmonary arterial hypertension.[19] In addition to its common pharmacological effects, it has been found that PDE-5 inhibitors such as sildenafil could affect other organs such as the liver, kidney, lung, and central nervous system (CNS).[19,20] Several studies indicate that the effects of PDE-5 inhibitors on oxidative stress and its linked adverse events play a crucial role in their organ protective properties.[21,22] PDE-5 inhibitors significantly decreased ROS formation, lipid peroxidation, and impaired tissue antioxidant capacity in various experimental models.[21,22] On the other hand, it has also been reported that PDE-5 inhibitors could significantly suppress inflammation and the release of inflammatory cytokines in different organs.[23,24] The effects of sildenafil on the respiratory system are one of the most interesting pharmacological effects of this drug.[25-28] Clinically, sildenafil is widely used for disorders such as pulmonary arterial hypertension.[25] It has been seen that sildenafil could blunt oxidative stress and its consequences in the pulmonary artery hypertension model.[29] Moreover, the effects of sildenafil on the immune system and inflammatory response have been studied in several experimental models.[30] Hence, sildenafil was evaluated for its potential anti-inflammatory and antioxidant properties in the lung of cholestatic animals in the current study.

As oxidative stress and inflammation play a significant role in the pathogenesis of cholestasis-induced lung injury, it is expected that sildenafil could play a positive role against this complication. The data obtained from this study could provide new insight into the therapeutic strategies against cholestasis-induced pulmonary damage.

Materials and Methods

Chemicals and reagents

4,2Hydroxyethyl,1-piperazine ethane sulfonic acid (HEPES), hexadecyl-trimethyl-ammonium bromide (HTAB), reduced glutathione (GSH), 2′,7′-dichlorofluorescein diacetate, hydrogen peroxide, 2,4,6-tripyridyl-s-triazine, methanol, sildenafil citrate, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma (St. Louis, MO, USA). Trichloroacetic acids, *m*-phosphoric acid, O-dianisidine hydrochloride, potassium chloride, sodium chloride, hydrochloric acid, ferric chloride, and hydroxymethyl aminomethane hydrochloride (Tris-HCl) were purchased from Merck (Merck KGaA, Darmstadt, Germany). Kits for evaluating serum biochemistry were obtained from ParsAzmoon® (Tehran, Iran). Kits for assessing immunoglobulin and cytokine in BALF were purchased from Shanghai Jianglai Biology® (China). BALF level of bile acids was analyzed by an EnzyFluo™ bile acids assay kit (BioAssay® Systems, USA).

Animals

Male Sprague-Dawley rats (n = 36, 250 ± 20 g) were obtained from Shiraz University of Medical Sciences, Shiraz, Iran. Animals were maintained in a standard environment (12 h photo schedule, ≈ 45±5% relative humidity, and temperature 24±1°C) with free access to tap water and a regular rat diet (RoyanFeed®, Isfahan, Iran). The ethics committee of experimental animal care and use at Shiraz University of Medical Sciences approved the current study (Approval code: IR.SUMS.REC.1399.1344).

Bile duct ligation surgery

Animals were randomly allotted into sham-operated and bile duct ligated (BDL) groups. Briefly, animals were anesthetized (8 mg/kg of xylazine and 70 mg/kg of ketamine, i.p), and a midline incision (≈ 2 cm) was made through the *linea alba*. The common bile duct was recognized and doubly ligated using a silk suture (# 04).[31-34] The sham operation involved laparotomy without bile duct ligation.

The validation of lung injury in BDL rats

Sham-operated and BDL rats (n = 6/group) were monitored at scheduled time intervals (3, 7, and 14 days after surgery).[12,33-40] The BDL model of cholestasis is an appropriate experimental tool for assessing the mechanisms involved in the pathogenesis of cholestasis-induced lung injury and evaluating pharmacological interventions in this complication.[8,15] In the current study, we found that all lung inflammation and fibrosis markers were significantly increased 14 days after BDL surgery. Therefore, in another round of experiments the following groups were added: BDL + Sildenafil (10 mg/kg/day, i.p, 14 consecutive days) and BDL + Sildenafil (20 mg/kg/day, i.p, 14 consecutive days). The sildenafil doses applied in the current study were selected based on the relevant literature in this field.[41] The time frame for evaluating tissue injury after BDL surgery was also selected based on previous studies, including our research on the BDL model of cholestasis.[12,33,35-40,42]

Broncho-alveolar lavage fluid (BALF) preparation

Animals were anesthetized using thiopental (80 mg/kg, i.p). Animals were placed in a dorsal position, and the trachea was exposed and cannulated using a 20 G catheter. The catheter was stabilized with a cotton thread. Then, 1 ml of ice-cooled saline-EDTA (2.6 mM EDTA in normal saline; 0.9% w:v NaCl) was injected into the lung, and the chest was gently massaged (10 sec).[43] The solution was re-aspirated and kept on ice. This procedure was repeated (5 times/animal and 1 ml each time). Then, the pooled lavage preparations were centrifuged (5 minutes, 300 *g*, 4 °C) to pellet cells. The supernatant was collected to analyze

TNF-α, IgG, bilirubin, and bile acids.[43,44] Then, 500 µL KCl (0.6 M) and 1.5 ml of ultrapure water were added to the cell pellet for erythrocyte lysis (10 sec). Samples were homogenized by inverting and centrifuged (5 min, 300 *g*, 4 °C). Finally, the supernatant was discarded, 1 ml of saline-EDTA was added to the cell pellet, and homogenized by inverting. The cell suspension was kept at 4 °C and used for cellular analysis.[43]

Serum biochemical measurements and BALF cellular analysis

Blood samples (5 ml) were obtained from the abdominal aorta, transported to serum preparation tubes (Improvacuter®; heparin-coated tubes), and centrifuged (4000 *g*, 15 min, 4 °C). Commercial kits (Pars-Azmoon®, Tehran, Iran) and a Mindray BS-200® autoanalyzer (Guangzhou, China) were employed to assess serum gamma-glutamyl transpeptidase (γ-GT), total bilirubin, alkaline phosphatase (ALP) alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST). Kits for assessing IgG and TNF-α in BALF were purchased from Shanghai Jianglai Biology® (China). BALF level of bile acids was analyzed by an EnzyFluo™ Bile Acids Assay Kit (BioAssay® Systems, USA). BALF total bilirubin was assessed using a Parsazmoon® kit (Tehran, Iran). A Prokan® automatic blood cell counter was used for the differential inflammatory cell count of BALF.

Reactive oxygen species in the lung of BDL rats

The level of reactive oxygen species (ROS) formation in the lung was measured using 2′, 7′dichlorofluorescein diacetate (DCF-DA) as a fluorescent probe.[31] For this purpose, 400 mg of the lung tissue was homogenized in 4 mL of ice-cooled Tris-HCl buffer (40 mM, pH = 7.4). Then, 100 µL of the resulted tissue homogenate was added to 1 ml of Tris-HCl buffer (40 mM, pH = 7.4) containing 10 µM of DCF-DA and incubated in the dark (10 min, 37°C incubator). Finally, the fluorescence intensity was assessed using a FLUOstar Omega® multifunctional fluorimeter (λ_{exc}= 485 nm and λ_{em}= 525 nm).[45]

Lung tissue lipid peroxidation

Lipid peroxidation in the lung tissue was assessed using the thiobarbituric acid reactive substances (TBARS) test. Briefly, 500 µL of the lung tissue homogenate (10% w:v in 40 mM Tris-HCl buffer, pH = 7.4) was treated with 2 mL of TBARS assay reagent (a mixture of 1 mL of thiobarbituric acid 0.375% w:v, 1 mL of 50% w:v of trichloroacetic acid, pH = 2). Samples were vortexed well (1 min) and heated (100 °C water bath, 45 min). Afterward, 2 mL of n-butanol was added, and samples were mixed and centrifuged (10000 *g*, 20 min, 4 °C). Finally, the absorbance of the n-butanol phase was measured (λ = 532 nm, EPOCH® plate reader, USA).[45]

The total antioxidant capacity of the lung tissue

The pulmonary tissue's ferric reducing antioxidant power (FRAP) was measured based on a previously reported procedure. Briefly, a working FRAP mixture was freshly prepared by mixing 10 mL of 300 mmol/L acetate buffer (pH = 3.6) with 1 mL of 10 mmol/L of 2, 4, 6-tripyridyl-s-triazine (dissolved in 40 mmol/L hydrochloric acids), and 1 mL of 20 mmol/L ferric chlorides. Then, 100 µL of tissue homogenate was added to 1.5 mL of FRAP reagent and incubated in a shaker incubator (37 °C, 5 min, protected from light). Finally, the absorbance was measured (λ = 593 nm, EPOCH plate reader, USA).[45]

Lung tissue gluthatione (GSH) content in cholestatic rats

The Ellman's (5, 5-dithio-bis-2-nitrobenzoic acid; DTNB) was used for assessing lung GSH content based on a previously reported protocol. Briefly, 1 mL of the lung tissue homogenate (10% w:v in 40 mM Tris-HCl buffer, 4°C) was added to 1 mL of deionized water (4°C) and 100 µL of trichloroacetic acid (50% w:v). The mixture was vortexed and centrifuged (10000 *g*, 4°C, 20 minutes). Then, the supernatant was mixed with 1 mL of Tris-HCl buffer and 100 µL of 10 mM DTNB solution (dissolved in methanol).[45] The absorbance was measured at λ = 412 nm (EPOCH® plate reader, BioTek®, USA).

Myeloperoxidase (MPO) activity in the lung tissue

The MPO activity was evaluated as an index of inflammatory cell infiltration in the lung tissue of cholestatic animals. Briefly, 100 mg of lung tissue was homogenized in 1 mL of hexadecyl-trimethyl-ammonium bromide (HTAB) solution (0.5% w:v; dissolved in 50 mM KH_2PO_4; pH= 6; 4°C) and centrifuged (4000 *g*, 15 minutes, 4 °C).[46] Then, 100 µL of the supernatant was added to 2.9 mL of 50 mM KH_2PO_4 buffer (pH = 6) containing O-dianisidine hydrochloride (16.7 mg/100 ml) and hydrogen peroxide (0.0005% v:v). After the incubation period (5 minutes, 25°C), the reaction was stopped by adding 100 µl of hydrochloric acid (1.2 M), and the absorbance was measured at λ = 400 nm (EPOCH® plate reader, USA).[46]

Lung tissue histopathology

Lung tissue samples were fixed in 10% v:v buffered formalin solution. Then, samples were fixed in paraffin blocks, and a 5-µm-thick slice of each sample was prepared by a microtome and stained with hematoxylin and eosin (H&E). A pathologist blindly analyzed tissue slides.

Statistical analysis

Data are represented as mean ± SD. Data sets were compared by the one-way analysis of variance (ANOVA) and Tukey's multiple comparison test as the *post hoc*. The analysis of pulmonary histopathological changes was performed by the Kruskal-Wallis, followed by the Mann-Whitney U test. Values of $P < 0.05$ were considered statistically significant.

Results

Monitoring serum levels of liver injury markers, including ALT, AST, LDH, bilirubin, bile acids, ALP, and γ-GT,

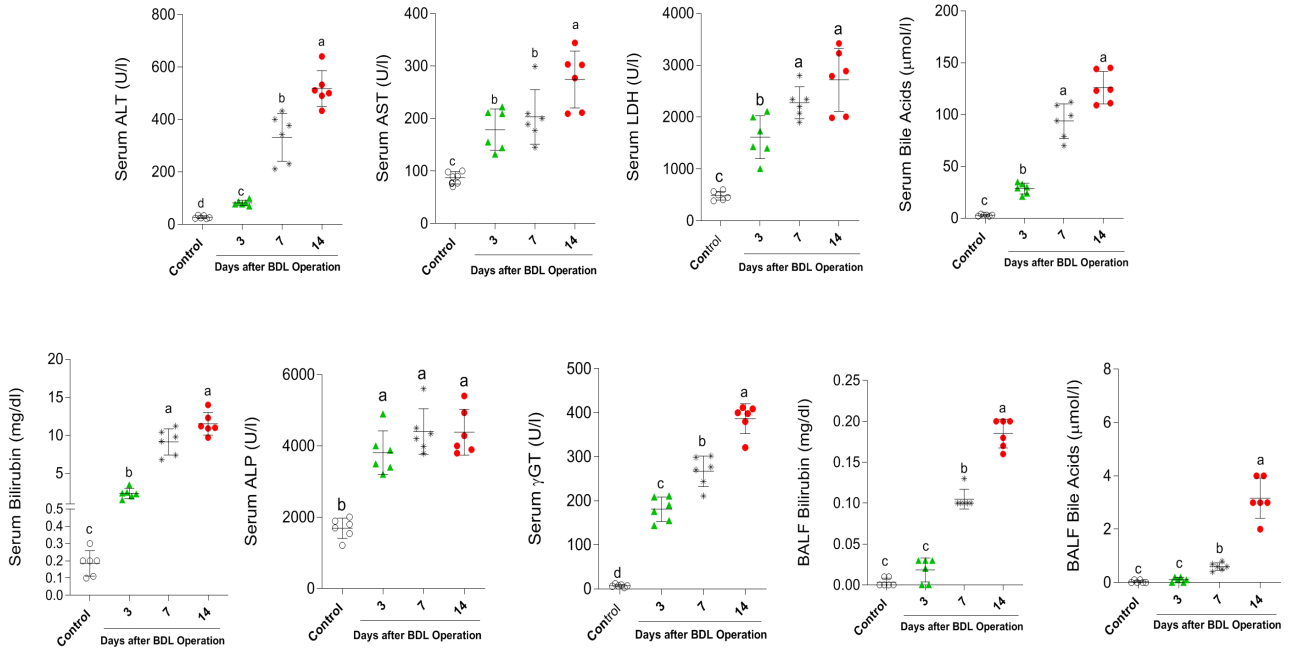

Figure 1. Serum biochemistry and broncho-alveolar fluid (BALF) level of bilirubin and bile acids in bile duct ligated (BDL) rats. Sildenafil treatment significantly decreased serum ALT, AST, and LDH levels in BDL animals (Supplementary file). Sildenafil had no significant effect on serum bile acids, bilirubin, ALP, and γ-GT in the current study (Supplementary file). Data are represented as mean±SD (n = 6). Data sets with different alphabetical superscripts are statistically different (P < 0.05).

indicated the proper induction of cholestasis in BDL animals (Figure 1, P < 0.001). On the other hand, a significant rise in BALF level of bilirubin and bile acids was also detected in cholestatic rats (P < 0.001, 7 and 14 days after BDL surgery) (Figure 1). It should be mentioned that sildenafil (10 and 20 mg/kg) significantly decreased serum ALT, AST, and LDH (Supplementary file). Meanwhile, sildenafil had no significant effect on serum ALP, γ-GT, bilirubin, and bile acids in BDL rats (Supplementary file).

BALF levels of IgG and TNF-α significantly increased in BDL rats (Figure 2). It was found that sildenafil (10 and 20 mg/kg, i.p, 14 consecutive days) significantly decreased IgG and TNF-α in the BALF of BDL animals (Figure 2, P < 0.05). The effect of sildenafil on TNF- α and IgG was not dose-dependent in the current study (Figure 2).

The level of inflammatory cells was monitored in the BALF of BDL rats (Figure 3). BALF level of lymphocytes, neutrophils, and monocytes was significantly increased on day 14 after BDL surgery (Figure 3, P < 0.01). No significant changes in the BALF level of eosinophils were detected in the current study (Figure 3). BALF level of inflammatory cells also showed no substantial changes on days 3 and 7

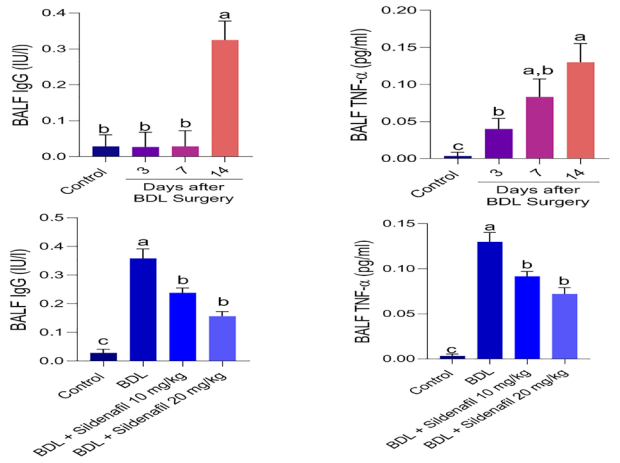

Figure 2. The level of IgG and TNF-α in the broncho-alveolar lavage fluid (BALF) of bile duct ligated (BDL) rats. Sildenafil (10 and 20 mg/kg) significantly decreased BALF levels of IgG and TNF-α. Data are represented as mean±SD (n = 6). Data sets with different alphabetical superscripts are statistically different (P < 0.05).

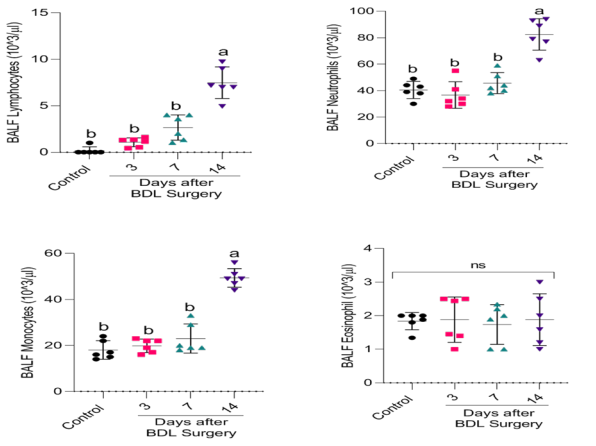

Figure 3. Monitoring the bronchoalveolar lavage fluid (BALF) of bile duct ligated (BDL) rats. Data are represented as mean±SD (n = 6). Data sets with different alphabetical superscripts are statistically different (P < 0.05).

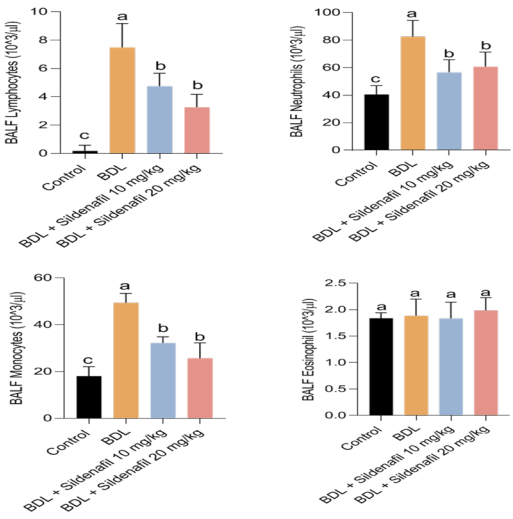

Figure 4. The level of inflammatory cells in the broncho-alveolar lavage fluid (BALF) of bile duct ligated (BDL) rats. Sildenafil significantly decreased inflammatory cells in the BALF of BDL animals. Data are represented as mean±SD (n = 6). Data sets with different alphabetical superscripts are statistically different (P < 0.05).

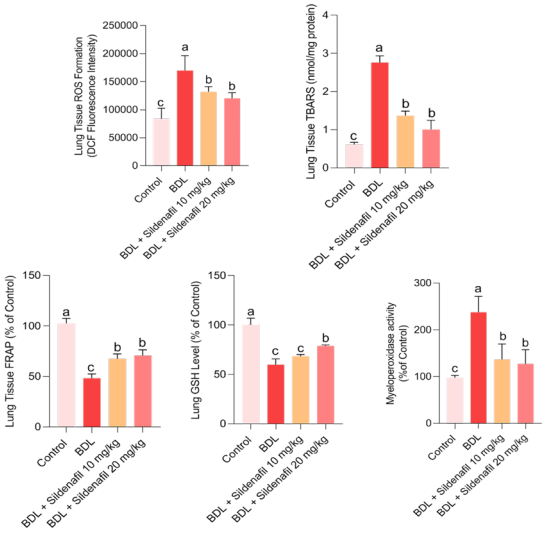

Figure 5. Biomarkers of oxidative stress markers in the lung tissue of bile duct ligated rats (14 days after bile duct ligation; BDL; operation). ROS: Reactive oxygen species; DCF: Di-chloro fluorescein; TBARS: Thiobarbituric acid reactive substances; FRAP: Ferric reducing antioxidant power; GSH: reduced glutathione. Data are represented as mean±SD (n = 6). Data sets with different alphabetical superscripts are statistically different (P < 0.05).

post-BDL operations (Figure 3). The effect of sildenafil (10 and 20 mg/kg) on BALF content of inflammatory cells revealed a significant decrease in these markers in drug-treated cholestatic animals (Figure 4, P < 0.05).

A significant increase in lung tissue level of reactive oxygen species (ROS) and lipid peroxidation was detected in the BDL rats (Figure 5, P < 0.01). Moreover, the current study showed a significant decrease in lung tissue glutathione (GSH) content and total antioxidant capacity (Figure 5, P < 0.01). It was found that sildenafil significantly blunted oxidative stress markers in the lung of BDL rats (Figure 5, P < 0.05). A significant increase in myeloperoxidase (MPO) activity was also detected in the lung of BDL rats (Figure 5, P < 0.01). Meanwhile, it was found that sildenafil decreased MPO activity in the lung tissue of BDL animals (Figure 5, P < 0.05).

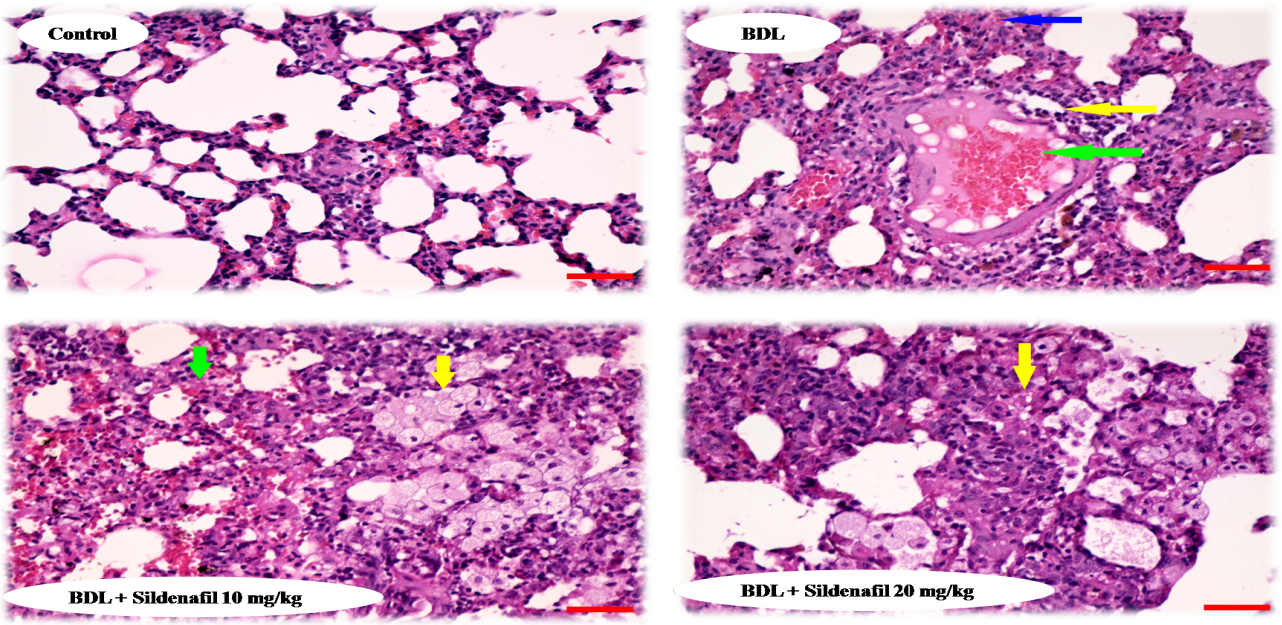

Figure 6. Lung histopathological alterations in cholestatic animals (14 days after bile duct ligation; BDL; surgery). Significant inflammatory cell infiltration (yellow arrow), vascular congestion (green arrow), and hemorrhage (blue arrow) were evident in the lung of BDL rats. Scores of lung histopathological changes and their statistical analysis are represented in Table 1. Scale bar = 100 µm.

Table 1. Pulmonary histopathological changes in bile duct ligated rats.

Treatments	Inflammation	Hemorrhage	Congestion
Control	0 (0, 0)	0 (0, 0)	0 (0, 0)
BDL	2 (2, 2)#	2 (1, 2)#	2 (1, 2)#
BDL + Sildenafil 10 mg/kg	1 (1, 1)a	1 (1,1)a	1 (1, 1)a
BDL + Sildenafil 20 mg/kg	1 (1, 1)a	0 (0, 0)a	0 (0, 0)a

0 = absent; 1 = mild; and 2 = moderate histopathological alterations.
Data are represented as median and quartiles for six random pictures per group (14 days after bile duct ligation; BDL; surgery).
# Indicates significantly different compared to the control group ($P < 0.05$).
a Indicates significantly different from the BDL group ($P < 0.05$).

Significant histopathological changes, including hemorrhage, vascular congestion, and the infiltration of inflammatory cells, were detected in the lung of BDL rats (Figure 6 and Table 1). It was found that sildenafil (10 and 20 mg/kg/day, i.p, for 14 consecutive days) significantly mitigated cholestasis-induced lung tissue histopathological alterations (Figure 6 and Table 1).

Discussion

Cholestasis is a multifaceted clinical complication affecting liver function and other organs such as the kidney, brain, heart, skeletal muscle, and lung.[12,40] Cholestasis-induced lung injury is a serious disorder that could lead to tissue fibrosis and respiratory complications.[8-10] Several studies mentioned the crucial role of oxidative stress, inflammatory response, and their linked adverse events in the pathogenesis of cholestasis-induced lung injury.[8,9,11-14] The current study found that sildenafil significantly mitigated cholestasis-induced lung injury. The effects of sildenafil on oxidative stress biomarkers and inflammation play a major role in its protective mechanisms.

Although the precise mechanisms of lung injury induced by cholestasis are not fully understood, some investigations revealed the pivotal role of inflammatory response and oxidative stress in the pathogenesis of this complication.[8,9,11-14] Therefore, the effects of sildenafil as the protective agent administered against cholestasis-induced lung injury are reviewed on these markers in the following parts.

Several investigations indicate that the effects of PDE-5 inhibitors such as sildenafil and vardenafil on oxidative stress markers play a crucial role in their cytoprotective mechanisms.[21,22] The activation of signaling pathways such as the nuclear factor-erythroid 2 related factor 2 (Nrf2) plays a fundamental role in the antioxidant effect of PDE-5 inhibitors.[21,22] The activation of Nrf2 is a basic mechanism in expressing the cellular antioxidant defense system.[21,41] It has been well-documented that cellular antioxidant systems are impaired in various tissues of cholestatic animals, namely in the liver.[31,39,47-60] In a recent study, we found that the Nrf2 signaling is significantly impaired in the liver and kidney of cholestatic animals.[33] It was found that the pharmacological intervention for activating Nrf2 signaling significantly blunted hepatic and renal injury in cholestasis.[33] As mentioned, oxidative stress plays a crucial role in bile acid-induced lung injury.[8,9,12] Significant ROS formation, lipid peroxidation, glutathione depletion, and decreased tissue antioxidants have been documented in the lung of cholestatic animals.[12] The accumulation of cytoprotective molecules, such as hydrophobic bile acids, seems to play a fundamental role in the occurrence of oxidative stress in the lung tissue during cholestasis.[10] In the current study, we found that sildenafil significantly decreased oxidative stress biomarkers in the lung of cholestatic animals (Figure 5). These data indicate that a significant part of the protective effects of sildenafil is mediated through its impact on blunting oxidative stress. The effects of sildenafil on oxidative stress in the lung of cholestatic animals could be mediated through its direct impact on signaling pathways such as Nrf2. On the other hand, it is well-known that inflammation and oxidative stress are two mechanisticallyinterrelated events.[61] Hence, the indirect effect of sildenafil on the accumulation of inflammatory cells in the lung could also be involved in its effects on oxidative stress markers.

It has been found that the PDE-5 inhibitors significantly suppress the activation of NFkB signaling.[22,62] These drugs have been widely used against inflammatory responses in various experimental models.[22,63] The activation of NFkB induces the expression of multiple genes involved in synthesizing pro-inflammatory cytokines.[38,64] The current study found that the BALF level of cytokines such as TNF-α was significantly decreased by sildenafil (Figure 2). Moreover, sildenafil significantly decreased the infiltration of inflammatory cells into the lung of cholestatic rats (Figure 3). These data align with previous studies indicating the anti-inflammatory role of PDE-5 inhibitors.[22,63] These data prove that a significant part of the protective effects of sildenafil against cholestasis-induced lung injury is mediated through its anti-inflammatory properties. The release of cytokines and inflammation is essential in lung injury pathogenesis in experimental cholestasis models.[15,65] Therefore, further evaluation of the inhibitory effects of sildenafil on various components of the NFkB signaling pathway in the lung of cholestatic animals could enhance our understanding of its precise mechanism of protective action.

The hepatoprotective properties of sildenafil in experimental cholestasis models is an important subject

that should be mentioned here. It has been noted that sildenafil could significantly improve liver function and blunt hepatic histopathological alterations in cholestasis.[21,41,66] The effects of sildenafil on oxidative stress and its associated complications in the liver seem to play a key role in its hepatoprotective properties.[21] Moreover, it has been found that sildenafil significantly suppressed inflammatory response in cholestasis models.[21,41,66] Based on these data, it may be emphasized that the positive effects of sildenafil on other organs (*e.g.*, the lung) could be linked, at least in part, with the role of this drug in improving liver function (Supplementary Data). However, as the bile duct is constantly obstructed in the BDL model, the main route for the excretion of cytotoxic molecules (*e.g.*, bile acids) is blocked. Hence, the tissue level of these molecules is significantly high (Figure 1). As shown in the supplementary file, sildenafil could not decrease the BALF level of bile acids (supplementary file). Hence, a significant part of the protective effects of sildenafil reported in the current model could be related to its direct impact on the lung tissue.

Based on previous studies, the BDL model is an appropriate model to evaluate the adverse effects of cholestasis on various organs.[12,45,67,68] The direct cytotoxic effects of compounds such as hydrophobic bile acids and bilirubin could be readily investigated in the BDL model of cholestasis.[12,45] On the other hand, this model closely resembles cholestatic liver disease in humans.[45] However, the severity of inflammation in various organs, the type of cytokines and growth factors released, and the mechanisms of tissue injury (*e.g.*, the mechanisms of tissue fibrosis) might be different in animal models compared with human subjects. On the other hand, a successful BDL operation and induction of cholestasis with minimal trauma and animals' mortality depends on the technical skills, surgeon expertise, post-operation cares, and available instrumental facilities.[45]

Conclusion

Sildenafil provides significant protective properties against cholestasis-induced lung injury. The role of sildenafil in mitigating oxidative stress and inflammation biomarkers plays a pivotal role in its protective mechanisms. These data provide new insight into the protective properties of sildenafil in the lung tissue of cholestatic animals. Therefore, repurposing sildenafil could offer a novel therapeutic option for cholestasis-induced lung injury in clinical settings.

Ethical Issues

The ethics committee of experimental animal care and use at Shiraz University of Medical Sciences approved the current study (Approval code: IR.SUMS.REC.1399.1344).

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Author Contributions

MMO, RH, and HN were involved in subject conceptualization, funding acquisition, methodology, data analysis, validation, project administration, resources, supervision, writing the original draft and review & editing the final version of the manuscript. NA was involved in data visualization, literature review, data analysis, and writing the original manuscript draft. MF, AM, and KM were involved in data collection, literature review, and preparing manuscript draft. All authors read and approved the final version of the manuscript.

Conflict of Interest

Non to be declared.

Supplementary Data

Supplementary data is available on the journal's web site along with the published article.

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