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Protective effects of *Nigella sativa* oil, thymoquinone and dexamethasone on bleomycin-induced lung fibrosis in rats

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Abstract

Pulmonary fibrosis (PF) is a chronic interstitial lung disease with a progressive damage to the air sacs and deposition of collagen fibers in the lung tissue. The study aimed to explore the effects of *Nigella sativa* oil (NSO) or thymoquinone (TQ), alone or in combination with dexamethasone (DEX), on the development of bleomycin (BLM)-induced PF. Forty-two male rats were divided into seven groups: Control (CTRL); BLM, received a single dose of BLM on day 0, intratracheally; all remaining groups received BLM, as well. DEX, received DEX daily, intraperitoneally, 1 day before BLM and continued for 14 days; NSO and TQ groups, received daily NSO and TQ, respectively, 7 days before BLM and continued for 35 days; DEX + TQ, received both DEX and TQ; DEX + NSO, received both DEX and NSO. At the end, lung tissues were used for histopathological and biochemical analyses. BLM significantly increased the severity of fibrosis and inflammation compared to the CTRL. Bleomycin also significantly increased the amount of hydroxyproline, however, decreased most antioxidant enzymes in the lung tissue compared to the other groups. Group TQ + DEX significantly reduced the severity of BLM-induced PF as well as alterations in biochemical parameters, lung weight and O2 saturation. *Nigella sativa* oil slightly reduced BLM-induced PF, however, it caused non-significant hyperemia in lung tissue. Thymoquinone potentiated the effects of DEX on most biochemical and pathological alterations of BLM-induced lung injury much better than NSO. More studies are needed to support the use of NSO and TQ as potential protective agents against PF.

Introduction

Pulmonary fibrosis (PF) is a chronic interstitial lung disease that begins with a progressive injury and inflammation in the air sacs and with the release of oxygen free radicals and pro-inflammatory mediators in the course of the disease. It leads to collagen deposition and fibrosis in the lung tissue and is associated with cough and shortness of breath.[1]

Several mechanisms have been proposed to cause PF including apoptosis in alveolar and bronchial cells, oxidative stress, inflammation and the release of pro-inflammatory factors as well as increasing the production and accumulation of extracellular matrices, especially collagen fibers.[2-4]

In studies conducted to investigate the effects of inducing PF by bleomycin (BLM) in rats, the results have shown that administration of intratracheally (IT) BLM leads to an increase in fibrotic changes, the amount of collagen and malondialdehyde (MDA) as well as a decrease in thiol and glutathione peroxidase (GPx) in rat lung tissue. The BLM increased inflammatory cytokines such as transforming growth factor-beta (TGF-β) and tumor necrosis factor alpha (TNF-α) in the bronchial alveolar fluid.[5]

Black seed plant with the scientific name *Nigella sativa* belongs to the Ranunculaceae family. It is a native plant in Asia and cultivated in Iran, Arabic countries, Europe and North Africa. The seeds of the *N. sativa* plant are used as spice and herbal medicine. It has been used as a traditional medicine for centuries offering natural treatments for a wide range of illnesses such as asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and even the flu.[6-9]

Thymoquinone (TQ) is one of the main active ingredients of *N. sativa* oil (NSO). It is a monoterpene and

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many properties of NSO have been attributed to this compound.[10] *Nigella sativa* and TQ produce a wide range of pharmacological effects including neuroprotective, nephroprotective, cardioprotective, gastroprotective, hepatoprotective and anti-cancer effects.[11] In addition, it has been reported that TQ reduces BLM-induced PF through antioxidant and anti-inflammatory effects.[12] Thymoquinone also inhibits the cyclooxygenase and 5-lipooxygenase pathways of the arachidonic acid metabolism including the formation of thromboxane B2 and leukotriene B4.[13]

Dexamethasone (DEX) is a commonly used synthetic corticosteroid that inhibits inflammation. It has been demonstrated that DEX could reduce collagen deposition and improve PF in animal models by IT injection of BLM. It has also demonstrated anti-inflammatory and anti-fibrotic effects by preventing the generation of inflammatory mediators, including TNF-α and interleukin-1β.[14-17]

Since the use of DEX can make some problems in patients due to its numerous side effects, research to find an effective combination that can simultaneously reduce the dose of DEX and enhance its effectiveness is crucial in order to improve the quality of life in PF patients.

Considering the importance of PF and the spread of Coronavirus disease 2019 (COVID-19) disease and its complications, including the occurrence of PF in some patients, this study was carried out to investigate the potential of herbal compounds such as NSO and TQ alone or in combination with DEX on prevention of PF induced by BLM in rats. Furthermore, since most pharmacological properties of NSO are associated with its active ingredient, TQ, in this project the effects of TQ and NSO were studied concurrently to investigate the effects of remaining ingredients of NSO on PF.

The present study aimed to evaluate the protective effects of NSO, TQ, DEX and their combination on the development of PF. Since PF is a chronic disease and after the establishment of the disease, there is no curative treatment for it, therefore, all of these compounds were used as preventative drugs. Therefore, the protective effects of NSO and TQ in comparison with DEX on lung fibrosis as well as any potentiation or synergy of these herbal medicines to the effects of the standard drug, DEX, were investigated.

Materials and Methods

Chemicals. Thymoquinone (2-isopropyl-5-methyl-1, 4-benzoquinone) was purchased from Sigma Aldrich (St. Louis, USA). Bleomycin sulfate was purchased from 13-Aban Pharmacy, Tehran, Iran. *Nigella sativa* oil was provided by Barij Essence Pharmaceutical Co Kashan, Iran. Dexamethasone was purchased from Iran Hormone Pharmaceutical Co Karaj, Iran.

Animals. Forty-two male Wistar rats, 8 - 12 weeks old and 200 - 250 g in weight, were used in seven experimental groups through random selection after a week of adaptation period. Rats were kept in metal cages under controlled environment conditions (25.00 ± 3.00 °C, 55.00 ± 5.00 % relative humidity, and 12:12 hr light/dark cycle). The protocol of this study was approved by the institutional Ethics Committee, Faculty of Vet Med, University of Tehran, Tehran, Iran (IR.UT.VETMED.REC.1402.038).

Induction of lung fibrosis. The animals were anesthetized by intraperitoneal injection of 90.00 mg kg^{-1} ketamine (Alfasan, Woerden, The Netherlands) and 7.00 mg kg^{-1} xylazine (Interchemie Co., Venray, The Netherlands). The PF model was performed with a single dose of 5.00 mg kg^{-1} BLM sulfate (Pfizer, Perth, Australia) dissolved in normal saline through IT administration as described below.[18]

Pilot study. A noninvasive laryngoscopic method was used to inject BLM into the trachea. Visualization of larynx was achieved using a modified human otoscope.[19] Initially, methylene blue solution (0.20 - 0.50 mL) was injected into the trachea using a sterilized gavage needle to develop the method and to ensure that the injected solution entered the trachea, not into the esophagus. Then, the established method was used for IT injection of BLM or normal saline in rats.

Experimental groups. Forty-two male Wistar rats were randomly divided into seven groups (n = 6) as follows: A) Control (CTRL) Group: They received normal saline during the test period when other groups were treated with different drugs. B) BLM Group: They received a single dose of BLM sulfate (5.00 mg kg^{-1}, BW) dissolved in normal saline by IT route. C) DEX Group: They received DEX (1.00 mg kg^{-1}, daily) by IP injection 1 day before BLM injection and continued the 13th day later. D) NSO Group: They received daily NSO (800 mg kg^{-1}), 7 days before BLM injection and continued to 28 days later.[20] E) TQ Group: They received daily TQ (2.50 mg kg^{-1}), 7 days before BLM injection and continued to 28 days later. F) TQ +DEX Group: They received daily TQ (2.50 mg kg^{-1}), 7 days before BLM injection and continued to 28 days later + received DEX (1.00 mg kg^{-1}, daily, IP), 1 day before BLM injection and continued the 13th day later. G) NSO + DEX Group: They received daily NSO (800 mg kg^{-1}), 7 days before BLM injection and continued to 28 days later + received DEX (1.00 mg kg^{-1}, daily, IP), 1 day before BLM injection -and continued the 13th day later.[20-23]

Sample collection. At the end of the experiment (day 35), the rats were euthanized by IP injection of a conventional dose of ketamine + xylazine, and cervical dislocation. The lungs were promptly removed and wet lung weights were measured and then the lungs were divided into two halves. In order to evaluate PF, the right lobe of the lung was kept in 10.00% formalin for histo-

pathological examination using a light microscope (KF2; Carl Zeiss, Oberkochen, Germany). The other lobe was frozen to measure the amount of hydroxyproline (HYP) and MDA as well as the activities of oxidative stress enzymes including catalase (CAT), GPx and superoxide dismutase (SOD).

Hydroxyproline assay. The HYP content in the lung was determined by the spectrophotometric method according to the HYP assay kit instruction (Kiazist, Hamedan, Iran). The data were expressed as µg of HYP *per* g of wet lung tissue weight.

Assay of oxidative stress enzymes. The lung homogenates were centrifuged at 12,000 rpm for 30min at 4.00 ˚C. The levels of SOD, GPx, CAT (Navand Salamat, Urmia, Iran) and MDA (Teb Pazhouhan Razi, Tehran, Iran) in lung tissue were measured according to the instructions of the diagnostic kits.

Histopathological analysis. Lung specimens were embedded in paraffin blocks and after preparation of 3.00 µm sections, they were stained with Hematoxylin and Eosin and Masson's trichrome (Polysciences, Warrington, USA).[24] To evaluate the severity of inflammation and fibrosis in lung interstitial tissue, the following scoring system (from zero to six) was used: Grade 0 represented normal lung tissue and the severity of inflammation or fibrosis increased from 1 to 6.

Measurement of body weight. During the study, the body weight of rats was measured every 7 days and the body weight alterations in each group was compared.

Assessment of blood oxygen saturation level. Blood oxygen level was estimated using a vital sign monitoring device before taking lung tissue samples from rats at the Department of Surgery and Radiology, Small Animal Hospital, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Statistical analysis. The data were analyzed using SigmaPlot software (14.0.0124; systat Software Inc., San Jose, USA). Depending on the type of data, statistical analysis methods such as Student's t-test, analysis of variance, and Tukey's test were used. The $p < 0.05$ was considered statistically significant. Non-parametric statistical analysis was done for histopathological data using the Kruskal-Wallis one-way analysis of variance on ranks, with multiple comparisons using Dunn's method.

Results

Biochemical findings. The HYP and MDA contents as well as the activities of CAT, SOD and GPx in the lung tissues of the experimental groups are shown in Table 1. The lung HYP content, as an indicator of collagen deposition, was increased significantly ($p < 0.01$) in BLM group compared to the other groups. However, in the NSO + DEX group, the amount of HYP showed a significant decrease compared to the DEX or NSO groups. Moreover, the TQ + DEX group decreased HYP content significantly compared to the DEX or TQ groups ($p < 0.05$). The amount of MDA in the lung tissue demonstrated a significant increase ($p < 0.05$) in the BLM group compared to the other groups except for the TQ group. The activity of CAT in the lung tissue indicated a significant decrease ($p < 0.05$) in the BLM group compared to the other groups. The activity of SOD in the lung tissue showed a significant decrease ($p < 0.001$) in the BLM group compared to the CTRL, DEX + NSO and DEX + TQ groups. The activity of GPx in the lung tissue was decreased significantly ($p < 0.05$) in the BLM group compared to CTRL, DEX + NSO, DEX + TQ and TQ groups, and the activity of this enzyme in DEX and NSO groups showed no significant difference ($p > 0.05$) with the BLM group (Table 1).

Body weight changes. The BW of rats was significantly decreased seven days after BLM treatment and increased gradually after that in BLM group as well as other BLM-treated groups except for NSO group. It was noted that on day 0, all groups were in the same BW range, however, on days 7, 14, and 21 there were significant weight losses in all groups compared to CTRL except for NSO group. In addition, there were significant differences between the BW values of TQ group on days 14 and 21 compared to the BLM group. On day 28, there was a significant difference between CTRL and all other groups, however, the BW values of NSO and TQ groups were significantly higher than those of the BLM group ($p < 0.05$). In addition, treatment with DEX alone or in combination with TQ and NSO did not change BLM-induced weight loss.

Blood oxygen saturation and wet lung weight. BLM treatment resulted in significantly decreased blood oxygen saturation and increased wet lung weight compared to

Table 1. Biochemical profile in all experimental groups.

Groups	HYP (µg mg^{-1} protein)	MDA (µmol mg^{-1} protein)	CAT (nmol mg^{-1} protein)	SOD (U mg^{-1} protein)	GPx (mU mg^{-1} protein)
CTRL	102.7 ± 20.62^{c}	0.741 ± 0.191^{d}	47.60 ± 5.67^{a}	538.30 ± 100.20^{a}	64.50 ± 10.90^{a}
BLM	217.00 ± 29.00^{a}	3.06 ± 0.463^{a}	19.10 ± 3.33^{d}	270.50 ± 42.80^{b}	30.50 ± 4.13^{b}
NSO	165.30 ± 21.06^{b}	2.13 ± 0.757^{bc}	29.80 ± 7.12^{c}	319.80 ± 52.40^{b}	44.50 ±7.92^{ab}
TQ	152.90 ± 8.97^{b}	2.60 ± 0.436^{ab}	30.20 ± 4.05^{bc}	317.40 ±67.10^{b}	50.50 ± 8.12^{a}
DEX	162.00 ± 11.44^{b}	1.80 ± 0.614^{bc}	29.20 ± 3.50^{c}	314.40 ± 52.90^{b}	44.50 ± 7.81^{ab}
NSO + DEX	123.40 ± 16.46^{c}	1.58 ± 0.443^{cd}	35.90 ± 3.45^{bc}	465.10 ± 56.90^{a}	54.00 ± 6.84^{a}
TQ + DEX	120.60 ± 14.11^{c}	1.36 ± 0.228 ^{cd}	38.70 ± 3.86^{b}	551.60 ± 42.40^{a}	60.90 ± 4.50^{a}

CTRL: Control, BLM: Bleomycin, NSO: *Nigella sativa* oil, TQ: Thymoquinone, DEX: Dexamethasone, HYP: Hydroxyproline, MDA: Malondialdehyde CAT: catalase, SOD: Superoxide dismutase, and GPx: Glutathione peroxidase.

$^{a\text{-}d}$ Values with different superscripts within one column differ significantly at $p < 0.05$.

CTRL group values ($p < 0.05$). Treatments with TQ and NSO alone or in combination with DEX significantly prevented BLM effects on blood oxygen saturation and wet lung weight of rats ($p < 0.05$), however, treatment with DEX could not change the effects of BLM ($p > 0.05$).

Histopathological findings. The results of histopathological analysis showed that normal lung alveoli in the CTRL group had no histological changes such as hemorrhage, chronic interstitial inflammation and interstitial PF (Fig. 1 and Table 2). However, all above mentioned pathological changes were observed in the BLM group; in particular, severe interstitial PF including marked loss of alveolar structure and over-proliferation of fibroblasts (Figs. 1A, C, D, E, F, G, and I). The NSO, NSO plus DEX and TQ treatment non-significantly ameliorated the BLM-induced pathological changes in the lungs, whereas, the DEX ($p < 0.05$), TQ plus DEX ($p < 0.01$) significantly improved BLM-induced PF (Table 2). Masson's trichrome staining of lung specimens of rats showed severe changes in lung structure due to BLM with accumulation of collagen fibers (Fig 1I). It depicted the marked deposition of deep blue-stained mature collagen fibers in the BLM group. Collagen depositions in the NSO, DEX, TQ, TQ plus DEX groups were reduced compared to that of the BLM group (Figs. 1H - 1N). Regarding NSO group, it was shown that although NSO treatment made non-significant improvements on most histopathological parameters in this model of BLM-induced PF, it caused non-significantly mild hyperemia in lung tissue. However, NSO significantly restored antioxidant activity such as SOD and CAT in lung tissue, and significantly decreased HYP levels (Tables 1 and 2).

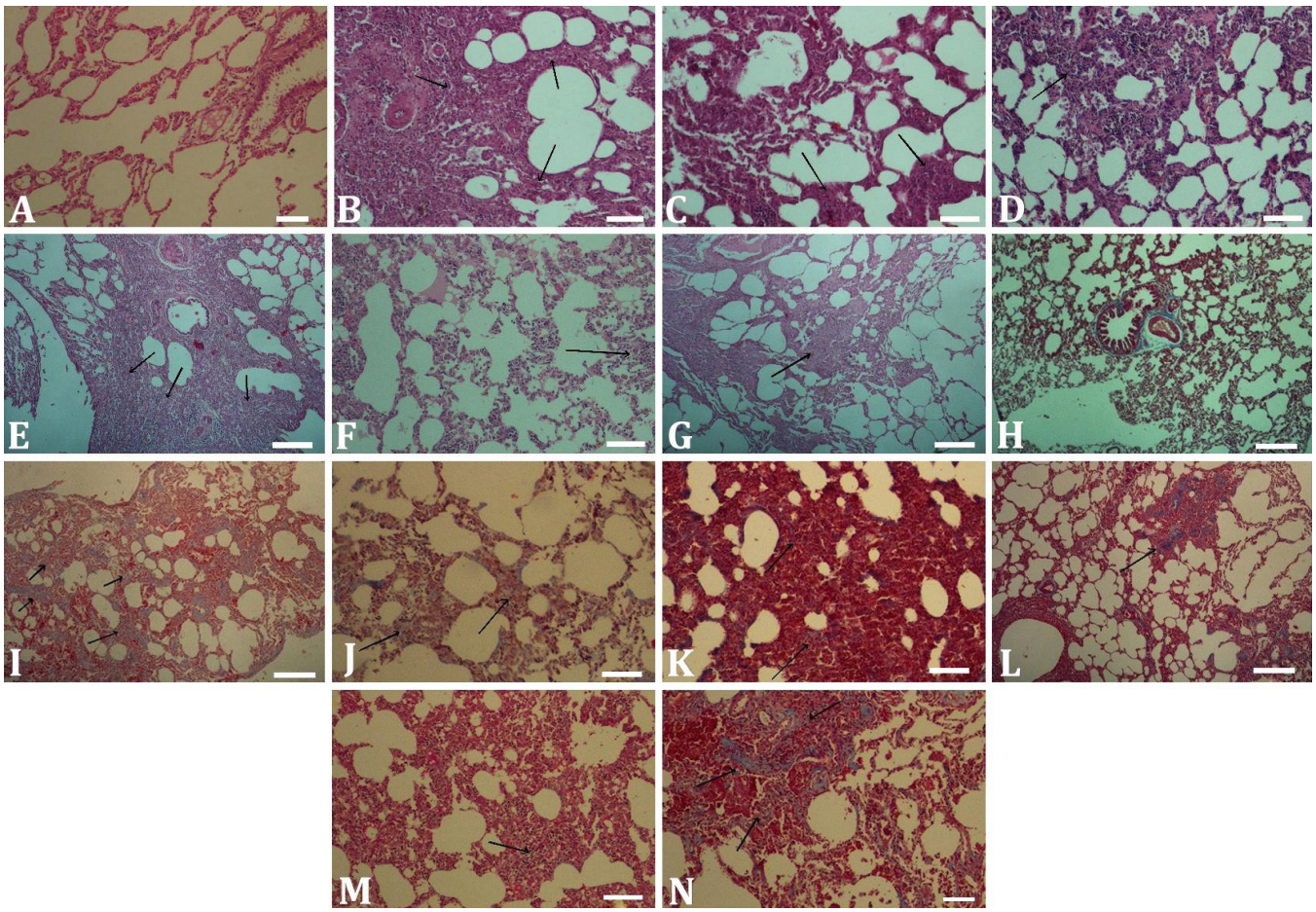

Fig. 1. Hematoxylin and Eosin staining of lung tissues in rats; **A)** Control group: Alveolar and bronchial tissues are normal, **B)** Bleomycin group: Lung tissue with chronic interstitial pneumonia, **C)** *Nigella sativa* oil (NSO) group: Lung tissue with moderate subacute interstitial pneumonia, **D)** Thymoquinone (TQ) group: Lung tissue with diffuse subacute interstitial pneumonia, **E)** Dexamethasone (DEX) group: Lung tissue with multifocal interstitial pneumonia, **F)** The DEX + TQ: Lung tissue with minimal interstitial pneumonia, **G)** The DEX + NSO group: Lung tissue with moderate subacute interstitial pneumonia. Black arrows indicate the interstitial pneumonia (Bar = 100 µm). Masson's trichrome staining of lung tissues in rats; **H)** Control group: Alveolar and bronchial tissues are normal, **I)** Bleomycin: Lung tissue with moderate fibrous tissue formation, **J)** *Nigella sativa* oil (NSO), **K)** Thymoquinone (TQ): Lung tissue with minimal foci of collagen tissue formation, **L)** Dexamethasone (DEX): Lung tissue with multiple small foci of collagen tissue formation, **M)** The DEX + TQ: Lung tissue with minimal foci of collagen tissue formation, and **N)** The DEX + NSO: Lung tissue with mild foci collagen tissue formation. Black arrows indicate the presence of fibrous tissue. (Bars = 100 µm in H, I, K-N, and 200 µm in J).

Table 2. The severity of histopathological lesions recorded in the lung tissues of experimental groups in rats. Data are expressed as median (25.00% - 75.00%).

Groups	Hemorrhage	Hyperemia	Edema	Chronic interstitial inflammation	Bronchitis	Bronchiolitis	Lymphatic follicle	Interstitial pneumonia	Purulent broncho-pneumonia	Emphysema	Atelectasis	Interstitial pulmonary fibrosis
CTRL	0.00	0.00	0.00	0.00	0.00	0.00	3.00	0.00	0.00	0.00	0.00	0.00
BLM	3.00	0.00	0.00	5.00*	3.00	0.00	3.00	3.00	3.00	3.00	3.00	6.00*
DEX	0.00	0.00	0.00	0.00 †	0.00	0.00	0.00	2.00	0.00	0.00	0.00	1.00 [b]
NSO	3.00	5.00	0.00	3.00 [a]	1.50	0.00	0.00	2.00	0.00	3.00	0.00	3.00 [a]
TQ	2.00	2.00	0.00	3.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	2.00
TQ + DEX	1.00	2.00	0.00	0.00	0.00	0.00	2.00	1.50	0.00	0.00	0.00	0.50 †
NSO + DEX	0.00	1.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.50 [a]

CTRL: Control, BLM: Bleomycin, NSO: *Nigella sativa* oil, TQ: Thymoquinone, DEX: Dexamethasone
[a] $p < 0.05$ vs. CTRL, * $p < 0.01$ vs. CTRL, [b] $p < 0.05$ vs. BLM, † $p < 0.01$ vs. BLM.

Discussion

The induction of PF by BLM is one of the common methods used for the evaluation of therapeutic agents and their mechanisms of action.[25] The IT injection of BLM induces pulmonary inflammation and increases fibroblasts in lung parenchyma which in turn causes the production and deposition of collagen fibers in the lung tissue.[26]

In general, two weeks after the IT injection of BLM, the normal alveolar structure is destroyed and collagen is widely substituted which can be detected by histopathological evaluations through staining with Masson's trichrome.[27]

Moreover, HYP content is commonly used to determine the amount of collagen deposition in tissues and is a common indicator to predict the effectiveness of the therapeutic agents in PF.[27]

In the present study, in addition to histopathological analysis of lung tissue and estimation of HYP content, the parameters of oxidative stress such as MDA, CAT, SOD, GPx were also studied to determine the effectiveness of various treatments which started before PF induction by IT injection of BLM up to 28 days later.

The results of this study showed that BLM significantly increased inflammation and fibrosis of the lung parenchyma as well as HYP and MDA levels while these parameters in the groups of DEX and TQ + DEX were associated with significant reductions compared to the BLM group. On the other hand, in these groups the activities of CAT, SOD, and GPx enzymes showed a significant increase compared to the BLM group.

Concerning body weight alterations, BLM resulted in a significant weight loss after 7 days. However, the group that was given NSO did not show any significant weight changes in this time point. Therefore, NSO could prevent weight loss in rats due to BLM injection.

Overall, the biochemical and histopathological findings of the present study confirmed that NSO and TQ could be effective in preventing fibrosis and lung inflammation and potentiate the effects of DEX when used together. It has not been studied how the combination of NSO, TQ and DEX can work together to reduce the inflammation and prevent PF. However, the effects of other compounds with DEX have been studied by Li *et al.*, which aimed to investigate the effectiveness of DEX in combination with berberine on BLM-induced PF. The results of their study showed that berberine enhanced the anti-fibrotic effects of DEX and the combined use of DEX with berberine was more effective than the use of DEX alone.[27]

On the other hand, recently it has been reported that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for causing COVID-19, increases the expression of host cell surface receptors either directly or *via* activating host signaling systems. This can lead to a cytokine storm which can trigger the activation

of host pro-fibrotic pathways. One of the most important benefits of TQ is its ability to block the angiotensin-converting enzyme 2 receptor which is the entry point for SARS-CoV-2. Moreover, TQ can also reduce cytokine release by inhibiting NF-kB which decreases oxidative stress and protects against PF.[21,28]

In the present study, BLM caused a significant increase in lipid peroxides and depletion in GPx, CAT and SOD. The results showed that NSO and TQ significantly reduced oxidative stress by increasing the lung tissue antioxidant enzymes such as CAT, SOD and GPx activities. These treatments also decreased lipid peroxidation which was demonstrated by a significantly reduced level of MDA compared to the BLM group. Our results clearly indicated the protective roles of NSO and its active ingredient, TQ, on lung injury *via* their antioxidant effects through the suppression of lipid peroxidation and enhancement of the antioxidant defense system.

A study conducted by Javadi *et al.*, regarding the protective effects of celery seed extract on PF caused by BLM showed that the compounds in celery seed such as flavonoids effectively reduced MDA and HYP levels and demonstrated protective effects against BLM. In the present study, NSO and its active ingredient TQ also significantly reduced the levels of HYP in lung tissue compared to the BLM group. Although, both NSO and TQ were able to reduce the levels of MDA, however, the reduction was significant just in the NSO group.[29]

Lipid peroxidation is induced by reactive oxygen species (ROS). Cell membranes are sensitive to oxidative damage due to the presence of polyunsaturated fatty acids. The elevated levels of ROS increase cell membrane lipid peroxidation and MDA levels.[30]

Amani *et al*., evaluated the protective effects of some herbal active ingredients including citral, silymarin and TQ against methotrexate-induced lung injury. They reported that TQ had a stronger effect in reducing lipid peroxidation and lung damage compared to the other compounds.[31]

Erboga *et al.*, found that BLM (at 2.50 mg kg^{-1}) caused significant histopathological changes with lung inflammation and fibrosis in 18 male Sprague-Dawley rats over 28 days. However, the infiltration of inflammatory cells and collagen deposition were reduced in the lung of the groups that received black seeds which was consistent with the finding of our study.[32]

The present study demonstrated that the use of NSO in combination with DEX, increased the levels of antioxidant enzymes in lung tissues such as GPx, CAT and SOD. Additionally, the levels of MDA and HYP were significantly reduced in NSO+DEX group in comparison with the BLM group. Experimental studies in animal models showed that BLM induced oxidative stress by production of free radicals which, in turn, can cause fibrotic changes in the lung parenchyma similar to that seen in PF patients.[33,34]

In another study by Samareh-Fekri *et al.*, the effects of methanol extract of fennel on PF caused by BLM was demonstrated. Their results showed a significant decrease in the degree of inflammation and fibrosis in the lung tissue parenchyma on days 14 and 28 compared to the BLM group. Moreover, the effect of the extract of fennel was significantly stronger than the methylprednisolone and the level of CAT enzyme activity in the lung tissue was also increased in the fennel group. In the present study, similar to DEX, the NSO group significantly decreased HYP and MDA and increased CAT enzyme activity in the lung tissue compared to the BLM group.[35]

The results of a research conducted by Almuhayawi, on the protective and therapeutic effects of NSO on the immunosuppressant effect of DEX showed that DEX caused a significant decrease in lymphocytes, leukocytes and the antioxidant enzyme GPx in the blood. However, DEX co-administeration with NSO for two weeks (5.00 mg kg^{-1} NSO, orally and 5.00 mg kg^{-1} DEX, IP) improved these parameters.[7] In this study, NSO was administered orally at a dose of 800 mg kg^{-1} for 35 days, while DEX was given I.P at 1.00 mg kg^{-1} for 14 days. The results of NSO, NSO+DEX, TQ and TQ+DEX groups indicated that the levels of antioxidant enzymes such as GPx, CAT, and SOD in the lung tissue were significantly higher compared to BLM group.

Histopathological findings in the present study demonstrated that BLM induced lung inflammation and PF. The anti-inflammatory mechanism of TQ is not fully known, however, previous studies in a recent study on LPS-induced inflammation showed that TQ decreased the synthesis of pro-inflammatory lipid mediators through cyclooxygenase -1 and 2 inhibitions.[36] Our study also elucidated that chronic interstitial inflammation was significantly reduced in DEX, TQ + DEX, NSO and NSO + DEX groups compared to BLM group.

Furthermore, the present study showed that BLM significantly decreased body weight and increased lung weight in rats. However, TQ, TQ + DEX and NSO groups increased body weight and groups of DEX, NSO, NSO + DEX, TQ and TQ + DEX decreased lung weight compared to BLM group. This was in accordance with the findings of Ahmad *et al.*, that TQ mitigated PF *via* down-regulation of pro-fibrotic cytokines and inhibition of oxidative stress.[12]

Dexamethasone is commonly used as an anti-inflammatory and anti-fibrotic agent in PF because of its preventive effects on generation of pro-inflammatory mediators including TNF-α and interleukin 1β.[14-17,27] In addition, DEX can induce apoptosis of lung inflammatory cells and alleviate acute forms of alveolitis and fibrosis in BLM -induced PF.[37]

In the present study, according to analysis sheet of NSO composition, the approximate amount of TQ in NSO was equal to 6.50 mg *per* 1,000 mg. The dose of TQ was 2.50 mg kg^{-1} but the dose of NSO was 800 mg kg^{-1}, therefore, the TQ equivalent dose that was used in the form of NSO was

about 5.20 mg kg^{-1}. As a result, the TQ and TQ + DEX groups which received about half of the amount of TQ compared to the NSO and NSO + DEX groups were able to increase the GPx, CAT and SOD levels in lung tissue, however, decrease the MDA and HYP levels.

The larger potentiative effects of TQ in combination with DEX may be due to the lack of interference from other pharmacologically active ingredients in NSO which might have negative impacts on the effectiveness of DEX in this experimental PF.

In conclusion, NSO, TQ and DEX when used alone could reduce the severity of PF and oxidative stress induced by BLM. These protective effects were attributed to their anti-inflammatory and anti-fibrotic potentials. Overall, TQ significantly potentiated the effects of DEX on most biochemical and pathological alterations of BLM-induced lung injury. However, more studies are needed to support the use of NSO and TQ alone or in combination with DEX as potential protective agents against PF in clinical settings.

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Conflict of interest

The authors declare there is no conflict of interest.

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