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Carvedilol attenuates experimentally induced silicosis in rats via modulation of P-AKT/mTOR/TGFβ1 signaling

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ABSTRACT

Silicosis is a well acknowledged occupational lung disorder with considerable negative impact on the patients' quality of life. Various signaling pathways have been reported to interplay in the pathogenesis of pulmonary fibro-proliferative disorders; of which, P-AKT/mTOR signaling pathway. The current study highlights the potential pulmonary protective effect of carvedilol; a non-selective α/β blocker against experimental silicosis-induced in rats by the intranasal installation of silica (50 mg/rat, 1 ml 0.9% NaCl). Carvedilol (20 mg/kg, orally) was administered for 8 weeks post intranasal silica installation. Carvedilol significantly attenuated silica-induced pulmonary damage on all the investigated scales. Inflammatory, oxidative/anti-oxidative and fibrotic incidences significantly improved with a significant histopathological restoration of lung architecture and attenuation of inflammatory and fibrotic biomarkers expression. Carvedilol significantly reduced lung contents of P-AKT and mTOR which, appears to be the main mechanism underlying the pulmonary protective effect of carvedilol. In conclusion; carvedilol attenuated silica-induced pulmonary fibrosis by modulating P-AKT/mTOR/TGFβ1 signaling and underlying inflammatory and fibrotic sequel.

1. Introduction

Silicosis is a worldwide occupational lung disorder. It is mainly induced by both long-term exposure to and inhalation of free crystalline silica particles [1]. Even though silicosis has long posed a serious respiratory health problem for probably centuries, it yet remains a chronic disease with no definite cure [2].

The lung is a vital organ whose physiologic function is dependent on that thin alveolar membrane. Continued deposition of the collagen-containing extracellular matrix (ECM) induces collapse of the normal alveoli with the replacement of the underlying parenchymal cells and impairment of gas exchange process [3].

The build-up and accumulation of fibrosing nodular lesions with progressive massive fibrosis and gradual loss of respiratory functions are the main charetrestic histopathological features associating pulmonary fibrosis [2,4]. Initially, the patients are usually asymptomatic except for dyspnea on exertion and later dyspnea develops at rest. With progressive lung damage; pulmonary hypertension, emphysema, and right-sided heart failure eventually supervene [5].

Given the fact that post silica exposure, the particles are engulfed by the alveolar macrophage, a series of pathological events occur including macrophages' activation, inflammatory and lipid mediators' induction, the formation of various reactive oxygen species (ROS) and activation of various fibrogenic cytokines. Such pathological events are mediated mainly via multiple signaling pathways [6].

The phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR)-dependent pathway is amongst the most integral pathways implicated in cellular differentiation, proliferation, metabolism, and survival. Its dysregulation has been associated with a variety of disorders, including neoplasia, immune-mediated diseases, and fibroproliferative disorders [7,8]. Interestingly, the PI3K/Akt pathway is reported to be amongst pathways implicated in myofibroblast differentiation in human lung fibroblasts [9].

Optimization and selection of an effective treatment for pulmonary fibrosis remain a significant challenge given the fact that pulmonary fibrosis is a chronic disorder underlined by multiple pathological mechanisms. Carvedilol; a β-arrestin based non-selective α/β-blocker has been reported to demonstrate antioxidant [10,11], anti-inflammatory and anti-fibrotic properties in experimental models of liver fibrosis [12,13].

The current study was elaborated to assess the modulatory effect of carvedilol on the progression of silicosis in a rat model with emphasis on its modulatory impact on the P-AKT/mTOR signaling pathway and underlying inflammatory and fibrotic axes. Various aspects of carvedilol activity were investigated including its impact on pulmonary inflammatory cells' infiltration, oxidants/antioxidants contents, lung

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transforming growth factor β1 (TGF β1) and hydroxyproline contents, lung nuclear factor κB (NFκB) expression and most importantly, lung P-AKT and mTOR contents.

2. Materials and methods

2.1. Animals

Thirty-six male Sprague Dawley rats (200–250 g) were purchased from the Holding Company for Biological Products and Vaccines, VACSERA (Giza, Egypt). Rats were allowed free water and food access throughout the whole experimental period. "Research Ethics Committee of Faculty of Pharmacy, Mansoura University" approved all the experimental work presented in the current study.

2.2. Drugs and chemicals

Carvedilol was used as Carvid, 6.25 mg tablets (*Multiapex Pharmaceutical industries Co., Badr City, Egypt*). It was suspended in 0.5% w/v carboxymethylcellulose (CMC) for oral administration and was immediately prepared before use. Crystalline silica particles (particle size of 0.5–10 μm) were purchased from Sigma (St. Louis, MO, USA).

2.3. Induction of silicosis and carvedilol administration

The experimental protocol and silicosis induction was in accordance with Hemmati et al., [14]. Silicosis was induced in 24 rats by the intranasal installation of (50 mg/rat, 1 ml 0.9% NaCl) of crystalline silica particles under light thiopental anesthesia (20 mg/kg, IP). Silica was suspended in saline (50 mg/rat, 0.1 ml) and was mixed with 20,000 IU penicillin. Rats in the normal control group (12 rats) received single intranasal instillate of 1 ml of sterile saline (0.9% NaCl) instead of silica suspension.

Twenty four hours post intranasal silica installation; the rats were allocated to three experimental groups; group (1): normal control, group (2): silica control and group (3): carvedilol treated group (12 rats/group).

Rats in the normal and silica control groups were orally gavaged once daily with a 0.2 ml of 0.5% w/v CMC (vehicle) for 8 weeks post-silica installation. The rats in the carvedilol treated group received carvedilol (20 mg/kg/day) [15], orally once daily for 8 weeks post-silica installation. By the end of the experimental period, rats in all the experimental groups were sacrificed using sodium pentobarbital (40 mg/kg).

2.4. Bronchoalveolar lavage fluid (BALF) collection, inflammatory cells quantification, and lactate dehydrogenase (LDH) activity assessment

BALF was collected as describe d by Abdelaziz et al., [16]. For BALF collection, rats were laid on their dorsal sides, the tracheal and thoracic area was shaved and was carefully opened, the tracheas were cannulated and the lungs were infused with sterile saline. The recovered BALF was centrifuged (4000 rpm, 10 min, 4 °C) and the sedimented pellets were re-suspended in 100 μl of saline and processed for differential determination of BALF's neutrophils', lymphocytes', monocytes', eosinophils' and basophils' contents. BALF'S LDH activity was determined kinetically using the commercially available assay kits (HumanGesllschaft fur Biochemica und Diagnostica, Germany) assay kits as instructed by the manufacturer.

2.5. Lung homogenate preparation and quantification of lung total protein, malondialdehyde (MDA), total nitric oxide (NOx) contents, reduced glutathione (GSH) concentration, catalase and myeloperoxidase (MPO) activities

The right lobes from all lungs were excised, rinsed in ice-cold saline and used for the preparation of lung homogenate as described by Abdelaziz et al., [16]. The homogenate was immediately used for quantification of lung MDA and NOx contents, GSH concentration and catalase activity using the commercially available Bio-Diagnostic (Giza, Egypt) assay kits according to the manufacturer's instructions. Lung total protein content was colorimetrically assessed using Thermo Scientific, assay kits (Rockford, USA) according to the manufacturer's instructions. The extent of neutrophils accumulation in the lungs was assessed by determination of MPO activity as described by [17,18].

2.6. Quantification of lung transforming growth factor β1 (TGF β1), protein kinase B (AKT) and mammalian target of rapamycin (mTOR) content

Lung homogenate was used for quantification of lung TGF β1 content using commercially available ELISA kits rat TGFβ1 platinum ELISA assay kits (Bender Med. systems Gmbh, Vienna, Austria). P-AKT and mTOR contents were quantified using ELISA assay kits, DRG International Inc. and LifeSpan Biosciences, Inc. (Seattle, USA) respectively as instructed by the manufacturer.

2.7. Quantification of lung hydroxyproline contents

Lung hydroxyproline and collagen contents were quantified as described by Abdelaziz et al., [16].

2.8. Histopathological examination

The left lobes from all lungs from all rats were harvested, rinsed in ice-cold saline and then immediately fixed in 10% buffered formalin solution for histopathological processing and immunohistochemical analysis. The first set of slides was stained with (H&E) to assess the extent of inflammation, silicosis and pulmonary architecture distortion. The second set was stained with Masson's Trichrome stain for assessment of the extent of pulmonary fibrosis, ECM deposition, and fibrosis score.

2.9. Immunohistochemical analysis of nuclear factor κB (NFκB) expression

For immunohistochemical analysis of NK κB expression, the serial sections were processed and were incubated with anti-NFκB (Thermo Fisher Scientific Inc., MA, USA), 1:100 dilution at 4 °C overnight. Then the slides were incubated with anti-rat IgG secondary antibodies (EnVision + System HRP; Dako) for 30 min at room temperature, visualized with di-aminobenzidine commercial kits (Liquid DAB + Substrate Chromogen System; Dako), and finally treated with Mayer's hematoxylin.

2.10. Statistical analyses

The results are presented as mean ± SEM and statistical significance was accepted at $p < 0.05$. The following statistical tests were used: 1-One-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison tests for statistical comparison between parametric data and Kruskal-Wallis test followed by Dunn's test for statistical comparison between non parametric data using Graph Pad Instat version 3.06 software packages.

3. Results

3.1. Effect of carvedilol (20 mg/kg) on BALF's total and differential inflammatory cells contents

Silicosis-induction significantly boosted lung total inflammatory cell contents by about 8 folds and pulmonary content of neutrophils, lymphocytes, monocytes, eosinophils, and basophils significantly escalated by about 10, 9, 7, 12 and 18 folds respectively compared to normal

Table 1
Effect of daily oral carvedilol (20 mg/kg) on lung total and differential inflammatory cells contents.

Group	Total leucocytes × 10^4	Neutrophil × 10^4	Lymphocyte × 10^4	Monocyte × 10^4	Eosinophil × 10^4	Basophil × 10^4
Normal control	23 ± 1.6	14.21 ± 0.9	6.8 ± 0.44	0.62 ± 0.1	0.34 ± 0.08	0.13 ± 0.05
Silica control	202 ± 17*	146 ± 8.4*	61.1 ± 6.0*	4.2 ± 0.34*	4.04 ± 0.40*	2.4 ± 0.13*
Silica/Carvedilol (20 mg/kg, orally)	38 ± 3.7$	23.7 ± 2.0$	12.36 ± 1.0$	0.92 ± 0.10$	0.66 ± 0.08$	0.32 ± 0.09$

Silicosis was induced by intratracheal instillation of silica particles 50 mg/rat, 1 ml 0.9% NaCl. Carvedilol treatment began 24 h post silica instillation and persisted for further 8 weeks. Statistical analysis was done using (ANOVA) followed by Tukey-Kramer test.
* Significantly different Vs normal control (n = 12; $p < 0.05$).
$ Significantly different Vs Silica control (n = 12; $p < 0.05$).

control. Daily oral carvedilol for 8 weeks significantly reduced total inflammatory cell content by approximately 81% and lung content of neutrophils, lymphocytes, monocytes, eosinophils and basophils significantly declined by about 84%, 80%, 78%, 84% and 87% respectively in comparison to silica control, (Table 1).

3.2. Effect of carvedilol (20 mg/kg) on BALF's LDH activity

In context, BALF's LDH activity significantly escalated by about 3 folds post intranasal silica installation in comparison to normal control. Daily oral carvedilol significantly attenuated lung LDH activity by 50% compared to silica control, (Fig. 1).

3.3. Effect carvedilol (20 mg/kg) on lung total protein content

Total lung protein content significantly increased by approximately 5 folds in the silica control in comparison to normal. Daily oral carvedilol significantly decreased lung total protein content by 70% compared to diseased silica control, (Fig. 2).

3.4. Effect of carvedilol (20 mg/kg) on lung MDA and NOx contents, reduced GSH concentration, catalase and MPO activities

Intranasal silica installation and progression of silicosis significantly increased lung MDA and total NOx contents by about 3.3 and 2.6 folds respectively and MPO activity by 3.3 folds with concomitant retraction in lung GSH concentration, catalase and MPO activities by 51% and 60% in comparison to normal. Carvedilol significantly decreased lung content of MDA and NOx by 50% and 35% and MPO activity by 54%

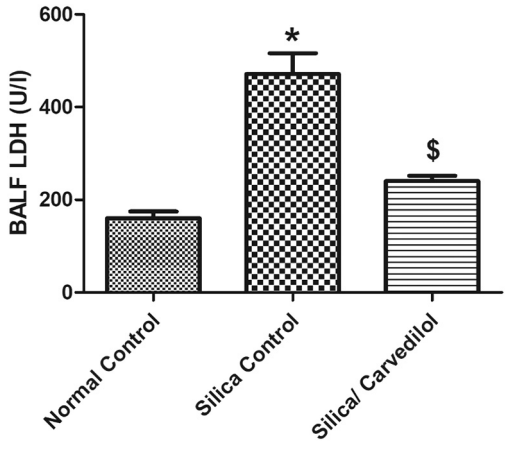

Fig. 1. Effect of daily oral carvedilol (20 mg/kg) on BALF's LDH activity: Silicosis was induced by intratracheal instillation of silica particles 50 mg/rat, 1 ml 0.9% NaCl. Carvedilol treatment began 24 h post silica instillation and persisted for further 8 weeks. Statistical analysis was done using (ANOVA) followed by Tukey-Kramer test.
*Significantly different Vs normal control (n = 12; $p < 0.05$)
$Significantly different Vs Silica control (n = 12; $p < 0.05$).

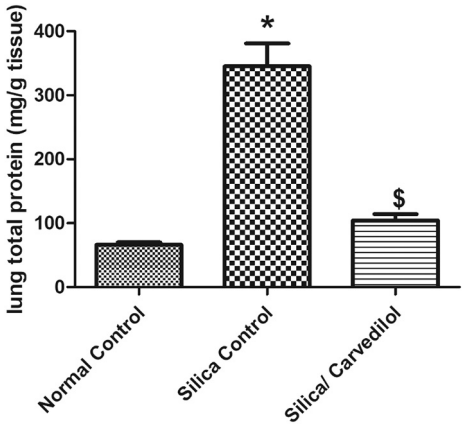

Fig. 2. Effect of daily oral carvedilol (20 mg/kg) on lung total protein content: Silicosis was induced by intratracheal instillation of silica particles 50 mg/rat, 1 ml 0.9% NaCl. Carvedilol treatment began 24 h post silica instillation and persisted for further 8 weeks. Statistical analysis was done using (ANOVA) followed by Tukey-Kramer test.
*Significantly different Vs normal control (n = 12; $p < 0.05$).
$Significantly different Vs Silica control (n = 12; $p < 0.05$).

with a significant escalation of both reduced GSH concentration and catalase activity by 50% and 73% compared to silica control, (Fig. 3).

3.5. Effect of carvedilol (20 mg/kg) on lung hydroxyproline and collagen contents

Silicosis progression was associated with a significant augmentation of lung hydroxyproline content by about 2 folds compared to normal. Concomitant carvedilol administration significantly reduced lung hydroxyproline and collagen contents by 39% in comparison to silica control, (Fig. 4: A).

3.6. Effect of carvedilol (20 mg/kg) on lung TGFβ1 content

Parallel to the significant enhancement in lung hydroxyproline content, silicosis progression was associated with a significant increase in lung TGFβ1 content by approximately 6 folds compared to normal. Oral carvedilol for 8 weeks significantly decreased lung TGFβ1 content by 65% in comparison to silica control, (Fig. 4: B).

3.7. Effect of carvedilol (20 mg/kg) on lung P-AKT content

Lung P-AKT content significantly escalated by approximately 2.7 folds in the silica control in comparison to normal upon silicosis progression. Carvedilol significantly reduced lung P-AKT content by 28% compared to the silica control, (Fig. 5).

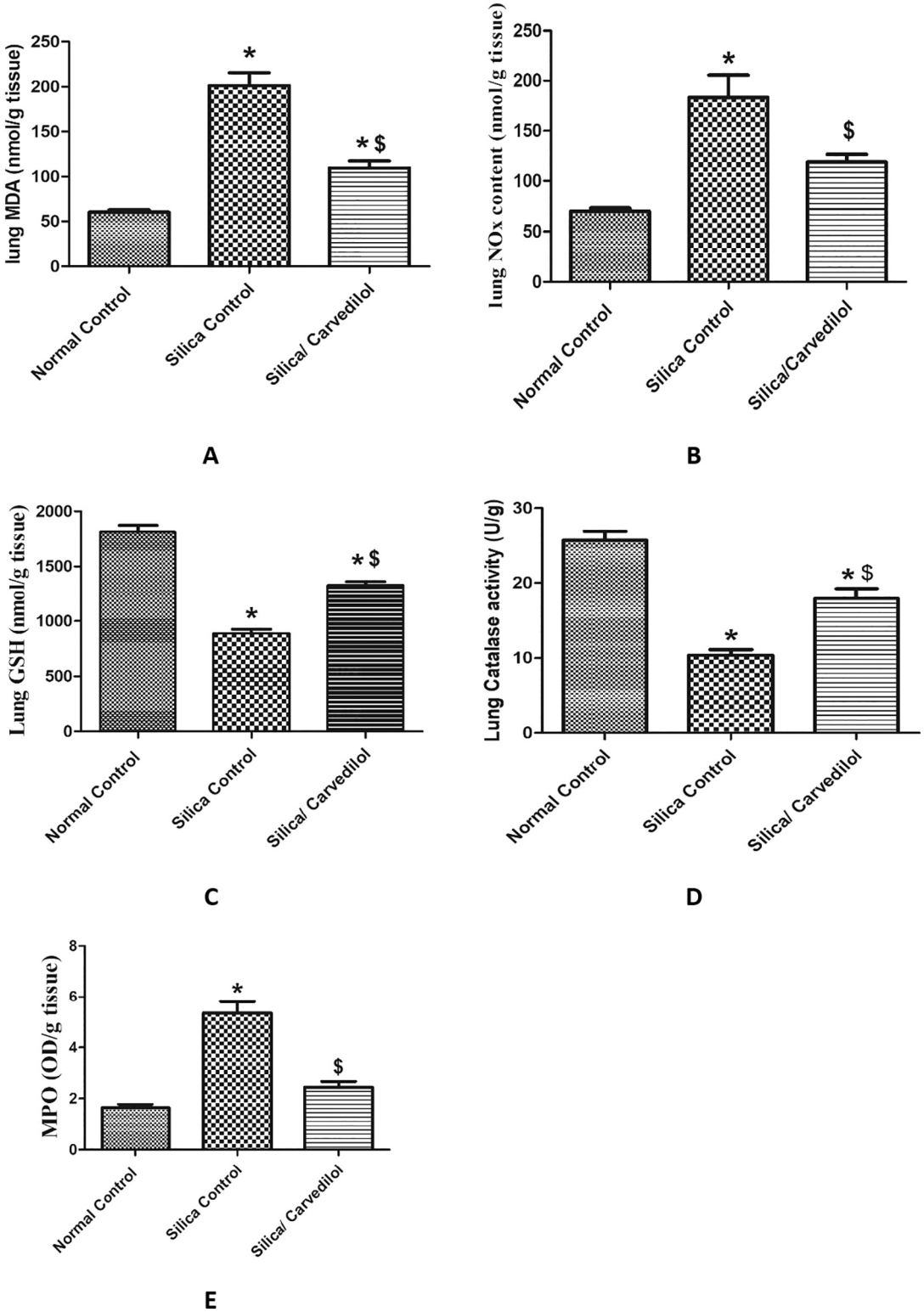

Fig. 3. Effect of daily oral carvedilol (20 mg/kg) on lung MDA content (A), NOx content (B), GSH concentration (C), Catalase activity (D) and MPO activity (E): Silicosis was induced by intratracheal instillation of silica particles 50 mg/rat, 1 ml 0.9% NaCl. Carvedilol treatment began 24 h post silica instillation and persisted for further 8 weeks. Statistical analysis was done using (ANOVA) followed by Tukey-Kramer test.
*Significantly different Vs normal control (n = 12; $p < 0.05$).
$Significantly different Vs Silica control (n = 12; $p < 0.05$).

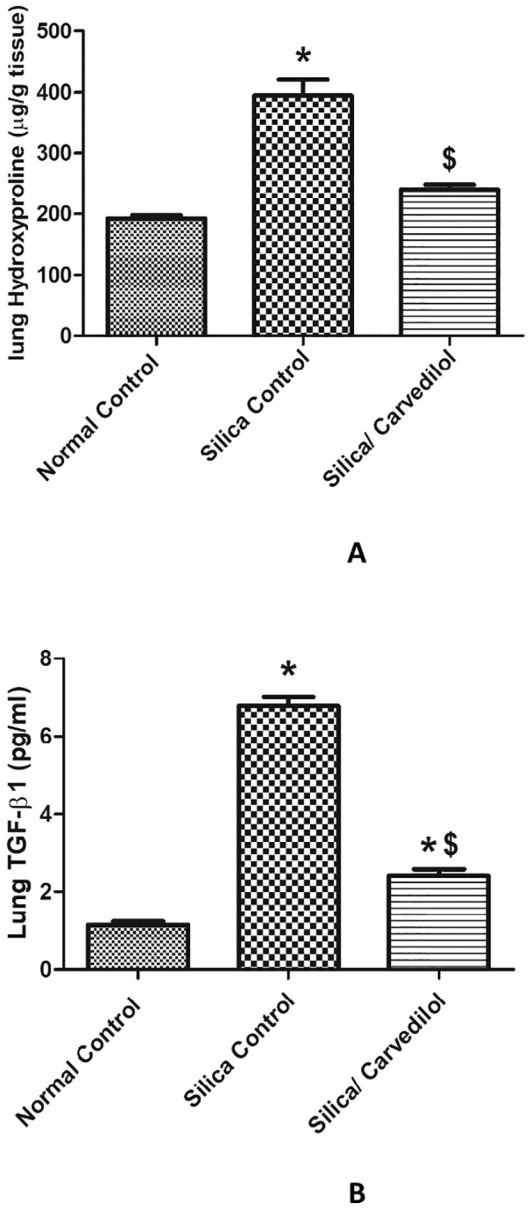

Fig. 4. Effect of daily oral carvedilol (20 mg/kg) on lung hydroxyproline content (A) and transforming growth factor β1 content (TGFβ1) (B): Silicosis was induced by intratracheal instillation of silica particles 50 mg/rat, 1 ml 0.9% NaCl. Carvedilol treatment began 24 h post silica instillation and persisted for further 8 weeks. Statistical analysis was done using (ANOVA) followed by Tukey-Kramer test.
*Significantly different Vs normal control (n = 12; $p < 0.05$)
$Significantly different Vs Silica control (n = 12; $p < 0.05$).

3.8. Effect of carvedilol (20 mg/kg) on lung mTOR content

In context, silicosis progression in the silica control was associated with a significant increase in lung mTOR content in comparison to normal by approximately 2 folds. Carvedilol, once daily orally for 8 weeks significantly suppressed lung mTOR content by about 24% compared to the diseased silica control, (Fig. 6).

3.9. Effect of carvedilol (20 mg/kg) on histopathological changes in H&E stained lung specimen

Macroscopical examination of H&E stained specimen from the normal control revealed ideal lung architecture with normal alveoli

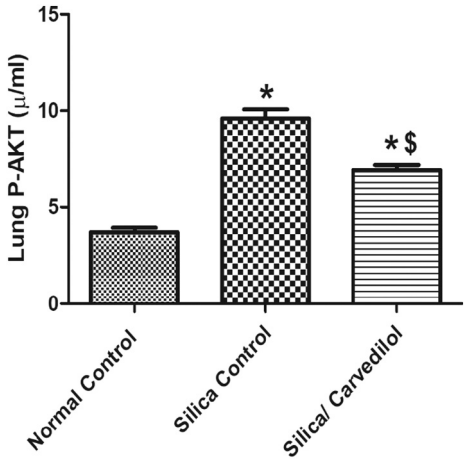

Fig. 5. Effect of daily oral carvedilol (20 mg/kg) on lung P-AKT content: Silicosis was induced by intratracheal instillation of silica particles 50 mg/rat, 1 ml 0.9% NaCl. Carvedilol treatment began 24 h post silica instillation and persisted for further 8 weeks. Statistical analysis was done using (ANOVA) followed by Tukey-Kramer test.
*Significantly different Vs normal control (n = 12; $p < 0.05$)
$Significantly different Vs Silica control (n = 12; $p < 0.05$).

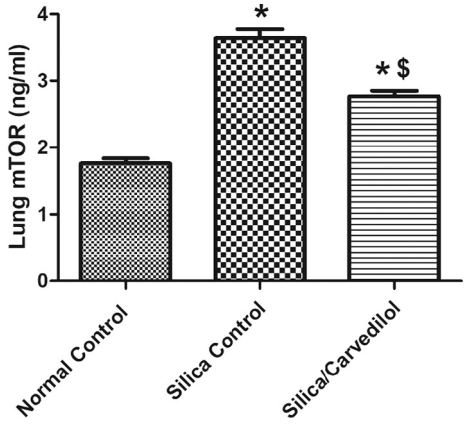

Fig. 6. Effect of daily oral carvedilol (20 mg/kg) on lung mTOR content: Silicosis was induced by intratracheal instillation of silica particles 50 mg/rat, 1 ml 0.9% NaCl. Carvedilol treatment began 24 h post silica instillation and persisted for further 8 weeks. Statistical analysis was done using (ANOVA) followed by Tukey-Kramer test.
*Significantly different Vs normal control (n = 12; $p < 0.05$).
$Significantly different Vs Silica control (n = 12; $p < 0.05$).

(arrow) and bronchi, (Fig. 7; A). Specimen from the silica control, on the other hand, revealed complete pulmonary collapse with epitheloid and lymphoplasmacytic infiltration (arrow), (Fig. 7; B). Carvedilol administration attenuated silica-induced damage where histopathological examination revealed significant resolution of inflammation and inflammatory infiltrates with restoration of normal pulmonary architecture (Fig. 7; C).

3.10. Effect of carvedilol (20 mg/kg) on histopathological changes in Masson's trichrome stained specimen

Meanwhile, a thin layer of perivascular collagen fibers with the absence of any evidence of any ECM accumulation was evident in the specimen examined from the normal control, (Fig. 8; A). Silicosis progression was associated with a significant degree of interstitial pulmonary fibrosis (arrow), (Fig. 8; B). On the other hand, carvedilol administration significantly reduced fibrosis where an only mild degree of perivascular fibrosis (arrow) was detected, (Fig. 8; C).

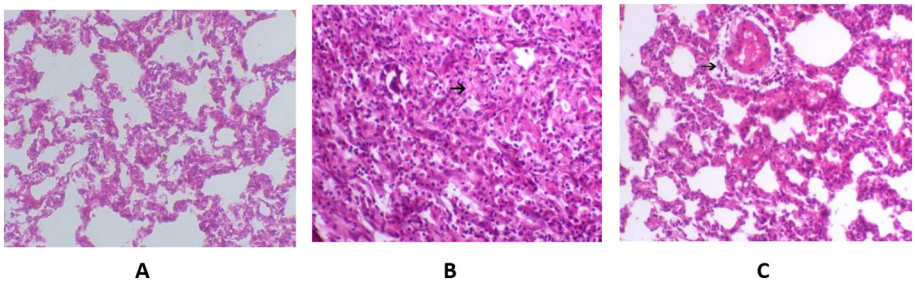

Fig. 7. Effect of daily oral carvedilol (20 mg/kg) on lung histopathological changes in H&E stained lung specimen: (A) Normal control revealed ideal lung architecture with normal alveoli (arrow) and bronchi, (B) Silica control, revealing complete pulmonary collapse with epitheloid and lymphoplasmacytic infiltration (arrow), and (C) Carvedilol treated group revealing significant resolution of inflammation and inflammatory infiltrates with restoration of normal pulmonary architecture, (Magnification 200×).

Fibrosis score significantly increased by approximately 17 folds in the diseased silica control in comparison to normal. Carvedilol administration significantly reduced fibrosis score by 39% in comparison to diseased silica control, (Fig. 8; D).

3.11. Effect of carvedilol (20 mg/kg) on NFκB expression in lung specimen

Immunohistochemical analysis of NFκB expression in lung specimen revealed a mild degree of NFκB expression within the alveolar cell lining in the normal control group, (Fig. 9; A). Silicosis progression was associated with a significant cytoplasmic expression of NFκB within the alveolar cells lining compared to normal, (Fig. 9; B). Carvedilol administration was associated with only mild NFκB expression within the alveolar cell lining compared to silica control, (Fig. 9; C).

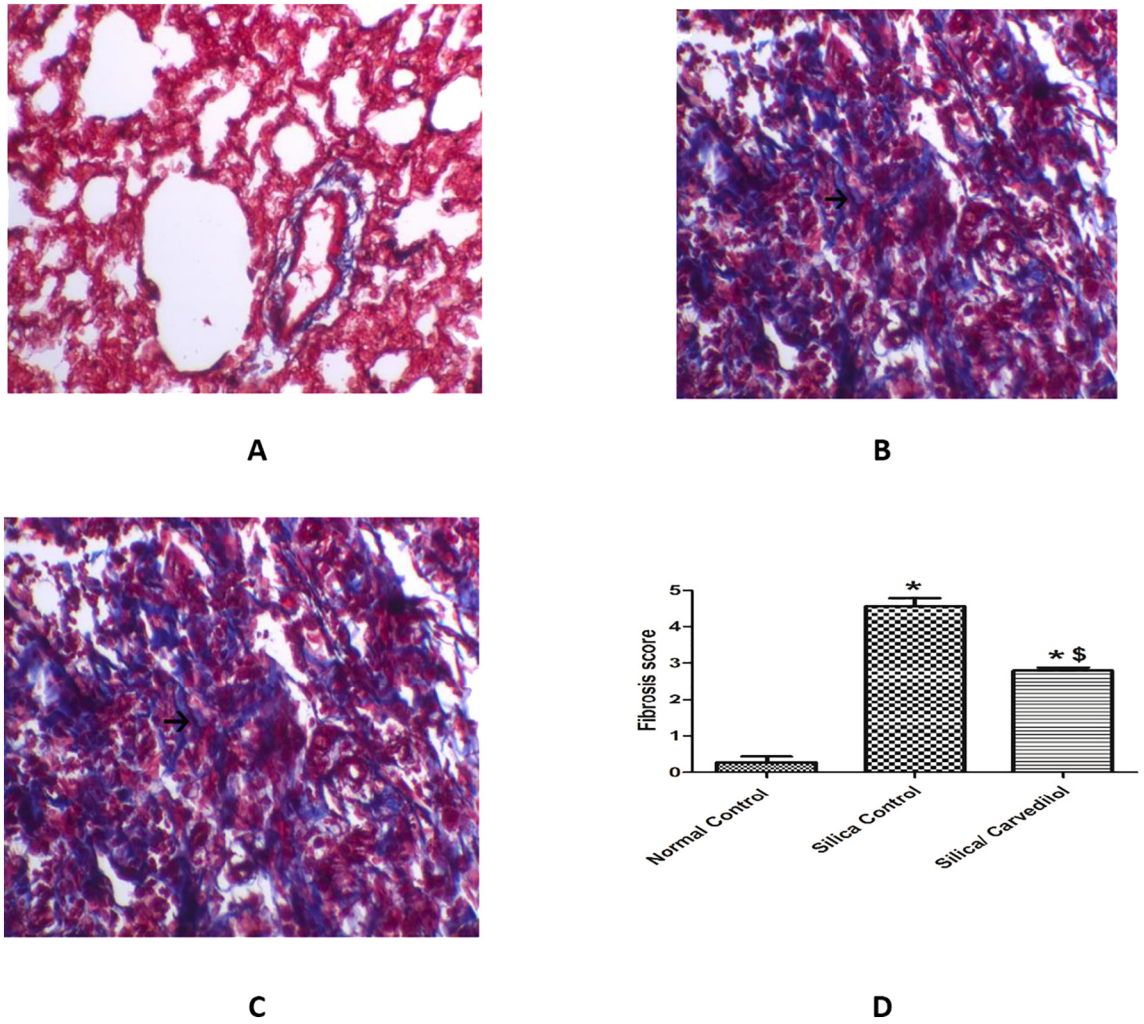

Fig. 8. Effect of daily oral carvedilol (20 mg/kg) on lung histopathological changes in Mason's Trichrome stained lung specimen: (A) Normal control revealing a thin layer of perivascular collagen fibers with the absence of any evidence of any ECM accumulation, (B) Silica control revealing significant degree of interstitial pulmonary fibrosis (arrow), (C) Carvedilol treated group revealing significantly reduced fibrosis where an only mild degree of perivascular fibrosis (arrow) was detected, (Magnification 200×) and (D), quantitative scoring of fibrosis score.
Silicosis was induced by intratracheal instillation of silica particles 50 mg/rat, 1 ml 0.9% NaCl. Carvedilol treatment began 24 h post silica instillation and persisted for further 8 weeks. Statistical analysis was done using Kruskal-Wallis followed by Dunn's test.
*Significantly different Vs normal control (n = 12; $p < 0.05$).
$Significantly different Vs Silica control (n = 12; $p < 0.05$).

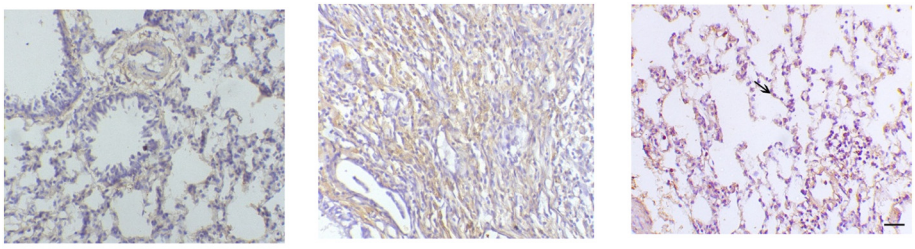

Fig. 9. Effect of daily oral carvedilol (20 mg/kg) on NF-κB expression: (A) Normal control revealing a mild degree of NFκB expression within the alveolar cell lining, (B) Silica control revealing significant cytoplasmic expression of NFκB within the alveolar cells lining and (C) Carvedilol treated group revealing only mild NFκB expression within the alveolar cell lining, (Magnification 200×, bar = 50 μm.).

4. Discussion

Given the nature of silicosis as a crippling respiratory disorder, with no definitive treatment, the investigation of new and effective therapies has become inevitable. The current study sheds light on the anti-fibrotic and pulmonary protective effect of carvedilol against silica-induced pulmonary damage and associated silicosis. Several parameters were investigated to verify the observed pulmonary protected effect of carvedilol and to highlight the underlying mechanisms implicated in the observed pulmonary protective effect.

Silicosis progression was associated with a significant impairment of lung biochemical hemostasis. Lung inflammatory cell content, LDH activity, total protein, MDA and total NOx contents, reduced GSH concentration, catalase and MPO activity, hydroxyproline, TGF β1, and NFκB expression revealed significant deviations compared to normal. This was associated with evident histopathological distortions.

The involvement of p-AKT/mTOR signaling pathway in silica-induced pulmonary fibrosis was investigated and indeed silicosis progression was associated with a significant enhancement in the lung p-AKT/mTOR content.

Carvedilol; a non-selective α/β blocker successfully restored lung hemostasis and silicosis-induced damage was significantly ameliorated. Carvedilol administration significantly restored lung p-AKT/mTOR content which appears to be the major mechanism underlying the pulmonary protective effect of carvedilol. The pulmonary protective effect of carvedilol observed in the current study was associated with a significant inhibition of P-AKT/mTOR/TGFβ1 axis. To the best of our knowledge, this is the first study reporting anti-fibrotic effect of carvedilol against silica-induced pulmonary fibrosis and verifying the underlined mechanism of action.

The results observed in the current study are in agreement with the study of Amirshahrokhi and Khalili., (2016) [15] reporting carvedilol to attenuate paraquat-induced lung injury. Carvedilol admiration was associated with a significant decrease in lung MDA, carbonyl protein, MPO and NOx, with increased levels of GSH, SOD, catalase and glutathione reductase. Moreover, lung NFκB, TGF β1, and hydroxyproline significantly declined with carvedilol administration giving credence to the results observed in the current study.

Several previously published literature have reported similar biochemical and functional alterations to those observed in the current study post intranasal instillation of silica and silicosis progression. Increased oxidants load, depletion of antioxidant stores, chronic inflammation, and tissue hypoxia have been reported to be interplay in silicosis progressions [6,16,19–21]. Nevertheless, inflammation and fibrosis have been repeatedly reported to be strongly associated. Increased levels of both of ROS/reactive nitrogen species [22] and inflammatory cytokines [23] were proposed as indicators of silicosis [16].

Once the inhaled silica particles reach the lungs, they activate the first line pulmonary defenses; alveolar macrophages, initiating lung injury, proliferation and fibroblasts' activation. Simultaneously, oxidative mechanisms are significantly up-regulated. Interestingly, acute and chronic inflammatory lesions, fibrosis progression and granuloma formation in experimentally-induced silicosis have been linked to NO [24].

The observed increase in MPO activity in the current study is suggestive of neutrophil infiltration which was further confirmed by quantification of BALF's inflammatory cells' content and histopathological examination. Macrophages and neutrophils are phagocytes that play major roles in the pathogenesis of many disorders. They belong to the innate immune system and can switch between different modes of activation upon cues received from their immediate microenvironment. Once activated, they secrete myriad of mediators that shape and regulate the microenvironment [25].

In the current study, Carvedilol administration managed to effectively reduce neutrophil infiltration within lung tissue as evidenced in BALF'S inflammatory cells quantification, histopathological examination and MPO activity with subsequent modulation of the fibrogenic cascade in the silicotic lung.

Amongst fibrogenic cytokines, TGFβ1 plays the most significant role. It induces various genetic programs that up-regulate collagen expression and ECM deposition with enhanced deposition of the type I collagen which eventually replaces lung epithelium and hinders normal gas exchange mechanics [8]. Under physiological conditions, a coordinated sequence of tissue repair is usually triggered by TGFβ1. Following proper repair and tissue healing, fibroblasts usually undergo apoptosis. In fibro-proliferative disorders, this repair cascade is disrupted with atypical repair and chronic lung fibrosis develops [26].

Identifying mTOR and PI3/AKT/mTOR pathway as a potential therapeutic target for pulmonary fibrosis is expected to minimize pulmonary fibrosis. In normal cells, the PI3K/AKT kinase pathway is a key regulator of various important processes including cell proliferation, differentiation, metabolism, and survival [27]. AKT signals to numerous downstream effectors of which; mTOR [8].

The importance of mTOR within this signaling matrix was confirmed by rapamycin's significant anti-proliferative properties [7,28]. mTOR has been reported by various investigators to be mediate inflammation, apoptosis, metabolism, and senescence. Such a key role of mTOR in mediating several disorders, including fibrosis and cancer, establishes it as a highly valuable target for manipulation and modulation [27].

It appears that PI3K/AKT/mTOR signaling is crucial for ATP-induced fibroblasts proliferation; which provides evidence confirming mTOR to be central to modulating fibroblast proliferation. Moreover, mTOR has been reported to affect DNA repair proteins either directly or indirectly by regulating a number of genes involved in the DNA damage repair and cell cycle machinery [8,29].

Regulation of inflammatory responses in certain cells such as monocytes and macrophages was reported to be mediated via mTORC1 [30] and mTORC2 signaling further enhances fibrosis by promoting the survival of fibrosis-associated secretory fibroblasts. In a study investigating granulomatous disorder, mTORC1 blockade suppressed apoptosis and enhanced macrophages proliferation to promote granuloma formation [31]. Palomid 529 (P529); a dual mTORC1 and mTORC2 inhibitor revealed anti-keloid disease (KD) properties [32].

Smad-mediated TGF β1 signaling may enhance mTOR signaling to promote cell survival and enhance fibrosis creating a hypoxic

environment [33]. Nevertheless, TGF β1-mediated PI3K/AKT signaling further enhancing mTOR signaling [34].

In light of the previously referred to evidences, in both experimental models of radiation and bleomycin-induced pulmonary fibrosis, mTOR inhibition was reported to be effective at suppressing fibrotic process. GSK2126458; a dual mTOR/PI3K inhibitor inhibited fibroblasts activation isolated from patients with fibrotic foci [35,36]. Moreover, MLN0128; another dual mTORC1 and mTORC2 inhibitor proved to be a potent anti-fibrotic in both in vitro and in vivo assays [37].

Given the significant central role of TGF β1 to the fibrotic process and its link to mTOR signaling, inhibition of both of mTOR and TGF β1 signaling can be proposed as a reasonable therapeutic approach for management of pulmonary fibrosis. mTOR inhibition can be presumed to modulate/suppress PI3K signaling in fibroblasts while TGF β1 inhibition may minimize AKT activation, thus decreasing the continued source for fibroblasts activation.

In the last few years, the anti-fibrotic action of carvedilol has been referred to by El-Demerdash et al., [13]. Carvedilol through its antioxidants and anti-inflammatory effects decreased hepatocyte damage, inhibited hepatic stellate cell activation, and reduced collagen formation and these effects were mediated primarily via suppression of NF-κB expression [38].

Indeed, in the current study, carvedilol administration was associated with a significant down-regulation of NF-κB expression, together with marked anti-oxidant properties and positive impact on the host anti-oxidant defenses. In the study conducted by El-Demerdash et al., [13], carvedilol administration to carbon tetrachloride intoxicated rats was associated with a significant reduction in hepatic TGFβ1 levels which gives credence to the observation of the current study where carvedilol administration was associated with a significant reduction in lung TGF β1 alongside the aforementioned parameters.

Interestingly, in the study of El-Wakeel et al., [39] investigating the anti-fibrotic properties of carvedilol against carbon tetrachloride-induced liver fibrosis, hepatic level of SMAD7 was up-regulated with carvedilol treatment which was associated with a decreased expression of TGF-β1. Activation of SMAD7 hinders TGF-β1-mediated miR-200 down-regulation [40].

An important issue to be taken into consideration is the effect of carvedilol as a nonselective α/β blocker on β2-mediated broncho-dilatory functions. Interestingly, patients with chronic obstructive pulmonary diseases and congestive heart failure demonstrated proper tolerance to carvedilol with no significant reversible airflow limitation, while, asthma patients did not [41]. Actually, there are a number of pieces of evidence advocating non-selective β blockers safety in chronic obstructive pulmonary diseases' patients. In the line of these observations, carvedilol's impact on β2-mediated broncho-dilatory is minimized making it a possible attractive approach for silicosis patients.

In conclusion: Carvedilol demonstrated promising results as an anti-fibrotic for management of experimentally-induced silicosis. Its inhibitory effect on macrophages' activation and neutrophil recruitment as well as its suppressive effect on P-AKT/mTOR/TGFβ1 signaling justify its anti-fibrotic and pulmonary protective effects. Further clinical studies are required to verify the anti-fibrotic impact of carvedilol and calibrate anti-fibrotic dose for silicosis patients.

Conflict of interest

None.

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