Comparative Analysis of Antifibrotic and Anti-inflammatory Effects of Metformin and Canagliflozin in Lung Fibrosis Models

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Abstract

This study investigates the comparative antifibrotic and anti-inflammatory effects of metformin and canagliflozin in lung fibrosis models, focusing on the potential effect of canagliflozin to mitigate fibrosis-related molecular and histopathological changes. ***Aim:*** *To evaluate the efficacy of metformin and canagliflozin in mitigating lung fibrosis and associated pulmonary complications in rat models by assessing their effects on SMAD2/3 signaling pathways, inflammatory markers, oxidative stress parameters, and histopathological alterations.* ***Methods:*** *This study utilized 40 adult male albino rats, divided into four groups: normal control, lung fibrosis control, metformin-treated lung fibrosis, and canagliflozin-treated lung fibrosis. Lung fibrosis was induced by intratracheal instillation of bleomycin. Treatments included oral metformin (50 mg/kg/day) or canagliflozin (40 mg/kg/day) for 14 days. Biochemical parameters, including fasting blood glucose (FBG), advanced glycation end products (AGE), lactate dehydrogenase (LDH), and protein content in bronchoalveolar lavage fluid (BALF), were measured. Histopathological and immunohistochemical analyses were performed on lung tissues to evaluate fibrosis markers, such as TGF-β expression and SMAD2/3 signaling.* ***Results:*** *The current study revealed significant pathological changes in the lung fibrosis control group compared to the normal control group across multiple parameters, including increased SMAD2/3 expression, AGEs, RAGE, MDA, protein, LDH, WBC counts, and lung weight, along with decreased SOD levels. These findings underscore the role of oxidative stress, inflammation, and fibrosis in lung fibrosis pathology. Treatment with Metformin and Canagliflozin significantly mitigated these changes, with both drugs reducing SMAD2/3, AGEs, RAGE, MDA, LDH, and WBC counts, while restoring SOD levels and reducing lung weight. Metformin demonstrated slightly stronger effects in reversing fibrosis, as reflected in histopathological staining and lung weight reduction, aligning with its known antifibrotic and anti-inflammatory properties. Metformin and Canagliflozin also exhibited differences in their systemic effects. Metformin-treated rats showed significant weight loss, potentially due to its metabolic effects, while Canagliflozin-treated rats maintained body weight closer to normal. Both treatments effectively reduced inflammatory and fibrotic markers, as shown by immune and Masson Trichrome stains, with significant group differences (KW p-values < 0.05).* ***Conclusion:*** *Metformin showed a slightly stronger antifibrotic effect, reflected in greater normalization of lung weight and histopathological markers, though it was associated with weight loss. In contrast, Canagliflozin preserved body weight while also demonstrating strong anti-inflammatory and antioxidant properties. These results suggest that Canagliflozin could be promising candidates for the management of lung fibrosis. Metformin may be preferred for its robust antifibrotic effects, while Canagliflozin offers additional benefits in metabolic regulation and weight preservation.*

Keywords: *Antifibrotic and Anti-inflammatory, Metformin and Canagliflozin , Lung Fibrosis Models*

Introduction

Idiopathic pulmonary fibrosis (IPF) is frequently associated with diabetes mellitus and is one of the most aggressive interstitial lung diseases that affects lung parenchyma, leading to lung fibrosis with a decline in lung function that progresses to respiratory failure with high mortality [1]. The incidence of IPF is 10.4 and 11.2 per 100,000 population, and the prevalence is 32.6 and 35.1 per 100,000 population worldwide [2], with a median survival period of 2–4 years [3]. Lung transplantation is the only intervention that has been proven to extend life expectancy [4]. The overall prognosis of the disease is poor. Existing oral therapies with pyridones (pirfenidone) and tyrosine kinase inhibitors (nintedanib) may improve the patient's quality of life and slow disease progression. However, these drugs have severe adverse effects. The lack of safe and effective treatment calls for further investigation to discover an optimum therapy for IPF [5].

Metformin is a biguanide antidiabetic drug used to lower blood glucose in type II diabetic patients with pleiotropic effects on cellular biology [6]. The drug has anti-inflammatory and antifibrotic effects. It is reported to inhibit cardiac fibrosis and reduce collagen synthesis in cardiac fibrosis, probably via inhibition of the TGF-β/Smad3 signaling pathway [7]. It can also reverse bleomycin-induced pulmonary fibrosis, suggesting that metformin has beneficial effects on idiopathic pulmonary interstitial fibrosis [8].

Canagliflozin is an orally administered sodium-glucose co-transporter-2 (SGLT2) inhibitor used in the treatment of patients with type 2 diabetes. It reduces renal glucose reabsorption, thereby increasing urinary glucose excretion and reducing blood glucose levels [9]. Canagliflozin can also regulate intracellular glucose metabolism and exert anti-inflammatory effects on immune cells [10]. Moreover, SGLT2 inhibition with canagliflozin is supposed to have antifibrotic effects and protect against extracellular matrix deregulation by regulating TGF-β1 production [11,12].

We aimed to evaluate the efficacy of metformin and canagliflozin in mitigating lung fibrosis and associated pulmonary complications in a diabetic rat model by assessing their effects on SMAD2/3 signaling pathways, inflammatory markers, oxidative stress parameters, and histopathological alterations

Materials and Methods

Animals:

The study utilized 40 adult male albino rats weighing 200–250 g, obtained from the animal house of the Faculty of Veterinary Medicine, Zagazig University, Egypt. The experimental protocols were approved by the Zagazig University Ethics Committee (approval no. ZU-IACUC/3/F/60/2023) and conducted following animal research guidelines. Rats were housed in cages with raised mesh bottoms to prevent coprophagy, with free access to standard food and water. Environmental conditions were maintained at 20–26˚C with a 12-hour light/dark cycle. Animals were acclimated for one week before starting the experiments.

Drugs and Chemicals:

The following were used: bleomycin (Thermo Fisher Scientific, USA), canagliflozin (Janssen Pharmaceuticals, UK), metformin (Sigma–Aldrich, USA), and 0.9% saline (Nile Co., Egypt). All drugs were supplied in powder form and freshly prepared before use.

Induction of Lung Fibrosis:

Lung fibrosis was induced by intratracheal instillation of bleomycin at a dose of 5 mg/kg [13].

Experimental Design:

Rats were divided into four groups (n = 10 per group):

1. **Normal Control:** Rats received sodium citrate buffer (0.5 mL/kg, I.P.) followed by normal saline (1 mL/kg, intratracheally).
2. **Lung Fibrosis Control:** Rats received A single dose of bleomycin, 5 mg/kg intratracheally
3. **Metformin-treated lung fibrosis group:** Rats received sodium citrate buffer (0.5 mL/kg, I.P.) followed by normal saline (1 mL/kg, intratracheally), administered metformin (50mg/kg) daily for 14 day by gavage from the first day of injection of bleomycin. [14].
4. **Canagliflozen-treated lung fibrosis group:** Rats received sodium citrate buffer (0.5 mL/kg, I.P.) followed by normal saline (1 mL/kg, intratracheally), administered canagliflozen, 40 mg/kg/day,by gavage from the first day of injection of bleomycin. [15].

At the end of the experiment, blood samples and bronchoalveolar lavage fluid (BALF) were collected for biochemical assays, and lung tissues were collected for histopathological and immunohistochemical analyses.

Measured Parameters:

Fasting Blood Glucose Levels: Measured using a OneTouch glucometer with blood obtained via tail vein puncture [16].

Body and Lung Weights: Measured using a digital weighing scale [17].

Bronchoalveolar Lavage Fluid (BALF): Performed by introducing ice-cold phosphate-buffered saline (PBS) into the lung and collecting it in conical tubes [18].

Biochemical Analyses:

Advanced Glycation Endproducts (AGE): Measured using ELISA kits (Cusabio, USA).

Receptors for Advanced Glycation Endproducts (RAGE): Measured using ELISA kits (MyBioSource, Canada).

Lactate Dehydrogenase (LDH): Assayed with a colorimetric kit (Abcam, UK).

Total Protein (TP): Quantified using a colorimetric kit (MyBioSource, Canada).

Histopathological Analysis:

Fresh lung tissues were rinsed with ice-cold saline and stored at −80°C. Formalin-fixed tissues were embedded in paraffin, sectioned (5 μm), and stained with hematoxylin-eosin (H&E) and Masson trichrome for histopathological assessment [18].

Immunohistochemistry:

Lung tissues were processed for TGF-β1 immunostaining using formalin-fixed, paraffin-embedded sections. Steps included antigen retrieval, application of primary and secondary antibodies, and detection using standard immunohistochemical techniques [19].

Statistical Analysis:

Data were analyzed using SPSS (v22). Results were expressed as means ± SEM and analyzed using one-way ANOVA with LSD post-hoc tests. Differences were considered significant at $p<0.05$ [31]. Histopathological results were analyzed using the Kruskal-Wallis test, followed by the Mann-Whitney U test for pairwise comparisons, with staining proportions scored according to the Modified Allred scoring system [20].

Results

Table 1 demonstrates a significant increase in SMAD2/3 relative expression in the lung fibrosis control group (6.62 ± 0.26) compared to the normal control group (1.03 ± 0.02), indicating the pathological role of SMAD2/3 in lung fibrosis. Both Metformin (2.28 ± 0.41) and Canagliflozin (2.51 ± 0.41) treatments significantly reduced SMAD2/3 expression compared to the lung

fibrosis control, highlighting their therapeutic effects. With a p-value < 0.05 and ANOVA confirming significant differences between groups, the results are statistically robust.

Table (1): changes (Mean ± SD) of SMAD2/3 relative expression for different groups of rats :

SMAD2/3	
	Mean ± SD
Normal Control	1.03 ± 0.02
lung fibrosis control	6.62 ± 0.26
Metformin-treated lung fibrosis	2.28 ± 0.41
Canagliflozen -treated lung fibrosis	2.51 ± 0.41
P value	**<0.05**

ANOVA Test, p value >0.05: nonsignificant, p value <0.05 significant Within the same column, values without common superscript capital letters are significantly different (p<0.05).

Table 2 shows a significant increase in AGE (UG/ML) levels in the lung fibrosis control group (101.14 ± 1.92) compared to the normal control group (28.7 ± 1.59), reflecting the pathological elevation of AGEs in lung fibrosis. Both Metformin (46.25 ± 2.13) and Canagliflozin (47.68 ± 3.72) treatments markedly reduced AGE levels compared to the lung fibrosis control, demonstrating their therapeutic effects.

Table (2): changes (Mean ± SD) of AGE (UG/ML) for different groups of rats:

AGE, UG/ML	
	Mean ± SD
Normal Control	28.7 ± 1.59
lung fibrosis control	101.14 ± 1.92
Metformin-treated lung fibrosis	46.25 ± 2.13
Canagliflozen -treated lung fibrosis	47.68 ± 3.72
P value	**<0.05**

ANOVA Test, p value >0.05: nonsignificant, p value <0.05 significant. Within the same column, values without common superscript capital letters are significantly different (p<0.05).

Table 3 highlights a significant elevation in RAGE (NG/ML) levels in the lung fibrosis control group (8.7 ± 0.72) compared to the normal control group (2.7 ± 0.1), emphasizing the role of RAGE in the progression of lung fibrosis. Treatment with Metformin (4.03 ± 0.33) and Canagliflozin (4.09 ± 0.14) significantly reduced RAGE levels compared to the lung fibrosis control group, indicating their in mitigating RAGE-mediated effects in lung fibrosis.

Table (3): changes (Mean ± SD) of RAGE (NG/ML) for different groups of rats :

RAGE, NG/ML	
	Mean ± SD
Normal Control	2.7 ± 0.1
lung fibrosis control	8.7 ± 0.72
Metformin-treated lung fibrosis	4.03 ± 0.33
Canagliflozen -treated lung fibrosis	4.09 ± 0.14
P value	**<0.05**

ANOVA Test, p value >0.05: nonsignificant, p value <0.05 significant. Within the same column,

values without common superscript capital letters are significantly different (p<0.05).
Table 4 shows a significant reduction in SOD (U/ML) levels in the lung fibrosis control group (59.94 ± 2.72) compared to the normal control group (141.6 ± 2), reflecting oxidative stress associated with lung fibrosis. Treatment with Metformin (122.25 ± 2.33) and Canagliflozin (123.92 ± 2.7) significantly increased SOD levels compared to the lung fibrosis control group, indicating their ability to restore antioxidant defenses.

Table (4): changes (Mean ± SD) of SOD (U/ML) for different groups of rats :.

SOD, U/ML	
	Mean ± SD
Normal Control	141.6 ± 2
lung fibrosis control	59.94 ± 2.72
Metformin-treated lung fibrosis	122.25 ± 2.33
Canagliflozen -treated lung fibrosis	123.92 ± 2.7
P value	<0.05

ANOVA Test, p value >0.05: nonsignificant, p value <0.05 significant Within the same column, values without common superscript capital letters are significantly different (p<0.05).
Table 5 demonstrates a marked increase in MDA (NMOL/ML) levels in the lung fibrosis control group (223.82 ± 5.87) compared to the normal control group (65.3 ± 1.11), highlighting increased lipid peroxidation and oxidative stress in lung fibrosis. Both Metformin (105.92 ± 3.22) and Canagliflozin (104.05 ± 5.23) treatments significantly reduced MDA levels compared to the lung fibrosis control group, indicating their effectiveness in mitigating oxidative damage

Table (5): changes (Mean ± SD) of MDA (NMOL/ML)for different groups of rats

MDA, NMOL/ML	
	Mean ± SD
Normal Control	65.3 ± 1.11
lung fibrosis control	223.82 ± 5.87
Metformin-treated lung fibrosis	105.92 ± 3.22
Canagliflozen -treated lung fibrosis	104.05 ± 5.23
P value	**<0.05**

ANOVA Test, p value >0.05: nonsignificant, p value <0.05 significant Within the same column, values without common superscript capital letters are significantly different (p<0.05).
Table 6 reveals a significant increase in protein (MG/ML) levels in the lung fibrosis control group (144.02 ± 15.18) compared to the normal control group (39.07 ± 0.51), indicating protein accumulation associated with lung fibrosis. Treatment with Metformin (77.22 ± 5.13) and Canagliflozin (73.53 ± 9.89) significantly reduced protein levels compared to the lung fibrosis control group, suggesting their effects in reducing protein accumulation and improving lung pathology.

Table (6): changes (Mean ± SD) of Protein (MG/ML) for different groups of rats :

Protein, MG/ML	
	Mean ± SD
Normal Control	39.07 ± 0.51
lung fibrosis control	144.02 ± 15.18
Metformin-treated lung fibrosis	77.22 ± 5.13
Canagliflozen -treated lung fibrosis	73.53 ± 9.89
P value	**<0.05**

ANOVA Test, p value >0.05: nonsignificant, p value <0.05 significant Within the same column,

values without common superscript capital letters are significantly different ($p<0.05$).

Table 7 shows a significant elevation in LDH (U/ML) levels in the lung fibrosis control group (179.16 ± 2.92) compared to the normal control group (51.6 ± 0.46), reflecting increased cellular damage and stress associated with lung fibrosis. Both Metformin (85.38 ± 8.28) and Canagliflozin (81.97 ± 7.42) treatments significantly reduced LDH levels compared to the lung fibrosis control group, indicating their ability to mitigate tissue damage.

Table (7): changes (Mean ± SD) of LDH (U/ML)for different groups of rats :.

LDH, U/ML	
	Mean ± SD
Normal Control	51.6 ± 0.46
lung fibrosis control	179.16 ± 2.92
Metformin-treated lung fibrosis	85.38 ± 8.28
Canagliflozen -treated lung fibrosis	81.97 ± 7.42
P value	**<0.05**

ANOVA Test, p value >0.05: nonsignificant, p value <0.05 significant Within the same column, values without common superscript capital letters are significantly different ($p<0.05$).

Table 8 shows a dramatic increase in WBC count ($X10^3$ CELL/ML) in the lung fibrosis control group (36.26 ± 7.92) compared to the normal control group (1.06 ± 0.01), indicating higher inflammation associated with lung fibrosis. Treatment with Metformin (14.05 ± 2.21) and Canagliflozin (14.22 ± 1.13) significantly reduced WBC levels compared to the lung fibrosis control group, reflecting their anti-inflammatory effects.

Table (8): changes (Mean ± SD) of WBCS (X103CELL/ML)for different groups of rats :.

WBCS, X103CELL/ML	
	Mean ± SD
Normal Control	1.06 ± 0.01
lung fibrosis control	36.26 ± 7.92
Metformin-treated lung fibrosis	14.05 ± 2.21
Canagliflozen -treated lung fibrosis	14.22 ± 1.13
P value	**<0.05**

ANOVA Test, p value >0.05: nonsignificant, p value <0.05 significant Within the same column, values without common superscript capital letters are significantly different ($p<0.05$).

Table 9 shows a slight increase in fasting blood glucose (FBG, mg/dl) levels in the lung fibrosis control group (86.4 ± 2.51) compared to the normal control group (80.33 ± 1.53). Metformin-treated lung fibrosis rats (88.5 ± 5.58) exhibited a minor further increase, while Canagliflozin-treated rats (83.67 ± 2.73) showed levels closer to the normal control group.

Table (9): changes (Mean ± SD) of FBG (mg/dl)for different groups of rats :.

FBG, mg/dl	
	Mean ± SD
Normal Control	80.33 ± 1.53
lung fibrosis control	86.4 ± 2.51
Metformin-treated lung fibrosis	88.5 ± 5.58
Canagliflozen -treated lung fibrosis	83.67 ± 2.73
P value	**<0.05**

ANOVA Test, p value >0.05: nonsignificant, p value <0.05 significant. Within the same column, values without

common superscript capital letters are significantly different ($p<0.05$).

Table 10 indicates that body weight (gm) remained relatively stable in the normal control group (215 ± 13) and the lung fibrosis control group (214.4 ± 11.59), suggesting that lung fibrosis itself did not significantly affect body weight. However, Metformin-treated lung fibrosis rats experienced a significant reduction in body weight (149.33 ± 28.56), which might indicate potential side effects of the treatment or its impact on metabolism. In contrast, Canagliflozin-treated rats maintained a body weight closer to the normal group (200.17 ± 16.69), suggesting a less pronounced effect on weight loss. With a p-value < 0.05, these differences are statistically significant. Superscripts and post-hoc analysis could further clarify the significance of differences between treatments. Overall, Canagliflozin appears to have a more favorable effect on preserving body weight in lung fibrosi

Table (10): changes (Mean ± SD) of body weight (gm)for different groups of rats :.

Body weight, gm	
	Mean ± SD
Normal Control	215 ± 13
lung fibrosis control	214.4 ± 11.59
Metformin-treated lung fibrosis	149.33 ± 28.56
Canagliflozen -treated lung fibrosis	200.17 ± 16.69
P value	<**0.05**

ANOVA Test, p value >0.05: nonsignificant, p value <0.05 significant Within the same column, values without common superscript capital letters are significantly different ($p<0.05$).

Table 11 shows a significant increase in lung weight in the lung fibrosis control group (2.76 ± 0.21) compared to the normal control group (1.63 ± 0.15), reflecting lung fibrosis-induced tissue remodeling and inflammation. Metformin-treated rats (1.67 ± 0.29) exhibited lung weights similar to the normal control group, indicating its effect to prevent or reverse fibrosis-related lung changes. Canagliflozin-treated rats (2.12 ± 0.28) showed a partial reduction in lung weight compared to the lung fibrosis control group but did not fully normalize lung weight like Metformin.

Table (11): changes (Mean ± SD) of lung weight (gm) for different groups of rats :.

Lung weight,	
	Mean ± SD
Normal Control	1.63 ± 0.15
lung fibrosis control	2.76 ± 0.21
Metformin-treated lung fibrosis	1.67 ± 0.29
Canagliflozen -treated lung fibrosis	2.12 ± 0.28
P value	<0.05

ANOVA Test, p value >0.05: nonsignificant, p value <0.05 significant Within the same column, values without common superscript capital letters are significantly different ($p<0.05$).

Histopathological Results

Both Metformin and Canagliflozin showed effective reductions in immune activity and fibrosis as reflected in the histopathological scoring, with Metformin displaying a marginally stronger impact on fibrosis.

Table 12: Histopathological scoring by Modified Allred score

Group	Immune stain Median expression	Score	Masson trichrome Median expression	score
Normal control	3.7	2	0.6	1
Lung fibrosis control	13	3	13.1	3
Metformin-treated Lung Fibrosis	2.4	2	2	2
Canagliflozin-treated Lung Fibrosis	4	2	3	2

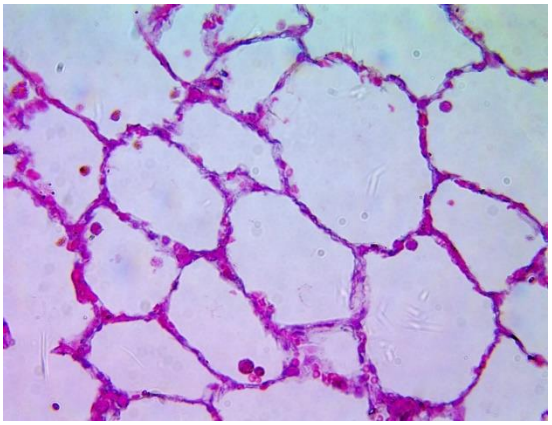

FIG. (1): Section of lung tissue of normal control group showed normal alveoli with thin alveolar wall and absent inflammation and fibrosis. (Trichrome x400)

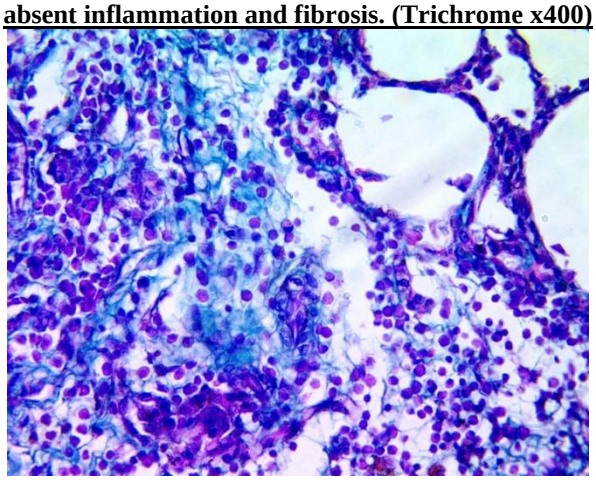

FIG (2): Section of lung tissue of lung fibrosis control group showed massive fibrosis with thickened alveolar walls and marked cellular infiltrate. (Trichrome x400).

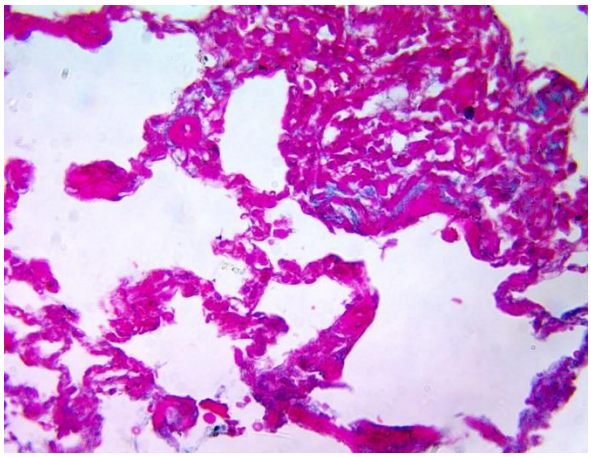

FIG. (3): Section of lung tissue of Metformin-treated lung fibrosis group showed mild fibrosis, focal cellular infiltrate and mild thickening of alveolar walls. (Trichrome x400)

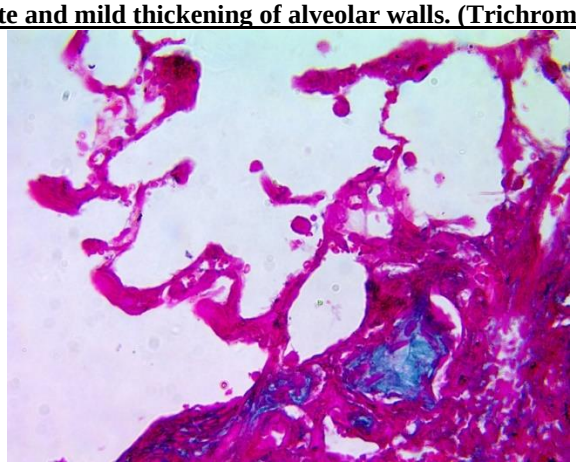

FIG. (4): Section of lung tissue of Canagliflozen-treated lung fibrosis group showed focal cellular infiltrate destruction of alveolar walls with focal fibrosis. (Trichrome x400)

Immunohistochemistry for TGF-β

Regarding immune stain: area stained demonstrated significant differences in both the immune stain and Masson Trichrome stain across the groups, as indicated by the KW p-values of 0.018 and 0.011, respectively)).

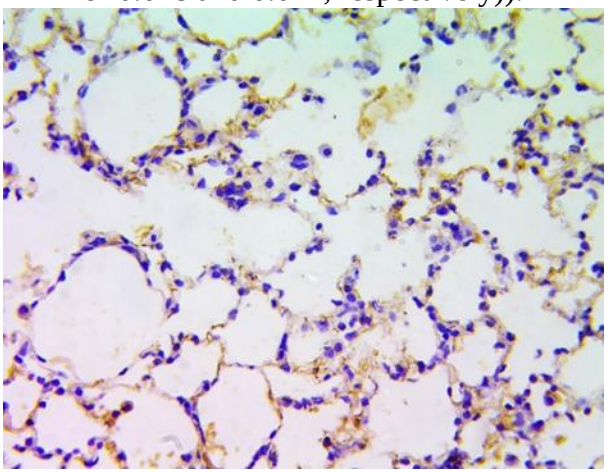

FIG. (5): Immunohistochemistry (IHC) for TGF-β in the lung tissue of Normal Control group showed negative staining. (TGF-β IHC x100) and (TGF-β IHC x400)

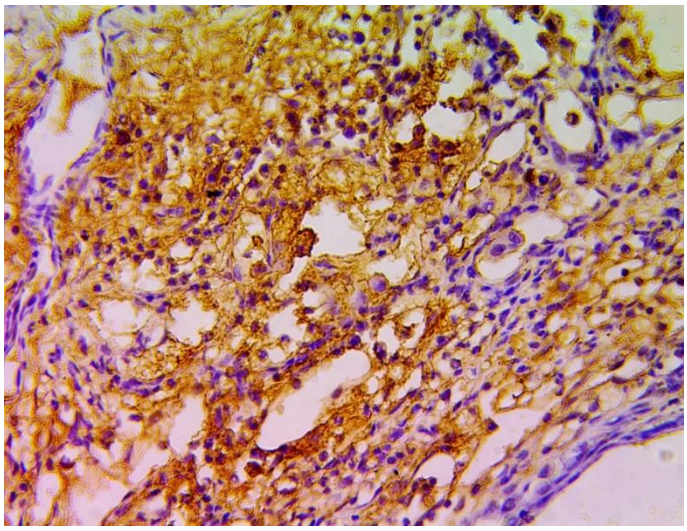

FIG. (6): Immunohistochemistry (IHC) for TGF-β in the lung tissue of Lung fibrosis control group showed marked positive staining. (TGF-β IHC x100) and (TGF-β IHC x400)

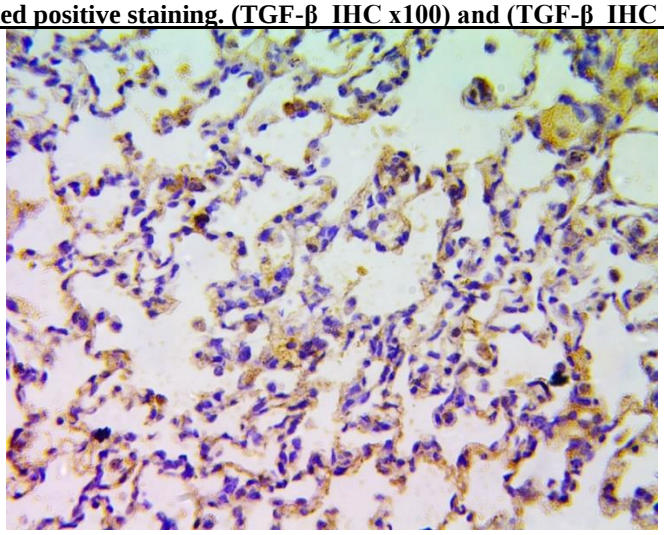

FIG. (7): Immunohistochemistry (IHC) for TGF-β in the lung tissue of Metformin-treated lung fibrosis group showed mild positive staining. (TGF-β IHC x100) and (TGF-β IHC x400)

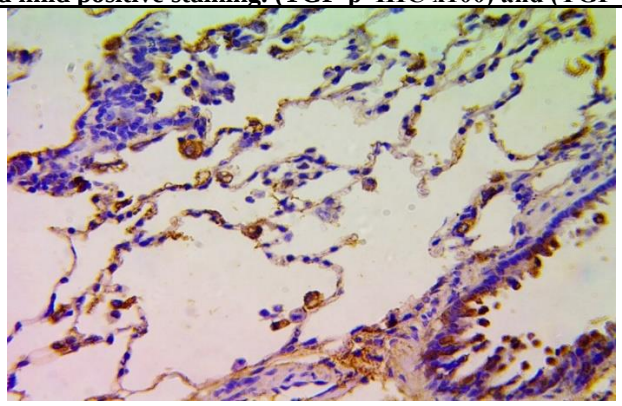

FIG. (8): Immunohistochemistry (IHC) for TGF-β in the lung tissue of Canagliflozen-treated lung fibrosis group showed focal mild positive staining. (TGF-β IHC x100) and (TGF-β IHC x400)

Table 13: Results: Area stained by Immune & Masson Trichrome stains

Group / Stain	Normal control	Lung Fibrosis control	Metformin Lung Fibrosis fibrosis	Canagliflozin Lung Fibrosis fibrosis	KW P-value
Immune stain	3.7(2.5-4.9)[a]	13(9.5-16.5)	2.4(2-2.8)[j]	4(2.6-7.8)[k]	.018*
Masson Trichrome stain	0.6(0.4-1.1)	13.1(12.8-13.5)[m,s]	2(1.5-3)[j,q,s]	3(1.1-3.5)	.011*

Variables are expressed as Median (IQR) *Significant (p-value <.05) Values in the same row with different letters (a-u) are significantly different (p-value <.05)

Discussion

This study explored the therapeutic effects of Canagliflozin in mitigating lung fibrosis and pulmonary complications in comparison with Metformin. The key findings demonstrated significant modulations in SMAD2/3 levels, protein content, LDH activity, WBC counts, and other parameters, highlighting the potential mechanisms of action for Canagliflozin.

Idiopathic pulmonary fibrosis (IPF) encompasses 17 to 37% of all interstitial lung disease diagnoses [21] and commonly affects patients over 60 years, with progressive failure of lung function causing death on average three years after diagnosis [22], categorizing it as a disease with poor survival. The pathogenesis is complex and involves multiple molecular pathways resulting from sustained or repetitive lung epithelial injury and subsequent activation of fibroblasts and myofibroblast differentiation [23]. Persistent myofibroblast phenotype contributes to excessive deposition of the extracellular matrix (ECM) and aberrant lung repair, leading to tissue scar formation, distortion of the alveolar structure, and irreversible loss of lung function [24]. Most clinical trials on IPF have failed to meet the primary endpoint, and an urgent need remains to identify agents or treatment strategies that can stop disease progression [25].

The current study revealed that SMAD 2/3 expression was significantly increased in the lung fibrosis control group compared to the normal control group, which aligns with findings by Gaikwad et al. [26], who demonstrated elevated SMAD 2/3 in IPF lungs. This supports the idea that the SMAD 2/3 pathway plays a central role in IPF progression. Metformin treatment significantly reduced SMAD 2/3 levels, consistent with Wang et al. [27], who reported that Metformin decreased SMAD 2/3 in bleomycin-induced fibrosis by inhibiting the TGF-β pathway. Similarly, Canagliflozin treatment also lowered SMAD 2/3 expression, in agreement with Zhang et al. [28], who found that SGLT2 inhibitors reduce SMAD activity, thus preventing fibrosis and tissue remodeling.

The current study revealed that RAGE expression was significantly increased in the lung fibrosis control group compared to the normal control group, highlighting the role of RAGE signaling in fibrosis, as supported by He et al. [29]. Treatment with Metformin significantly reduced RAGE levels, consistent with Lin et al. [30], who showed that Metformin improves inflammation by inhibiting RAGE expression. Similarly, Canagliflozin reduced RAGE levels, aligning with Sourris et al. [31], who reported that SGLT2 inhibitors mitigate RAGE expression through improved glycemic control, oxidative stress reduction, and AGE regulation.

The current study revealed that AGE levels were significantly increased in the lung fibrosis control group compared to the normal control group, consistent with Kyung et al. [32], who

identified AGE accumulation as a hallmark of IPF. Metformin reduced AGE levels, consistent with Salazar et al. [33], who explained that Metformin prevents AGE formation due to its antioxidant and anti-inflammatory properties. Similarly, Canagliflozin treatment lowered AGE levels, in agreement with Sourris et al. [31], who highlighted the role of SGLT2 inhibitors in reducing AGE formation by improving glycemic control and oxidative stress.

The current study revealed that oxidative stress markers were significantly altered in the lung fibrosis control group, with increased MDA and decreased SOD levels compared to the normal control group. These findings are consistent with Gungor et al. [34] and Karamalakova et al. [35], who linked bleomycin-induced fibrosis to oxidative stress and antioxidant enzyme depletion. Metformin treatment reduced MDA levels and increased SOD, aligning with Liu et al. [36], who demonstrated Metformin's ability to enhance antioxidant defenses and reduce oxidative damage through inhibition of NOX4. Similarly, Canagliflozin reduced MDA and increased SOD levels, supported by Luna-Marco et al. [37], who reported enhanced antioxidant enzyme activity with SGLT2 inhibitors.

The current study revealed that inflammatory markers, including protein, LDH, and WBC levels in BALF, were significantly elevated in the lung fibrosis control group compared to the normal control group, indicating inflammation and tissue damage. These findings agree with Li et al. [38] and Kreuter et al. [39], who reported increased inflammatory markers in IPF. Metformin treatment significantly reduced these markers, consistent with Kolieb et al. [40], who demonstrated Metformin's anti-inflammatory effects in reducing inflammatory cell infiltration. Similarly, Canagliflozin decreased protein, LDH, and WBC levels, aligning with Bastawy et al. [41], who noted reduced LDH levels and inflammation with SGLT2 inhibitors.

The current study revealed that fasting blood glucose (FBG) was significantly higher in the lung fibrosis control group compared to the normal control group. Both Metformin and Canagliflozin significantly reduced FBG levels, with no significant difference between the two treatments. These findings are compatible with Mabrouk Gabr et al. [42], who demonstrated Canagliflozin's effectiveness in improving glycemic control by increasing urinary glucose excretion, and Yang et al. [43], who found no superiority between the two drugs in regulating glucose levels.

The current study revealed that lung weight was significantly increased in the lung fibrosis control group compared to the normal control group. Metformin treatment significantly reduced lung weight, consistent with Sarrafha and Javadi [44], who attributed this reduction to Metformin's ability to inhibit oxidative stress and inflammation. Canagliflozin also reduced lung weight, although to a lesser extent, in agreement with Kabel et al. [45], who highlighted SGLT2 inhibitors' role in reducing oxidative stress and fibrotic markers.

The current study revealed that histopathological findings using Masson Trichrome stain showed marked fibrosis in the lung fibrosis control group, which improved significantly with both Metformin and Canagliflozin treatments. These results align with Xiao et al. [46], who demonstrated Metformin's antifibrotic effects by inhibiting fibroblast activation, and Wang et al. [47], who showed Canagliflozin's antifibrotic effect through the inhibition of TGF-β/p-Smad3 signaling. Immunohistochemical staining for TGF-β1 further confirmed strong positive staining in the lung fibrosis control group, which was significantly reduced with both treatments. These findings align with Ma et al. [48] and Chen et al. [49], who demonstrated the antifibrotic roles of Metformin and SGLT2 inhibitors in reducing TGF-β1 expression and fibrotic protein deposition.

Conclusion

The findings of the current study highlight the significant pathological alterations in lung fibrosis, including increased oxidative stress, inflammation, fibrosis markers, and lung remodeling. Both Metformin and Canagliflozin demonstrated notable therapeutic potential by mitigating these pathological changes, as evidenced by their ability to reduce SMAD2/3 expression, AGEs, RAGE, MDA, protein levels, LDH, and WBC counts while enhancing antioxidant defenses (SOD). Metformin showed a slightly stronger antifibrotic effect, reflected in greater normalization of lung weight and histopathological markers, though it was associated with weight loss. In contrast, Canagliflozin preserved body weight while also demonstrating strong anti-inflammatory and antioxidant properties. These results suggest that both Metformin and Canagliflozin could be promising candidates for the management of lung fibrosis. Metformin may be preferred for its robust antifibrotic effects, while Canagliflozin offers additional benefits in metabolic regulation and weight preservation. Further studies are recommended to explore the mechanisms of these therapies and their potential synergistic effects in combination treatments.

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