Original Article

Investigation of the effect of metformin and aloe vera on the reduction of pulmonary fibrosis caused by bleomycin in rat models

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Abstract

Background and aim: Bleomycin is an antibiotic with anti-tumor activity. The main problem with the use of this drug is the occurrence of dangerous complications of pulmonary fibrosis. The present study investigated the effect of metformin as an anti-inflammatory and anti-fibrosis and aloe vera gel with antioxidant properties and the combination of the two on the inhibition of pulmonary fibrosis due to bleomycin injection. **Materials and Methods:** Seven groups of adult male Wistar rats were selected with a weight range (20,200%). In addition to the control group who received a single intra-tracheal dose of 0.3 ml of sterile saline, the other groups were prescribed intra-tracheal bleomycin 1 ml per 100 g body weight in the volume of 0.3 ml. Therapeutic groups included: (1) group metformin 500 mg/kg daily, (2) aloe vera 500 mg/kg daily, (3) aloe vera 700 mg /kg daily, (4) the combined group received 500 mg/kg drug and 500 mg/kg plant simultaneously intraperitoneal as treatment in a 21-day treatment period after intra-tracheal administration of bleomycin. Data were analyzed using 22spss software to analyze the overall comparison of the completely randomized variance analysis test and Duncan's multiple range test was used to compare the groups. In all experiments, $p < 0.05$ was considered as a significant difference. **Results:** The final weight of the animals during 21 days of experiments in negative control group (normal saline) was 191.55±2.13, positive control group (bleomycin) 192.85±2.63, in treatment group (1) 198.50 ±8.61, treatment group (2) 197.50 ±1.51, treatment group (3) 217.75 ±2.19, treatment group (4) 219.50±1.51 that treatment groups have shown a decrease compared to the control groups, but this decrease is not statistically significant ($P< 0.05$). The following results were obtained for the average lung weight of each group in grams: Negative control group (normal saline) = 1.2, positive control group (bleomycin) = 2.82, treatment group (1) = 1.62, treatment group (2) = 1.42, treatment group (3) = 1.43 and treatment group (4) is 1.25. **Conclusion:** According to the results, metformin, as a potent anti-fibrosis agent, which is probably due to reduced synthesis of prostaglandins, as well as aloe vera with high antioxidant effect, are able to inhibit collagen accumulation. According to the results obtained in the combined group of these two substances, a sharp decrease in the amount of collagen accumulation can be observed, which indicates its greater effect.

Keywords: Bleomycin, Metformin, Pulmonary Fibrosis, Aloe Vera Gel

Introduction

Bleomycin is a chemotherapeutic antibiotic produced by the bacterium Streptomyces verticillus and is used to treat testicular, head and neck squamous cells, throat, cervix, skin and kidneys cancers. It may also be effective in peritoneal malignant tumors and pleural and in the treatment of Hodgkin's and non-Hodgkin's lymphoma [1]. Unlike many cytotoxic agents, bleomycin has little effect on the bone marrow and immune system and rarely has significant suppressive effects. Due to its excellent performance, it is one of the most suitable drugs for the treatment of these cancers, but the main problem with the use of this drug is its side effects, i.e. pulmonary fibrosis in many cases [2]. Several mechanisms have been proposed for pulmonary fibrosis, including the following: 1) Apoptosis in the alveolar and bronchial epithelial cells, which triggers the fibrosis cycle. 2) Inflammation and increased cyclooxygenase activity, secretion of inflammatory cells into the alveolar space, releasing of proteolytic enzymes and inflammatory mediators by neutrophils and lung macrophages. 3) Pre-inflammatory chemokine and cytokines such as interleukins, tumor necrosis factor (TNF-α), growth factors such as beta growth factor

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(TGF-β) and NF-KB transcription factor. 4) Oxidative and peroxidation stress of membrane lipids. 5) Discharge of natural antioxidant systems such as glutathione and discharge of ATP, NAD. 6) Iron presence in the lungs: Iron as the main cofactor for oxidative damage of bleomycin and cofactor is essential for the activity of the enzyme prolyl-hydroxylase, which is involved in the formation of collagen. 7) Increase and accumulation of extracellular collagen matrix by fibroblasts and over-expression of factors that prevent the breakdown of collagen such as metalloproteinase (TIMP) [3]. The causes of pulmonary fibrosis and pathogenesis of the disease have not yet been determined, but various factors such as reactive oxygen species, growth factors, inflammatory cells such as lymphocytes and neutrophils, cytokines and chemokine play an important role in a fibrotic process [4, 5]. It also occurs in some bone marrow transplant recipients and in people with chronic inflammatory diseases such as scleroderma and rheumatoid arthritis [6]. Metformin is actually a dimethyl biguanide that targets cell metabolism and can be effective in treating fibrosis [7]. In recent years, much attention has been paid to the antioxidant role of metformin [8]. Metformin is not able to reduce the effects of free radicals such as reactive oxygen species (ROS) by storing antioxidants and clearing superoxide, and does not react with hydrogen peroxide [9]. Metformin has an anti-inflammatory effect by reducing the secretion of interleukin 6, mediating TNF-α (alpha tumor necrosis factor) and preventing the production of IL1, IL6 and IL8 [10]. Metformin activates AMPK through two separate mechanisms, namely inhibiting the mitochondrial respiratory chain and subsequently increasing the AMP / ATP and ADP / ATP increase ratios or direct AMPK activation. AMPK protects cellular processes such as autophagy, fatty acid oxidation, inflammation, fibrosis, oxidative stress, and reactive oxygen species (ROS) and the production of FGF23 in bone cells, against AKI and CKD [11].

Aloe vera is a plant made up of 96% water and 4% dry matter, 75 of which are known to contain dry matter, including vitamins, non-organic salts, minerals, enzymes, sugars, phenolic compounds, anthraquinone, lignin, emodin, saponins, sterols, amino acids, salicylic acid, sulfates, tannins and steroids [12]. The compounds found in aloe vera gel are polysaccharide compounds that can reduce and repair inflammation. These compounds also have antimicrobial properties. Antioxidants in the form of vitamins A, E, amino acids and essential fatty acids are also found in it. Aloe vera gel contains powerful antioxidants that belong to a large and well-known family of polyphenols. Aloe vera strengthens and modulates the immune system in viral diseases such as herpes, and is anti-inflammatory [13]. Therefore, research on the possible beneficial or harmful effects of this plant on the reduction of pulmonary fibrosis caused by the anti-cancer drug of bleomycin seemed necessary, and one of the useful strategies to investigate this possibility is to use different animal models. Therefore, the aim of this study was to investigate the effect of aloe vera and metformin extract on the reduction of pulmonary fibrosis caused by the anti-cancer drug bleomycin in rats.

Methods

The changes caused by the injection of bleomycin in a rat laboratory animal are almost identical to those in humans [14]. This paper used a Wistar rat male laboratory animal. Because of the importance of the animal's weight during the test and also to eliminate the effect of weight changes, the selected animals must all have a certain weight range. It is best to weigh less than 150 grams or no more than 220 grams. Less than 150 grams of the animal is very young, and more than 220 grams of the animal is fully grown, and the body's fatty tissue increases, dispersing the results. In this study, rats were selected with a weight range of 200 ± 20. The rats were divided at 23 ± 2°C and 12 hours of darkness and suitable humidity. Standard water and food of rat were provided free of charge to the animals. The rows were randomly divided into six groups of four and placed in separate cages, and the floor of the cage was covered with clean, coarse tracheal.

To evaluate the antioxidant and anti-fibrotic effects of metformin pulmonary protection in bleomycin-induced pulmonary fibrosis, a dose of 500 kg /mg was selected [15]. To investigate the antioxidant and pulmonary protective effects of aloe vera gel in pulmonary fibrosis caused by bleomycin, a dose of 500 kg / mg and 700 kg / mg 700 was selected [16]. In this study, animals were classified into six groups, with four rats in each group. 1) Negative control group (normal saline): recipient of 0.5 cc drug carrier (normal saline) for 21 consecutive days after administration of a single dose of intra-tracheal bleomycin (normal saline), 2) Positive control group (bleomycin): recipient of 5.0 cc drug carrier (normal saline) for 21 consecutive days after administration of a single dose of 0.3 cc intra-tracheal bleomycin, 3) Metformin treatment group: recipient of 500 mg/kg intra-tracheal metformin for 21 consecutive days, 4) Aloe vera treatment group: recipient of 500 mg/kg of intra-tracheal aloe vera gel for 21 consecutive days, 5) Aloe vera group treatment: recipient of 700 mg / kg aloe vera gel intraperitoneally for 21 consecutive days, 6) Combination treatment group: recipient of 500 mg of Kg/mg metformin and 500 mg/kg of aloe vera gel simultaneously intraperitoneally for 21 consecutive days.

On the second day of the experiment, treatment groups received a single dose of 0.3 cc bleomycin through an intra-tracheal.

During the experimental period, animals should be considered for general and respiratory status as well as hair condition, hair loss and daily animal activities. As mentioned, the weight of the animal is an important factor and should be considered. After a period of 21-days, each of the animals anesthetized and their chests were opened with the help of surgical instruments. After disconnecting the trachea and arteries that connect to the lungs and the appendages around it, the lungs were quickly removed from the chest and washed with normal saline, dried with sterile gauze, and then the total

weight of each animal's lungs was calculated by a laboratory scale [17]. Then, a lobe was removed from each lung for biochemical tests and a lobe for pathological tests and weighed. For each group of rats, the mean of variable was expressed as mean ± SEM, and the statistical variance analysis (ANOVA) was used to compare the means and to evaluate and determine the difference between the means and to make the results significant, Duncan's multiple range test test was analyzed using SPSS software and in all experiments, $P<0.05$ was considered as a significant difference.

Data analysis

Graph of body weight change trend in grams in different days before and after injection into bleomycin trachea.

As the results show, weight changes during the experimental period in the control groups showed a gradual increase until the twenty-first day of the experiment, in the bleomycin group have increased in weight until the day of injection into the trachea, but have decreased sharply from the day of injection to four days and then there was a gradual weight loss until twenty-first day. In the drug, plant, and combination groups, a weight gain is seen until the day of injection, and from the day of injection of intra-tracheal bleomycin, a weight loss is seen for 4 days, and then there is a gradual weight gain until the end of the test.

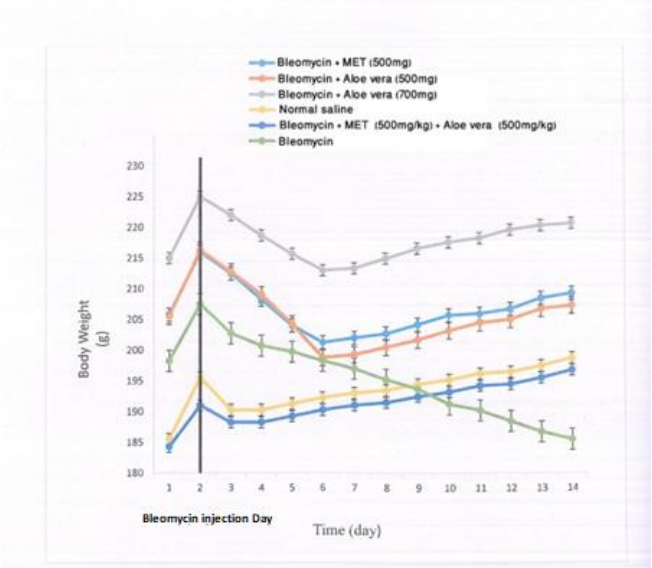
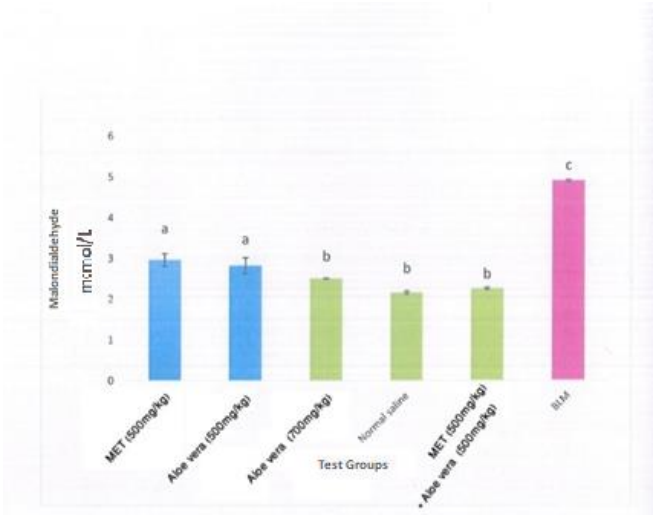
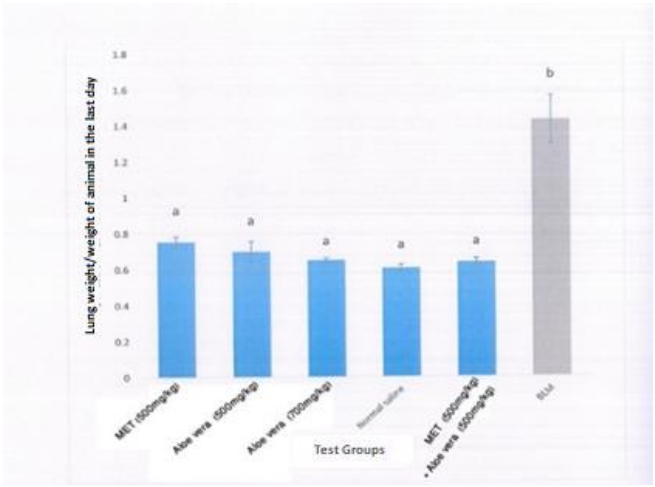


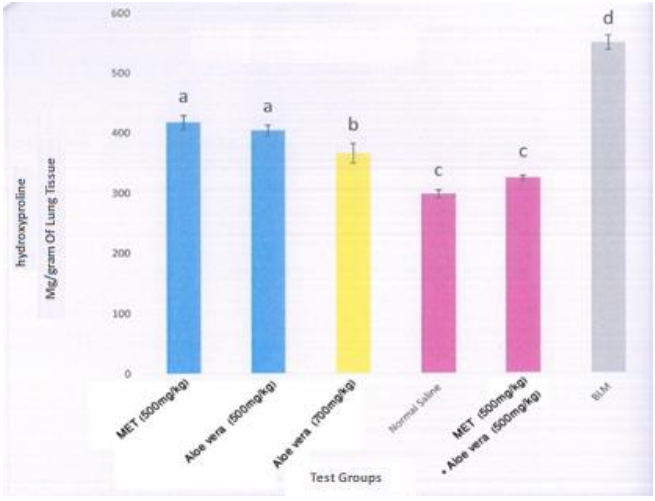
Diagram of the effect of treatment groups on lung weight gain in different groups tested



The effect of treatment groups on the ratio of lung weight to the weight of the last day of the animal in different groups tested



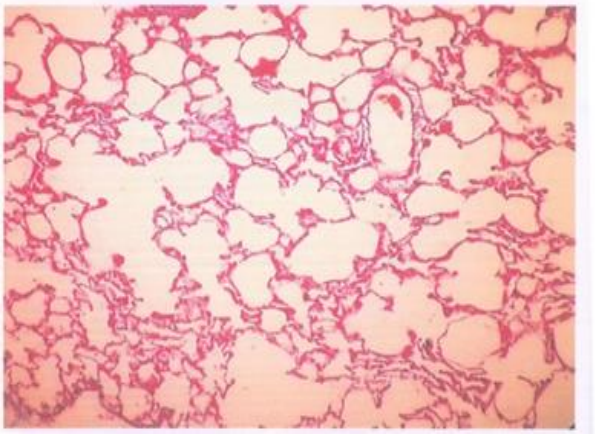
The effect of treatment groups on the level of hydroxyproline in the whole lung (mg/g tissue) in different groups tested



The effect of therapeutic groups on the total level of malondialdehyde (µm/l) in different groups tested

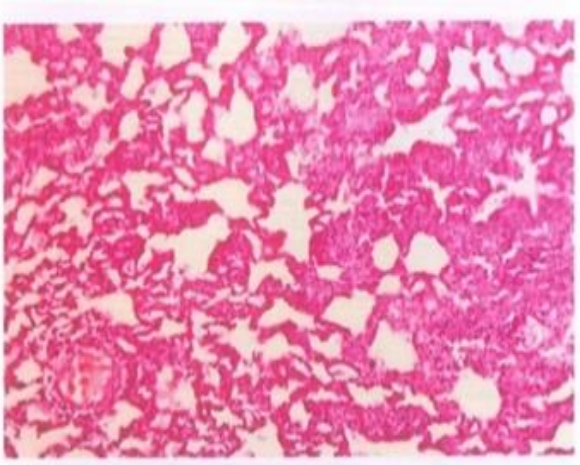
Using the one-way analysis of variance (ANOVA) and the Duncan test, groups with similar letters and colors have similar effects according to the Duncan test (P <0.05) and are not significantly different. According to statistical studies of analysis of variance and comparison of means with Duncan's test method, the amount of hydroxyproline and malondialdehyde of the whole lung of bleomycin group increased by 83% and 126%, respectively, compared to the control group, which this increase was statistically significant (P<0.05). In the metformin group, in terms of these factors, there was an increase of 40% and 37%, respectively, compared to the control, which was statistically significant (P <0.05). In the same group, these factors were reduced by 23% and 39%, respectively, compared to the bleomycin group, which was statistically significant. In the Aloe vera gel group, the hydroxyproline and malondialdehyde levels of the whole lung increased by 35% and 30%, respectively, compared to the control group, this increase was statistically significant (P <0.05) and the same factors decreased by 26% and 42%, respectively, compared to the bleomycin group, which was statistically significant (P <0.05). In the metformin + aloe vera group, the amount of hydroxyproline and malondialdehyde in the whole lung increased by 22% and 16%, respectively, compared to the control group, which was not statistically significant, and the same factors were reduced by 33% and 48%, respectively, compared to the bleomycin group, which was statistically significant (P <0.05).

The results of histological tests performed in the control group show that the alveolar walls are thin and natural, the accumulation of collagen and fibrosis is not seen in the alveolar areas (H&E X).

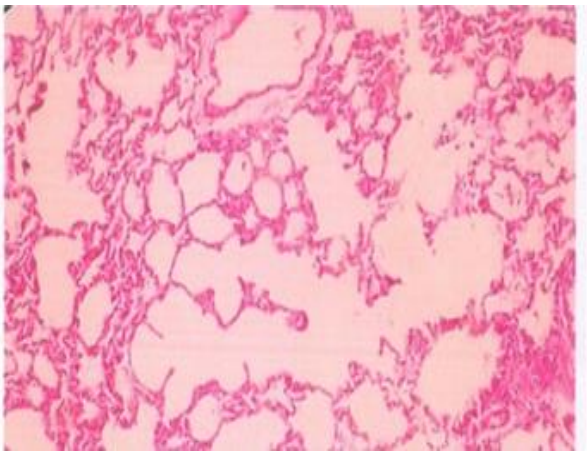


The apparent cross-section of the rat's lung tissue, four weeks after intraperitoneal injection of normal saline.

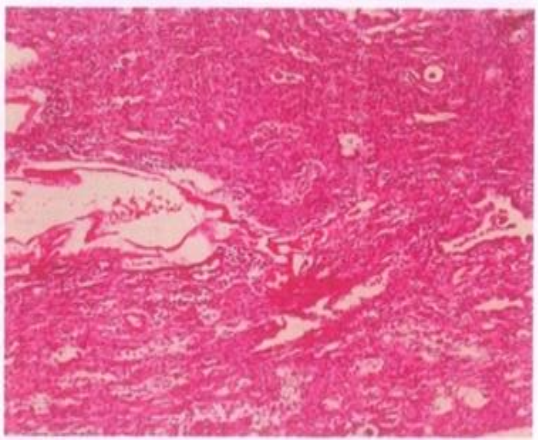
The results of histological tests performed on the positive control group (bleomycin) show that in most areas, the walls of the alveolus have ruptured and become open and irregular spaces. In other words, the emphysema area can be clearly seen. In fact, in this sample, level 4 of fibrosis is observed. It should be noted that various lesions, including emphysema and fibrosis caused by bleomycin, have been observed on a slide.



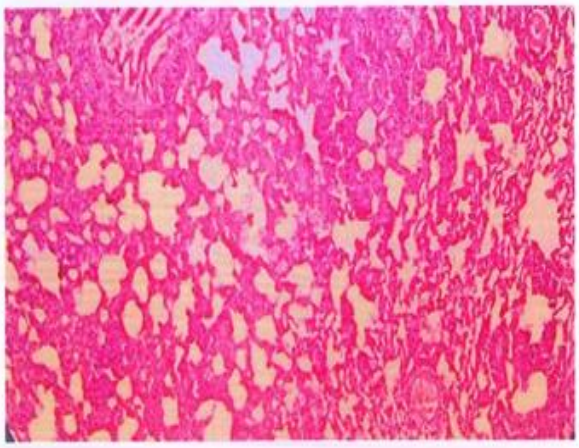
The apparent cross-section of the rat's lung tissue, 21 days after intra-tracheal administration of bleomycin, as a single dose.



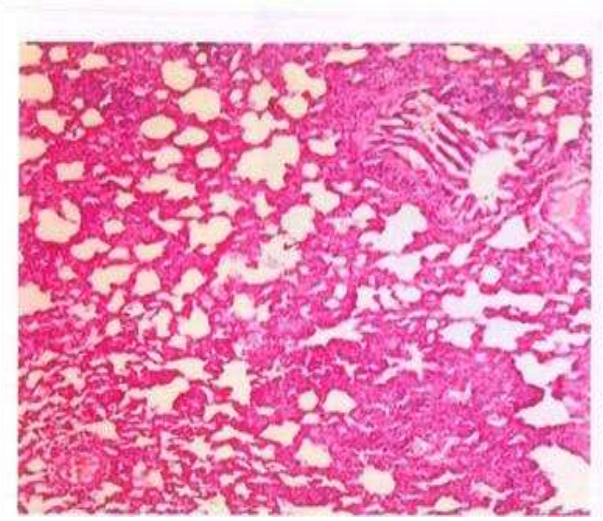
The apparent cross-section of the rat's lung tissue, 21 days after intra-tracheal administration of bleomycin, metformin treatment group.



The apparent cross-section of the rat's lung tissue, 21 days after intra-tracheal administration of bleomycin, treatment group of 500 aloe vera gel.



The apparent cross-section of the rat's lung tissue, 21 days after intra-tracheal administration of bleomycin, treatment group of 700 aloe vera gel.



The apparent cross-section of the rat's lung tissue, 21 days after intra-tracheal administration of bleomycin, Metformin + Aloe Vera gel treatment group

In the drug groups, it was observed that most of the alveoli were healthy and were much less visible in the interstitial tissue and around the bronchioles compared to the group of bleomycin fibrosis, and the severity of fibrosis and tissue damage was reduced. Also, with the coloring of Masson's trichrome, the amount of collagen fibers in the drug groups is very small compared to the bleomycin group.

Discussion and Conclusion

In the present study, according to the mechanism of fibrosis, metformin and aloe vera compounds and the combination of the two were considered together, which according to the results, as well as studies, it seems that while not damaging the body's tissues, each of them can inhibit this fibrosis through a specific mechanism or with the help of.

According to a study by Sun Mi Choi et al. (2016) metformin in rat was reduced by decreasing the pulmonary fibrosis caused by bleomycin. The results showed a decrease in mRNA levels of collagen, procollagen, fibronectin in rat treated with metformin on the 21st day, compared with rat of the bleomycin group [18], which is consistent with the results obtained in this study. Nanda Gamad et al. (2018) in study of "decreased pulmonary fibrosis from bleomycin by metformin" was performed on the rat animal model. In the treatment group with metformin, there are histopathological changes, increased inflammatory cells, lipid peroxidation, decreased reserve antioxidants and increased inflammatory mediators (TNF-α) as well as high levels of TGF-β, Smad2/3, ERK1/2, p38, JNK, fibronectin, hydroxyproline and type 1 collagen enhancement resulting from the administration of bleomycin, all improved with high doses of metformin. Structural, biochemical, and molecular changes are natural. This protective effect may be attributed to the activation of AMPK by metformin and thus to the reduction of oxidative stress and TGF-β [19].

In a study by Cahova M et al. (2015), metformin prevented oxidative stress caused by ischemic perfusion in fatty liver by weakening the formation of reactive oxygen species, the results showed that metformin reduced inflammation expression (TNF-α, TLR4, IL-1β, Ccr2), and infiltrative monocytes (Ly6c) and macrophages (CD11b). The data show that metformin reduces mitochondrial function but also protects against liver damage from I/R. It has also been suggested that the beneficial effect of metformin on a combination of three mechanisms is helpful: increasing the activity of the antioxidant enzyme, producing ROS less than mitochondria, and reducing ischemic inflammation [20].

In a study by Yebpella G. G et al. (2011), "Photochemical screening and comparative study of antimicrobial activity of various aloe vera extracts," the results of phytochemical screening showed the following metabolites: saponins, alkaloids, glycosides, tannins, proteins and flavonoids. These antimicrobial activities in plant gel at 10% DMSO, which showed that green skin gel has the most properties [21].

A study by Lawrence et al. (2009) examined the isolation, purification, and evaluation of Aloe vera antibacterial agents, the results show that ethanol, methanol and acetone extracts from aloe vera gel were tested for their antimicrobial effect against four gram-positive and gram-negative bacteria using the Agar diaphragm diffusion method. Various extracts showed the level of antimicrobial activity compared to the tested pathogens. The results showed that ethanol and methanol extracts were more active, while acetone extract was less active than most of the tested pathogens. This study shows that the antimicrobial activity of A. vera jelly extract depends on the synergistic effect of different compounds. Aloe vera gel can be further recommended in the treatment of various bacterial diseases due to its broad antimicrobial effects [22].

The drugs and plants used with their antioxidant and anti-inflammatory effects have been able to prevent the

occurrence or progression of some of these reactions and thus slow down the progression of fibrosis, although the effect of these substances in the prescribed dose has not been 100%, it can be hoped that by using these compounds in high doses, it is possible to control or delay the unwanted process of fibrosis. Histological studies of the lungs in the treated groups showed that the pulmonary damage caused by bleomycin decreased compared to the positive control group (bleomycin). Comparison of the degree of inflammation and fibrosis in these groups is quite significant. Histological results show a better performance of the selected treatment materials than biochemical results.

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