Original Article

Effect of Moringa Leaf Extract on Inhibiting Lung Fibrosis in Wistar Rats with Diabetic Model

Zahrina Isnandia Zaenab[1], Suryono[2], Ayu Munawaroh Aziz[3]

[1]Faculty of Medicine, University of Jember, Jember, Indonesia
[2]Department of Cardiovascular, Faculty of Medicine, University of Jember, Regional General Hospital dr. Soebandi, Jember, Indonesia
[3]Department of Histology, Faculty of Medicine, University of Jember, Jember, Indonesia

Abstract

Background: Uncontrolled diabetes can lead to macrovascular and microvascular complications in various organs. Organ fibrosis is one of the microvascular complications due to diabetes. Hyperglycemia triggers ROS formation thereby causing oxidative stress. Oxidative stress enhances pro-inflammatory and pro-fibrotic activities. The lungs have a lot of vascularization and connective tissue, making them susceptible to diabetes complications. Moringa is called 'The Miracle Tree' because it has many properties. Moringa leaves are the most studied part, for their efficacy as antioxidants. This study examines the effect of moringa leaf extract (MLE) in inhibiting lung fibrosis in diabetic Wistar rats.

Methods: A total of 27 male Wistar rats were randomized into three groups, K0, K1 and P. Group K0 was given saline (ip) and saline (orally); K1 was given Streptozotocin (STZ) (ip) and saline (orally); P was given STZ (ip) and MLE (orally). MLE was administered daily at 1000 mg/Kg BW dose for four weeks. Lung fibrosis assessment was carried out using a modified Ashcroft scale.

Results: Lung fibrosis assessment showed that the K1 group had the highest mean of lung fibrosis and K0 had the lowest mean of lung fibrosis. Kruskal-Wallis test showed that there was a significant difference in the data comparison between the three groups. The post-hoc test showed that there was a significant difference in the data comparison between groups. Comparison of the K0-K1 group data showed the smallest significance value and comparison of the K0-P group data showed the highest significance value.

Conclusion: MLE can inhibit lung fibrosis in diabetic Wistar rats.

Keywords: diabetes, hyperglycemia, lung fibrosis, MLE, STZ

Corresponding Author:
Suryono | Department of Cardiovascular, Faculty of Medicine, University of Jember, Regional General Hospital dr. Soebandi, Jember, Indonesia | suryonofiha@gmail.com

Submitted: May 21st, 2023
Accepted: July 27th, 2024
Published: July 31st, 2024

J Respirol Indones. 2024
Vol. 44 No. 3: 207–12
https://doi.org/10.36497/jri.v44i3.534

INTRODUCTION

Indonesia is ranked fifth as a country with the largest number of diabetics in the world.[1] In Indonesia, there are still many diabetics who are not even aware that they have diabetes and also have low awareness to undertake routine control.[2]Uncontrolled diabetes can lead to complications in various organs. Diabetes complications can be in the form of microvascular and macrovascular disorders. Complications also vary, depending on the duration of suffering and diabetes control. Organ fibrosis is one of the microvascular complications of diabetes.[3,4]

Hyperglycemia plays an important role in activating the fibrogenic cascade in diabetes.[4] Hyperglycemia induces excessive reactive organ species (ROS) production in mitochondria and causes DNA damage. During the DNA repair process, PARP will be activated. PARP will inhibit GAPDH. GAPDH activation will disrupt the glycolysis process. Thus, the glycolysis process is routed through pathogenic signaling pathways: the polyol pathway, the hexosamine pathway, activation of protein kinase C and formation of advanced glycation end products (AGEs). These pathways will enhance ROS generation. The imbalance between ROS and antioxidants triggers oxidative stress. Oxidative stress induces the release of pro-inflammatory and pro-fibrotic mediators and results in the formation of fibrosis in various organs.[5]

The lungs are susceptible to diabetes complications due to their abundant vascularity and connective tissue.[6] Cohort studies show that diabetic patients have a higher risk of lung disease including

lung cancer, tuberculosis, asthma, lung fibrosis and chronic obstructive pulmonary disease (COPD).[7]

Moringa is called "*The Miracle Tree"* because it has many benefits.[8] Among other parts, moringa leaves are the most studied for their nutritional content and function, especially as an antioxidant.[9] Previous studies have found the highest antioxidant activity in Moringa leaves.[10] Antioxidant mechanisms by increasing antioxidant enzymes and inhibiting pro-oxidant enzymes.[11]

Among several doses tested, the dose of 1000 mg/Kg BW showed the best results to inhibit glomerular damage due to streptozotocin (STZ) induced.[12] Other studies have also examined the toxicity of moringa leaf extract (MLE) in various organs and concluded that MLE is safe to use.[13] The description above underlies researchers to examine the effect of MLE in inhibiting lung fibrosis in Wistar rats with diabetic models.

METHODS

This is a true experimental study with a post-test-only control group design conducted from November 2021 to March 2022. The procedure has been approved by the Ethics Commission of the Faculty of Medicine, University of Jember. Twenty-seven male Wistar rats (weight 200–300 g, 2–3 months old) were housed for seven days and randomized into three groups, normal control group (K0), diabetes control group (K1) and MLE group (P).

The K1 and P group rats were injected with STZ at a dose of 45 mg/Kg BW intraperitoneally (ip), while the K0 group was injected with saline. After injection with STZ, rats were provided with dextrose 10% overnight to prevent hypoglycemic shock. Three days later, fasting blood glucose levels were measured. The treatment was carried out for four weeks by giving MLE at 1000 mg/Kg BW dose orally to group P. Meanwhile, groups K0 and K1 were given saline. MLE was obtained by maceration with 96% ethanol for three days.

After four weeks, the rats were terminated and necropsied to collect lung organs. Histopathological preparations were made using Masson's trichrome staining and lung fibrosis was assessed using the modified Ashcroft scale.[14]

The Kruskall-Wallis test was chosen to compare data between three groups. Then, followed by a post hoc test to compare data between groups.

RESULTS

Kruskal-Wallis test results showed a significance value of *P*=0.0001. This means that there is a significant difference in the data comparison between the three groups (Table 1).

Table 1. Kruskal-Wallis test results

Detail	Results
Kruskal-Wallis	22,799
Df	2
Significance	0.0001

Data analysis was followed by a post-hoc test to see comparisons between groups (Figure 1). Post-hoc test results showed significant differences in all comparisons between groups. Comparison of the K0-K1 group showed no significant difference value, while the comparison of the K0-P group showed the highest significance value.

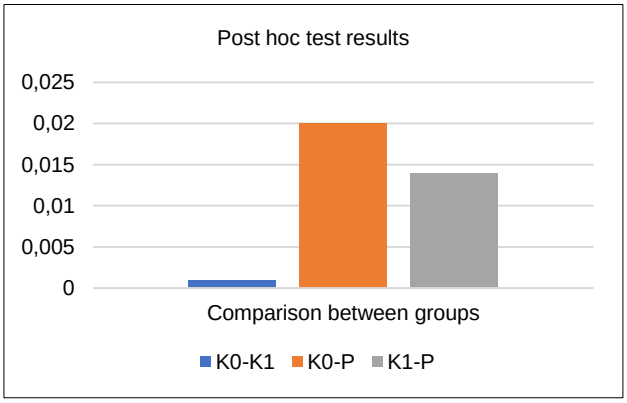

Figure 1. Post hoc test results

The modified Ashcroft scale is used as a parameter to assess the histopathology of lung fibrosis. The mean score of lung fibrosis for each group can be seen in Figure 2. The K1 group showed the highest mean score of lung fibrosis and K0 showed the lowest score.

The histopathology of rats' lungs can be seen in Figure 3. The K0 group showed that the lung tissue structure was still intact, although septal thickening could be seen in several parts. The K1 group showed

fibrous masses >50% of the field of view and in some rats, fibrous masses were seen covering the entire field of view. Group P showed fibrotic mass ≤50% of the field of view.

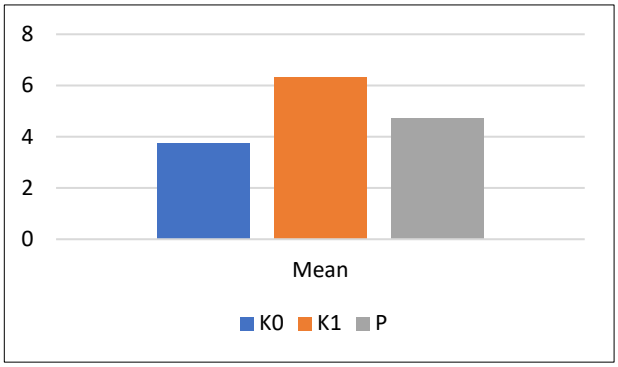

Figure 2. The mean score of lung fibrosis for each group

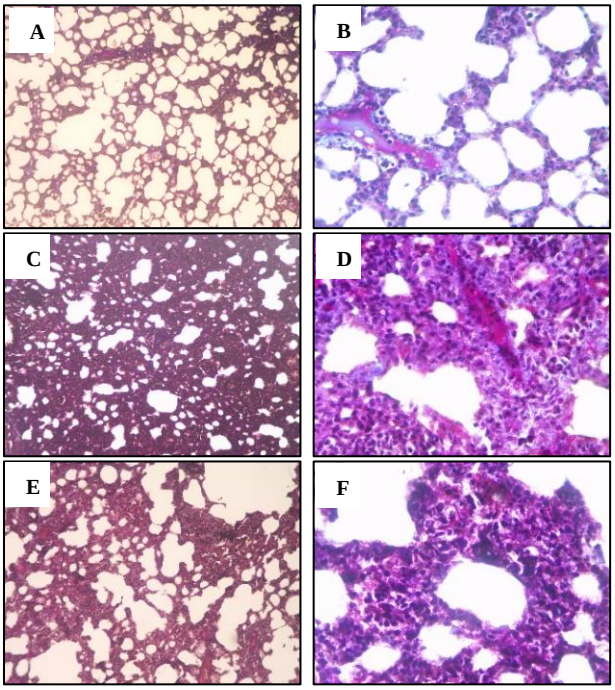

Figure 3. Histological features observed in lung tissue of experimental group, magnification: 100x dan 400x. K0 group (A & B); K1 group (C & D); P group (E & F).

DISCUSSION

The diabetic group showed the highest mean of lung fibrosis. Data analysis also showed significant differences when tested with the other two groups. This supports the idea that lung fibrosis is a complication of diabetes and that lungs are vulnerable to the effects of hyperglycemia.[4,6] Previous studies have also found changes in lung function and structure due to complications from diabetes.[15–17]

The MLE-treated group showed a lower mean of lung fibrosis than the K1 group. Data analysis for the P group also showed a significant difference when tested with the K1 group. These prove that MLE can inhibit the development of lung fibrosis. These results were also strengthened by previous studies that discussed the ability of moringa leaf extract to inhibit fibrosis and damage to several organs, such as the liver and kidney.[12,17–19] Those studies state that the antioxidant effect of moringa leaf extract will reduce the incidence of oxidative stress and prevent the release of pro-inflammatory signals thereby reducing pro-fibrotic activity in organs.[12,17–19]

Data analysis between the MLE and normal groups also showed significant results. We are trying to find out whether MLE at 1000 mg/Kg BB dose is toxic to lung tissue. Previous studies evaluated MLE toxicity in various organs, especially the lungs.[20,21] The results of this study showed no signs of toxicity from the use of MLE in rats and it was concluded that MLE is safe for consumption. Other studies also stated that the use of ethanol solvents in the manufacture of moringa leaf extract showed a safer effect compared to using water solvents.[22]

In the normal group, thickening of the alveolar septum was seen. We expect that this group will not develop fibrosis. We tried to examine the reasons for lung fibrosis in the normal group from several things. Histologically, the lungs have abundant capillary-alveolar tissue and connective tissue, making them susceptible to microvascular damage due to diabetes.[23] In terms of age, the rats used in this study were young adults. Previous researched rats from postnatal age to adulthood. The results stated that with age, there is an increase in the interstitial tissue and the number of collagen fibers. This increase is seen quite significantly in adulthood and young adults.[24]

Another study compared the expression of fibrosis-related genes and collagen in young to old adult rats. The results found expression of genes related to fibrosis in young adult rats.[25] This study used normal saline as a placebo. We tried to find out whether administration of normal saline can cause lung fibrosis. Normal saline is often used as a placebo

in rat models of lung fibrosis. Previous studies induced lung fibrosis using bleomycin and gave normal saline as a placebo to the control group. The results showed normal lung histology in the control group.[26]

Moringa leaf extract is administered orally, so this method has the potential to cause aspiration in the lungs. There are several reasons why the peroral method can cause lung aspiration. First, the tube inserted can reduce integrity and interfere with the function of the upper and lower esophageal sphincter. Second, chronic stimulation of the pharynx can trigger reflux, thereby increasing the aspiration potential of gastric juices into the lungs. Last, malposition when inserting the tube into the stomach has the potential to cause lung aspiration.[27,28] Previous studies found inflammation and fibrosis in the lungs of rats due to chronic microaspiration of gastric juice into the lung and chronic microaspiration of gastric juice can stimulate inflammation, migration and differentiation of fibroblast cells in the lung.[29,30]

LIMITATION

Limitations of this study were not testing the antioxidant activity of the moringa leaves and not examining the fasting blood glucose levels of the rats after four weeks of MLE administration.

CONCLUSION

MLE can inhibit lung fibrosis in rats with the diabetic models but has not been able to maintain lung tissue to match the condition of the normal group.

ACKNOWLEDGMENTS

The authors would like to thank everyone who assisted in this research and publication.

CONFLICT OF INTEREST

None.

FUNDING

None.

REFERENCES

1. International Diabetes Federation. IDF diabetes atlas 10th edition. 10th ed. Magliano D, Boyko E, editors. Vol. 102, International Diabetes Federation. 2021.
2. Kemenkes RI. Pola Hidup Sehat dan Deteksi Dini Bantu Kontrol Gula Darah Pada Penderita Diabetes. Serina IV. Jakarta; 2021.
3. Chawla A, Chawla R, Jaggi S. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? Indian Journal of Endocrinology and Metabolism. 2016;20:546–51.
4. Tuleta I, Frangogiannis NG. Diabetic fibrosis. Biochimica et Biophysica Acta - Molecular Basis of Disease. 2021;1867:166044.
5. Shah MS, Brownlee M. Molecular and cellular mechanisms of cardiovascular disorders in diabetes. Circulation Research. 2016;118:1808–29.
6. Mameli C, Ghezzi M, Mari A, Cammi G, Macedoni M, Redaelli FC, et al. The diabetic lung: Insights into pulmonary changes in children and adolescents with type 1 diabetes. Metabolites. 2021;11(2):69.
7. Ehrlich SF, Quesenberry CP, Van Den Eeden SK, Shan J, Ferrara A. Patients diagnosed with diabetes are at increased risk for asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, and pneumonia but not lung cancer. Diabetes Care. 2010;33(1):55–60.
8. Essa MM, Subash S, Parvathy S, Meera A, Guillemin GJ, Memon MA, et al. Brain health benefits of Moringa oleifera. In: M. M. Essa MAMMA, editor. Food and Brain Health. New York: Nova Science Publishers; 2014. p. 113–8.
9. Aminah S, Tezar R, Yanis M. Kandungan nutrisi dan sifat fungsional tanaman kelor (Moringa oleifera). Buletin Pertanian Perkotaan Balai Pengkajian Teknologi Pertanian Jakarta. 2015;5(2):35–44.
10. Shih MC, Chang CM, Kang SM, Tsai ML. Effect of different parts (leaf, stem and stalk) and seasons (summer and winter) on the chemical

compositions and antioxidant activity of Moringa oleifera. Int J Mol Sci. 2011;12(9):6077–88.
11. Hernández-Rodríguez P, Baquero LP, Larrota HR. Flavonoids: Potential therapeutic agents by their antioxidant capacity. In: Bioactive Compounds: Health Benefits and Potential Applications. Woodhead Publishing; 2019. p. 265–88.
12. Gianosa G, Suryono S, Santosa A. Renoprotective activity of Morinaga oleifera lamk toward kidney injury rats induced by streptozotocin. NurseLine Journal. 2022;7(1):21–7.
13. Kasolo JN, Bimenya GS, Ojok L, Ogwal-Okeng JW. Sub-acute toxicity evaluation of Moringa oleifera leaves aqueous and ethanol extracts in Swiss Albino rats. International Journal of Medicinal Plant Research. 2012;1(6):75–81.
14. Hübner RH, Gitter W, El Mokhtari NE, Mathiak M, Both M, Bolte H, et al. Standardized quantification of pulmonary fibrosis in histological samples. Biotechniques. 2008;44(4):507–11.
15. Schuyler MR, Niewoehner DE, Inkley SR, Kohn R. Abnormal lung elasticity in juvenile diabetes mellitus. American Review of Respiratory Disease. 1976;113(1):37–41.
16. Davis TME, Knuiman M, Kendall P, Vu H, Davis WA. Reduced pulmonary function and its associations in type 2 diabetes: The Fremantle Diabetes Study. Diabetes Res Clin Pract. 2000;50(2):153–9.
17. Hu Y, Ma Z, Guo Z, Zhao F, Wang Y, Cai L, et al. Type 1 diabetes mellitus is an independent risk factor for pulmonary fibrosis. Cell Biochemistry and Biophysics. 2014;70:1385–91.
18. Susanto H, Yunisa DT, Taufiq A, Putra WE, Jannah NR, Putri SA, et al. Anti fibrogenesis effect of green materials Moringa oleifera leaf powder (MOLP) on the progression of hepatocellular carcinoma. In: AIP Conference Proceedings. 2021. p. 2353.
19. Wilujeng LK, Safitri FN, Supriono S, Kalim H, Poeranto S. The effect of Moringa oleifera (Lam) leaves ethanol extracts as anti-inflammatory and anti-fibrotic through TNF-α and p38-MAPK expression: In vivo model of liver fibrosis approach. In: AIP Conference Proceedings. 2021. p. 2353.
20. He B sai, Wang X, Zhang Y, Gao C, Wu C kai, Guo S rui, et al. Anti-oxidant, anti-inflammatory, and anti-fibrotic effects of Moringa oleifera seeds on renal injury diabetic induced by streptozotocin. J Funct Foods. 2022;95(4):105168.
21. Awodele O, Oreagba IA, Odoma S, Teixeira Da Silva JA, Osunkalu VO. Toxicological evaluation of the aqueous leaf extract of Moringa oleifera Lam. (Moringaceae). J Ethnopharmacol. 2012;139(2):330–6.
22. Saleem A, Saleem M, Akhtar MF, Baig MMFA, Rasul A. HPLC analysis, cytotoxicity, and safety study of Moringa oleifera Lam. (wild type) leaf extract. J Food Biochem. 2020;44(10):e13400.
23. Alia F, Putri M, Anggraeni N, Syamsunarno MRAA. The potency of Moringa oleifera Lam. as protective agent in cardiac damage and vascular dysfunction. Frontiers in Pharmacology. 2022;12:724439.
24. Margiana R, Liem IK, Pawitan JA. Microscopic study of the lungs of sprague-dawley rats from various ages of postnatal and aging periods. Online J Biol Sci. 2015;15(2):74–82.
25. Calabresi C, Arosio B, Galimberti L, Scanziani E, Bergottini R, Annoni G, et al. Natural aging, expression of fibrosis-related genes and collagen deposition in rat lung. Exp Gerontol. 2007;42(10):1003–11.
26. Thrall RS, McCormick JR, Jack RM, McReynolds RA, Ward PA. Bleomycin-induced pulmonary fibrosis in the rat. Inhibition by indomethacin. American Journal of Pathology. 1979;95(1):117–30.
27. Gomes GF, Pisani JC, Macedo ED, Campos AC. The nasogastric feeding tube as a risk factor for aspiration and aspiration pneumonia. Current Opinion in Clinical Nutrition and Metabolic Care. 2003;6:327–33.
28. Blumenstein I, Shastri YM, Stein J. Gastroenteric tube feeding: Techniques, problems and solutions. World Journal of Gastroenterology. 2014;20:8505–24.

29. Samareh Fekri M, Poursalehi HR, Najafipour H, Dabiri S, Shokoohi M, Siahposht Khacheki A, et al. Pulmonary complications of gastric fluid and bile salts aspiration, an experimental study in rat. Iran J Basic Med Sci. 2013;16(6):790–6.
30. Chiang CC, Chen CM, Suen JL, Su HH, Hsieh CC, Cheng CM. Stimulatory effect of gastroesophageal reflux disease (GERD) on pulmonary fibroblast differentiation. Digestive and Liver Disease. 2020;52(9):988–94.