**JURNAL KEDOKTERAN DIPONEGORO**
**(*DIPONEGORO MEDICAL JOURNAL*)**
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v12i6.39824
**JKD (DMJ), Volume 12, Number 6, November 2023 : 362-367**



# THE EFFECT OF MORINGA LEAF EXTRACT (MORINGA OLEIFERA) ON THE MICROSCOPIC STRUCTURE OF THE LUNGS OF MALE WISTAR RATS GIVEN INHALATION OF E-CIGARETTE LIQUID VAPOR

Alif Akbar Bialangi[1*], Akhmad Ismail[2], Ratna Damma Purnawati[2], Farmaditya Eka Putra Mundhofir[2]
[1]Undergraduate Program, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[2] Department of Histology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
Corresponding Author's email: akbarbialangi@gmail.com

## ABSTRACT

**Background:** Moringa leaf extract is proven to have high antioxidant bioactive compounds and acts as an anti-inflammatory and prevents cell damage due to exposure to free radicals or oxidative. In the respiratory system exposed to Electronic Delivery Nicotine System liquid vapor, an inflammatory response and oxidative stress occur. **Objective:** This study aimed to determine the effect of Moringa leaf extract (Moringa oleifera) on the microscopic structure of the lungs of male Wistar rats given inhalation of e-cigarette liquid vapor. **Methods:** This research is an experimental laboratory with Post Test Only Control Group Design. Rats were divided into 4 groups containing 6 rats in each group, determined based on simple random sampling with the following divisions: Aquadest 3 mL (K1), gave inhalation of liquid vapours of electric cigarettes (K2), gave inhalation of liquid vapours of electric cigarettes + Moringa leaf extract with the dekoxy method dose of 500 mg/kgBW (P1), administration of vapor inhalation of e-cigarette liquid + Moringa leaf extract with the dekoxy method at a dose of 500 mg/kgBW (P2). Termination of the experimental animals was carried out on the 15th day and the rat pulmonary organs were taken to assess the microscopic structure of the rat lung. **Results:** The results of the Kruskal-Wallis test and continued with the Mann-Whitney test showed a significant difference ($p < 0.05$), however, there was no significant difference in the degree of pulmonary damage in the P1 to P2 groups. **Conclusion:** There is an effect of Moringa leaf extract (Moringa oleifera) on the microscopic structure of the lungs of male Wistar rats given inhalation of liquid vapor of electric cigarettes.

***Keywords:*** *Moringa leaf extract, electric cigarette, Lungs, Rattus norvegicus*

## INTRODUCTION

Electronic Nicotine Delivery System (ENDS) or so-called e-cigarette is a battery-powered cigarette device and liquid ENDS. ENDS are combined with liquid flavoring instead of tobacco leaf. The composition of liquid ENDS includes nicotine, water, glycerol, propylene glycol, and optional flavorings.[1] ENDS users are increasing in contemporary society, and tend to compete with traditional tobacco cigarettes.[2]

The Prevalence of ENDS users among adults in the United States (US is stimated to reach 8.1 million people in 2018 based on the National Center for Health Statistics (NCHS).[3,4] Based on smoking status, adults who quit smoking in the past year were most likely to have ever (57.3%) and current ENDS users This is (25.2%).[3] The results of the Global Youth Tobacco Survey (GYTS) survey, of the total youth surveyed, 4.1% were adolescents using ENDS, consisting of 3% boys and 1.1% girls. A study from Istiqomah et al (2016) regarding the lifestyle of the Semarang Vaper Corner e-cigarette community, 82.2% were ENDS users aged 17-25 years, 86.7% were male.[5]

In use, ENDS is activated, ENDS liquid burns and aerosolization occurs so that vapor is formed. Inhaled ENDS liquid vapor can act as a stimulator or affect the respiratory system.1 In the respiratory system exposed to ENDS liquid vapor, an inflammatory response and oxidative stress occur.[1] In the respiration system exposed to liquid vapor ENDS arises inflammatory response and oxidative stress.[6] The inflammatory response in the respiratory system results in increased secretion production and decreased mucociliary clearance.[7,8] Studies from Garcia-Arcos I (2016) in rats found an increase in respiratory tract hyperactivity, mucin production, and cytokine and protease expression. The results of this study found the potential dangers of nicotine inhalation during the use of ENDS.[9] Increased hyperactivity in the respiratory tract of rats and increased inflammation of epithelial cells in the lungs can occur due to exposure to nicotine ENDS liquid vapor.[10]

**JURNAL KEDOKTERAN DIPONEGORO**
**(*DIPONEGORO MEDICAL JOURNAL*)**
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v12i6.39824
**JKD (DMJ), Volume 12, Number 6, November 2023 : 362-367**



This inflammatory response and oxidative stress can occur in the cerebrum, vasa, and lungs. In the vasa, endothelial dysfunction, neutrophil activation, and release of cytokines and inflammatory mediators occur. Increased oxidative stress and impaired immune defenses against bacterial and viral infections.[6]

In the Tan WS study, high concentrations of antioxidant bioactive compounds were found in Moringa (Moringa oleifera) leaves and can be used as alternative inflammatory drugs. The results of this research found that Moringa leaf extract can act as an anti-inflammatory through pro-inflammatory mediators and cytokines.[11]

The genus Moringa Adans. consists of 13 species, including the Moringa plant (Moringa oleifera Lam). This plant is native to India and cultivated for traditional medicine as well as a source of nutrition. The wide range of phytochemicals among leaves, flowers, fruit, seeds, seed oil, bark, and roots depends on the variety, season, and location.[12]

Moringa is grown as a vegetable for consumption. Moringa leaves contain phytonutrients including phenolic compounds. Phenolic compounds have the effect of preventing damage to normal cell deoxyribonucleic acid (DNA) and apoptosis of cancer cells, so that phenolic compounds can be used as chemotherapy modalities.[13]

This research will be conducted by researchers to determine the effect of Moringa leaf extract (Moringa Oleifera) on the microscopic structure of the lungs of male Wistar rats given e-cigarette liquid vapor inhalation.

## METHODS

The research was conducted at the Experimental Animal Laboratory, Faculty of Mathematics and Science, UNNES. The research design is purely experimental with the Post-test Only Control Group Design approach. The subject of this research are wistar rats which were selected randomly and have complied with the inclusion and exclusion criteria. 6 rats per cage were accommodated for one week in experimental animal cages. The standard feed for rats was chicken chow and given tap water. No mice died during the acclimation process.

**Table 1.** Average Body Weight of Rats

| Group | Mean Body Weight of Rats |
|---|---|
| K1 | 198,9 |
| K2 | 199 |
| P1 | 202,1 |
| P2 | 194,5 |

6 rats per cage were acclimated to experimental animal cages For one week. The standard diet for the rats was chicken chow and given tap water. No rats died during the acclimation period.

The rats were divided into four groups, with 6 rats in each group and determined based on simple random sampling. Group K1 was given 3 ml of distilled water orally by gastric probe for 14 days. Group K2 was given 3 ml of distilled water orally by gastric probe and given aroma-free vapor inhalation of electric cigarettes. Group P1 was given inhalation of liquid vapours of electric cigarettes and given Moringa leaf extract with the dekoxy method at a dose of 500 mg/kgBW for 14 days. Group P2 was given steam inhalation of electric cigarettes and given maceration method Moringa leaf extract at a dose of 500 mg/kgBW for 14 days.

Termination of the experimental animals was carried out on day the 15th day by placing wistar rats into glass cubes filled with chloroform/liquid ether cotton. Wistar rat pulses were collected and placed in 10% formalin preservative. The samples were then processed for hematoxylin and eosin (HE) staining.

Readings of the specimens were made in five fields of view at 400x magnification and observed whether there was a change in the microscopic structure of the male Wistar rat pulmonary structure. The assessment was carried out based on the criteria for the degree of alveolar damage according to Hansel and Barnes.

The data hypothesis testing was carried out using the Kruskal-Wallis test. Furthermore, the analysis of the effect of Moringa leaf extract on the microscopic structure of the lungs of Wistar rat between treatment groups was performed using the Mann-Whitney test. Significance is indicated when the p value 0.05.

JURNAL KEDOKTERAN DIPONEGORO
(*DIPONEGORO MEDICAL JOURNAL*)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v12i6.39824
**JKD (DMJ), Volume 12, Number 6, November 2023 : 362-367**



## RESULTS

**Table 2.** Preparate Reading Result

| | | | Degree of Damage | | | |
|---|---|---|---|---|---|---|
| | | | **Mild** | **Moderate** | **Severe** | **total** |
| Group | K1 | Count | 6 | 0 | 0 | 6 |
| | | % within group | 100.0% | 0% | 0% | 100.0% |
| | K2 | Count | 0 | 0 | 6 | 6 |
| | | % within group | 0% | 0% | 100.0% | 100.0% |
| | P1 | Count | 0 | 6 | 0 | 6 |
| | | % within group | 0% | 100.0% | 0% | 100.0% |
| | P2 | Count | 3 | 3 | 0 | 6 |
| | | % within group | 50.0% | 50.0% | 0% | 100.0% |
| Total | | Count | 9 | 9 | 6 | 24 |
| | | % within group | 37.5% | 37.5% | 25.0% | 100.0% |

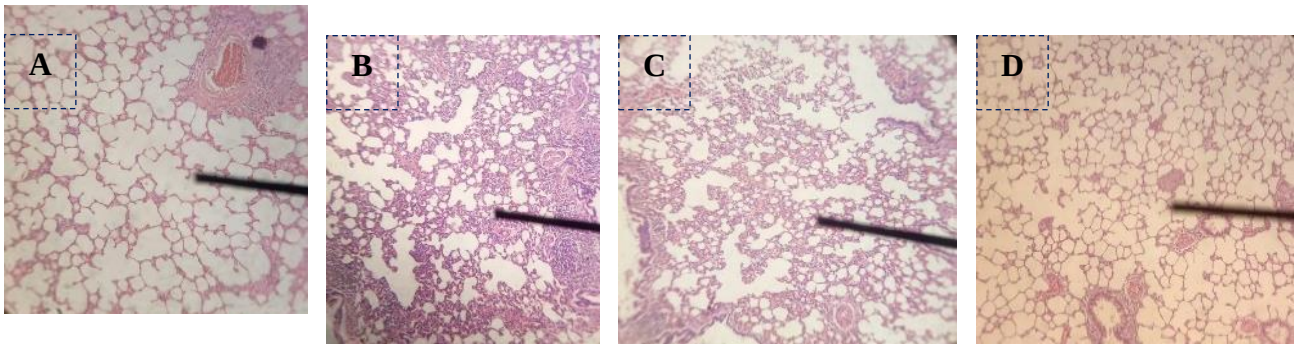


**Figure 1.** A. K1 group, B. K2 group, C. P1 group, D. P2 group

The results of the Kruskal Wallis test obtained a value ($p < 0.05$), so it can be concluded that the degree of damage based on the treatment group had a significant difference. To find out the differences between the treatment groups, the test was continued with the post hoc Mann Whitney test.

**Table 3.** Kruskal-Wallis test results

| Group | Degree of Damage | | | p |
|---|---|---|---|---|
| | **Mild** | **Moderate** | **Severe** | |
| K1 | 6 (100%) | 0 (0%) | 0 (0%) | <0,001 |
| K2 | 0 (0%) | 0 (0%) | 6 (100%) | |
| P1 | 0 (0%) | 6 (100%) | 0 (0%) | |
| P2 | 3 (50%) | 3 (50%) | 0 (0%) | |

* Significance ($p < 0,05$)

The results of the Kruskal Wallis test obtained a value ($p < 0.05$), so it can be concluded that the degree of damage based on the treatment group had a significant difference. To find out the differences between the treatment groups, the test was continued with the post hoc Mann Whitney test.

**Table 4.** Mann-Whittney test result

| Group | | p |
|---|---|---|
| **I** | **II** | |
| K1 | K2 | 0,002* |
| | P1 | 0,001* |
| | P2 | 0,056 |
| K2 | P1 | 0,001* |
| | P2 | 0,002* |
| P1 | P2 | 0,056 |

*significance ($p < 0,05$)

JURNAL KEDOKTERAN DIPONEGORO
(*DIPONEGORO MEDICAL JOURNAL*)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v12i6.39824
**JKD (DMJ), Volume 12, Number 6, November 2023 : 362-367**



The results of the Mann Whitney test showed that there was a significant difference in the degree of lung damage in the K1 to K2 and P1 groups, while there was no significant difference in P2. There was a significant difference in the degree of lung damage in the K2 group to P1 and P2 groups. While the degree of lung damage in the P1 to P2 there was no significant difference.

## DISCUSSION

### Differences in Pulmonary Microscopic Structure of Male Wistar Rats Between Treatment Groups

Based on the results of the microscopic evaluation, there were differences between the treatment groups. In the K1 group, the pulmonary organ damage in Wistar rats was found with a score of 5-10% of the degree of damage in 5 fields of view per specimen. On microscopic examination, there was slight haemorrhage, but no widening of the interalveolar septum, widening of the alveolar lumen, inflammatory cell infiltration, or granulomas. In the K2 group, the score of damage to the pulmonary organs of wistar rats was 60-70%. On microscopic examination, there was severe pulmonary damage in the form of granulomas, thickening of the interalveolar septum, inflammatory cell infiltration, and haemorrhage.

The P1 group obtained a score of 40 - 50% damage to the pulmonary organs of wistar rats. Microscopic evaluation showed moderate lung damage in the form of haemorrhage, inflammatory cell infiltration, thickening of the interalveolar septum, widening of the alveolar lumen, and edema. The P2 group obtained a score of damage to the lungs of wistar rats 20-30%. Microscopic evaluation showed mild lung damage, only haemorrhage, inflammatory cell infiltration without interalveolar septal thickening and alveolar lumen thickening.

The differences that occurred between groups P1 and P2 were in accordance with the theory that the ethanol solvent in the maceration method of Moringa leaf extract contained more phytochemical components with antioxidant and anti-inflammatory properties. Phytochemical components, such as flavonoids, phenolic acids, tannins and saponins can be extracted more in ethanol solvent than water solvents in Moringa leaf extract using the dekoxy method. In the study of Tutik et al (2018), the percentage yield of Moringa leaf extract using the maceration method was 12.69%.[14] In the study of Rachmawati et al. (2019), the percentage yield of Moringa leaf extract using ~~the~~ dekoxy method was 10.31%. The results of the yield test of Moringa leaf extract with the dekoxy method are lower than the maceration method.[15] 5 The results of the study of Tutik et al. (2018) found that the $IC_{50}$ antioxidant activity value in the maceration method of Moringa leaf extract was 103.98 g/mL, while in Tukiran et al.'s study (2020) obtained the value of antioxidant activity $IC_{50}$ in Moringa leaf extract dekoksi method of 122,742 g/mL.[14,16] $IC_{50}$ is a measure of the effectiveness of a compound in inhibiting biological/chemical functions. A compound is said to be a very potent if the $IC_{50}$ value is <50, strong if the $IC_{50}$ value is 50-100, moderate if the $IC_{50}$ value is 100-250 and weak if the $IC_{50}$ value is 250-500. The lower the $IC_{50}$ value, the higher the antioxidant activity of a compound.[16]

### Histomorphological changes in the pulmonary rats due to exposure to e-cigarette liquid vapor

This study used male wistar rats as a representative model for exposure to e-cigarette liquid vapor in humans. Many pathological changes, such as increased thickness of the alveolar septum, dilation of the alveolar lumen, infiltration of eosinophils and macrophages, and haemorrhage were observed.

The study by Schweitzer et al found that nicotine contained in e-cigarette liquid had an effect on endothelial layer damage and inflammation in the lung by inducing oxidative stress mechanism. inflammation and macrophages, and haemorrhage in the treatment group. In the study by Ardy C et al (2020) it was found that damage to the pulmonary organs of rats had been exposed to e-cigarette vapor at a dose of 12 mg/ml nicotine with a moderate degree of damage category.[18]

Based on the results of this study, the lungs of wistar rats exposed to e-cigarette liquid vapor without giving Moringa leaf extract resulted in severe damage scores. This can happen because the dose of nicotine used is a toxic dose. Based on the conversion dose from animals to humans, the conversion factor for rats (200gr) to humans (70kg) is 0.018 so the conversion value is 55 mg/day. However, the dose used in the treatment group of this study was 12 mg, which when converted to humans it would be worth 666 mg. If the

JURNAL KEDOKTERAN DIPONEGORO
(*DIPONEGORO MEDICAL JOURNAL*)
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v12i6.39824
**JKD (DMJ), Volume 12, Number 6, November 2023 : 362-367**



average use of 1 refillable liquid bottle of 30 ml (15 mg) can be used up within 7 days, then the liquid can be used 4 times a day. The toxic dose of nicotine is 44 times the usual dose. Therefore the reasonable limit of nicotine use when using the conversion value is 14 times per day. This research is evidence of the effect of giving Moringa leaf extract. This is indicated by the maximum damage to the pulmonary organs, but a significant effect was obtained after administration of Moringa leaf extract.

**The effect of moringa leaf extract (Moringa Oleifera) on the microscopic structure of the lungs of male Wistar rats given inhalation of e-cigarette liquid vapor**

Moringa leaf extract has several benefits, including antioxidant, anti-inflammatory and antimicrobial activities. In the researches that has been done, there is a significant difference between the group without Moringa leaf extract (K2) and the group given Moringa leaf extract (P1 and P2). In the K2 group, severe damage to the pulmonary organs was found with alveolar damage of 60% of the total field of view. In groups P1 and P2 groups, there was mild to moderate damage to the pulmonary organs with 0-30% and 30-60% of alveolar damage of the entire visual field. The results of this study support the theory that Moringa leaf extract contains several phytochemical compounds with antioxidant, anti-inflammatory and protective properties against oxidative tissue damage. Tissue damage caused by free radicals and oxidative damage may be prevented by the antioxidant properties found in Moringa leaves.[19]

## CONCLUSION

1. There is an effect of traditional method of Moringa leaf extract orally 500 mg on the microscopic structure of the lungs of male wistar rats given inhalation of liquid vapours of electric cigarettes compared to the control group.
2. There is an effect of moringa leaf extract by maceration method orally 500 mg on the microscopic structure of the lungs of male wistar rats who were given inhalation of liquid vapors of electric cigarettes compared to the control group.
3. Moringa leaf extract with maceration method has more effect on the microscopic structure description of male Wistar rat pulmo which is given inhalation of e-cigarette liquid vapor compared to the traditional method of Moringa leaf extract group.

## ETHICAL APPROVAL

This research has obtained an Ethical Clearance from the Health Research Ethics Commission, Faculty of Medicine, UNDIP with No. 34/EC/H/FK-UNDIP/VI/2022.

## REFERENCES

1. Brandon TH, Goniewicz ML, Hanna NH, Hatsukami DK, Herbst RS, Hobin JA, et al. Electronic nicotine delivery systems: a policy statement from the American Association for Cancer Research and the American Society of Clinical Oncology. Clin Cancer Res [Internet]. 2015 Feb 1 [cited 2021 Mar 25];21(3):514–25. Available from: www.aacrjournals.org
2. D'Amario D, Migliaro S, Borovac JA, Vergallo R, Galli M, Restivo A, et al. Electronic cigarettes and cardiovascular risk: caution waiting for evidence. Eur Cardiol Rev. 2019 Dec 1;14(3):151–8.
3. Villarroel MA, Cha AE, Vahratian A. Electronic cigarette use among U.S. Adults, 2018. NCHS Data Brief. 2020;(365):1–8.
4. Creamer M, Wang T, Babb S, Cullen K, Day H, Willis G, et al. Tobacco product use and cessation indicators among adults. United States, 2018. Morbidity and Mortality Weekly Report 2019. MMWR Morb Mortal Wkly Rep. 2019;68(45):1013–9.
5. Comparison of forced expiratory volume among the smokers and non-smokers in brick kiln workers. Int J Community Med Public Heal. 2016;3(12):3399–402.
6. Lerner CA, Sundar IK, Yao H, Gerloff J, Ossip DJ, McIntosh S, et al. Vapors produced by electronic cigarettes and e-juices with flavorings induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung. PLoS One. 2015;10(2):1–26.
7. Reidel B, Radicioni G, Clapp PW, Ford AA, Abdelwahab S, Rebuli ME, et al. E-cigarette use causes a unique innate immune response

**JURNAL KEDOKTERAN DIPONEGORO**
**(*DIPONEGORO MEDICAL JOURNAL*)**
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
DOI : 10.14710/dmj.v12i6.39824
**JKD (DMJ), Volume 12, Number 6, November 2023 : 362-367**



in the lung, involving increased neutrophilic activation and altered mucin secretion. Am J Respir Crit Care Med. 2018;197(4):492–501.
8. Laube BL, Afshar-Mohajer N, Koehler K, Chen G, Lazarus P, Collaco JM, et al. Acute and chronic in vivo effects of exposure to nicotine and propylene glycol from an e-cigarette on mucociliary clearance in a murine model. Inhal Toxicol [Internet]. 2017 Apr 16;29(5):197–205. Available from: https://doi.org/10.1080/08958378.2017.1336585
9. Garcia-Arcos I, Geraghty P, Baumlin N, Campos M, Dabo AJ, Jundi B, et al. Chronic electronic cigarette exposure in mice induces features of COPD in a nicotine-dependent manner. Thorax. 2016;71(12):1119–29.
10. Xu Y, Zhang Y, Cardell LO. Nicotine exaggerates LPS-induced airway hyperreactivity via JNK-mediated up-regulation of toll-like receptor 4. Am J Respir Cell Mol Biol. 2014;51(3):370–9.
11. Tan WS, Arulselvan P, Karthivashan G, Fakurazi S. Moringa oleifera flower extract suppresses the activation of inflammatory mediators in lipopolysaccharide-stimulated RAW 264.7 macrophages via NF- κ B pathway. Mediators Inflamm. 2015;2015.
12. Dhakad AK, Ikram M, Sharma S, Khan S, Pandey V V., Singh A. Biological, nutritional, and therapeutic significance of moringa oleifera lam. Phyther Res. 2019;33(11):2870–903.
13. Lin M, Zhang J, Chen X. Bioactive flavonoids in Moringa oleifera and their health-promoting properties. J Funct Foods [Internet]. 2018;47(June):469–79. Available from: https://doi.org/10.1016/j.jff.2018.06.011
14. Tutik, Dwipayana INA, Elsyana V. Identifikasi dan perbandingan aktivitas antioksidan ekstrak daun kelor pada variasi pelarut dengan metode DPPH. J Farm Malahayati. 2018;1(2):80–7.
15. Rachmawati SR. Characterization of moringa (moringa oleifera lam.) leaf water extracts by chemical and microbiology. 2019;10(2):102–16.
16. Tukiran, Miranti MG, Dianawati I, Sabila FI. Aktivitas antioksidan ekstrak daun kelor dan buah bit sebagai bahan tambahan minuman suplemen.pdf. Akt antioksidan ekstrak daun kelor (moringa oleifera lam) dan buah bit (beta vulgaris l) sebagai bahan tambah minuman suplemen. 2020;5:113–9.
17. Schweitzer KS, Chen SX, Law S, Van Demark M, Poirier C, Justice MJ, et al. Endothelial disruptive proinflammatory effects of nicotine and e-cigarette vapor exposures. Am J Physiol - Lung Cell Mol Physiol. 2015;309(2):L175–87.
18. Ardy C, Hamid IS, Yudhana A, Widjiati W, Purnama MTE, Fikri F. Pengaruh paparan uap rokok elektrik terhadap gambaran histopatologi organ pulmo tikus putih (rattus norvegicus). J Med Vet. 2020;3(1):38.
19. Sreelatha S, Padma PR. Antioxidant activity and total phenolic content of moringa oleifera leaves in two stages of maturity. Plant Foods Hum Nutr. 2009;64(4):303–11.