

ORIGINAL ARTICLE

Nephrolepis exaltata Herbal-mask Better than Regular Cloth-mask in Decreasing Nasal Mucociliary Transport-Time of Textile Industry Workers

Masker Herbal Nephrolepis Exaltata Menghambat Pemanjangan Waktu Transpor Mukosilier Hidung Pekerja Industri Tekstil


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ABSTRACT

Background

Herbal masks are made from lamination-spraying of *Nephrolepis exaltata* (NE) extract on regular medical masks, which may absorb *Volatile Organic compounds* (VOC) in the occupational exposure of the textile industry's dyeing area. Therefore, clinically, the effect on nasal mucociliary transport time (TMSH) needed to be tested and compared with ordinary cloth-mask users used by textile industry workers as their daily personal protective equipment.

Methods

Pre- and post-test randomized control trials on 30 textile-dyeing areas of textile industry workers in Bawean Indonesia, divided into two groups; 1) users of NE herbal masks (N=17) as the treatment group and 2) regular cloth mask users (N=13) as control-group, for eight weeks. First, the TMSH time (seconds) was carried out before and after treatment using the saccharin test by an ENT specialist, followed by a different test on delta.

Results

The TMSH-time of NE herbal mask users before vs after treatment was 1169.60 ± 644.55 seconds vs 1075.75 ± 677.36 ($p = 0.102$). On subjects wearing regular cloth masks was 1113.75 ± 479.43 vs 1187.40 ± 544.96 ($p = 0.003$). The mean delta's difference before and after treatment was 25.5 seconds, with a significant difference in mean delta TMSH-time between the treatment and control groups ($p = 0.048$).

Conclusions

Textile-industry workers who wear NE herbal masks have better TMSH times than regular cloth mask wearer

Keywords: *Nephrolepis exaltata* herbal mask; TMSH-time; regular cloth mask; textile-industry workers

ABSTRAK

Latar Belakang

Masker herbal dibuat dari semprotan laminasi ekstrak *Nephrolepis exaltata* (NE) pada masker medis reguler, mampu menyerap *Volatile Organic Compound* (VOC) di lingkungan kerja sektor pencelupan warna industri tekstil. Secara klinis, efeknya terhadap waktu transport mukosiliar hidung (TMSH) perlu diuji dan dibandingkan dengan masker kain biasa yang dipergunakan pekerja industri tekstil sebagai alat pelindung diri sehari-hari.

Metode

Uji kontrol acak pre dan post-test pada 30 pekerja sektor pencelupan warna industri tekstil di Bawen Indonesia, yang dibagi 2 kelompok; pemakai masker herbal NE (N=17) dan pengguna masker kain biasa (N=13), selama 8 minggu. Pemeriksaan waktu TMSH (detik) dilakukan pada sebelum dan sesudah perlakuan menggunakan uji sakarin oleh spesialis THT, dilanjutkan uji beda terhadap delta.

Hasil

Waktu TMSH pemakai masker herbal NE sebelum vs sesudah perlakuan didapatkan $1169,60 \pm 644,55$ detik vs $1075,75 \pm 677,36$ ($p = 0,102$). Subyek pemakai masker kain biasa selama $1113,75 \pm 479,43$ vs $1187,40 \pm 544,96$ ($p = 0,003$). Selisih delta *mean* sebelum dan sesudah perlakuan selama 25,5 detik, dengan perbedaan bermakna delta *mean* waktu TMSH antara kelompok perlakuan dan kontrol ($p = 0,048$).

Kesimpulan

Pekerja industri tekstil yang memakai masker herbal NE memiliki waktu TMSH lebih baik dibanding pemakai masker kain biasa.

Kata Kunci: Herbal-mask *Nephrolepis exaltata*; waktu TMSH; masker kain biasa; pekerja industri tekstil

INTRODUCTION

The textile-industry development in Indonesia is resulting in occupational chemicals exposure to their workers.ⁱ In 2021, the International Labour Organization (ILO) released that occupational exposure is a potential risk for workers, especially chemicals, gases, fumes, aerosols, particles and other substances. The most common in sectors that include textile industry processes such as dyeing accompanied by the absence of good ventilation, indoor air pollutants can be more concentrated, putting workers at higher risk for harmful levels of exposure (WHO 2018), which caused as many as 3 billion workers were living and working in places where the air quality was below WHO standards. The most common pollutants considered in air pollution estimates include fine (PM_{2.5}) and course (PM₁₀) particulate matter, ozone, nitrogen dioxide (NO₂), sulfur dioxide (SO₂), as well as VOC, i.e., benzene, formaldehyde or carbon monoxide.ⁱⁱ

Formaldehyde exposure may cause hyperreactivity of mucosal membranes, occurring inflammation of the nasal mucosa,ⁱⁱⁱ and occupational air pollution, especially the PM₁₀ and PM_{2.5} affect the IL-6 and SOD-3 levels in the textile industry workers^{iv} in our previous research, so it also may reduce the mucociliary nasal function. A positive correlation between length of work with nasal mucociliary transport time reflected the chronic inflammatory response that was clinically measured by cilia-malfunction and structural pathological damages or mucosal adaptations (hyperplasia and hypertrophy of the turbinate and nasal mucosa),^v as well age and the workplace, location of exposure; indoor or outdoor.^{vi}

The previous research showed an inconsistent effect on nasal mucociliary transport-time exposure by wood-dust, outdoor inhalation of gas/fuel station workers and iron-dust,^{3,5,6} however, it was strong evidence that occupational air pollutants surrounding the indoor dyeing sector of the textile industry increased IL-6 and SOD3.⁴ It was suggested that inflammation and stress oxidative effect might also change the nasal-cilia function and prolonged the nasal mucociliary transport time.

The standard saccharine test detects nasal mucociliary clearance time in healthy individuals in adults and children. The mean nasal mucociliary clearance time for healthy individuals is 8.2 minutes in children and 9.5 minutes in adults. The mean nasal mucociliary clearance rates were 11.1 mm/min for children and 12.7 mm/min for adults.^{vii} Deviated nasal septum, chronic sinusitis, allergic rhinitis, atrophic rhinitis, chronic smokers and patients with recent nasal packings were taken as diseased conditions in adults, whereas children with adenoid hyperplasia were taken for the study. In all of these, nasal mucociliary clearance was significantly prolonged. ^(viii) The nose physiologically has the first-line defence function of the body in clearing the inspiratory air from dust particles, bacteria, viruses, allergens, toxins and carrying particles caught in the mucosal lining toward the nasopharynx and oropharynx. The function is carried out by cilia and mucous blankets, known as the mucociliary system.^{7,8} The nasal mucociliary system is affected by various factors, including physiological/anatomical, disease and environmental factors.⁷

Nephrolepis exaltata originally came from America, Mexico, West India, and Africa, was classified as an invasive plant and may be found in humid areas such as in forests and swamps. It had natural decomposition effects on formaldehyde to prevent mucosa in the respiratory tract from being damaged.^{ix,x} Our previous research made innovative medical masks laminated with *Nephrolepis exaltata* herbal extracts to protect against occupational inhaled exposure, which later received patent No. IDM000921225 as Acchadana®-herbal masks.^{xi} In daily practice, textile industry workers use cloth-mask as their personal protective equipment, and how effective compared to herbal-mask in protecting upper-respiratory tract pathogenesis remains questioned.

METHODS

Research design

Pre- and post-test randomized control trials used the textile industry employees at Bawen Semarang Indonesia who was exposed to the occupational chemical in the dyeing process section between October and November 2018.

Study subjects

The subjects fulfilled the inclusion criteria, i.e. 20-55 years old, having a good health condition, willing to be a research subject and also included subjects having complaints of mucus or watery discharge out of the nose and a working time (in a textile factory). The exclusion criteria, i.e. having a history of alcohol consumption, history of liver disease, history of autoimmune disease, history of cancer, pulmonary and heart disease. They were employees of a textile industry in Bawen, Semarang, Indonesia, exposed to occupational chemicals in the dyeing process section.

The sample size determination comprised 30 male workers and was based on the total number of male workers in the dangerously polluted area of the factory (dyeing area). The subjects were

randomized into the groups wearing; i) NE-herbal masks and ii) cloth masks (single-ply). All subjects were explained how to use the masks correctly. The masks were given to the supervisor of the subjects every ten days, and then he distributed them to all subjects, ensuring compliance with their use.

The masks were worn and changed every day for eight weeks. The duration of wearing masks per day is about 6-7 hours on weekdays, based on their active working-hour. Compliance with the use of masks is ensured and monitored daily using a monitoring card. Follow-up by the researcher was carried out every ten days to evaluate the use of masks and the health condition of the research subjects. The control group used regular cloth masks, which were washed and changed every month for eight weeks.

Preparation of NE-herbal masks

Making extracts of *Nephrolepis exaltata* was carried out at the Laboratory of Science and Mathematics, Diponegoro University, Semarang. The NE-herbal masks were made by CV. Beauty Kasatama, Surabaya, prepared by a lamination technique (three-ply surgical masks were laminated with NE-HRS extracts). The factory itself produced the cloth masks.

Nasal mucociliary transport time measurement

After eight weeks, a second measurement of NMCTT was performed by ENT specialists. Then the subject's nose was examined using the nasal speculum to see the structure inside the nose. Then, a forceps alligator inserted the lactic saccharin tablet into the lower front of the inferior concha. The examination was done twice, i.e. pre-test and post-test. Pre-test was done on all research subjects before the treatment was given.

The nasal mucociliary transport time (NMCTT) was measured using saccharin test methods.^(xii) Particles of 1 mm diameter of saccharin were placed in 1 cm anterior and inferior nostrils, verified by inspection that there was no obstruction. Subjects were asked not to smoke, eat, cough, drink, or sneeze during the examination and with a flexure head position of 10°. The NMCTT was calculated by how long (seconds) it took for the saccharin tablet to become sweet after being put in for the first time.^{xiii}

Statistical analysis

The data were analyzed with SPSS for Windows version 25. The Shapiro-Wilk test analysed primary data on mucociliary-transport times to determine the data distribution, which turned out to be normal. In addition, the *Wilcoxon Signed Ranks Test and Paired T-test* were used to analyze the data. Ethical clearance by Commission on Health Research Ethics Faculty of Medicine Diponegoro University and Dr Kariadi Hospital Semarang Indonesia No. 401/EC/FK-RSDK/III/2018.

RESULTS

Subject Characteristics

Analysis of research subjects showed that variables such as age, sex, length of work, rhinitis allergy and smoking were not statistically different ($p > 0.05$) between control and treatment groups. Therefore, the subjects were eligible for different tests conducted. Thus, the number of research samples that met the inclusion criteria was 30 subjects. Table 1 shows the research

subjects of 30 subjects divided into the *Nephrolepis exaltata* herbal treatment group (N = 17) and the regular cloth mask control group (N = 13).

Table 1. Characteristics of the subject (N=30)

Characteristics	Groups		p
	Treatment (%)	Control (%)	
Age			
<40 y	10 (58.8%)	8 (61.5%)	0.59*
>40 y	7 (41.2%)	5 (38.5%)	
Sex			
Man	16 (94.1%)	10 (76.9%)	0.20*
Woman	1 (5.9%)	3 (23.1%)	
Length of working			
0-5 y	3 (17.6%)	3 (23.1%)	0.47**
6-10 y	1 (5.9%)	1 (7.7%)	
11-15 y	0 (0.0%)	0 (0.0%)	
16-20 y	12 (70.6%)	6 (46.2%)	
21-25 y	1 (5.9%)	3 (23.1%)	
Rhinitis Allergy			
Positive	0 (0.0%)	2 (15.4%)	0.17*
Negative	17 (100.0%)	11 (84.6%)	
Smoking habit			
Positive	9 (52.9%)	3 (23.1%)	0.10*
Negative	8 (47.1%)	10 (76.9%)	

*Fisher's exact test

**Pearson chi-Square

The youngest research subjects in the treatment group were 23 years old, and the oldest was 4d, 36.5 ± 7.01 mean. However, the most youthful research subjects, rs old and the oldest, were 53 years old, $36.3 \pm$ an. The sex of research subjects in both groups was male-dominated. As many as 16 (94.1%) male subjects and a female subject was only one subject (5.9%) in the treatment group. In comparison, the control group had 10 (76.9%) male subjects and the female subjects were three people (23.1%). The years of service mean in the treatment group was 15.6 ± 6.66 , with the highest distribution in the range of 16-20 years for as many as 12 subjects (70.6%) and the control group also had the highest distribution in the range of 16-20 years as many as six subjects (46.2%) with the mean years of service was 15.8 ± 8.34 . There was no history of allergies in the treatment group. However, two subjects with a history of allergies were in the control group (15.4%). Nine subjects (52.9%) were also obtained in the treatment group, and three subjects (23.1%) in the control group with a positive smoking history. After Fisher's Exact Test had been done, it was found that the comparison between the treatment group and control group was not significantly different in age and sex distribution, history of allergies and history of smoking. The Pearson Chi-Square test found that the comparison between treatment and control groups was not significantly different ($p = 0.47$) with a 95% confidence interval.

Normality test for NMCTT

The data normality test was done along with the Shapiro-Wilk test (Table 2). The normality test was done for NMCTT before and after treatment. The Shapiro-Wilk normality test had a significantly different data distribution ($p < 0.05$) in the treatment group. The mean of mucociliary-transport time before treatment was 1169.60 ± 644.55 ; after eight weeks, the rate of mucociliary-transport time was better (1075.75 ± 677.36). In the control group, the mean of mucociliary-transport time before treatment was 1113.75 ± 479.43 , and at the end of the research was improved by 1187.40 ± 544.96 .

Table 2. Normality test for mucociliary-transport time

Groups	Mean \pm SD	Max	Min	P*
Treatment				
Before	1169.60 \pm 644.55	2400	451	0,004*
After	1075.75 \pm 677.36	2400	372	0.001*
Control				
Before	1113.75 \pm 479.43	2150	500	0.186
After	1187.40 \pm 544.96	2400	512	0.143

*Shapiro-Wilk ($P < 0.05$), significant

NMCTT before and after the treatment

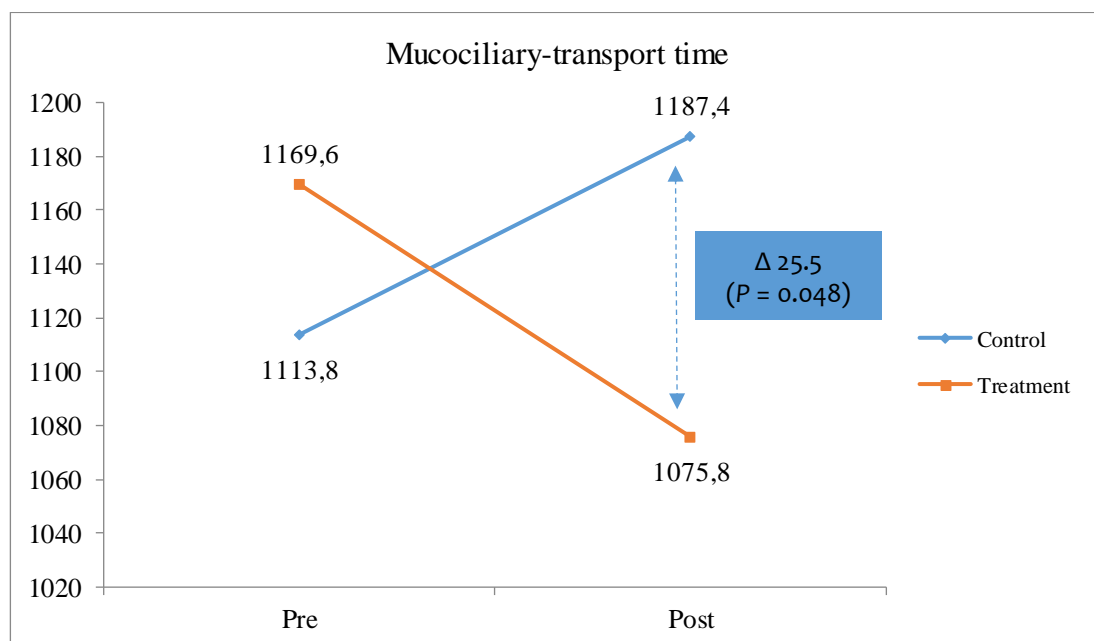
Table 3 shows a significant decrease of NMCTT in the treatment group after being given the treatment for eight weeks compared to the initial time, with the delta was -93.85. The control group had an insignificant improvement, with a delta score of 73.65. The mean difference of delta for NMCTT before and after the treatment was 25.5. Statistically, there was a significant difference in the mean delta for NMCTT between the treatment and control groups ($p = 0.048$) (Fig 1).

Table 3. Mean for a mucociliary-transport time before and after treatment

Mucociliary-transport time	Before	After	P
	Mean \pm SD	Mean \pm SD	
Treatment	1169.60 \pm 644.55	1075.75 \pm 677.36	0.102*
Control	1113.75 \pm 479.43	1187.40 \pm 544.96	0.003**

*Wilcoxon Signed Ranks Test ($p > 0,05$), not significant

**Paired t-test ($p < 0,05$), significant

**Fig 1.** The difference in mean delta for a mucociliary-transport time before and after treatment

DISCUSSION

The *Nephrolepis exaltata* may absorb and neutralize VOC.^{9,xiv} Consistent with these findings, and our previous research showed that this plant significantly reduces the number of sinonasal-goblet cells in *Sprague Dawley* rats which were exposed to VOC.^{xv} In those studies, fifteen control

subjects used cloth masks which failed to provide sufficient protection from small pollutants. Usually, pollutants with small diameters ($\leq 2.5\text{mm}$) are inhaled until they reach the lungs. A study also proved that cloth-mask could only filter 28% of pollutants in highly polluted areas, such as highways in China. Otherwise, a combined *Nephrolepis exaltata* - *Hibiscus rosa sinensis* herbal mask protects the sinonasal immune system and increases the lung function of motorcycle taxi drivers.^{xvi}

Nephrolepis exaltata is a cheap, easy-to-obtain, maintain, and increase plant. Thus, it can be independently cultivated. Furthermore, this plant contains substances useful to neutralize air pollutants, such as formaldehyde, benzene, toluene, xylene, and many other VOCs. Our previous study on *Sprague Dawley* rats also demonstrates the potency of *Nephrolepis exaltata* in filtering BTX compound, which belongs to VOC,^{14,16} as well as IgA levels and pulmonary function, decreasing the level of reactive oxygen species.^{xvii}

Chronic exposure to chemicals causes respiratory edema and increased mucous secretion, slowing down the NMCTT. The upper respiratory tract mucosa also gets inflamed, reducing its function. The saccharin test may measure the reducing part of the respiratory tract. The dyeing sector work environment typically has high humidity, where the effects of exposure to hazardous chemicals can be further increased. The effect of such exposure on upper respiratory tract function is reversible and returns to normal after four weeks without exposure. The control group's decreased upper respiratory tract function was suspected due to constant inhalation of chemicals in the work environment.

The masks used by the control group were regular cloth masks, which were only able to prevent the entry of large particles. At the same time, chemical pollutants in gaseous form could penetrate and enter the respiratory tract of the workers. The results obtained are in accordance with the research hypothesis stating that there is no decrease in the upper respiratory tract function after using a regular cloth mask. Therefore, the above hypothesis can be accepted. The treatment group used herbal masks that contained essential oils and amines. It is well known that essential oils act as antioxidants and anti-inflammatory agents that can be used in fighting against oxidative stress and inflammation caused by inhalation of chemicals. This is consistent with the findings that reported inhibiting inflammatory processes in pulmonary and hepatic cells exposed to chemical pollutants after adding essential oils.⁽²³⁾ Amines in *Nephrolepis exaltata* can react with formaldehyde in the work environment to form imines and water. As a result, formaldehyde potentially damages the potency of *Nephrolepis exaltata* herbal mask in upper respiratory mucosa function.

The decrease of upper respiratory mucosa function in the control group was suspected due to constant inhalation of chemicals in the work environment. The masks used by the control group were regular cloth masks, which could only prevent the entry of large particles. At the same time, chemical pollutants in gaseous form could penetrate and enter the upper respiratory tract of the workers. The results obtained are in accordance with the research hypothesis stating that there is no increase in the upper respiratory tract function after using a regular cloth mask. Therefore, the above hypothesis can be accepted.

Mucociliary transport is a local defence function of the nasal mucosa by a self-cleansing mechanism transporting unknown particles trapped in the mucous membrane towards the nasopharynx.^(xviii) This NMCTT can be affected by various factors such as physiology or anatomy,

disease and environment.^{5,7} The NMCTT extension can be occurred through the inflammatory process due to VOC substances exposure in the respiratory system of workers. This inflammatory process can be prevented by using *Nephrolepis exaltata* herbal mask.

The saccharine test was used to measure the NMCTT in this research as it was easier to use and did not require any complicated equipment, as well as did not occur any discomfort to the subjects. The saccharine test was affected by subjective factors, so subjects were not informed what materials were used in this study. The NMCTT extension in the control group was suspected due to continuously inhaling chemicals in the working environment. The regular cloth mask, which the control group used, can only inhibit the entering of substantial particles, while the chemical pollutants in gas formed and small particles would pass by and could be inhaled by the employees.

The result of the research shows the difference in NMCTT before and after using the *Nephrolepis exaltata* herbal mask in 8 weeks. The result of Paired T-test shows a significant decrease in NMCTT before and after the treatment in the treatment group. The control group shows a significant increase ($p = 0.003$) after being tested with paired t-test. The results of this study are in accordance with the research hypothesis, which states that there is a difference in the speed of NMCTT in those employees who use *Nephrolepis exaltata* herbal masks and in those who use regular cloth masks, so that the proposed hypothesis can be accepted.

The research aims to observe the potency of *Nephrolepis exaltata* herbal mask to reduce mucociliary-transport time. The decrease of mucociliary-transport time can be observed from the difference in the delta before and after the treatment. The result of this research shows a significant difference in mucociliary-transport time before and after treatment with the statistical analysis. Furthermore, there is a difference in the mean of NMCTT delta between the treatment and control groups ($p = 0.046$).

A significant decrease in the mean delta treatment group showed that *Nephrolepis exaltata* herbal mask potentially prevents inflammation of the mucociliary clearance system in the upper respiratory tract, especially in the nose, resulting in the shortening of NMCTT on saccharin test examination. *Nephrolepis exaltata* has a high amount of essential oils. Essential oil is an antioxidant and anti-inflammation agent which can be used to fight against oxidative stress and inflammation due to chemical inhalation.^{xix,xx} Amines compound from *Nephrolepis exaltata* can react with formaldehyde pollutants in the working environment to form imine and water.^{xxi} As an effect of this reaction, formaldehyde which is destructive to the structure and function of cells is inhibited from entering the respiratory tract. According to previous studies, the genus plants *Nephrolepis exaltata* was known to have pharmacological activity as anti-inflammatory and anti-nosiseptic,^{xxii} and also reported some chemical compounds such as flavonoid, terpenoid, phenol compound, and xanthone.^{xxiii} This is confirmed by the results of phytochemical analysis showing that the *Nephrolepis exaltata*, like all other ferns, contains alkaloids, flavonoids, steroids and methanol fractions. The *Nephrolepis biserrata* sample was a potential antioxidant.^{xxiv} Another research using microscope-microchemistry methods shows that *Nephrolepis* contains flavonoid in free form and glycosides which are useful as antioxidants.

The research result shows a significant decrease of NMCTT since the herbal mask is made from *Nephrolepis exaltata*, which contains chemical compounds that can absorb VOC substances in a textile manufacturing environment in the form of formaldehyde and BTX. The result is in

accordance with the *National Aeronautics and Space Administration (NASA)* 's 's research result mentioning that *Nephrolepis exaltata* can absorb formaldehyde, xylene, trichloroethylene, and carbon monoxide in the air.^{10,14} The protection ability of *Nephrolepis exaltata* herbal mask was supported by the research using *Sprague Dawley* rat which was exposed to BTEX. The other study result shows that *Nephrolepis exaltata* herbal mask is better than the activated carbon mask.⁹

The time for data collection could not be equated to all samples. It may have been affected by the physical and mental conditions of the subjects, as well as the limited population of subjects suggested to be the limitation of the study. Another potential limitation was anticipated by adherence control via direct interviews with some research subjects and dyeing supervisors every two weeks, along with the distribution of *Nephrolepis exaltata* herbal mask to the treatment group.

CONCLUSION

From the research, the result can be concluded that the use of *Nephrolepis exaltata* herbal mask is better in reducing NMCTT of textile industry employees compared with the use of regular cloth mask. However, *Nephrolepis exaltata* herbal masks must be well-controlled and changed only once a day.

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AUTHORS CONTRIBUTION

All authors will take public responsibility for the manuscript's content submitted to Jurnal Biomedika dan kesehatan. The contributions of all authors must be as described: TN : collecting data, data analysis, data interpretation, preparing the manuscript; AP: concept, design; AP,IPM: data analysis, data interpretation, final approval of the version to be published

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CONFLICT OF INTEREST

Competing interests: No relevant disclosures.

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