Effect of tiger milk mushroom 
(Lignosus rhinocerus) 
supplementation on respiratory
health, immunity and antioxidant 
status: an open-label prospective study

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Tiger milk mushroom (TMM; Lignosus rhinocerus) have been used for a long time by indigenous 
communities in South East Asia regions as traditional medicine for different ailments, including 
respiratory disorders. The beneficial effects of TMM have been proven through in vivo and in vitro 
models, but these effects have yet to be validated in a clinical study. In this study, the beneficial 
effects of TMM supplementation were investigated in 50 voluntary participants. Participants 
were required to take 300 mg of TMM twice daily for three months. Level of interleukin 1β (IL‑1β), 
interleukin 8 (IL‑8), immunoglobulin A (IgA), total antioxidant capacity, malondialdehyde (MDA), 
3-nitrotyrosine (3-NT), 8-hydroxydeoxyguanosine (8-OHdG), pulmonary function and respiratory 
symptoms were assessed during baseline and monthly follow-up visits. Results demonstrated that 
supplementation of TMM significantly (p < 0.05) suppressed the level of IL‑1β, IL‑8, MDA, as well as 
respiratory symptoms. In additional to that, TMM also significantly (p < 0.05) induced the level of IgA, 
total antioxidant capacity, as well as pulmonary function. Analyses of data indicated that gender and 
BMI were factors influencing the outcomes of antioxidant status. Collectively, our findings suggested 
that TMM supplementation effectively improves respiratory health, immunity and antioxidant status.

Tiger milk mushroom (TMM; Lignosus rhinocerus) was first mentioned in the “The Diary of John Evelyn” about 
400 years ago. It is an important medical product which was received as repository’s collection by the Order 
at Paris from Jesuits of Japan and China¹. This mushroom is commonly known as “cendawan susu rimau” or 
“kulat susu rimau” meaning “Tiger Milk mushroom”. TMM was successfully cultivated in 2009; thus, making it 
commercially available and spurring researches on its therapeutic uses. Its sclerotium extracts had been shown 
to be antioxidant², antimicrobial³, anti-inflammatory⁴, anti-asthmatic⁵, and able to enhance immunomodulatory 
activities⁶. Recent in silico absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis 
revised that most compounds (31 from 36 compounds) from TMM extracts were orally active, and high 
absorption rates were demonstrated by at least ten of the compounds⁷. Along with that, a preclinical toxicology 
study also showed that there was no treatment-related sub-acute toxicity following 28-days oral administration 
of 1000 mg/kg TMM. The no-observed-adverse-effect-level (NOAEL) dose was higher than 1000 mg/kg⁸.

Recently, World Health Organisation (WHO) had stressed on the importance of respiratory health and 
protecting the vulnerable lung from external stressors such as particles, chemicals and infectious organisms⁹. 
Respiratory diseases such as chronic obstructive pulmonary disease (COPD), asthma, acute lower respiratory 
tract infections, tuberculosis and lung cancer represents global health burden¹⁰. Upon exposure to external 
stressor, human’s first-line filtration involves nasal vibrissae, mucociliary escalator and cough reflex. Salivary 
immunoglobulin A (IgA) is an immunity defence at mucosal surfaces protecting against pathogens and smaller

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Airway inflammation is body’s natural defence mechanism. In the lung, toll-like receptors (TLRs) activates inflammatory cells to produce growth, chemokines and pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin 1β (IL-1β), and interleukin 8 (IL-8). These cytokines evokes the neutrophils to migrate out of pulmonary capillaries into air spaces and performs phagocytosis. As the lung is an vital organ responsible for providing oxygen, excessive inflammation can be detrimental to human health and it calls for need to establish lung homeostasis. Anti-inflammatory properties of TMM was investigated in Sprague Dawley rats and results reported strong inhibitory effects on TNF-α production in macrophage cells. To add, extracts of TMM also proven to be effective in reducing the infiltration of eosinophil into lungs of ovalbumin (OVA)-sensitized asthmatic rats. Thus, suggesting TMM as alternative treatment for acute asthma and to reduce airway inflammation.

Respiratory diseases like asthma and COPD are also linked to oxidative stress. Reactive oxygen species (ROS) formed damages cellular molecules, causing cell injury, and could induce respiratory cell death. Modulation of oxidative stress can aid in therapeutic management and prevention of disease. Several studies had associated respiratory diseases with markers of oxidative stress such as malondialdehyde (MDA) for lipid damage, 8-hydroxy-2′-deoxyguanosine (8-OHdG) for DNA damage and 3-Nitrotyrosine for protein damage. Elevated levels of markers had been reported for elderly and smokers with COPD as well as allergic asthmatic children. These markers of oxidative stress had been proposed to be biomarkers of respiratory diseases; such MDA for asthma monitoring, 8-OHdG for COPD and lung cancer as well as 3-NT for acute respiratory distress syndrome (ARDS) and viral infection. Alongside, the Nasal Symptom Questionnaire (NSQ) is another useful clinical utility for scoring of nasal symptoms and allergic rhinitis.

Indigenous communities in South East Asia regions traditionally used TMM for improvement of respiratory health and yet is known about its efficacy. As such, this study aims to evaluate effects of TMM on improvements of respiratory health utilising various indicators such as immunity, symptoms and oxidative stress.

### Results

#### Characteristics of participant.

Fifty participants with no background of chronic respiratory disease, non-smokers and not undergoing any supplementation targeting respiratory health have been recruited into this study. No dropout during follow up period (Fig. 1). Most participants were at the age of 30–35 years old (n = 27, 54%); followed by 41–45 years old (n = 14, 28%), 46–50 years old (n = 5, 10%) and lastly 36–40 years old (n = 4, 8%). There were more male participants (n = 28, 56%) as compared to female participants (n = 22, 44%). The majority of the participants were in the category of normal weight (n = 30, 60%); followed by overweight (n = 12, 40%), lastly were underweight and obese with 10% and 6% respectively (Table 1).

#### Changes in vital signs.

All the 3 vital signs showed no significant changes throughout the whole study (Table 2). Blood pressure of participant was within normal range, even after taking into account of the standard deviation. Variation of blood pressure between each visit was within 5 mmHg, which returned to be statistically not significant. Similarly, heart rate and temperature of participants were also showing very minimal changes between each visit.

#### Changes in respiratory health and immunity.

Almost all of the respiratory health parameters showed significant changes (p < 0.05) after three-months supplementation of TMM, except FVC (Table 3). FVC changes were insignificant with some fluctuations in between the visits. On the other hand, FEV₁ showed significant changes (p < 0.01) with 19.7% improvement by the end of study. Together with that, ratio of FEV₁ to FVC was also significantly (p < 0.001) improved from 67.40 ± 19.76 to 85.73 ± 13.82, which is equivalent to 27.2% improvement. Respiratory symptoms as assessed by NSQ showed significant changes by the end of study; scoring was decreased from 8.96 ± 4.89 to 2.32 ± 0.79, equivalent to 74.1% of drastic changes. Likewise, VAS score as self-administrated by participants was also showing significant reduction (p < 0.001) from 2.54 ± 1.54 to 0.68 ± 0.49. Results showed that inflammatory condition of participant’s respiratory system was significantly improved after supplementation. Reduction in IL-1β and IL-8 content were 54.9% and 40.8% respectively. IgA level increased from 4.83 ± 0.28 ng/mL to 10.26 ± 1.79 ng/mL, which reflected a significant improvement after supplementation. Reduction in IL-1β and IL-8 content were 54.9% and 40.8% respectively. IgA level increased from 4.83 ± 0.28 ng/mL to 10.26 ± 1.79 ng/mL, which reflected a significant improvement after supplementation.

#### Changes in antioxidant status of respiratory system.

Results showed that total antioxidant capacity of participant was significantly (p < 0.001) increased from 0.703 ± 0.321 mmol/L during baseline visit to 1.188 ± 0.525 mmol/L in last follow up visit, which is equivalent to 68.9% of improvement. MDA content as the indicator of lipid peroxidation due to oxidative damage was significantly reduced (p < 0.001) from 1.10 ± 0.87 mmol/L to 0.34 ± 0.18 mmol/L, equivalent to 68.3% reduction. In contrast, 3-NT and 8-OHdG content as indicator of oxidative damage on protein and DNA were not affected with the supplementation of TMM (Table 4). When demographic factors were tested as between-subject effect, it was found that gender and BMI played a role in influencing the outcome of antioxidant status. Male was more responsive to supplementation as compared to female in increasing total antioxidant capacity. Result also showed that normal weight participant was statistically more responsive in suppressing production of MDA as compared to other categories of BMI.
Discussion
This study is the first to report the beneficial effects of TMM on respiratory health through clinical approach. Our findings revealed that TMM supplementation is effective in improving overall respiratory health and immunity of the participants. Few parameters being assessed as the indicator of respiratory health had shown a significant improvement by the end of this study. Those parameters included pulmonary function, respiratory symptoms, VAS, interleukin level and immunoglobulin level. Ratio of FEV₁ to FVC that reflects pulmonary function has shown 27.2% improvement after three months of TMM supplementation. Meanwhile, respiratory symptoms as assessed by NSQ and VAS have shown a drastic drop by the end of study, with the reduction of 74.1% and
73.2% respectively. The underlying mechanism responsible for such changes was likely due to the dropped in IL-1ß and IL-8 level, with 54.9% and 40.8% reduction respectively in this study. IL-1ß and IL-8 are both the major cytokines involved in the initiation and persistence of inflammation in airway and lung\(^28,29\). IL-1ß is an inducible cytokine and generally only expressed in damaged cells or triggered by pathogen product through the activation of pattern recognition receptors (PRRs)\(^{30}\). In the lung, IL-1ß is produced by epithelial cells, alveolar

### Table 1. Characteristics of participant.

| Characteristic          | Frequency |
|-------------------------|-----------|
| **Gender (n/%)**        |           |
| Male                    | 28 (56.0) |
| Female                  | 22 (44.0) |
| **Age (years) (n/%)**   |           |
| 30–35                   | 27 (54.0) |
| 36–40                   | 4 (8.0)   |
| 41–45                   | 14 (28.0) |
| 46–50                   | 5 (10.0)  |
| **Body mass index (BMI) (n/%)** |         |
| Underweight (<18.5)    | 5 (10.0)  |
| Normal weight (18.5–24.9) | 30 (60.0) |
| Overweight (25–29.9)    | 12 (24.0) |
| Obese (≥30)             | 3 (6.0)   |

### Table 2. Changes in vital sign of participant during study.

| Parameter                  | Baseline | First follow-up | Second follow-up | Third follow-up |
|-----------------------------|----------|-----------------|------------------|-----------------|
| **Blood pressure (mmHg)**   |          |                 |                  |                 |
| Systolic                    | 122.66 ± 20.62 | 120.52 ± 21.80 | 121.86 ± 16.56 | 123.25 ± 18.10 |
| Diastolic                   | 82.28 ± 13.56 | 81.24 ± 12.33  | 80.38 ± 13.26  | 82.34 ± 13.70  |
| **Heart rate (BPM)**        |          |                 |                  |                 |
|                             | 80.8 ± 13.6 | 84.6 ± 10.7     | 81.6 ± 11.5     | 81.1 ± 11.1     |
| **Temperature (°C)**        | 36.64 ± 0.39 | 36.74 ± 0.27   | 36.75 ± 0.24   | 36.80 ± 0.22   |

Values were expressed as means ± SD. Statistically significant p values are marked in asterisks (*). \(^{a}\) p-value was calculated using general linear model (GLM) for repeated measures model, with sampling time point as within-subjects factor.

### Table 3. Changes in respiratory health and immunity of participant during study as indicated by clinical assessment and laboratory outcomes.

| Parameter                  | Baseline | First follow-up | Second follow-up | Third follow-up |
|-----------------------------|----------|-----------------|------------------|-----------------|
| **Pulmonary function**      |          |                 |                  |                 |
| FVC, mean ± SD              | 5.75 ± 2.28 | 5.07 ± 1.94     | 5.12 ± 2.16      | 4.91 ± 1.92     |
| FEV\(_1\), mean ± SD        | 3.50 ± 0.96 | 3.60 ± 1.07     | 3.97 ± 1.39      | 4.19 ± 1.74     |
| FEV\(_1\)/FVC ratio, mean ± SD | 67.40 ± 19.76 | 75.81 ± 18.72   | 82.05 ± 16.98   | 85.73 ± 13.82   |
| **Respiratory symptoms**    |          |                 |                  |                 |
| NSQ, mean ± SD              | 8.96 ± 4.89 | 5.96 ± 3.81     | 3.96 ± 1.19      | 2.32 ± 0.79     |
| VAS, mean ± SD              | 2.54 ± 1.54 | 1.62 ± 0.89     | 1.12 ± 0.67      | 0.68 ± 0.49     |
| **Inflammation**            |          |                 |                  |                 |
| IL-1ß (pg/mL), median (IQR)| 3.38 (2.99–3.99) | 3.06 (2.86–3.40) | 1.70 (1.48–2.42) | 1.49 (1.27–1.92) |
| IL-8 (pg/mL), median (IQR)  | 15.27 (13.10–18.49) | 13.69 (11.15–16.55) | 10.15 (8.73–12.38) | 9.33 (7.50–10.81) |
| **Immunity**                |          |                 |                  |                 |
| IgA (ng/mL), mean ± SD      | 4.83 ± 0.28 | 5.05 ± 1.30     | 7.06 ± 1.54      | 10.26 ± 1.79    |

Statistically significant p values are marked in asterisks (*). \(^{a}\) p-value was calculated using general linear model (GLM) for repeated measures model, with sampling time point as within-subjects factor. \(^{b}\) p-value was calculated using general linear model (GLM), with gender tested as between-subject effect. \(^{c}\) p-value was calculated using general linear model (GLM), with age tested as between-subject effect. \(^{d}\) p-value was calculated using general linear model (GLM), with BMI tested as between-subject effect.
function, but studies have shown that macrophage dysfunction in many respiratory diseases is highly prevalent. IL-1β, as well as pro-IL-1β gene expression. Although macrophages played a critical role in immune function and inflammation, they are also upregulated by IL-1β.

Previous studies have shown that TMM exerted the effect of modulating inflammatory properties in both in vivo and in vitro studies. In vivo study showed that methanol extracts of TMM exhibits acute anti-inflammatory activities, by using carrageenan-induced paw edema test. These anti-inflammatory activities were likely attributed by high-molecular-weight protein in TMM which exerts inhibitory effect on lipopolysaccharide (LPS)-tumour necrosis factor (TNF)-α production. TNF-α was reported to be the key modulator that recruits inflammatory cells, stimulating the generation of inflammatory mediators such as IL-1β and IL-8, increasing oxidative stress, and inducing airway hyperresponsiveness. Apart from inhibitory action against TNF-α production, TMM also exhibited regulatory effect against macrophages. The regulatory effect of TMM was suggested to be attributed by its high linoleic acid content. Linoleic acid inhibits inflammatory responses from macrophage through inactivation of nuclear factor (NF)-κappaB and activator protein-1 (AP-1) by suppressing oxidative stress and signal transduction pathway of signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK)-1. In addition, oral administration of linoleic acid was found to effectively reduced the autoinduction suppression in mucosal tissue played its part through three main mechanisms; immune exclusion, intracellular neutralization and virus excretion. Studies have demonstrated that TMM supplementation could be attributed by its anti-inflammatory and immunomodulatory properties. This finding also concurred with a recent study that reported airway relaxation effects of TMM.

Sinonasal disease was mainly triggered by persistent or recurrent episodes of infection or inflammation of one or both sinus cavities. Thus, the significant improvement in NSQ scoring was most likely attributed by the anti-inflammatory effect of TMM supplementation. In addition to that, the antiproliferative effect of TMM could also contributed the improvement in sinonasal symptoms.VAS is a psychometric scale that proven to be useful in recording sinonasal symptom severity. Finding from VAS was well correlated with NSQ outcome, both of the scales showed similar degree of reduction in the end of study. Impaired peak lung function has been highlighted as one of the risk factor that contributed to development of chronic lung disease. Low FEV1 and FEV1/FVC between 18 and 30 years of age predicted airflow obstruction in 20 years later, and that prediction is independent from smoking status. In addition to that, impaired pulmonary function in early adulthood also leads to other health consequences in later life. As such, TMM supplementation that can effectively improve pulmonary function suggested a preventive effect against development of pulmonary diseases. The protective effect of TMM supplementation could be attributed by its anti-inflammatory and immunomodulatory properties. This finding also concurred with a recent study that reported airway relaxation effects of TMM.

Mucosal membranes are critical as the first line of immunity defence in respiratory tract in preventing the invasion of pathogens through epithelial barrier. IgA being the most predominant immunoglobulin isotype in mucosal tissue played its part through three main mechanisms; immune exclusion, intracellular neutralization and virus excretion. Studies have demonstrated that IgA level of participants was doubled after three months of TMM.

| Parameters | Baseline | First follow-up | Second follow-up | Third follow-up | \(p\)-value | \(p\)-value | \(p\)-value | \(p\)-value |
|------------|----------|----------------|----------------|----------------|------------|------------|------------|------------|
| TAC (mmol/L), means ± SD | 0.703 ± 0.321 | 0.867 ± 0.426 | 0.986 ± 0.360 | 1.188 ± 0.525 | < 0.001* | < 0.001* | 0.28 ± 0.26 (0.33) | 0.26 (0.18–0.37) |
| MDA (mmol/L), median (IQR) | 0.94 (0.81-1.21) | 1.03 (0.91–1.22) | 0.29 (0.19–0.45) | 0.47 (0.13–0.77) | 0.94 (0.81–1.21) | 0.50 (0.38–0.61) | 0.70 (0.23–0.98) | 0.139 |
| 3-NIT (ng/mL), median (IQR) | 0.50 (0.38–0.61) | 0.54 (0.44–0.69) | 0.55 (0.12–0.72) | 0.70 (0.23–0.98) | 0.50 (0.38–0.61) | 0.54 (0.44–0.69) | 0.55 (0.12–0.72) | 0.70 (0.23–0.98) |
| 8-OHdG (ng/mL), median (IQR) | 0.91 (0.58–1.05) | 0.70 (0.23–0.98) | 0.55 (0.12–0.72) | 0.70 (0.23–0.98) | 0.91 (0.58–1.05) | 0.70 (0.23–0.98) | 0.55 (0.12–0.72) | 0.70 (0.23–0.98) |

### Table 4. Changes in antioxidant status of participants during study as indicated by laboratory outcomes. Antioxidant status was analysed from salivary sample. TAC Total Antioxidant Capacity; MDA Malondialdehyde; 3-NIT 3-Nitrotyrosine; 8-OHdG 8-hydroxy-2-deoxyguanosine. Statistically significant * values are marked in asterisks (*). \(p\)-value was calculated using general linear model (GLM) for repeated measures model, with sampling time point as within-subjects factor. \(p\)-value was calculated using general linear model (GLM), with gender tested as between-subject effect.
supplementation, suggesting a strengthen respiratory immunity. Low IgA level is often being associated with increased risk of developing respiratory tract infection52. Although the underlying mechanism of IgA induction by TMM still remains unclear, but study done on Ganoderma lucidum, type of mushroom with close evolutionary relationship with TMM, have proved that IgA can be induced through the up-regulation of Toll-like receptor 4 (TLR4) dependent pathway53. Interestingly, the same study also reported that natural immunity can be activated without promoting inflammation, which is concurred with our present findings. Another study on medicinal mushroom highlighted that proteoglycan, which is a common constituents of medicinal mushroom enhanced the secretion of mucosal IgA by up-regulating the expression of polymeric immunoglobulin receptor (pIgR)54. PlgR had a dual role of transporting locally produced IgA across mucosal epithelial, as well as being the precursor for production of glycopolymers which improved immune functions of IgA55. As the first liner in immunity defence, enhanced production of IgA by TMM supplementation is important to prevent colonization of respiratory tract by pathogens and penetration of antigens through epithelial cells. The antiviral properties of IgA were well established by studies on Sendai virus, influenza virus, and rotavirus56. Most recent studies also suggested the potential role of IgA in fighting SARS-CoV-2 infection12,13.

Free radicals were generated in every single second and majority of them were generated as by-product from respiration process. In other words, respiratory system is particularly more susceptible to oxidative stress-mediated injury; mainly due to high endogenous oxygen concentration in lung and inhalation of exogenous air pollutants. Antioxidant as therapeutic alternatives to improve respiratory disease has gained much attention lately due to its easy accessibility through dietary intake. Recent review pointed out that although definitive data is still lacking, but available evidences suggested that dietary intake of antioxidant is closely associated with better pulmonary function, less lung function decline and reduced risk of COPD57. Our result indicated that three months of TMM supplementation can effectively increase total antioxidant capacity by almost 70%. This observation is presumably due to the high phenolic content in TMM58. Although phenolic compounds in TMM may possess the ability to scavenge radicals, but these compounds are yet to be identified warranting further studies for validation59. Epidemiological studies and associated meta-analyses had suggested long term consumption of diets rich in plant polyphenols to significantly improve antioxidant capacity and to offer protection against cancers, cardiovascular diseases, respiratory diseases and neurodegenerative diseases59.

High polyphenol diet was associated with better pulmonary function in population based study60. In addition, TMM supplementation could potentially exert a protective effect against inflammation-related respiratory diseases as polyphenols had been proven to effectively reduce inflammation, by (1) up-regulating antioxidant gene expression, (2) attenuating endoplasmic reticulum stress signalling, (3) blocking pro-inflammatory cytokines, (4) suppressing inflammatory gene expression by stimulating histone deacetylase activity, or (5) activating transcription factors that antagonize chronic inflammation61. It is worth noting that male participants in our study is showing more significant changes in antioxidant capacity as compared to female participants. Similar observation has been discussed previously in view of the differences in the level of oxidative stress between male and female62.

Lipid peroxidation was found to be positively associated airway inflammation; reactive oxygen species (ROS) were generated by several inflammatory cells that participate in airway inflammation and their production will in turns amplify the inflammation in airway, thus created a positive looping63. MDA is the main product of lipid peroxidation often being used as biomarker to assess oxidative-mediated lipid damage. Its correlation with pulmonary function and respiratory disease severity was well established64. Our result indicated TMM supplementation exerted a suppressive effect on MDA production, which implicated its potential protective effect against oxidative damage. Such observation was likely attributed by improved total antioxidant capacity in TMM65. Reduced lipid peroxidation often being used as biomarker to assess oxidative-mediated lipid damage. Its correlation with pulmonary function and respiratory disease severity was well established66. Our result indicated TMM supplementation exerted a suppressive effect on MDA production, which implicated its potential protective effect against oxidative damage. Such observation was likely attributed by improved total antioxidant capacity in TMM67. Although phenolic compounds in TMM may possess the ability to scavenge radicals, but these compounds are yet to be identified warranting further studies for validation58. Epidemiological studies and associated meta-analyses had suggested long term consumption of diets rich in plant polyphenols to significantly improve antioxidant capacity and to offer protection against cancers, cardiovascular diseases, respiratory diseases and neurodegenerative diseases59.

Conclusion

Although TMM is well-known for its ethnomedicinal uses in curing many ailments and to improve respiratory health, but scientific evidences that supporting its therapeutic uses were limited to in vivo and in vitro models. Present work is the first to study the efficacy of TMM supplementation to improve respiratory health through a clinical approach. Findings revealed that TMM supplementation can effectively improve the respiratory health, immunity, as well as overall antioxidant status. Thus, suggesting TMM supplementation as a potential adjuvant therapy to the current drugs used for the management of respiratory diseases. Nonetheless, the lack of a placebo group is one important limitation in this study. Randomized controlled trial is recommended for future study to...
validate our findings. In addition, this study was not registered with any Clinical Trial Registry and will consider registering future studies.

**Methods**

**Study design.** This is an open-label, single-arm, prospective study that involved three-months period of supplementation. The study was conducted with full compliance to the principles of Helsinki Declaration, as well as criteria outlined in Malaysian Guidelines for Good Clinical Practice\(^7\). Eligibility was confirmed in accordance to protocol-checklist and written informed consent was obtained from each participant. The study was approved by principal investigator’s Institutional Ethics Committee (UCSI University, Malaysia, approval code IEC-2020-FMHS-026).

**Participants selection.** This open-label study enrolled 50 volunteers (aged 30- to 50-year-old) with good general healthy condition. Recruitment was conducted in UCSI University, Kuala Lumpur, Malaysia. The following inclusion criteria were applied: (1) participants who were not undergoing any supplementation targeting respiratory health; (2) non-smoker; (3) participants who were able to fully understand protocol and study information given by investigators; and (4) participants who willing to give informed consent. Participants were exclude based on these exclusion criteria: (1) undergoing medication plan targeting respiratory system; (2) taking antimicrobial or antiviral medication during study entry; (3) undergone major surgical procedures within 6 months prior to study entry; and (4) pregnant or lactating woman. All participants were being provided with a participant information sheet and explained by investigator. A written informed consent was sought from each participant.

**Supplementation.** At baseline visit, demographic characteristic and medical history of participants were collected. Participants were then started on three-months oral supplementation of TMM fine powder given in the form of capsule (TigerPro\(^-\), Nexus Wise, Selangor, Malaysia), at the dosage of 300 mg, twice daily. Three follow up visits were conducted with one month interval between each visit. A paper case report form (CRF) was used to capture the information of vital signs, pulmonary function, respiratory symptoms and self-evaluated severity of symptoms during each visit. Nasal lavage and saliva were also collected during each visit, at week 0, 4, 8 and 12 for laboratory investigation.

**Clinical assessment.** Blood pressure and heart rate were measured using Omron automatic blood pressure monitor HEM 7120 (Omron Healthcare, Kyoto, Japan). Temperature was measured using Braun forehead infrared thermometer NTF 3000 (Braun GmbH, Kronberg, Germany). Pulmonary function was tested using spirometry method. Force Vital Capacity (FVC), Forced Expiratory Volume (FEV1) and FEV1/FVC ratio were assessed in triplicate using Contec SP70B handheld digital spirometer (Contec Medical Systems, Hebei, China). FVC (in liters) is the greatest total amount of air an individual can forcefully breathe out after breathing in as deeply as possible. FEV1 (in liters) is the amount of air an individual can force out of lungs in one second. It is a useful indicator for breathing problem. FEV1/FVC ratio was served as an indicator of general wellness of pulmonary function.

Validated nasal symptom questionnaire (NSQ) developed by Saito and his colleagues was being adopted with slight modification to evaluate participants sinonasal symptoms in this study\(^27\). NSQ is a self-administered questionnaire consisting of 10 items with 2 parts (I–II): (I) 8 items related to nasal symptoms and (II) 2 quality of life related items. Each item of the NSQ was divided into 6 levels: no symptoms at all (0 points); very mild (1 point) to very severe (5 pointes). Total points (NSQ score) (ranging from 0 to 50) were analysed. In addition, visual analogue scale (VAS) with the scale of 1 to 10 was being used to self-evaluate the severity of nasal symptoms.

**Laboratory examination.** Nasal lavage was collected using NeilMed Sinus Rinse (NeilMed Pharmaceuticals, Inc., Santa Rosa, Canada), a low-pressure nasal irrigation tool with isotonic saline. Participant was required to bend forward their body to a comfort level and tilted their head down. Keeping their mouth open without holding breath, placed the cap snugly against nasal passage. Squeezed the bottle gently until the solution starts draining from the opposite nasal passage. The lavage fluids were collected using a 10 mL sterile tube. Lavage fluids were treated with N-acetyl cysteine in lab to disrupt mucus, supernatant was then collected after centrifugation for further analysis. The levels of the proinflammatory cytokines interleukin (IL)-1β and IL-8 were quantified in duplicate using BioLegend enzyme-linked immunosorbent assay (ELISA) kits (BioLegend CNS trifugation for further analysis. The levels of the proinflammatory cytokines interleukin (IL)-1β and IL-8 were quantified in duplicate using BioLegend enzyme-linked immunosorbent assay (ELISA) kits (BioLegend CNS trifugation for further analysis. The levels of the proinflammatory cytokines interleukin (IL)-1β and IL-8 were quantified in duplicate using BioLegend enzyme-linked immunosorbent assay (ELISA) kits (BioLegend CNS trifugation for further analysis. The levels of the proinflammatory cytokines interleukin (IL)-1β and IL-8 were quantified in duplicate using BioLegend enzyme-linked immunosorbent assay (ELISA) kits (BioLegend CNS trifugation for further analysis. The levels of the proinflammatory cytokines interleukin (IL)-1β and IL-8 were quantified in duplicate using BioLegend enzyme-linked immunosorbent assay (ELISA) kits (BioLegend CNS 

Unstimulated saliva samples were collected using a sterile 2.0-mL vial. Participants uncapped the vial, placed the straw into the vial, and passively drooled down the straw for 90s. All samples were assayed for different parameters in duplicate using respective kits. Immunoglobulin (IgA) was assayed using Elabscience QuicKey Human IgA ELISA Kit (Elabscience Biotechnology Co. Ltd, Texas, United States), total antioxidant capacity was assayed using Elabscience total antioxidant capacity (T-AOC) colorimetric assay kit (Elabscience Biotechnology Co. Ltd, Texas, United States), malondialdehyde (MDA) as lipid peroxidation biomarker was assayed using Elabscience MDA colorimetric assay Kit (Elabscience Biotechnology Co. Ltd, Texas, United States), 3-nitrotyrosine (3-NT) as biomarker of oxidative stress-derived protein damage was assayed using Elabscience 3-NT ELISA Kit (Elabscience Biotechnology Co. Ltd, Texas, United States) and 8-hydroxydeoxyguanosine (8-OHdG) as biomarker of oxidative stress-derived DNA damage was assayed using Elabscience 8-OHdG ELISA Kit (Elabscience Biotechnology Co. Ltd, Texas, United States). Key component that constitute the first line of immunological defence of respiratory tract is secretory IgA, and it is predominantly found in salivary secretions. Recent studies...
have suggested the potential role of salivary IgA as predictor of susceptibility to respiratory infections\(^{27,28}\). Levels of salivary oxidative stress were also found to be associated with the development of asthma\(^{29,30}\) and COPD\(^{31}\).

**Statistical analysis.**  Demographic characteristics were presented as categorical data, expressed in frequency and percentage. All outcomes were analysed as continuous dependent variables, presented as mean ± SD for normally distributed data or median (interquartile range) for non-normally distributed data. The changes in clinical and laboratory outcomes from baseline visit to last follow-up visit were analysed using general linear model (GLM) for repeated measures model. Within-subjects factors were defined as the sampling time point. Gender, age and BMI were tested as between-subject effect. Homogeneity of the variance and covariance structure of the dependent variables was assessed by Levene and Box M tests. Sphericity test of the residual covariance matrix was assessed using Mauchly’s sphericity test. Results considered significant if \(p < 0.05\) with 95% of confidence interval. Statistical analysis was performed using SPSS 26.0 (IBM Corp., New York, United States) for MacOS.

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**References**

1. Evelyn, J. The diary of John Evelyn 1st edn. (Nabu Press, Charleston, 1994).

2. Lau, B. F. et al. The potential of mycelium and culture broth of *Lignosus rhinocerotis* as substitutes for the naturally occurring sclerotium with regard to antioxidant capacity, cytotoxic effect, and low-molecular-weight chemical constituents. *PLoS ONE* **9**(7), e102509-e (2014).

3. Shopana, M., Sudhahar, D. & Anandarajagopal, K. Screening of lignosus rhonoceros extract as antimicrobial agents against selected human pathogens. *J. Pharm. Biomed. Sci.* **18**(11), 1–4 (2012).

4. Lee, S. S. et al. Anti-inflammatory effect of the sclerotium of *Lignosus rhinocerotis* (Cooke) Ryvarden, the Tiger Milk mushroom. *BMC Compl. Altern. Med.* **14**(1), 359 (2014).

5. Johnathan, M., Gan, S. H., Ezumi, M. F. W., Faezahtul, A. H. & Nurul, A. A. Phytochemical profiles and inhibitory effects of Tiger Milk mushroom (*Lignosus rhinocerotis*) extract on ovalbumin-induced airway inflammation in a rodent model of asthma. *BMC Compl. Altern. Med.* **16**, 167 (2016).

6. Wu, K.-H., Lai, C. K. M. & Cheung, P. C. K. Immunomodulatory activities of mushroom sclerotal polysaccharides. *Food Hydrocolloids* **25**(2), 150–158 (2011).

7. Sillapachaiyaporn, C. & Chuchawankul, S. HIV-1 protease and reverse transcriptase inhibition by tiger milk mushroom (*Lignosus rhinoceros*) sclerotium extracts: In vitro and in silico studies. *J. Tradit. Compl. Med.* **10**(4), 396–404 (2020).

8. Lee, S. S., Tan, N. H., Fung, S. Y., Paloor, J. & Sim, S. M. Evaluation of the sub-acute toxicity of the sclerotium of *Lignosus rhinocerus* (Cooke), the Tiger Milk mushroom. *J. Ethnopharmacol.* **138**(1), 192–200 (2011).

9. Cox, M. J., Ege, M. J. & von Mutius, E. Challenges, impact and the future. *Lung Microbiome*. 83, 240 (2019).

10. Soriano, J. B. et al. Prevalence and attributable health burden of chronic respiratory diseases, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Respir. Med.* **8**(6), 385–396 (2020).

11. Maurer, M. A. et al. Glycosylation of human IGA directly inhibits influenza a and other sialic-acid-binding viruses. *Cell Rep.* **23**(1), 90–99 (2018).

12. Wang, Z. et al. Enhanced SARS-CoV-2 neutralization by dimeric Iga. *Sci. Transl. Med.* **13**(577), eaab1555 (2021).

13. Sterlin, D. et al. IgA dominates the early neutralizing antibody response to SARS-CoV-2. *Sci. Transl. Med.* **13**(577), eaab223 (2021).

14. Tiernan, C., Lyons, M., Comyns, T., Nevill, A.M., Warrington, G. Salivary IgA as a predictor of upper respiratory tract infections and relationship to training load in elite rugby union players. *J. Strength Condition. Res.* **34**(3) (2020).

15. Garth, J., Barnes, J.W., Krick, S. Targeting cytokines as evolving treatment strategies in chronic inflammatory airway diseases. *Int. J. Mol. Sci.* **19**(11) (2018)

16. Aghasaref, P., George, U. & Pidaparti, R. A review of inflammatory mechanism in airway diseases. *Inflamm Res.* **68**(1), 59–74 (2019).

17. Pizzino, G. et al. Oxidative Stress: Harms and Benefits for Human Health. *Oxid. Med. Cell Longe*. 2017, 8416763 (2017).

18. Yang, W. & Omaye, S. T. Air pollutants, oxidative stress and human health. *Mutation Res. Genetic Toxicol. Environ. Mutagen.* **674**(1), 45–54 (2009).

19. Bortey-Sam, N. et al. Oxidative stress and respiratory symptoms due to human exposure to polycyclic aromatic hydrocarbons (PAHs) in Kumasi, Ghana. *Environ. Pollut.* **228**, 311–320 (2017).

20. Lee, J. S., Shin, J. H., Hwang, J. H., Baek, J. E. & Choi, B. S. Malondialdehyde and 3-nitrotyrosine in exhaled breath condensate in retired elderly coal miners with chronic obstructive pulmonary disease. *Sci. Health Work*. **5**(2), 91–96 (2014).

21. Baraldi, E. et al. 3-Nitrotyrosine, a marker of nitrosative stress, is increased in breath condensate of allergic asthmatic children. *Allergy* **61**(1), 90–96 (2006).

22. Liu, X. et al. 3-Nitrotyrosine, a marker of nitrosative stress, is increased in breath condensate of allergic asthmatic children. *Allergy* **61**(1), 90–96 (2006).

23. He, L. et al. Malondialdehyde in nasal fluid: a biomarker for monitoring asthma control in relation to air pollution exposure. *Environ. Sci. Technol.* **54**(18), 11405–11413 (2020).

24. Jin, H. et al. Smoking, COPD, and 3-nitrotyrosine levels of plasma proteins. *Environ. Health Perspect.* **119**(9), 1314–1320 (2011).

25. Hsiao, Y. et al. 3-Nitrotyrosine, a marker of nitrosative stress, is increased in breath condensate of allergic asthmatic children. *Allergy* **61**(1), 90–96 (2006).

26. Pease, J. E. & Sabroe, I. The role of interleukin-8 and its receptors in inflammatory lung disease: implications for therapy. *Am. J. Respir. Crit. Care Med.* **163**(1), 19–25 (2002).

27. Piconi, M., Fabris, D., Cucchi, M. & Santin, M. 3-Nitrotyrosine, a marker of nitrosative stress, is increased in breath condensate of allergic asthmatic children. *Allergy* **61**(1), 90–96 (2006).

28. Lappalainen, U., Whitsett, J. A., Wert, S. E., Tichelaar, J. W. & Bry, K. Interleukin-1beta causes pulmonary inflammation, emphysema, and airway remodeling in the adult murine lung. *Am. J. Respir. Cell Mol. Biol.* **30**(5–6), 296–306 (2018).

29. Ricci, G. et al. 3-Nitrotyrosine, a marker of nitrosative stress, is increased in breath condensate of allergic asthmatic children. *Allergy* **61**(1), 90–96 (2006).

30. Pease, J. E. & Sabroe, I. The role of interleukin-8 and its receptors in inflammatory lung disease: implications for therapy. *Am. J. Respir. Crit. Care Med.* **163**(1), 19–25 (2002).

31. Piconi, M., Fabris, D., Cucchi, M. & Santin, M. 3-Nitrotyrosine, a marker of nitrosative stress, is increased in breath condensate of allergic asthmatic children. *Allergy* **61**(1), 90–96 (2006).

32. Kolb, M., Margetts, P. J., Anthony, D. C., Pittos, F. & Gauldie, J. Transient expression of IL-1beta induces acute lung injury and chronic repair leading to pulmonary fibrosis. *J. Clin. Invest.* **107**(12), 1529–1536 (2001).
73. Manna, P. & Jain, S. K. Obesity, oxidative stress, adipose tissue dysfunction, and the associated health risks: causes and therapeutic strategies. *Metab. Syndr. Relat. Disord.* **13**(10), 423–444 (2015).
74. Sutherland, E. R. *et al.* Cluster analysis of obesity and asthma phenotypes. *PLoS ONE* **7**(5), e36631 (2012).
75. Guideline, I. H. T. Guideline for good clinical practice. *J. Postgrad. Med.* **47**(3), 199–203 (2001).
76. Nikasinovic-Fournier, L. *et al.* Nasal lavage as a tool for the assessment of upper-airway inflammation in adults and children. *J. Lab. Clin. Med.* **139**(3), 173–180 (2002).
77. Lee, K.-M. *et al.* A pilot study on the association between job stress and repeated measures of immunological biomarkers in female nurses. *Int. Arch. Occup. Environ. Health* **83**(7), 779–789 (2010).
78. Nakamura, D., Akimoto, T., Suzuki, S. & Kono, I. Daily changes of salivary secretory immunoglobulin A and appearance of upper respiratory symptoms during physical training. *J. Sports Med. Phys. Fitness* **46**(1), 152 (2006).
79. Bentur, L., Mansour, Y., Brik, R., Eizenberg, Y. & Nagler, R. M. Salivary oxidative stress in children during acute asthmatic attack and during remission. *Respir. Med.* **100**(7), 1195–1201 (2006).
80. Yigla, M., Berkovich, Y. & Nagler, R. M. Oxidative stress indices in COPD—Broncho-alveolar lavage and salivary analysis. *Arch. Oral Biol.* **52**(1), 36–43 (2007).

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Author contributions
E.S.S.T. involved in the conduct of study, data analysis and manuscript writing. T.K.L. involved in the data collection and data analysis. C.K.T. is the principal investigator of this study, oversee the conduct of whole study and involved in manuscript writing.

Competing interests
The authors declare no competing interests.

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