ORIGINAL ARTICLE

# Implementation of the "Health-triangle Concept" for a "Biosmart and Safe Bus" as a Solution in the Pandemic Era: An Observational and Randomized Clinical Trial

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## ABSTRACT

**Introduction:** The "Health Triangle Concept", implemented in "Biosmart and Safe Bus", balances interactions between the host, agent, and environment. This concept results in a safe eco-biological environment in the bus cabin, enhances immune system function, and maintains oxidative stress homeostasis. This study aimed to prove the effectiveness and efficacy of implementing the "Health Triangle Concept" in bus transportation through the development of a bus called the "Biosmart and Safe Bus." **Materials and methods:** The "Challenge tests" were conducted at Laboratory for Development of Agro-industrial and Biomedical Technology (LAPTIAB). The pre- and post-test samples were taken from seventy passengers (19-23 years old), divided randomly to control group (n = 35) in a regular bus, and intervention group rode the "Biosmart and Safe Bus" (n = 35). **Results:** The delta levels of IgA improved (17.89±30.19 mg/mL) in buses adopting the concept, as well as delta levels of IL-6 (13.38±0.96 pg/mL). A significant difference (p = 0.016) and a strong negative correlation (r = -0.611) in delta levels of SOD3 (54.69±163.91 U/ml) and ROS (24.17±45.62 (U/ml) in intervention group.**Conclusion:**The "Health-Triangle Concept" for the "Biosmart and Safe Bus" resulted in an eco-biological safe bus cabin environment, improved passenger's airway immune systems, and enhanced oxidative stress homeostasis.
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**Keywords:** Biosmart and Safe Bus, Health-Triangle Concept, IgA, IL-6, ROS, SOD3

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## INTRODUCTION

Aerogenic viruses may circulate, evaporate, and move through the air, and remain indoors for several hours. Closed rooms with a high density of people can store large viral loads, as viruses are more stable in such environments. Epidemiological studies have shown an association between aggravating factors such as fine particles and viruses and increased oxidative stress in the respiratory tracts of bus passengers. The upper airway mucosa, which is exposed to pollutants, can experience an inflammatory response and generate reactive oxygen species (ROS) through mitochondria, potentially leading to nasal epithelium cell death [1]. This inflammatory response also triggers the immune system's humoral and cellular mechanisms to maintain the body's integrity against various hazards [2]. Despite the increasing use of motor vehicles, the impact of airborne pollutants and their health risks inside public transportation, such as commuter buses, is not well understood.

Public transportation is one of the major causes of the spread of the coronavirus disease 2019 (Covid-19) virus.

The Indonesian government has made numerous efforts to combat the Covid-19 pandemic, including large-scale social and public transportation restrictions. However, these restrictions have posed challenges for bus operators, who have raised concerns about passenger capacity limitations. Meanwhile, the ongoing threat of viral transmission and the potential for new waves or mutations, technological innovations and collaborations across scientific fields, including medicine, have been crucial in developing a healthy mode of bus transportation using the "Health Triangle Concept" [3]. The impact of airborne pollutants and their health risks inside public transportation, such as commuter buses, is not well understood, and as global pandemic might not be only caused by viruses such as the coronavirus SARS-CoV-2, but also by the agent's-change triggered by climate change as well as host-lifestyle. Thereby, this conceptual approach is able to protect the potential-disaster of those global epidemic, by controlling host, agent and environmental-homeostasis [1–3].

The risk of airborne infections in public transport cabins poses a significant challenge, which could result in a significant decrease in passenger numbers. A systematic review presents a comprehensive overview of measures and strategies to protect passengers and ensure smooth operations by identifying four key strategies to prevent virus transmission in public transport, including improving air quality, cleaning, wearing masks, and maintaining social distancing in vehicles. However, the implementation of technology-based solutions, such as efficient screening systems, automatic mask detection mechanisms, ultraviolet disinfection devices, is needed to implement these strategies in a holistic, integrative and effective manner [4]. Other reviews also stated that there were evidences of transmission and presence of viral RNA on public transportation for SARS-CoV-2, but further exploration of transmission factors and the effectiveness of mitigation strategies has not been researched [5].

Currently, there were also no research on the safety and security of bus passengers regarding the risk of disease transmission, although there are studies on the effect of airflow in closed spaces on the risk of inhalation transmission. However, under certain conditions, a good ventilation system in public transportation affects passenger comfort and health. Passengers who travel by bus may be exposed to the transmission and spread of infectious agents, including viruses and bacteria and their spread is influenced by factors such as humidity, ventilation size and quantity, and the density of people present. Viral pathogens can form aerosols during sneezing and coughing, which can travel up to 2 m. Under closed conditions, aerosol particles containing pathogenic microorganisms can persist longer and are more easily inhaled. An awareness of the risk of transmission has increased since the World Health Organization officially announced that the Covid-19 virus could be transmitted through the air. The risk of transmission is greater if people are in a room where the airflow is restricted. Therefore, developing air-conditioning systems for public transportation to prevent infection of new bus passenger clusters during the spread of Covid-19 is crucial [6]. The existing strategy to help reduce the transmission and spread of the virus on public transport during the pandemic is a multilayer network approach, namely; identification of key critical places that will be prioritized in interventions, vaccination campaigns where critical bus stops are priority vaccination points, availability of health care units, optimization of public transportation or exploring various points of interest to gather insights from various other interesting issues [7].

The "Health Triangle Concept" is a strategic option for the transmission and spread of the virus, that emphasizes the importance of maintaining an equilibrium between the "host" (the bus passenger), the "agent" (the cause, namely, the virus), and the "environment" (the bus cabin). This concept highlights the necessity for the "Health Triangle" to remain balanced and stable to ensure the individual's health and prevent illness. In the context of bus transportation, the "Health Triangle Concept" was developed collaboratively by the bus body industry (Karoseri Laksana), bus entrepreneurs (PO. SUMBER ALAM EXPRES), and Diponegoro University. It regulates the balance of the equilateral triangle between the health conditions of passengers, the bus cabin environment, and the number and density of viruses (viral load) in the bus cabins by regulating the number of passenger seats, circulating air conditioners, and the use of herbal masks.

The "Biosmart and Safe Bus" is an implementation of the "Health Triangle Concept," engineered to provide a healthy eco-biological environment within the bus cabin. This involves "smart" cabin design to enable "physical distancing" (passenger seat arrangement), efficient air circulation, and the application of "nanosilver" liquid coating on the entire surface of the bus cabin to reduce viral load. "Biosmart" refers to the bus's ability to control the ecological and biological balance of the environment, where the number and density of viruses in the cabin are minimized, filtered with "high-efficiency particulate air (HEPA)-filter" technology, and degraded by ultraviolet (UV) light. A "Safe Bus" means that passengers should also wear ACCHADANA® herbal masks to prevent exposure to and transmission of pathogens between passengers. The effectiveness and efficacy of the concept may be proved by its capacity in reducing airborne microbes as well as stabilizing the immune system and oxidative stress to all passengers [1,2,8].

This study aimed to scientifically demonstrate the effectiveness and efficacy of implementing the "Health Triangle Concept" in bus transportation through the development of a bus called the "Biosmart and Safe Bus." The goal was to provide a bus cabin environment with low viral loads, resulting in a reduced risk of bacterial and viral transmission. By assessing the eco-biological aspects of the bus cabin, this study sought to establish the scientific validity of the concept in creating a safe and healthy environment for passengers.

## MATERIALS AND METHODS

A parallel theoretical studies and serial delivery of test results conducted by the author and team conclusively demonstrated that the "Biosmart and Safe Bus" is a safe and healthy mode of transportation during the pandemic era. The observational "Challenge tests" were conducted at the BPPT Agro and Laboratory for Development of Agro-industrial and Biomedical Technology (LAPTIAB). The blocked-allocation randomized controlled trial included 80 passengers (19-23 years old, homogenization of the age-range recommended by the ethics committee) that met inclusion criteria as participants. All were not involved in the design, or conduct, or reporting, or dissemination plans of our research. Self-drawls randomly separated them into two groups. The inclusion criteria were good health (no history of allergic rhinitis, asthma, cancer, liver disease, lung and heart disease, autoimmune and congenital diseases), and Covid-19 vaccination history. The exclusion criteria were alcohol consumption, smoking, pregnancy or menstruation, and the use of an inhaler during the study period. The control group (n = 35) rode in a regular bus, and the intervention group rode the "Biosmart and Safe Bus" (n = 35). All passengers were subjected to pre- and posttest sampling using the nasal wash method. The posttest samples were taken after an approximate 25-h bus journey. Nasal washing was performed by an ear, nose and throat (ENT) specialist, and enzyme-linked immunosorbent assays (ELISA) were performed at the Biomolecular Laboratory of Muhammadiyah University, Semarang to demonstrate changes in the immune system due to inflammatory responses and oxidative stress. This report complies with the CONSORT checklist.

### Manufacture of the "Biosmart and Safe Bus"

Two prototype buses were built, one in 2020 and the other in 2021, by the CV Karoseri Laksana, bus body company in Semarang. The passenger seat configuration is 1-1-1, using an HEPA filter with a laminar airflow regulator, UV-C lamps, and nanosilver-coatings on all cabin surfaces.

### ACCHADANA® Herbal Mask

CV Beauty Kassatama produces a herbal mask with the ACCHADANA® brand patent owned by the Diponegoro University (brand certificate No: IDM000921225), which has been shown to improve the sinonasal immune system and lung functions.

### Challenge Test

In 2021, the BPPT and LAPTIAB tested the effectiveness of the HEPA filter and nanosilver coating applied to the "Biosmart and Safe Bus," using a bio-indicator approach and Staphylococcus aureus airborne test bacteria. It was confirmed and to catalogue the most often used methods and conditions of bioaerosol sampling to identify the bacteria, commonly available, well-described, and relatively inexpensive methods of sampling, identification, and subtyping guarantee a high reliability of results and allow to obtain fast and verifiable outcomes in environmental studies on air transmission routes. Testing was performed by inoculating the test bacteria in bus cabins and subsequently sampling the air to detect the presence of test bacteria at specific sampling sites, and at certain time intervals. Testing was conducted for 8 h, with the first sampling taking place 30 min after inoculation, and subsequent sampling every 1 h. The test method was based on the total plate count of the agar media in Petri dishes. The resulting data were the number of bacteria in colony-forming units (cfu). The resulting data were analyzed to determine the effectiveness of the air system and nanosilver-coating in reducing airborne bacteria.

### Nasal wash Sampling

Nasal wash fluid was used as the sample for the ELISA test [8]. Immune system function and sinonasal oxidative stress were measured. A syringe containing 10 cc of warm isotonic saline was poured into one nostril. Prior to this, the person would sit with their head tilted at a 45-degree angle, take a deep breath, and hold it while the saline was administered into the nostril. After the fluid entered, the individual held the position for a few seconds and then bent down to collect the sinonasal fluid in a container. The same procedure was sequentially performed for the other nostril. Nasal wash fluid was analyzed using ELISA for the levels of IgA, interleukin-6 (IL-6), ROS, and superoxide dismutase (SOD3).

### IgA, IL-6, ROS and SOD3 Levels

ELISA was performed to detect IgA and IL-6 levels using the Human IgA 96-well ELISA Kit (ABCLONAL RK00200), and Human IL-6 ELISA 96-well kit (ABCLONAL RK00004), respectively. For ROS and SOD3, we used Human ROS ELISA 96-well (SUNLONG BIOTECH SL2046 Hu and Human SOD3), and Extracellular ELISA (SOD3) 96-well kits (ABCLONAL RK02313) respectively.

### Statistical Analysis

All data were analyzed using SPSS version 25 and tested using the chi-square and Kruskal–Wallis tests. Primary data for IgA, IL-6, SOD3, and ROS levels were analyzed using the Kruskal-Wallis test, paired t-test, Wilcoxon rank-sum test, and one-way ANOVA. The distribution of SOD3 and ROS data was tested for normality using the Shapiro–Wilk and Pearson correlation tests.

## Ethical Clearance

This study was approved by the Ethics and Health Research Commission of the Faculty of Medicine Diponegoro University No. 412/EC/KEPK/FK-UNDIP/XI/2021.

## RESULTS

We excluded 10 participants that met the exclusion criteria were alcohol consumption, smoking, menstruation, and the use of an inhaler during the study period. Figure 1 shows the flow CONSORT diagram with detailed information on the excluded participants. Allocated-randomization resulted 35 subjects in each group that analyse the IgA and IL-6 levels, as well as ROS and SOD3 levels, before and after intervention, and all did not received the feedback results of the measurement.

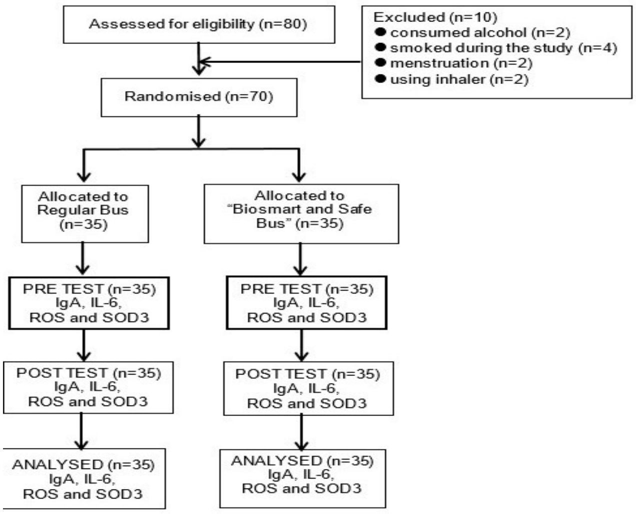


**Figure 1: Consort flow diagram**

## Challenge Test

The primary observational outcome of the challenge test revealed that 108 cells/ml of S. aureus test bacteria were introduced into the bus cabin at 0 and 4 h. Sampling was performed using the Petri dish exposure and swab method, and the number of bacteria in each sample was counted. The bacterial population was relatively high during the first 30 min, but then decreased. The bacterial population increased again after the second inoculation at 4 h, specifically for the mannequin face, and was higher than that of the first inoculation (0 h) (Figure 2).

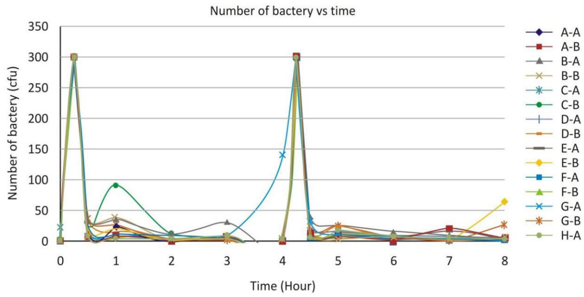


**Figure 2: Number of bactery vs time exposed in challenge test**

IgA and IL-6 Levels in "Biosmart and Safe Bus" Passengers

A comparative test of IgA levels between the "Biosmart and Safe Bus" and regular bus passengers both showed an increase in posttest levels compared to the pretest (Table I). IgA is an essential component of the humoral immune system and plays a role in the host's defense against pathogens [9].

**Table I: Levels of IgA and IL-6 ELISA nasal wash pre-test, post-test, and their deltas**

| Markers | Group Control (±SD) | Intervention (±SD) | p |
|---|---|---|---|
| **IgA (mg/mL)** | | | |
| Pre-test | 91,32±56,07 | 49,50±15,76 | 0,000 * |
| Post-test | 121.50±79.48 | 67.40±33.95 | 0.002 * |
| p | 0.014†* | 0.021†* | |
| Delta | 30.18±76.09 | 17.89±30.19 | 0.182 |
| **IL-6 (pg/mL)** | | | |
| Pre-test | -17.72±0.92 | -18.15±1.10 | 0.083§ |
| Post-test | -3.83±1.21 | -4.77±0.95 | 0.001 * |
| p | <0.000¶* | <0.000¶* | |
| Delta | 13.90±1.27 | 13.38±0.96 | 0.013 * |

*Significant (p<0.05); Mann–Whitney; † Wilcoxon; § Independent t-test; ¶ Paired t-test

A comparative test of IL-6 levels between the "Biosmart and Safe Bus" and regular bus passengers showed an increase in posttest levels compared to pretest levels (Table I). Delta levels of IL-6 differed, implying that the "Biosmart and Safe Bus" cabin engineering concept was more effective in preventing excessive exposure to pollutants in the environment.

## ROS and SOD3 Levels in "Biosmart and Safe Bus" Passengers

Oxidative stress is an injury to cells caused by an imbalance of free radicals and antioxidants produced by cells, exceeding the cell's ability to neutralize them. Elevated levels of ROS indicate an increase in oxidative stress, whereas increased SOD3 levels reflect the formation of antioxidants that help protect cells against free radical damage. A previous study reported a significant difference (p = 0.016) and a strong negative correlation (r = -0.611) in delta levels of SOD3 and ROS in "Biosmart and Safe Bus" passengers wearing ACCHADANA® herbal masks and medical mask using the Pearson correlation test (Table II).

**Table II: Delta of SOD3 and ROS levels of passengers on 'Biosmart and Safe Bus' wearing ACCHADANA® herbal and medical mask.**

| Delta | ACCHADANA® herbal mask | | | Medical mask | | |
|---|---|---|---|---|---|---|
| | Mean±SD | p | r | Mean±SD | p | r |
| SOD3 (U/ml) | 54.69±163.91 | 0.016* | -0.611* | -333.33±1793.54 | 0.712 | -0.100 |
| ROS (U/ml) | 24.17±45.62 | | | 28.37±50.26 | | |

*Pearson correlation test (p<0.05, significant)

## DISCUSSION

The intention of physical restrictions on 'Biosmart and Safe Bus' is to reduce the spread and transmission of the virus between passengers and it was proved by the challenge test that lowered the bacterial population (Figure 2). A study that evaluated the impacts of 6 months physical distancing on Brazilian older women upon biological markers resulted that the serum concentration of IFN-γ, IGF-1, and IFN-γ/IL-5 were significantly higher, while lower concentration of IL-2 and IL-5. Nevertheless, evidence-based public health measures to reduce the spread of the virus include—self-isolation, physical distancing, wearing face masks, frequent hand washing with soap and running water, avoiding touching the face, vaccination, etc. are recommended as evidence-based lifestyle interventions in the management of Covid-19 [7].

A model bus cabin environment was created to minimize the risk of airborne transmission in a closed-room environment. The engineering focused on improving the air circulation control system to achieve a more laminar airflow direction. This allowed for the air to be effectively drawn into HEPA filters and exposed to UV-C lights. Furthermore, the entire surface of the bus cabin was coated with a nano-silver coating. The HEPA filters played a crucial role in filtering and purifying the air within the cabin. They were capable of capturing particles as small as 0.3 µm, including fine particles, smoke, and bacteria with a 99.97% efficiency. UV-C light has a disinfectant effect through radiation energy at a wavelength of 254 nm and deactivates microorganisms, thereby reducing the concentration of microbes and endotoxins by approximately 99% [10,11].

Transport-related microenvironments often have one of the highest air pollution levels, where commuters have significant exposure to air pollutants. A study in Kathmandu Valley (KV), Nepal, analyzed cabin air quality and ventilation rates in public buses reported that ventilation levels in a majority of bus trips were inadequate, especially considering the recommended thresholds to reduce the transmission risk of airborne diseases [12]. Other research underscores the need for further investigation into the cumulative effects of stable compounds in cabin air and provides insights for developing healthier public transportation systems, because it revealed that the ventilation system of the bus significantly reduced the infiltration of outdoor particulate matter and water vapor [13].

The individuals on our study, remained in the bus cabin for approximately 10 h, with the temperature and humidity set at 25 C and 66%, respectively, so that it did not affect the pollutant concentration [14]. Appropriate humidity reduces viral transmission and inactivation. The higher the humidity, the greater the virus inactivation. With the right air humidity, the virus can be inactivated by up to 28% within 10 min, affecting changes in droplet size. This study demonstrated that the nanosilver coating was effective in reducing the bacterial population on the surface, which was indicated by the decrease in the bacterial population as the contact time increased. However, the results of the challenge test should be verified with those of the smoke test and Computational Fluid Dynamics.

The secondary outcomes were IgA and IL-6 levels as nasal immune system indicators, and ROS and SOD3 levels as nasal-stress oxidative markers. Seventy nasal washes from participants were analyzed, and all participants experienced no adverse events during the treatment. A cohort of 11 older adults during Covid-19, found that their unstimulated saliva samples secretory IgA, IgG and IgM by ELISA and cytokines (IL-2, IL-5, IL-6, IL-8 and IL-10, TSLP, IFN-γ, TNF-α) significantly differences by social isolation. A tendency to increase salivary levels of IgA and no differences in salivary levels of IL-6 before and after 6 and 20 months of the start of social isolation from the Covid-19 pandemic [15].

Our study found an increase in IgA levels reflects an increase in the number of pathogens infecting the body [9,14,16]. Previous research has also demonstrated that post-test IgA levels in regular bus passengers increased significantly compared to pre-test levels, while IgA levels in "Biosmart and Safe Bus" passengers only increased slightly, implying that the airborne pollutant exposure control system in the bus cabin is more effective.

The delta (posttest difference minus pretest) levels of IgA further support the notion of improved bus cabin conditions in buses adopting the “Biosmart and Safe Bus” concept [17].

Infection or inflammation in the upper airway increases mucosal IL-6 products, which act as pro-inflammatory cytokines by stimulating macrophages and neutrophils to produce cytotoxic ROS. Serum IL-6 levels can serve as a responsive indicator of acute inflammatory reactions, surpassing the sensitivity and specificity of conventional inflammatory indicators, namely, C-reactive protein. IL-6 may contribute to the host’s defense against tissue injury and pathogen infection, but excessive synthesis and accumulation of IL-6 can cause pathological abnormalities; thus, IL-6 plays a dual role in the on-off system in controlling inflammatory response [2,18]. The observed differences in delta IL-6 levels are influenced by the cabin engineering concept of “Biosmart and Safe Bus,” which effectively reduces the potential for pathogen-induced injury. These results are consistent with a study on the differences in turbulent and laminar airflows applied to hospital operating rooms [19]. Air particles containing <10 cfu/$m^3$ are in the clean air category, and the rate and direction of airflow affect the density (cfu) of particles in the room. Therefore, a laminar airflow technique is needed, which is more efficient than turbulent flow in reducing the spread of bacteria. As the main pro-inflammatory mediator, IL-6 induces an acute-phase inflammatory response, causing local and systemic changes. Increased IL-6 levels are important in individuals with allergies to air and dust, or microbes entering the respiratory system [20]. The study about the effect of social isolation imposed by Covid-19 on the immune/inflammatory responses in the mucosa of the upper airways in the older adult population showed that a controlled deviation of Th1 and Th2 immune responses, both during infection and after vaccination, that may generate favorable conditions to combat this viral infection without exacerbation of the immune response, and aggravation of the pathology [15].

The results of this research also support the theory that Interleukin-6 appears to play an active role in the post-injury immune response, making it an attractive therapeutic target in attempts to control hyperinflammatory provoked organ injury. Homeostasis control of the cellular inflammatory response by controlling the microenvironment in the bus cabin, results in a smaller increase in IL-6 levels, which also shows evidence that IL-6 is a multifunctional cytokine that regulates acute-phase response and inflammation [21].

By controlling air flow homeostasis and the micro environment in the bus cabin based on the health triangle concept, the opportunity for inhalation injury that can expose the airway mucosa in bus passengers can be eliminated. In the circulation, IgA is the second most abundant Ig class, its normal concentration (  2 mg/ml), the results of this study show an increase in IgA levels 15 times normal, however the existing interventions are only half that. This proves that the "Health Triangle Concept" intervention is able to suppress IgA fluctuations which are believed to reduce the activity of potentially detrimental phlogistic humoral immune defense mechanisms and contribute to mucosal homeostasis [22].

SOD3 plays a role in oxidative stress homeostasis. SOD3 is an extracellular enzyme that is abundant in the airway mucosa. The distribution of SOD3 in the extracellular compartment indicates that this enzyme protects cells against free radical damage and chronic inflammation [23,24].

There is a notable correlation between the anthocyanin content in herbal masks derived from extracts of *Nephrolepis exaltata* and *Hibiscus rosa-sinensis*, and a corresponding gradual change in the level of SOD3. This indicates that the levels of endogenous antioxidants are steadily increasing as well. Passengers wearing medical mask also show an increasing trend in SOD3 levels due to the adaptive response of cells exposed to acute infectious agents within a 24-h period [25,26]. SOD3 is abundant in the epithelial cells of the mucosa of the ducts, [18] and plays an important role in reducing potential superoxide radicals. A study by Wu et al. (2016) [19] on the effects of exposure to air pollutant PM2.5 for 4–8 h, showed that the content of nitrate, chloride, and heavy metals, increased reactive oxygen or nitrogen species (ROS, RNS) and oxidative stress [25,27–30]. However, if exposure is less than 1 h, the reaction is different, and the pollutants can inhibit the activity of antioxidant enzymes. Prolonged exposure to pollutants for over 48 h has a significant impact on the activity of antioxidant enzymes, leading to their inhibition. A study on textile factory workers who were exposed to pollutant vapors during the dyeing process also found evidence of a decrease in SOD3 levels over a course of 2 months, demonstrating that brief exposure to pollutants not only causes oxidative stress but can also cause adaptive responses [26,27,31].

Airway mucosal cells produce more ROS when individuals inhale air pollutants [29]. For example, ROS that starts to form due to viral infection triggers the initial pathway of interferon-gamma (IFN-γ) secretion, which plays an important role in fighting viral infections. Previous studies have noted that a significant increase in ROS levels occurs medical mask users. [19] Textile industry workers who wore herbal masks also experienced a significant decrease in ROS levels; therefore, herbal masks proved to be more effective than medical mask in preventing exposure to air pollutants in the factory environment [32].

Passenger seat position also significantly affected ROS levels. Passengers who sat in the back seat experienced greater exposure to air pollutants, indicated by higher ROS levels. It has been elucidated that the "Biosmart and Safe Bus" incorporates a cabin air ventilation system strategically positioned in the middle to adhere to the challenge test requirements. This test indicates that the proximity of passengers to the air ventilation system influences the risk of transmission associated with exposure to airborne particles [31,33,34].

**"Biosmart and Safe Bus": A Transportation Solution in Pandemic Era**

This concept may reduce the viral load using HEPA filter technology, UV-C light, and the application of nanosilver coatings on the cabin surface, producing differences in IL-6 levels. The HEPA filter is used against bacteria and viruses, with a 99.97% filtering percentage [33]. UV-C light technology has also shown to inhibit the transmission of viruses and bacteria by >99% to 100% [35]. Nanosilver coating has many benefits, including antimicrobial properties, its use in water and air filters, biosensors, and in vivo diagnostic biomarkers. Rostami et al. (2021) proved that paint combined with silver nanoparticles were more effective in reducing the level of fungal contamination in hospital wards than paint without a nanoparticle combination [32]. Another study demonstrated that very small nano-silver particles (<10 nm) easily released ions from their oxidized surfaces, which enhanced antibacterial performance [36,37].

The "Biosmart and Safe Bus" socially distanced the seats of their passengers, which may have inhibited the increase in IL-6 levels by reducing the spread of infectious pathogens. This study is consistent with research showing that physical distancing and compliance policies can slow viral transmission with varying time lags [38]. Another study on the effect of social distancing on the spread of infectious diseases found that social distancing can reduce the spread of infectious diseases and slow the rate of infection and death [39].

Bus passengers in the "Biosmart and Safe Bus" concept wear herbal masks, with the ACCHADANA® brand patent. Previous studies have demonstrated that wearing herbal masks containing extracts of *Nephrolepis exaltata* and *Hibiscus rosa-sinensis* results in a higher humoral immune system (IgA levels) and cellular immune response (IL-6 levels) than using medical mask. Moreover, the utilization of herbal masks has been associated with more balanced biomarkers of nasal wash oxidative stress in online motorcycle taxi riders.

The potential biases in sample selection were adequately controlled to avoid the factors that can affect the levels of IgA, IL-6, ROS, and SOD3, i.e., age range, gender proportion, health screening to assess pre-existing health conditions, so that homogeneous participants could be produced. However, the length of travel and rest time were influencing factors. Prolonged travel time produces a noticeable decline in physical conditions, which creates a more conducive environment for pathogens to infect individuals [24]. Individuals sitting or standing close together in a closed environment pose a high risk of disease transmission. The spread of pathogens or other inflammatory traces, including pollutants, can pose a risk to bus passengers. However, the engineering of "Biosmart and Safe Bus" based on the "Health Triangle Concept" can reduce the risk of pathogens and pollutants, and the possible long-term physiological effects on passengers using the bus can protect their health status and the risk of infection.

## CONCLUSION

The "Health Triangle Concept" in the "Biosmart and Safe Bus" produces a safe bus cabin eco-biological environment. The passenger airway immune system improved, characterized by increased levels of IgA and IL-6. Passenger oxidative stress homeostasis also improved and was characterized by a balance between ROS and SOD3 levels. Therefore, this concept can be applied as a transportation solution during the pandemic, specifically for the prevention of viral transmission.

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