ORIGINAL ARTICLE

The efficacy and safety of electrolyzed water against Covid-19 as an alternative hand sanitizer

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ABSTRACT

Aim To investigate the efficacy of electrolyzed water against viruses and its safety to the skin.

Methods Virus culture was carried out at level-3 Bio-Safety (BSL-3) facilities. The test material was prepared at room temperature mixed with one part virus suspension and one organic load. As an antiseptic and disinfectant control, 0.7% formaldehyde was used. Cytotoxic effects of electrolyzed water were performed on Vero cells. In order to assess the safety of electrolyzed water, a skin sensitivity test was conducted for electrolyzed water exposure.

Results Electrolyzed water has a higher value of reduction factor than antiseptic and disinfectant control, formaldehyde, and it was statistically different from control. Cytotoxicity test results on Vero cells showed that electrolyzed water demonstrated safety in Vero cell viability. As many as 58 participants who met the inclusion criteria took electrolyzed water sensitivity test to the skin. The sensitivity test showed that participants with reactions to electrolyzed water were all female, with a mean age of 32.6 years. The patch-test was positive in 3 of 4 participants who reacted to the product.

Conclusion Electrolyzed water is effective as a new antiseptic and disinfectant against viruses and safe for human skin.

Key words: cell survival, disinfectants, local anti-infective agents, Vero cells, viruses

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INTRODUCTION

During the pandemic, the need for antiseptics and disinfectants increases to break the chain of virus spread and transmission. COVID-19 pandemic has become a significant health problem experienced by the world. This pandemic originated from discovering a novel virus from SARS-CoV (SARS coronavirus) in Wuhan, Hubei Province, China, at the end of December 2019 (1). Infection spread very quickly and has become a significant pandemic throughout the world. Also, the virus can transmit from human to human via secretions from patients or carriers (2). The fact that makes the problems caused by this virus even more remarkable is that the virus can survive outside the host cell. It can survive in the air and where droplets or secretions stick for several hours to several days (3-4). This causes this virus' virulence to be very high and very difficult for all world citizens.

The socioeconomic impacts of this pandemic are very severe due to lockdown policies that have been implemented in all parts of the world. The world economy has stopped, economic activity was stagnant, factories and large companies have ceased to operate and fired their employees, which has led to high unemployment and poverty everywhere. Citizens of the world are quarantined in their homes and cities, resulting in emotional and psychological disorders due to limited social relationships (5-6).

Exploring and developing antiseptics and disinfectants can be one way to break the chain of the virus spreading (7). Antiseptics and disinfectants are expected to play a role in killing viruses that roam the air or stick to parts of the human body to prevent the further spread of the virus. Antiseptics and disinfectants commonly used, such as ethanol, glutaraldehyde, or sodium hypochlorite, have severe irritation levels or mucosa and eyes when used for persistent periods and direct contact with the mucosa or eyes (8-9). This shows that existing antiseptic and disinfectants irritate the skin and mucous membranes (10). If used continuously and for a long time, it can cause skin problems (eczema and dermatitis) (11).

Electrolyzed water results from bioengineering using the electrolysis process of sodium chloride to produce ions with the ability as an antiseptic and disinfectant without using a mixture of bleaching or ethanol (12). The absence of ethanol or bleaching makes this antiseptic and disinfectant have advantages in safety against the skin, mucosa, and eyes if used continuously (12).

The coronavirus pandemic has become an emerging disease. The need for hand sanitizers that effectively reduce the spread of the coronavirus and are safe to use are essential during a pandemic. Electrolyzed water can be a safe and effective alternative to hand sanitizers in preventing the spread of the coronavirus (6,12).

This study is the first research that explores the efficacy of electrolyzed water against coronaviruses and explores the safety of electrolyzed water on human skin.

MATERIAL AND METHODS

Material and study design

SARS-CoV-2 isolates were obtained from sputum of patients diagnosed with SARS infection at the Moh Hoesin General Hospital, Palembang, Indonesia, during the period April to August 2021.

To assess the safety of electrolyzed water, a skin sensitivity test was conducted for electrolyzed water exposure in September 2021. Participants who took part in this study were all nurses 20-40 years old and willing to participate in the study who worked at the Palembang Primary Care Centre, where they often used hand sanitizer for daily service activities. Institutional review board approval was obtained, and all participants signed informed consent for the skin patch test.

Participants completed an initial interview in which they reported their usual hand-hygiene practices and skin condition. Participants were instructed not to apply any products to the skin of the back of the hand for 72 hours before testing. Also, they were not allowed to ingest antihistamines or anti-inflammation drugs or wash the area during the test.

The procedure of this study complied with the principles of the Declaration of Helsinki. This study was approved by the Ethical Committee of the Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia (No: 155/kptfkunsri-rsmh/2021).

Methods

Culture of viruses. SARS-CoV-2 was grown on Vero cell culture (African green monkey kidney, ATCC num. CCL-81, Virginia, USA) (13). The medium used was minimum essential medium (MEM) without foetal calf serum (FCS), 100 ug/mL of streptomycin and 100 IU/mL of penicillin added. The virus culture was then stored at -80^{o}C. Determining cultures infected with the SARS-CoV-2 virus were determined if more than 50% of cell cultures were infected with viruses. The initial log_{10} virus titers were between 8.92 ± 0.25 and 9.4 ± 0.38. This virus culture was carried out based on WHO recommendations at level-3 Biosafety (BSL-3) facilities (13).

The following test material was prepared at room temperature mixed with one part virus suspension and one organic load. The organic load used was 0.3% albumin, 10% FCS and 0.3% albumin with 0.3% sheep erythrocytes. After incubation, proceed with dilution with 1:10 ice-cold MEM. Next, a three-day incubation was carried out at 37 ^{o}C in a CO_2 incubator. As an antiseptic and disinfectant control, 0.7% formaldehyde was used (dilution 1:10 as a disinfectant control). Next, electrolyzed water's cytotoxic effects were performed on suspended Vero cells in 96-well plates using MTT cell Proliferative Kit I (Roche, Basel, Switzerland). Cytotoxicity tests to assess electrolyzed water's toxic effect on cells were carried out by testing electrolyzed water against Vero cells without adding a virus.

Electrolyzed water preparation. Electrolyzed water is an antiseptic and disinfectant that was made by utilizing electrophoresis technology from sodium chloride. Electrolysis was carried out on a solution of 10% sodium chloride (Sigma Aldrich, Singapore). Furthermore, ions that have the potential to be antiseptic and disinfectant will be produced. Electrolyzed water used in this study was the concentration of 0.05%, 0.5%, and 5%.

Calculation of reduction factor (RF). The reduction factor (RF) was calculated as the difference between infection titration before incubation (control titration) and infection titration after incubation with viruses and electrolyzed water. Next, the log_{10} titer and standard deviation are calculated as the variance of RF.

Evaluation of electrolyzed water safety. On the first day, 0.1 mL of electrolyzed water was applied to clean skin of the back of the hand and covered with nonstick plaster. The test site was assessed on the third day for erythema and papules, the same dressing reapplied, and on day four the site was reevaluated. Results were read by a dermatologist and scored as it is recommended by the International Contact Dermatitis Research Group using a rating of negative (-), 1+ if erythema was present, and 2+ if both erythema and vesicles/papules or blistering were present (14).

Statistical analysis. The assessment of each parameter's mean expression levels was made. The Fisher exact or Mann-Whitney test was used to compare selected variables between those with and without reactions. The p=0.05 was considered statistically significant.

RESULTS

Electrolyzed water had reduction factors (RF) value higher than the RF value of antiseptic and disinfectant control, formaldehyde, and it was statistically significantly different (p=0.01). The higher RF values indicate the better efficacy of the test material to reduce viral load. Electrolyzed water with a higher concentration can effectively increase the RF value, which directly shows the ability of electrolyzed water as an antiseptic and disinfectant (Table 1).

Table 1. Antiseptic and electrolyzed water disinfectant tests against viruses

Test material	Reduction factors (and standard deviation)		
	0.3% BSA	10% FCS	0.3% BSA and sheep erythrocytes
Electrolyzed water 0.05%	4.15 (0.12)*	4.15 (0.12)*	4.15 (0.12)*
Electrolyzed water 0.5%	4.45 (0.21)*	4.45 (0.21)*	4.45 (0.21)*
Electrolyzed water 5%	5.76 (0.35)*	5.76 (0.35)*	5.76 (0.35)*
Formaldehyde	3.25 (0.23)	3.25 (0.23)	3.25 (0.23)

*independent T test: $p<0.05$ versus formaldehyde;
BSA, bovine serum albumin; FCS, foetal calf serum

Cytotoxicity test results on Vero cells demonstrated that anolyte water was safe in Vero cell viability. Vero cell viability was above 90%, which indicates that electrolyzed water with a concentration of 0.05%-5% was safe against normal cells. The higher electrolyzed water concentration showed a slight decrease in Vero cell viability.

Among 58 participants in the skin test, 51 (87.9%) had no reactions. Seven (12.1%) had reactions to electrolyzed water and all were females with a mean age of 32.6 years (range: 21-40 years). There were no significant differences between those with or without a reaction in age (p=0.11) (Table 2).

Table 2. Characteristics of 58 participants with and without skin reactions

Variable	Reactions (n=7)	No reactions (n=51)	p
Mean age (years)	32.6	39.0	0.11
History of itchy, sore hands (No, %)	2 (21.1)	2 (2.9)	0.047

Three of seven participants who showed reactions to electrolyzed water chose not to follow the study follow-up. The remaining four participants with reactions were patch tested to electrolyzed water. The patch test was positive in three of the four participants who reacted to electrolyzed water (Table 3).

DISCUSSION

Electrolyzed water with a higher concentration can increase the reduction factor value and show the ability of electrolyzed water as an antiseptic and disinfectant against coronaviruses. The results of this study are supported by research by Takeda, which states that the use of acidic electrolyzed water as a contact disinfectant can inactivate virucidal activity in SARS-CoV-2 (15).

Electrolyzed water contains a variety of ions that function as antiseptics and disinfectants (7). Electrolyzed water is rich in hypochlorite ions and sodium hydroxide ions, producing these ions from sodium chloride and water electrolysis. Hypochlorite ions have anti-bacterial, anti-viral, and anti-fungal effects (16,17). Ion hypochlorite can damage bacterial cell wall composed of complex proteins, lipids and carbohydrates (18). The viral capsid consists of simpler proteins than the bacterial cell wall, so the hypochlorite ion can quickly destroy the capsid. (16,17). The ability of hypochlorite ions is strengthened by sodium hydroxide ions that fight as a detergent and can accelerate the lysis of the viral protective wall (capsid) (16).

Electrolyzed water has other advantages in terms of safety of use. In our study, electrolyzed water was found safer for the skin than former antiseptic, and in various concentration proved safe on Vero cells and human skin applications. Electrolyzed water is rich in ions, where these ions are easily degraded when they come in contact with the skin (19). This is different from chemical substances found in antiseptics in general, where these substances are generally not easily degraded. This slow degradation intends to make chemical contact with the skin and mucosa sufficiently. However, the side effect is that chemicals in antiseptics can generally trigger a hypersensitivity reaction on the skin. Inflammatory reactions in the skin will damage the skin barrier and facilitate the infection of microorganisms on the skin (20).

In conclusion, electrolyzed water is effective as a new antiseptic and disinfectant against the virus and is safe for human skin.

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TRANSPARENCY DECLARATION

Conflict of interest: None to declare.

Table 3. Summary of skin reactions in four participants

Age (years), ethnicity	Time before reaction	Description	Allergies	Patch-test results description
25, Sumatran	Immediately on contact	Red, blotchy, itching, progressing to cracks.	History of eczema	Negative
31, Javanese	Immediately on contact	Fine white rash with red centre, itching.	History of eczema and asthma	2+ with blisters
22, Sumatran	Immediately on contact	Itching, progressing to dry.	Amoxicillin	2+ with blisters
35, Sumatran	Immediately on contact	Itching progressing to excessive dryness; cracked.	History of eczema	1+, raised erythema, no blisters

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