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IN VITRO VIRUCIDAL ACTIVITY OF DABUR RED TOOTHPASTE, AN AYURVEDIC PREPARATION, AGAINST SARS-COV-2

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ABSTRACT

With the outbreak of novel coronavirus disease (COVID-19), importance of maintaining good oral health to prevent a severe course of the disease has been stressed. WHO has claimed that transmission of the disease primarily occurs through droplet spread and contact routes. This pose a high risk of cross infection between dental health care personnel and patients. Worldwide research has been conducted for treatment of COVID-19 especially using herbs/ medicinal plants. However, at present there is no specific treatment available for SARS-CoV-2 infection and other diseases such as respiratory diseases. Therefore, we investigated the virucidal potential of Dabur Red toothpaste, an ayurvedic preparation comprised of potent Ayurvedic herbs, preliminary verified towards coronavirus SARS-CoV-2. The efficacy was determined at 2 test concentrations by incubating the virus with the toothpaste for definite contact time (2 min). Subsequently, virus was neutralized and added to confluent layer of host VERO cells. Endpoint titers (50% cell culture infectious dose, CCID50) and the log reduction value (LRV) was compared with the negative control (LRV<1 indicates no virucidal activity, LRV>1 indicates virucidal activity). In conclusion, the data demonstrated that Dabur Red toothpaste possess potent virucidal activity against SARS-COV-2, with a Log reduction value (LRV) of >2 log (99% reduction).

KEYWORDS: SARS-CoV-2, transmission, droplets, Dabur Red toothpaste, virucidal.

1. INTRODUCTION

As per the World Health Organization (WHO) about 4 billion people (80% of the world population) use herbal medicine for some aspect of their primary health care.[1] Herbal medicine is a common element in Ayurvedic, Homeopathic, Naturopathic, Traditional oriental and Native American Indian medicine.[2] Ayurveda is traditional science based upon holistic treating methods where ailments are treated by balance of three biological humors called doshas *i.e.* Vata, Pitta, and Kapha.[3] The balance among the three doshas determine health care in Ayurveda including dental health. Nowadays, the hike of demand is more towards the usage of various ayurvedic products for treatment and management of various diseases. With the rise of Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) pandemic, evidence-based research in Ayurveda is receiving larger acceptance in India and abroad.[4] Moreover, use of indigenous plants has a long history in different parts of the world for various types of diseases including respiratory diseases.

Respiratory infections are primarily transmitted through contact transmission, droplet transmission and airborne transmission, which are corelated with each other. According to current evidence, COVID-19 is primarily transmitted between people through respiratory droplets and contact routes. As per Centre for Disease Control, the principal mode by which people are infected with SARS-CoV-2 (the virus that causes COVID-19) is through exposure to respiratory droplets carrying infectious virus.[5] WHO also claimed the spread of novel coronavirus SARS-CoV-2 by salivary droplets or discharge from the nose.[6] The salivary droplets are generated while breathing, talking, coughing, or sneezing and are formed as particles in a mixture of moisture and droplet nuclei of microorganisms. The strength and transmission of infection by salivary droplets differ among people due to variation in amount, size and the distance of the transmission by salivary droplets. Saliva could form aerosols and reach a distant host along the airflow when in a favourable environment. In a recent study, SARS-CoV-2 has been detected in 91.7% of the saliva samples studied, indicating that saliva as a potential source of SARS-CoV-2 spreading.[7] Hence individual protection measures help to a greater extent to mitigate the risk of transmission to healthcare staff, patients and the remaining population.

Since, oral cavity is an entrance and an outlet of body and saliva is a common medium for transmission of infectious diseases, a good oral hygiene is important for reduced infectivity. The present study aimed to evaluate the virucidal potential of oral care product, Dabur Red

toothpaste (DRT). DRT is an ayurvedic preparation which has a unique blend of traditional Indian Medicine and modern pharmaceutical technology for keeping the gums and teeth healthy as well as for maintaining oral hygiene by preventing the formation of infectious salivary droplets and thereby halting the spread of the virus through droplet transmission.

2. MATERIALS AND METHODS

21 Materials: SARS-CoV-2 (USA-WA1/2020 strain), Vero 76 cells (ATCC), Culture media- MEM (ATCC), MEM (ATCC), Fetal bovine serum (ATCC) and Gentamicin (ATCC) were used in the study. Dabur Red toothpaste (DRT) was obtained from Dabur India Limited, Ghaziabad, Uttar Pradesh, India. The composition details of DRT are given in Table 1.

Table 1: Active ingredients of Dabur Red toothpaste.

S. No.	Active ingredients
1	Maricha (*Piper nigrum*)
2	Pippali (*Piper longum*)
3	Shunti (*Zingiber officinale*)
4	Tomar beej (*Zanthoxylum armatum*)
5	Lavang oil (*Oil of Syzygium aromaticum*)
6	Karpoor (*Cinnamomum camphora*)
7	Pudina Satva (*Mentha piperita*)
8	Gairic Powder (*Red Ochre*)

22 Preparation of test sample: SARS-CoV-2 virus stock solution was prepared by growing the virus in Vero 76 cells. Culture media used was minimum essential medium (MEM) supplemented with 10 U/mL trypsin, 1 µg/mL EDTA, and 50 µg/mL gentamicin. DRT was mixed with sterile water in a ratio of 1:2 to make a slurry. Slurry was added to virus stock to attain final screening concentrations of 30% and 17%.

23 Method: Virucidal Assay- DRT was tested for virucidal activity by liquid-liquid contact with virus solution. DRT was mixed with virus solution, so that there was 90% test sample and 10% virus (v/v). The experiment was performed in triplicates for each test concentration. Culture media was added to one tube of each concentration which served as toxicity control. Ethanol was used as positive control and sterile water was used as control.

Test sample and virus (mixture) were incubated at room temperature for 2 min. as indicated in Table 2. Following the incubation, solutions were neutralized by test media in 1/10 dilution.

Table 2: Product information, concentration, and contact time for testing.

Product Name	Active Ingredients	Contact Time	Concentrations
Dabur Red Toothpaste	Paste with Ayurvedic actives	2 minutes	1:2 (paste: water) slurry mixed to make uniform mix

2.4 Virus Quantification: Neutralized samples were pooled for quantification. Samples were serially diluted using log dilutions (7 dilutions) in culture medium. Each dilution was added to 4 wells of a 96-well plate with 80-100% confluent Vero 76 cells. The cytotoxicity control (no virus) was added to an additional 4 wells. Two of the cytotoxicity wells were infected with virus to serve as neutralization control, ensuring that residual sample in the titer assay plate did not inhibit growth and detection of surviving virus. All plates were incubated at 37±2°C, 5% CO_2 for 5 days. On day 6 post-infection, plates were scored for presence or absence of viral Cytopathic Effect (CPE). The Reed-Muench method was used to determine endpoint titers (50% cell culture infectious dose, $CCID_{50}$) of the sample, as well as the log reduction value (LRV) of the test sample and compared with the negative control (water). Virus titers were determined by inhibitory concentrations (CCID50 – 50% cell culture infectious doses). LRV (Log reduction value, *i.e.* virus titer in Control - virus titer in Test Item) was also calculated for the test sample. The Virucidal activity was reflected by LRV of test sample.

3. RESULTS AND DISCUSSION

The virucidal activity of DRT was performed by incubating the virus with DRT for a definite time period of 2 minutes (called as the Contact time) followed by neutralization of the test solution. Virus titer was determined by endpoint dilution in 96-well microplates of host cells. The parameter evaluated is CCID50 (50% cell culture infectious dose) and the log reduction value (LRV) of the test sample. Virus titers and LRV of SARS-CoV-2 after incubation with DRT for 2 min. are shown in Table 3.

The data represents that DRT exhibited significant cytotoxicity to Vero 76 cells, affecting detection of virus. At both the test concentration of DRT (30% and 17%, full cytotoxicity was observed in the 1/10 and 1/100 dilutions, limiting detection of virus to >2.7 log CCID50 per 0.1 mL.

The Virucidal activity of test sample was reflected by LRV (Virus titer of Control – Virus titer of sample). Virus control sample had titers of 4.7 log CCID50 per 0.1 mL and test sample were compared to this for determining LRV. DRT exhibited virucidal activity (LRV>2,

>99% reduction of virus).

Neutralization controls demonstrated that residual sample did not inhibit virus growth and detection in the endpoint titer assay in wells where full cytotoxicity was not observed. The positive control performed as expected.

Table 3: Virucidal efficacy against SARS-CoV-2 after a contact with virus at 22 ± 2°C.

Sample	Concentration	Contact Time	Toxicity[a]	Neut. Control[b]	Virus Titer[c]	LRV[d]
DRT	30%	2 minutes	1/100	None	<2.7	>2.0
DRT	17%	2 minutes	1/100	None	<2.7	>2.0
Ethanol	63%	2 minutes	1/10	None	<1.7	>3.0
Virus Control	n/a	2 minutes	None	None	4.7	-

[a] *Toxicity indicates the highest dilution of the endpoint titer where full (80-100%) cytotoxicity was observed.*

[b] *Neutralization control indicates the highest dilution of the endpoint titer where compound inhibited virus CPE in wells after neutralization (ignored for calculation of virus titer and LRV).*

[c] *Virus titer of test sample in log10 CCID50 of virus per 0.1 mL.*

[d] *LRV (log reduction value) is the reduction of virus in test sample compared to the virus control.*

Based on the results presented in this study, DRT inactivated SARS-CoV-2 by 99% within a contact time of 2 minutes. No cytotoxicity was observed for tested concentrations of DRT. The viral titer reductions are indicative of virucidal activity of DRT, which also shows promising results. These results demonstrate that DRT can be effective as virucidal agent, opening a possibility of the application of the oral care products against SARS-CoV-2 and other viral diseases of mouth. The study provides an insight that oral care products might reduce the viral load of saliva and could thus lower the transmission of SARS-CoV-2. For the ongoing pandemic scenario, the present study can be a route to recommend infection control strategy and optimum dental care as well as preventing infections in dental settings.

4. CONCLUSION

This short communication reports about the virucidal activity, preliminary verified towards Coronavirus SARS-CoV-2. The objective of the study was to determine the virucidal

potential of the Dabur Red toothpaste against SARS-CoV-2. Test sample was tested for virucidal activity by liquid-liquid contact with virus solution. The test sample was tested at two test concentrations (30% and 17%). Viral titre and the log reduction value (LRV) of the test sample was compared with the negative control. Based on the results obtained, it can be concluded that DRT demonstrated virucidal activity against SARS-CoV-2, with a Log reduction value (LRV) of >2 log (99% reduction). Hence Dabur Red toothpaste possess virucidal activity against SARS-CoV-2 (a causative agent of COVID-19) with LRV >2 suggesting 99.0% effectiveness against SARS-CoV-2.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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