Evaluation of Virucidal activity of Acid based disinfectant toilet cleaner

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ABSTRACT:
The primary step towards preventing communicable diseases comprises of maintaining good hygiene in one’s surroundings. Toilet seat/bowl is one example which is expected to be highly contaminated with germs/microbes and improper cleaning of such places may lead to occurrences of infectious agents-associated diseases Human adenoviruses cause an array of illnesses in humans, including upper respiratory symptoms, fever, pneumonia, gastroenteritis, and conjunctivitis and are more common in places daycares, schools, children’s camps, hospitals, military settings, and other health care centres. Hence, proper disinfection of the surroundings such as toilet/restrooms is a necessity in order to kill these pathogenic organisms. Accordingly, the present study was undertaken to evaluate the Virucidal activity of Disinfectant toilet cleaner, which comprised of 10.5%HCl (Hydrochloric acid), against the test organism Adenovirus. The test item ‘Sani Fresh Advanced Disinfectant Toilet Cleaner (10.5% HCL)’ manufactured by Dabur India Limited, was found to exhibit a high virucidal activity. This study, therefore, highlights the potency of the test item in reducing the viral load of the toilet bowl.

Keywords: toilet disinfectant, adenovirus, hydrochloric acid, toilet cleaner

INTRODUCTION:
Maintaining good hygiene in one’s surroundings is the initial step towards preventing communicable diseases spread by a variety of microbes. Microbes need nutrients and water to grow and thus such places are expected to be highly contaminated with germs. One such example is the toilet seat/bowl which serve as reservoirs for the growth of microbes. Outbreaks of infectious agents-associated diseases from toilets have been documented, largely from improper cleaning and disinfection of restroom facilities[1,2]. An infected person may shed pathogenic microbes while defecating or urinating as bacteria and viruses may be present in the urine during infection. Infectious viruses that cause insect-borne encephalitis have been known, but other viruses such as smallpox and adenoviruses, SARS-CoV-2 virus have also been detected in the urine[3,4]. The areas outside of the toilet bowl/urinal can get contaminated from aerosols generated from flushing leading to potential transmission by inhalation and indirectly by fomite contamination[5]. It was found in a study that in an outbreak of hepatitis A virus (HAV) within a middle school, the use of a particular toilet for defecation was linked to the source of infection. This toilet had been used by the index case during a bout of diarrhoea[6].

Also, it has been found that the air quality of the air regulated through ventilation systems of large buildings is poor if the system is placed closer to the restrooms. Verani et al. (2014) [6] detected adenoviruses in 77% of air samples collected from the restrooms in hospitals and 62% in air from offices. Human adenoviruses are double-stranded DNA viruses that cause a range of illnesses in humans, including upper respiratory symptoms, fever, pneumonia, gastroenteritis, and conjunctivitis[7]. Adenoviruses are associated with numerous disease outbreaks, particularly those involving daycares, schools, children's camps, hospitals, military settings, and other health care centres. Children and immunocompromised individuals are more severely impacted by adenovirus infections[8]. Adenovirus infection accounts for up to 10% of respiratory infections in children[7]. The common occurrence of adenovirus probably results from prolonged shedding from the respiratory and urinary tracts as well as fomites[9]. Therefore, it is very essential to clean and disinfect the surroundings such as the toilet/restrooms. Disinfection refers to the chemical inactivation or killing of microbes. Moreover, as the viruses and bacteria may remain for a prolonged period of time on the surface. Therefore, there exists a need for a disinfectant which is able to kill these pathogenic organisms effectively.

Accordingly, a study was undertaken to evaluate the Virucidal activity of Disinfectant toilet cleaner. The test item comprised of 10.5%HCl (Hydrochloric acid). The test item was found to exhibit a high virucidal activity. This study, therefore, highlights the
potency of the test item ‘Sani Fresh Advanced Disinfectant Toilet Cleaner (10.5% HCL)’ manufactured by Dabur India Limited, in reducing the viral load of the toilet bowl.

MATERIALS AND METHODS:

Test substance details:
- **Test substance name:** Sanifresh Advanced Disinfectant Toilet Cleaner
- **Test Substance ingredients:** Active ingredient – Hydrochloric acid 10.5% w/v, water. Oleyl Amine Ethoxylate, Alkyl Trimethyl Ammonium Chloride, Fragrance, Butylated Hydroxy Toluene.

**Materials:** Distilled water, PBS, Neutral Red (1:1000 solution), Foecal calf serum, Trichloroacetic acid (10% solution), hard water for dilution, Culture Media-MEM, water bath, pH-meter, CO₂ incubator, stopwatch, graduated pipettes, volumetric flasks, centrifuge (400gN to 1000gN), magnetic stirrer, refrigerator, freezer, container, inverted microscope.

**Cell Line and Culture Medium**
Vero (ATCC-CCL-81) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM high Glucose supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml), and amphotericin B (5 µg/mL) in a humidified atmosphere of 5% CO₂ at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm² culture flasks and all experiments were carried out in 96 well microtitre plates.

**In vitro Cytotoxicity Assay**
The monolayer cell culture was trypsinized and the cell count was adjusted to 100,000 cells/ml using DMEM High Glucose containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension was added. After 24h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100µl of different test concentrations of test drugs (i.e., test substance and the standard Acyclovir) were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO₂ atmosphere. Microscopic examination was carried out and observations were noted at every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µL of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO₂ atmosphere. The supernatant was removed and 100 µL of DMSO (Dimethyl sulfoxide) was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated, and concentration of test drug needed to inhibit cell growth by 50% (CTC₅₀) values was generated from the dose-response curves for each cell line.

**Virucidal assay**

**Test organism:**
The virucidal activity was evaluated using the test organism *Adenovirus* type 5, strain Adenoid 75, ATCC VR-5.

**Method:**
The study was undertaken as per the guidelines prescribed in EN 14476: 2013+A2:2019: Chemical disinfectants and antiseptics - Quantitative suspension test for evaluation of Virucidal activity in the medical area. Details on the test conditions are given in Table no. 1.

<table>
<thead>
<tr>
<th>Table no. 1: Test conditions</th>
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</thead>
<tbody>
<tr>
<td><strong>Neutralization Method</strong></td>
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<tr>
<td><strong>Experimental test Conc.</strong></td>
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<tr>
<td><strong>Contact Times</strong></td>
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<tr>
<td><strong>Experimental Conditions</strong></td>
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<tr>
<td><strong>Interfering Substance</strong></td>
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<tr>
<td><strong>Test Temperature</strong></td>
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<tr>
<td><strong>Temperature of Incubation</strong></td>
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</tbody>
</table>

A sample of the test product as specified in Table no. 1 was added to a test suspension of viruses in a solution of an interfering substance. The mixture was maintained at 20°C ± 1°C temperatures for a contact time of 5 Minutes ± 10 seconds. At the end of this contact time, an aliquot is taken; the virucidal action in this portion is immediately suppressed by a validated method (dilution of the sample in ice-cold cell maintenance medium). The dilutions are transferred into cell culture units (petri dishes, tubes or wells of microtitre plates) either using monolayer or cell suspension. Infectivity tests are done either by plaque test or quantal tests. After incubation, the titres of infectivity are calculated according to Spearman and Kärber (quantal tests, C.1) and evaluated. Reduction of virus infectivity is calculated from differences of lg virus titres before (virus control) and after treatment with the product.

**RESULTS**

The virucidal activity of the test item ‘Sanifresh Advanced Disinfectant Toilet Cleaner’ was evaluated against the test organism *Adenovirus*. The results are as shown in Table no. 2.

**Table no. 2: Virucidal activity of Test Substance against Adenovirus**
<table>
<thead>
<tr>
<th>Product Name</th>
<th>Virus</th>
<th>Virus control (log TCID50)</th>
<th>Test product (log TCID50)</th>
<th>Log Reduction</th>
<th>% Reduction of Viral load</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sani Fresh Advanced Disinfectant Toilet Cleaner (10.5% HCL)</td>
<td>Adenovirus</td>
<td>7.04</td>
<td>3.92</td>
<td>3.13</td>
<td>99.9</td>
</tr>
</tbody>
</table>

The results of the test item ‘Sani Fresh Advanced Disinfectant Toilet Cleaner (10.5% HCL)’ treated with Virus viz., *Adenovirus* after a contact time of 5 min showed 3.13 log reduction of antiviral activity at Neat (80%) product concentration respectively under the tested conditions set out in this study. This corresponds to a 99.9% reduction in the viral load. Hence, it is concluded that the test item exhibited high virucidal activity against *Adenovirus*. Thus, the results demonstrate that the test item acts as a potent disinfectant.

**DISCUSSION**

It is of primary importance to keep the restrooms, specifically the toilet bowls, surface in proper hygienic condition by cleaning with an effective disinfectant in order to reduce the spread of pathogenic microorganisms. An environmental monitoring study concluded that toilets are an important source of viral contamination, mainly in health care settings, where disinfection can have a crucial role in preventing virus spread\(^\text{[26]}\). The present study was undertaken to evaluate the Virucidal activity of a disinfectant toilet cleaner. The test item was ‘Sani Fresh Advanced Disinfectant Toilet Cleaner which contained 10.5% hydrochloric acid. The virucidal activity of the test item was evaluated at Neat (80%) product concentration. It is evident from the results that the test item exhibited a high virucidal activity against *Adenovirus* with an approximate 99.9% reduction in the viral load. This study, therefore, demonstrates the potency of the test item ‘Sani Fresh Advanced Disinfectant Toilet Cleaner (10.5% HCL)’ manufactured by Dabur India Limited, in reducing the viral load of the toilet bowl.

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

**REFERENCES:**