WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 5, 1795-1800. Research Article ISSN 2277– 7105

VIRUCIDAL EFFECT OF DABUR RED TOOTHPASTE, AN AYURVEDIC PREPARATION, ONENVELOPED HERPES SIMPLEX VIRUS TYPE 1 IN VITRO

Amit Sirdesai[1], Amit Gupta[1], Ranjan Mitra[1] and Prasun Bandyopadhyay[1], Kumari Sneha[1] and Jyoti Sree Unnithanh[2]*

[1]Dabur Research Development Centre, 22-Site IV, Sahibabad, Ghaziabad-201010, Uttar Pradesh.

[2]Dabur Research Foundation, 22-Site IV, Sahibabad, Ghaziabad-201010, Uttar Pradesh.

Article Received on
17 March 2021,

Revised on 07 April 2021,
Accepted on 27 April 2021

DOI: 10.20959/wjpr20215-20430

***Corresponding Author**
Dr. Jyoti Sree Unnithanh
Dabur Research Foundation, 22-Site IV, Sahibabad, Ghaziabad-201010, Uttar Pradesh.

ABSTRACT

HSV infections are one of the most common viral infections all over the world and infects a variety of host tissue. Herpes simplex viruses is an enveloped virus and is very contagious leading to diseases ranging from superficial infections to life-threatening ones. This virus is transmitted from person to person through infected saliva (either directly, or by drinking fromthe same glass or cup) or by skin contact. Signs of infection comprise of painful red fluid-filled blisters on the mouth, lips, and tongue, depicting the importance of oral health care. Though there are approved conventional oral antiviral therapies, such as acyclovir (Zovirax) and others, emergence of resistant virus in recent years due to frequent use led to focus on employing traditional approach for treatment of HSV-1. Therefore, the effect of Dabur Red toothpaste was investigated, which is an ayurvedic preparation comprised of potent Ayurvedic herbs against HSV-1 to kill the virus. The efficacy was determined 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 titer (50% Tissue culture infectious dose, TCID50) and the log reduction value (LRV) was determined. In conclusion, the data demonstrated that Dabur Red toothpaste possess potent virucidal activity against Herpes Simplex Virus Type-1, with a Log reduction value (LRV) of 3.04 log (99.9% reduction).

KEYWORDS: Herpes, antiviral therapy, ayurvedic herbs, Dabur Red toothpaste, virucidal.

1. INTRODUCTION

Oral health is essential to general health and well-being. Maintaining good oral health is extremely important to keep infections at bay. In general, a good oral care practice such as daily brushing and flossing along with our natural defence system, helps to keep outgrowth of microorganisms under control, preventing the occurrence of oral infections. One of the most prevalent oral infection worldwide is Herpes simplex caused by Herpes simplex virus Type 1 (HSV-1). As per WHO, an estimated 3.7 billion people under age 50 (67%) have HSV-1 infection globally.[1] It commonly causes infections in or around the mouth (sometimes called orolabial, oral-labial or oral-facial herpes). Symptoms of herpes include painful Red fluid-filled blisters or ulcers at the site of infection (on the mouth, lips, and tongue), excruciating pain, tingling, fever, difficulty in eating. When inside the mouth, it can cause damage to the soft tissue of the gums. This can cause the teeth and gums to separate and create gaps for bacterial growth leading to further complications. In some cases, HSV-1 infection can also lead to more severe complications such as encephalitis (brain infection) or keratitis (eye infection).[2] HSV-1 is mainly transmitted by oral-to-oral contact via contact with the HSV-1 virus in sores, saliva, and surfaces in or around the mouth.[1] However, the greatest risk of transmission is when there are active sores. In Ayurveda, Herpes is called as "Visarpa or Parisarpa", caused by viruses including Herpes Simplex Type-1[3] According to Ayurveda, there are three energies or doshas in our body- vata, pitta and kapha. These energies are responsible for the good health of an individual. Any imbalance even in one of these energies may lead to health issues. In Herpes, all these three energies or doshas are vitiated in the body. Symptoms are manifested according to the doshas or dhatus affected.[3]

Conventional treatment for Herpes consists of antivirals such as Acyclovir, Famciclovir, and Valaciclovir, which are FDA approved. However, these antiviral drugs have numerous side effects and pose greater drug resistance.[4] The virus lies dormant behind the blood-brain barrier where the immune system and antiviral drugs cannot reach[5], leading to probability of recurrence of the infection. Thus, treatment of herpes infection is a cause of major concern owing to the difficulty in eliminating it from the ganglion, high cost of treatment and increasing drug resistance. Traditional medical practitioners have been using herbal medicines for management of diseases including viral infections. Plant extracts from *Hypericum mysorense*, *Hypericum hookerianum* and *Usnea complanta* showed significant anti-viral efficacy.[6] Ayurvedic texts mentions various herbs medicines and treatment for HSC-1. One such example is the Eugenol in clove, which has shown potent efficacy against HSV-1, by damage the viral

envelopes of freshly formed virions thereby inhibiting the viral replication at the very initial stage.[7] Other medicinal plant extracts such as *Zingiber officinale* (Ginger), *P. nigrum*, *Zanthoxylum armatum* etc. are also very well reported for their antiviral activity.[8,9]

The present study aimed to evaluate the virucidal potential of oral care product, Dabur Red toothpaste (DRT) against Herpes simplex virus Type-1. DRT is an ayurvedic preparation consist of a unique blend of traditional Indian Medicine and modern pharmaceutical technology for keeping the gums and teeth healthy as well as keeping the infections at bay. The ingredients in the DRT has been selected based on their traditional usage and recommended use from the *Ayurvedic text* and used them in the forms as used traditionally. This study highlights the potential of oral care products based on ayurvedic formulations in eliciting the antiviral activity or oral cavity.

2. MATERIALS AND METHODS

1.1 Materials: Herpes Simplex Virus - Type-1 (ATCC), Vero 76 cells ((NCCS), Pune, India.). Culture media-MEM (HiMedia, India) Fetal bovine serum (Gibco, USA) 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*)

2.2 Preparation of test sample: HSV-1 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 1200 µg/ml.

2.3 Method: *In vitro* Cytotoxicity Assay- DRT was evaluated for its *in vitro* cytotoxicity activity by MTT assay in Vero cells. The test sample was evaluated for cytotoxicity with

different concentrations ranging from 4000µg/ml to 250µg/ml.

Virucidal Assay- Vero cells were cultivated as 1×10^5 cell/well in MEM culture medium at 37 °C in a humidified 5 % CO_2 atmosphere for 24 hrs. One non-toxic concentration of test sample, i.e., lower than CTC50 was tested for antiviral property by virucidal assay against virus challenge dose of 10 TCID50.The virus suspension (10TCID50) with test sample were incubated at 37°C for 2 min (Test sample+ Virus suspension). In addition, the virus without test sample was kept as virus control (Pathogen Control). The experiment was performed in triplicates for each test concentration. Acyclovir was used as positive control.Test sample and virus (mixture) were incubated at room temperature for 2 min as indicated in Table 2. After incubation 2.5% cell culture solution containing 10% inactivated fetal bovine serum was added into each tube to neutralize the test sample at room temperature.

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 uniformmix (1200 µg/ml)

2.4 Virus Quantification

Neutralized samples were pooled for quantification. The neutralized solution was diluted 10to 10^8 times with cell culture solution, and 100 µl of each mixture (Test compound+ Virus suspension) were added to the monolayer cultures. All plates were incubated at 37□2□C, 5% CO_2. The CPE was observed every 24 hours for 72 hours. The plates were then scored for presence or absence of viral Cytopathic Effect (CPE) and compared with controls, which was expressed as the protection offered by the test samples to the cells and the virus titer was estimated by endpoint titration method as TCID 50/ml. The Reed-Muench method wasused to determine endpoint titers of the sample by inhibitory concentrations (TCID50 – 50%cell culture infectious doses. LRV (Log reduction value, i.e. virus titer in Control - virus titer in Test sample) was also calculated for the test sample. The Virucidal activity was reflected by LRV values of Test sample.

3 RESULTS AND DISCUSSION

The Test sample demonstrated a dose dependent toxicity against Vero cell as mentioned in Table 3. The CTC50 value for which resulted to be >2000 µg/ml, and hence the concentration 1200 µg/ml was taken for *in vitro* virucidal activity.

Table 3: Cytotoxic activity of test sample against Vero cell line.

Name of TestCompound	Test Conc. (µg/ml)	% Cytotoxicity
Dabur Red paste	4000	86.58±1.3
	2000	33.78±1.4
	1800	32.14±3.1
	1600	29.79±1.0
	1400	27.19±0.5
	1200	24.98±1.2
	1000	23.60±1.2
	500	11.52±0.8
	250	6.52±1.0

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 TCID50 (50% tissue culture infectious dose) and the log reduction value (LRV) of the test sample. The data mentioned in Table-4 represents that DRT exhibited significant cytotoxicity to Vero 76 cells, affecting detection of virus. At the testing concentration of DRT (1200 µg/ml), full cytotoxicity was observed in the 1/10 dilutions.

Table 4: Virucidal efficacy against HSV-1 after a contact time of 2 minutes with virusat 22 □ 2□C

Virus	Name of Test Sample	Viral Load (TCID)	Test Conc. (µg/ml)	Log TCID50 reduction	% Reduction
HSV - I	Dabur Red Paste	10	1200	3.04	99.9
	Acyclovir (Std)	10	10	3.58	99.9

The Virucidal activity of test sample was reflected by LRV values (Virus titer of Control – Virus titer of sample). DRT exhibited virucidal activity (LRV: 3.04, indicating 99.9 % reduction of virus).

4 CONCLUSION

This short communication reports about the virucidal effect towards the commonly infected virus of oral cavity. The objective of the study was to determine the virucidal effect of the Dabur Red toothpaste against HSV-1. Based on the results of Viral titer and the log reduction value (LRV) of the test sample, it can be concluded that DRT demonstrated virucidal activity against HSV-1, with a Log reduction value (LRV) of 3.04 log (99.9 % reduction). Hence Dabur Red toothpaste possess virucidal activity against HSV-1 with LRV of 3.04 suggesting 99.9 % effectiveness against HSV-1.

ACKNOWLEDGMENTS: Authors are grateful to Radiant Research Services Pvt. Ltd, Bangalore, India for conducting the experiment.

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

REFERENCES

1. Herpes simplex virus. fact-sheets details (WHO) https://www.who.int/news-room/fact-sheets/detail/herpes-simplex-virus
2. Luisa F. Duarte *et al*. Herpes Simplex Virus Type 1 Infection of the Central Nervous System: Insights into Proposed Interrelationships with Neurodegenerative Disorders. Front. *Cell. Neurosci*, 2019; 13 (46).
3. Ayurvedic medicine, treatment and remedies for Herpes. Compiled by D. Laxmidutta Shukla. https://www.myupchar.com/en/disease/herpes-simplex-virus/ayurveda
4. Kimberlin DW, Whitley RJ. Antiviral therapy of HSV-1 and -2. In: Arvin A, Campadelli-Fiume G, Mocarski E, *et al*.(eds). Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis. Cambridge: Cambridge University Press; 2007. Chapter 64. Available from: https://www.ncbi.nlm.nih.gov/books/NBK47444/.
5. Anthony N. Van den Pol. Viral infection leading to brain dysfunction: more prevalent than appreciated? *Neuron*, 2009; 64(1): 17–20.
6. P. Vijayan *et.al*. Antiviral activity of medicinal plants of Nilgiris. *Indian J Med Res*, 2004; 120: 24-29.
7. Kannissery Pramoda, Shahid H. Ansarib and Javed Alia. Eugenol: A Natural Compound with Versatile Pharmacological Actions. *Natural Product Communications*, 2010; 5(12).
8. Sulochana Kaushik *et al*. Anti-viral activity of Zingiber officinale (Ginger) ingredients against the Chikungunya virus. *VirusDis*, 2020; 31(3): 270–276.
9. Raimundas Lelesius *et al*. In vitro antiviral activity of fifteen plant extracts against avian infectious bronchitis virus. *BMC Veterinary Research*, 2019; 15: 178.