**Journal of Pharmaceutical and Scientific Innovation**
**www.jpsionline.com**
**Research Article**

# ASSOCIATING A HYPERTONIC SOLUTION WITH SPECIFIC PLANT PROCYANIDINS FOR THE TREATMENT OF GENITAL HERPES

Ravi Shrivastava*
VITROBIO Research Institute, ZAC de Lavaur 63500 Issoire, France
Email: vitrobio@orange.fr

Received on: 21/01/13 Revised on: 25/02/13 Accepted on: 27/02/13

**ABSTRACT**
Presence of free Herpes Simplex Virus (HSV) particles in the vaginal cavity and consequent continuous infection of new healthy cells delay the recovery from Genital herpes (GHSV). As the HSV envelop contains several surface proteins and since some plant tannins have a strong affinity for these proteins, we incorporated specific tannins into an osmotically active solution to evaluate clinical efficacy.
60 women having visible lesions of genital herpes were treated with RPEX (10ml per day) for 14 consecutive days. Product was administered daily into the vaginal cavity and the symptoms of GHSV were evaluated before treatment and on days 1 (2 h), 4, 7, and 14. Smears from GHSV lesions were also collected to evaluate the number of virus-loaded multinucleated giant cells using Tzanck test. Data were analyzed using CFR21 USFDA software and SAS9.1.3. statistical program.
The mean amount of free virus was diminished by nearly 17%, 44%, 65%, and 100% on days 1, 4, 7, and 14, respectively, with a corresponding reduction in the size of the lesions, and complete recovery on day 14.
HSV surface glycoprotein inhibitors incorporated into an osmotically active solution represents a new approach to treat GHSV.
**Keywords:** Genital herpes, plant procyanidins, virus glycoprotein inhibition.

## INTRODUCTION

Genital herpes (GHSV) is a sexually transmitted disease caused by the double-stranded DNA herpes simplex virus Type 2 (HSV-2) and occasionally by Type 1 (HSV-1). The virus enters into the body through microscopic tears and remains dormant indefinitely. The virus is activated periodically under certain conditions of stress or pregnancy.[1] When the infected person has a herpes outbreak, the virus travels down the nerve fibers to the genital area (vagina or penis), enters into a few cells by attaching with specific cell membrane receptors such as nectin-1, HVEM or 3-O heparan sulfate, and then multiplies.[2] As the cell lyses liberate free virus particles onto the infected surface, these virions begin attacking adjacent new healthy cells, leading to the formation of blisters and inducing pain, inflammation, itching, vaginal dryness, turbid discharge, and abnormal vaginal flora due to alkaline pH. When the blisters rupture, an even greater amount of free HSV particles is liberated, attacking more new cells and maintaining the state of infection. Symptoms usually last between 15 and 30 days depending upon the functioning of the immune system of the infected person.[3]

Since this presence of a large amount of infective free virus particles on the infected area is the primary cause of maintaining and further developing the infection, any treatment strategy must be directed to inactivate and to stop new host cell infection. Unfortunately, in absence of any topical antiviral or topical virus entry-blocking drug, only symptomatic treatments are currently used to treat HSV-2 infections.[4]

The HSV envelop contains at least 10 virus encoded glycoproteins (GPs),[5] and some of these GPs, presumably GPC, GPB, and GPD, interact with the host cell surface heparan sulfate receptors to trigger pH-dependent fusion of the viral envelop with the host cell plasma membrane.[6,7] Due to the multiplicity of the HSV virus GPs and poor antigenicity, it is also extremely difficult to elaborate an efficient vaccine. Several approaches including subunit vaccines, peptide vaccines, live virus vectors and DNA vaccine technology have been used to develop prophylactic and therapeutic vaccines but their efficacy remains limited.[8] It is also postulated that HSV virus use certain topically available matrixmetalloproteins (MMPs) for proteolytic degradation of host cell membrane and infection.

The HSV envelop GPs and MMPs are proteinacious in nature, and since certain plant procyanidins (PCDs) have a strong affinity for proteins, we searched specific plant PCDs having strong affinity for these particular proteins using *in vitro* methods as described by Shrivastava.[9] The hypothesis was that the PCDs may bind to virus GPs, thereby inhibiting virus–cell interaction and new infection. As PCDs are big molecules, they cannot enter into the cells and are naturally eliminated through vaginal secretion. To enhance vaginal secretion, the PCDs were incorporated into an osmotically active, hypertonic solution, as described by Shrivastava,[10] and the resulting solution was named RPEX. After initial *in vitro* selection of the best antiviral PCDs, a pilot clinical trial was conducted to evaluate the efficacy of RPEX in women with acute GHSV infection.

## MATERIALS AND METHODS

### Selection of anti-GHSV PCDs

Several tannin-rich plants went through extraction process, and the PCD-rich plant extracts were prepared as described by Khanal et al.[11] Extracts were atomized for drying and diluted in the culture medium or in the test product base before use. Vero cells were grown *in vitro* following the method published by Shrivastava.[12] The minimum tissue culture infective dose of HSV2 virus capable of killing 50% or 100% vero cells *in vitro* ($TCID_{50}$ or $_{100}$) was determined. A fixed amount of PCD-rich plant extract (50 µg/ml) was prepared in Dulbecco's Modified Eagle's Medium (DMEM, PEE, France) and pre-incubated in a test tube with $TCID_{100}$ virus concentration for 1h at 37°C to allow PCD–virus GP interaction. HSV-sensitive vero cells were then infected with

the PCD–virus suspension and virus titer was measured. Active plant extracts were then associated with each other at half the concentration (25 µg/ml) and the PCD association (VB-PCDs) capable of neutralizing 90% to 100% virus growth was selected for the preparation of RPEX.

**Preparation of test product batches**

The test product containing 0.54% VB-PCDs in glycerol as an osmotically active solution (pH 4.5) was prepared as described by Shrivastava et al.[13] This transparent and viscous solution was filled into 10ml low-density polyethylene tubes with a 4cm long canula for vaginal application.

**Table 1:** Distribution of External Genital Herpes Lesions in the Patients (n=60).

| Total number of patients | Patients presenting outer skin lesions | 1 outer genital herpes lesion | 2 outer genital herpes lesion | >2 outer genital herpes lesions |
|---|---|---|---|---|
| 60 | 42/60 | 16 | 17 | 9 |

**Table 2:** Number of Patients Rating the Sensation of Vaginal Itching, Redness, Pain, Dryness, Discharge and Presence of Blisters, on a 0 to 3 Scale ( 0 = No Symptom, 1= Mild, 2 = Moderate, 3 = Severe) at the Start of Treatment, 2h after 1st Product Application and on Days 4,7, and 14. The p-Values Indicate Statistical Significance Compared to the Before Treatment Values.

| Parameter | Number of replies (n=60) | | | | |
|---|---|---|---|---|---|
| **Vaginal itching** | **None** | **Mild** | **Moderate** | **Severe** | **p-value** |
| Day -1 before treatment | 19 | 18 | 11 | 12 | 0.3430 |
| + 2h | 28 | 21 | 8 | 3 | <.0001 |
| Day 4 | 47 | 11 | 2 | 0 | <.0001 |
| Day 7 | 60 | 0 | 0 | 0 | . |
| Day 14 | 60 | 0 | 0 | 0 | . |
| **Vaginal redness** | | | | | |
| Day -1 before treatment | 07 | 18 | 14 | 21 | 0.0620 |
| + 2h | 11 | 19 | 18 | 12 | 0.3430 |
| Day 4 | 11 | 29 | 20 | 0 | 0.0174 |
| Day 7 | 27 | 30 | 3 | 0 | <.0001 |
| Day 14 | 58 | 1 | 1 | 0 | <.0001 |
| **Vaginal pain** | | | | | |
| Day -1 before treatment | 8 | 12 | 21 | 19 | 0.0620 |
| + 2h | 13 | 8 | 32 | 7 | <0.001 |
| Day 4 | 29 | 12 | 13 | 06 | <0.0002 |
| Day 7 | 36 | 13 | 09 | 02 | <0.0001 |
| Day 14 | 55 | 4 | 1 | 0 | <0.0001 |
| **Vaginal dryness** | | | | | |
| Day -1 before treatment | 5 | 11 | 25 | 19 | 0.0015 |
| + 2h | 14 | 28 | 18 | 0 | 0.0743 |
| Day 4 | 17 | 34 | 09 | 0 | 0.0003 |
| Day 7 | 20 | 40 | 0 | 0 | 0.0098 |
| Day 14 | 57 | 3 | 0 | 0 | <.0001 |
| **Vaginal discharge** | | | | | |
| Day -1 before treatment | 16 | 15 | 18 | 11 | 0.6295 |
| + 2h | 21 | 10 | 20 | 9 | 0.0433 |
| Day 4 | 36 | 12 | 8 | 4 | <.0001 |
| Day 7 | 42 | 15 | 1 | 2 | <.0001 |
| Day 14 | 51 | 9 | 0 | 0 | <.0001 |
| **Presence of blisters** | | | | | |
| Day -1 before treatment | 04 | 18 | 16 | 22 | 0.0074 |
| + 2h | 04 | 18 | 16 | 22 | 0.0074 |
| Day 4 | 16 | 17 | 15 | 12 | 0.8174 |
| Day 7 | 23 | 24 | 13 | 0 | 0.1572 |
| Day 14 | 57 | 2 | 1 | 0 | <.0001 |
| **Vaginal pH** | **Normal 3.6-4.6** | **Acidic <3.6** | **Slightly Alkaline 4.6 -7.0** | **Highly alkaline >7.0** | **p-value** |
| Day -1 before treatment | 10 | 2 | 31 | 17 | <.0001 |
| + 2h | 21 | 0 | 36 | 3 | <.0001 |
| Day 4 | 39 | 0 | 19 | 2 | <.0001 |
| Day 7 | 51 | 0 | 7 | 2 | <.0001 |
| Day 14 | 57 | 0 | 3 | 0 | <.0001 |