

## A Clinical Pilot Study of Lignin–Ascorbic Acid Combination Treatment of Herpes Simplex Virus

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**Abstract.** Antiviral drugs as well as natural remedies have been used to reduce symptoms and the rate of recurrences of herpes simplex virus type 1 (HSV-1) infection, a common disease. To evaluate anti-HSV-1 activity of a pine cone lignin and ascorbic acid treatment, a clinical pilot study was carried out. Forty-eight healthy patients of both genders between 4 and 61 years old (mean:  $31 \pm 16$  years), with active lesions of HSV-1, took part in the study. According to the HSV-1 stage at the presentation, the patients were classified into the prodromic (16 patients), erythema (11 patients), papule edema (1 patient), vesicle/pustule (13 patients) and ulcer stages (7 patients). One mg of lignin-ascorbic acid tablet or solution was orally administered three times daily for a month. Clinical evaluations were made daily the first week and at least three times a week during the second week after the onset and every six months during the subsequent year to identify recurrence episodes. The patients who began the lignin-ascorbic acid treatment within the first 48 hours of symptom onset did not develop HSV-1 characteristic lesions, whereas those patients who began the treatment later experienced a shorter duration of cold sore lesions and a decrease in the symptoms compared with previous episodes. The majority of the patients reported the reduction in the severity of symptoms and the reduction in the recurrence

episodes after the lignin-ascorbic acid treatment compared with previous episodes, suggesting its possible applicability for the prevention and treatment of HSV-1 infection.

Herpes simplex virus 1 (HSV-1), a neurotropic virus, commonly infects the skin and mucous membranes, and remains latent before erupting in response to different stimuli (1). Lesions are often painful, burning or pruritic and tend to recur in most patients (2). The treatment of HSV-1 is based on the control, but not on the cure of the disease, since the virus cannot be eradicated (3). Despite extreme variation in the severity of the lesion from patient to patient, symptoms are normally resolved in one or two weeks without treatment (4). Nevertheless, it has been suggested that antiviral drugs such as nucleoside analogs could reduce the symptoms and the recurrence rate (5-7). Clinical trials of different topical and systemic antiviral drugs, have demonstrated an effective treatment in HSV-1 infections (8-10). Acyclovir has been used as a standard regimen for the control of oral HSV-1 (5, 7, 11, 12), but produced controversial efficacy (13, 14) and a wide array of side-effects (6, 15), and even failed to prevent the secondary sunlight exposure-induced recurrence (16). Hamuy and Berman reviewed the use of a number of antiviral agents with diverse results, however, the authors emphasize that the different methods researchers had used made direct comparison difficult (17).

Lignins are major cell components formed by the dehydrogenation polymerization of three monolignols, *p*-coumaryl, coniferyl and sinapyl alcohols. Lignin carbohydrate complexes, Fr VI and Fr VII, from the alkaline extract of pine cones, have shown potent antiviral activity in mice *via* direct interaction with virus (18). Lignin carbohydrate complexes, such as alkali-lignin, lignin sulfonate and Fr VI or Fr VII, at higher concentrations, strongly enhanced both the radical intensity and cytotoxic activity of ascorbic acid (19). However,

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Table I. Stages of HVS lesions in the 48 study patients.

Stage	Description of the stage	Duration (days)	No of patients
0 Prodrome	Normal skin; tingling, burning pain or itching sensation.	1	16
1 Erythema	Redness of the skin.	1-2	11
2 Papule/ edema	Small solid raised lesions, inflammation.	1-2	1
3 Vesicle/pustule	A blister-like skin elevation in which fluid is visible through the outer layer of the skin.	3-6	13
4 Ulcer/soft crust	Shallow gray yellow ulcer.	4-7	7

Adapted from Paterson and Kwong (20).

there has been no clinical report of the antiviral activity of lignin carbohydrate complex. The purpose of this study was to evaluate the effect of pine cone lignin and ascorbic acid combination treatment on the duration with lesions and the decrease of symptoms in HSV-I infected patients.

### Patients and Methods

**Patients.** Participants were recruited among students and outpatients from the clinics of the Faculty of Dentistry of the Universidad Autonoma del Estado de Mexico. A total of 1,300 people were face to face interviewed; those with a history of HSV-1 recurrent episodes were invited to participate in the study. They were requested to attend the Oral Pathology Laboratory, when presenting a new onset. From 139 people originally invited to participate, 73 people did not attend the laboratory during the study period. Eighteen patients were lost after the treatment was started. A total sample of 48 patients, 17 male (35%) and 31 female (65%), aged from 4 to 61 years (mean: 31±16 years) with active lesions detected on presentation, participated in the study. None of the patients reported systemic disease or medicine use. This study was approved by the Institutional Ethical Committee and all the participants signed a document of informed consent.

At the start of treatment/on presentation at the laboratory, the patients were classified, according to the criteria of Paterson and Kwong (20) (Table I). To record the evolution of the HSV lesions, during the first week the patients attended the clinic daily from Monday to Friday, and during the second week they attended three times. Those patients who lived quite far from the Dental School were contacted by telephone. The patients were followed up for a year with the purpose of evaluating the recurrence rate after combination treatment with pine cone lignin and ascorbic acid. Photographs of the lesions were taken (not shown). Due to the difficulty of detecting HSV-1 patients, their previous clinical history and treatment experience were used as the control. The studied variables were: age, gender, physical findings, history of recurrent episodes, duration of the lesions, family cases, prodromal symptoms (burning, itching, pain, tingling sensation, sore throat and general malaise), reactivation stimuli (fever, sun exposure, trauma, physical or emotional stress, food allergy, respiratory tract infections and gastrointestinal diseases), location of the lesions (lips, and skin around the mouth and oral mucosa), number of lesions, evolution, reduction of symptoms and treatment previously used.

**Interview procedure.** Previous explanations of the nature of the study were given to the control group. Students and outclinic patients were asked if they had ever suffered ulcerations in the mouth with the following characteristics: ulceration in mobile

mucosa with gum inflammation during early childhood, presence of ulcerations in lip skin or face, recurrence episodes associated with triggers. The subjects slides and clinical photographs of lesions in different stages of HSV-1 were also shown to them.

Patients and/or their companions were interviewed in the waiting rooms of clinics of the School of Dentistry of the Universidad Autonoma del Estrado de Mexico, while the students of the School of Dentistry were interviewed in their classrooms. Patients were interviewed about 35 semi-structured survey items in the dental office of the Oral Pathology Laboratory. The clinical review was conducted by using dental mirror and the established protection barriers.

**Preparation of lignin-ascorbic acid tablets.** The pine cone lignin fraction was prepared by a modification of the method previously reported (18). In brief, the cones of *Pinus densiflora* Sieb. et. Zucc. (obtained in Ehime, Japan) were washed with hot water and the residue was extracted with 1% NaOH. The NaOH extract was neutralized with hydrochloric acid, concentrated under reduced pressure and treated with ethanol. After centrifugation, the precipitate was dissolved in hot water to remove the ethanol, concentrated, mixed with dextrin and then finally dried to obtain the crude lignin fraction (pine cone extract powder JS) at an approximate yield of 50%.

Each lignin-ascorbic acid tablet contained a mixture of 50 mg pine cone extract powder JS, 50 mg ascorbic acid (BASF, Tokyo, Japan), 83 mg maltitol (Mitsubishi Shoji Foodtech Co., Ltd., Tokyo, Japan), 13 mg potato starch (Shirokuma Hokkai Food Co., Ltd., Sapporo, Japan) and 13 mg calcium stearate (Shinagawa Chemical Industry Co., Ltd, Tokyo, Japan).

**Treatment schedule.** The patients were administered one tablet of one mg before each meal daily for one month, or one mg of powder (tip of a coffee spoon), dissolved in a glass of boiled water or juice, before each meal daily also for one month. In the patients with recurrent onset, the treatment was repeated.

**Statistical analysis.** SPSS version 10.0 (Copyright© SPSS Inc., Chicago, USA) was used to analyze the association between variables by means of Chi-square Student's *t*-test and Kendall correlation. Differences were considered significant at  $p \leq 0.05$ .

### Results

**HSV-1-infected patients.** In total, 48 HSV-1 patients were enrolled between January 2006 and December 2006; these patients were followed until December 2007. The female to male ratio was 1.82, most of the patients were in the first three decades of life, 29% were more than 40 years old (Figure 1).

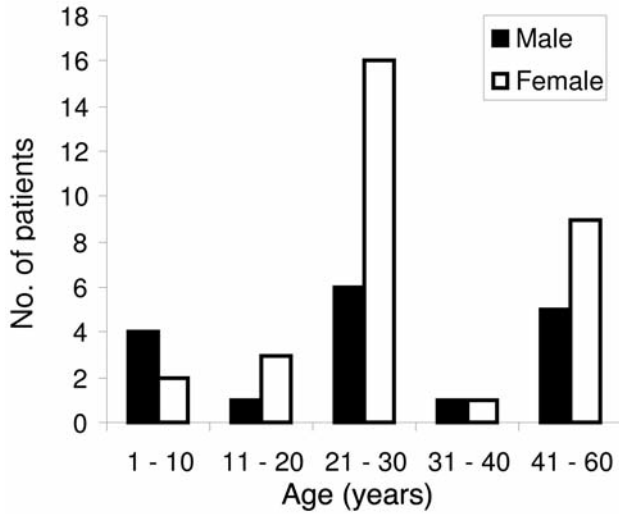


Figure 1. Distribution by age of HSV-1-infected patients studied according to gender.

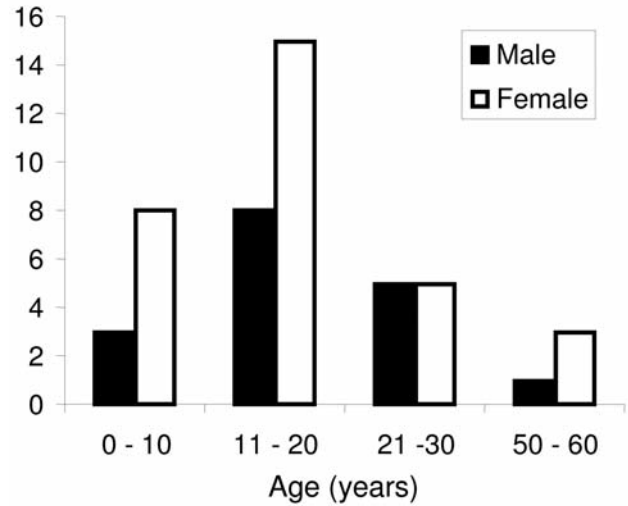


Figure 2. Distribution by age at first outbreak among HSV-1-infected patients according to gender.

Twenty-three percent of the total (11 patients out of 48 patients) started HSV-1 outbreaks in the first decade of life, 48% (23 patients) in the second decade and 29% (14 patients) after 21 years of age (Figure 2). Of them six patients, relatives of patients under study, developed HSV-1 infection for the first time, four were children and two adolescent, only one of them showed the typical features of primary herpetic gingivostomatitis. There were significantly more patients (65%) who had affected relatives living at the same house, than those who had unaffected relatives (35%) ( $p=0.014$  Chi-square test).

According to the patients' information, episode frequency varied from one recurrence episode to more than seven episodes per year (Figure 3).

All of the patients reported a combination of internal and external triggers of viral reactivation, as follows: fever (31%, 15 patients out of 48 patients), respiratory diseases (52%, 25 patients), gastrointestinal disorders (4%, 2 patients), emotional stress (48%, 23 patients), menstruation (6%, 3 patients), allergy (15%, 7 patients) and sun exposure (65%, 31 patients). Common prodromal symptoms were: tingling (15%, 7 patients), burning (15%, 7 patients), swelling (15%, 7 patients), itching (17%, 8 patients) and a combination of them including sore throat and malaise (40%, 19 patients). The skin of the lips was involved in 81% of the patients (39 patients), and the vermillion border in 19% (9 patients). A single lesion was observed in 31% (15 patients) of the patients with clinical evidence of lesion, while multiple lesions were noted in 36% (17 patients). The clinical manifestations are shown in Table II and were similar to those previously reported (1-4, 9). The mean size of the lesions was  $0.938 \pm 0.483$  cm (SD).

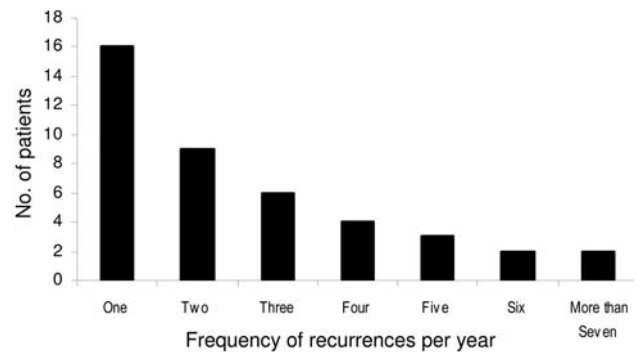


Figure 3. History of episodes per year. N=42 patients. Six patients reported a first outbreak.

*Anti-HSV-1 activity of lignin ascorbic acid combination treatment.* The mean duration of the lesions in the patients who were treated with pine cone lignin and ascorbic acid in the first two days was significantly shorter ( $4.2 \pm 3.7$  days) than that of the patients treated three to twelve days ( $8.3 \pm 4.9$  days) (Figure 4). Fifty percent (24 patients) of the patients reported previous use of topical acyclovir, but 33% of them (8 out of the 24 patients) reported no reduction of the symptoms with that treatment or reduction in the recurrence episodes.

The pine cone lignin and ascorbic combination was administered to 58% (28 out of the 48 patients) in the first 48 hours of onset, to 21% (10 patients) between 48 and 72 hours and to 21% (10 patients) after 72 hours. In 81% (39 patients) of the cases, the symptoms decreased; the extent of the decrease depended on the day that the lignin-ascorbic

Table II. Clinical features of HSV lesions.

Clinical variables	N	%
<b>Number</b>		
None*	16	33
Single	15	31
Multiple unilateral	11	23
Multiple bilateral	6	13
Total	48	100
<b>Contour</b>		
None*	16	33
Round	11	23
Irregular	21	44
Total	48	100
<b>Margins</b>		
None*	16	33
Indurations	19	41
Erythematic	13	26
Total	48	100
<b>Color</b>		
None*	16	31
Yellow	6	15
Red	16	33
Red-gray	10	21
Total	48	100

\*Lesions without clinical manifestations were those in prodromal stage.

acid combination treatment was started (Figure 5). Also 90% (43 patients) reported a reduction in the recurrence rate.

Only five patients presented a recurrence episode during the study (Figure 6). In four of these patients, there was a reduction in the frequency of the usual recurrence episodes; in one patient, the usual recurrence rate (1/year) did not change. In all five patients, the duration of the onsets was shorter than usual.

### Discussion

The present study demonstrated that HSV-1 patients who took the pine cone lignin and ascorbic acid combination treatment in the first 48 hours after onset clearly manifested the efficacy of this treatment in reducing the length of the outbreak and reducing the symptoms. Even the patients who took the treatment on the subsequent days reported a beneficial effect, in comparison with their previous experience. It is possible that the treatment could benefit these latter patients to a small extent by accelerating healing. It seems that the inhibitory effect of pine cone lignin and ascorbic acid combination treatment on HVS-1 infection might depend on the antioxidant and immunopotentiating activities of lignin and ascorbic acid (18). The free radical

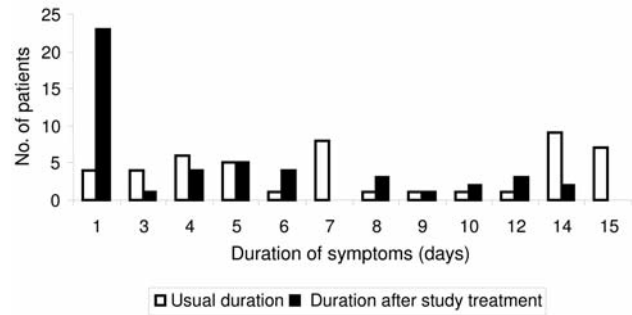


Figure 4. Duration of lesions previous versus current treatment. Usual duration is based on patients' reports. Student's t-test,  $p=0.001$ .

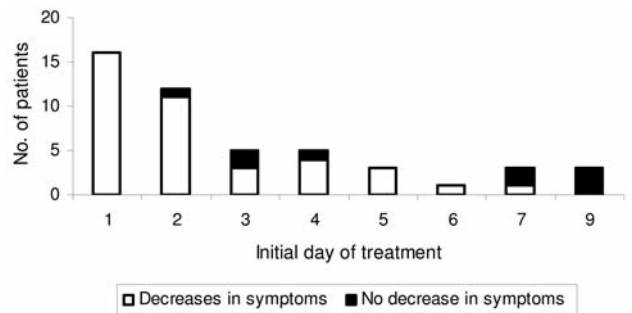


Figure 5. Symptoms reduction according to the day after onset, of starting pine cone lignin and ascorbic acid complex treatment. Symptoms were reduced significantly when the treatment was taken in the first 48 h; Kendall's Tau-b,  $p=0.001$ .

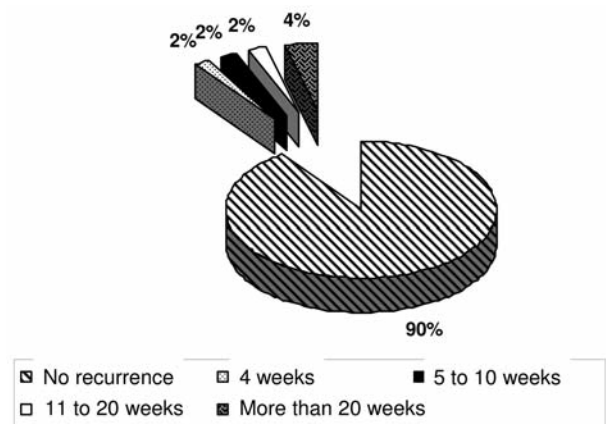


Figure 6. Recurrence after the pine cone lignin-ascorbic acid complex treatment.  $N=48$  patients.

scavenging activity of lignin (21) could attenuate oxidative stress and consequently induce antiviral protection. Although it has been reported that the use of herbal medicines can lead to severe adverse effects (22, 23), no patients in the present study presented any side-effects.

A number of alternative medicines and dietary modifications have been administered with reproducible or conflicting results. The use of topical zinc for facial and circumoral herpes infection has been recommended, though zinc oxide can cause adverse effects (24, 25). The topical use of *Melaleuca alternifolia* (tea tree oil) reduced the time of re-epithelialization (26); also the dried leaf extract of lemon balm (*Melissa officinalis*), when administered locally, promoted the healing process and reduced the recurrence frequency (27). Ascorbic acid has been suggested to be useful for prevention of HSV-1 reactivation (28), and according to Terezhalmay *et al.* (29), 600 mg of water-soluble bioflavonoid and 600 mg of ascorbic acid reduced the bladder stage of HSV-1 and prevented the disruption of the vesicular membrane. Essential oils of ginger, thyme hyssop and sandalwood inactivated HSV before it entered cells, even in acyclovir-resistant HSV-1 (30). Propolis inhibited the viral entry *in vitro* (31) and interfered with the viral replication cycle *in vivo* (32).

In general, all studies agree that the chances of treatment success are correlated with lesion age, the first 24 to 48 hours being the most effective. This is the time during which the viral replication temporarily dominates the host immune response (8) and the response diminishes considerably with older lesions. The optimum treatment period is very brief, so to increase the chances of success, medication should be prospectively dispensed and treatment self-initiated by the patient at the first indication of a new episode (7). Patients in this study who received the treatment in the first 24 hours did not develop the typical symptoms of the onset; even those patients whose treatment was administrated within 72 hours reported a shorter duration of the episode.

The clinical aspects reported by the patients were similar to those reported in developing countries (11). The majority of the patients were in the third decade of life, the prevalence was low in children and adolescents. Twenty-nine percent of the patients were between forty and sixty years old, although it has been reported that the frequency of reactivation appears to decline after 35 years of age (33). These observations could be explained as 27% of the patients were students of the Dental School and the rest were patients or individuals accompanying patients to the Dentistry School's clinics. The prevalence of HSV is associated with low socioeconomic status (9), which is a common situation among patients who attend the Dentistry School's clinics.

Most cases of primary herpetic gingivostomatitis are subclinical, therefore children and adolescents, reporting a first outbreak in this study could be examples of previously undiagnosed primary episodes as they presented clinical manifestations of a recurrent onset rather than those described in primary herpetic gingivostomatitis (13).

The identification of familial cases living at the same house is not surprising since HSV infection could be spread *via* respiratory droplets or direct exposure to infected saliva (34). In addition to this internal trigger of HSV outbreak, external triggers, such as sun exposure, for possible enhanced HSV outbreak have been described previously (20, 30).

Although there were significant differences between the previous and current duration after the pine cone lignin and ascorbic acid combination treatment and differences in the reduction of symptoms taking into account historical data provided by the patients, the small number of patients and the lack of a control group limited our ability to generalize the results to a larger population. To evaluate the effectiveness of pine cone lignin and ascorbic acid combination treatment, subsequent randomized placebo-controlled studies must be conducted in a representative sample of the pediatric population to treat herpetic gingivostomatitis as well as in adults who suffer from recurrent HSV-1.

In conclusion, pine cone lignin and ascorbic acid combination has a potent antiviral activity, promoting faster healing, reduction of symptoms and reducing the frequency of recurrence episodes in HSV-1. Pine cone lignin and ascorbic acid combination could be useful in preventing and treating HSV infection.

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