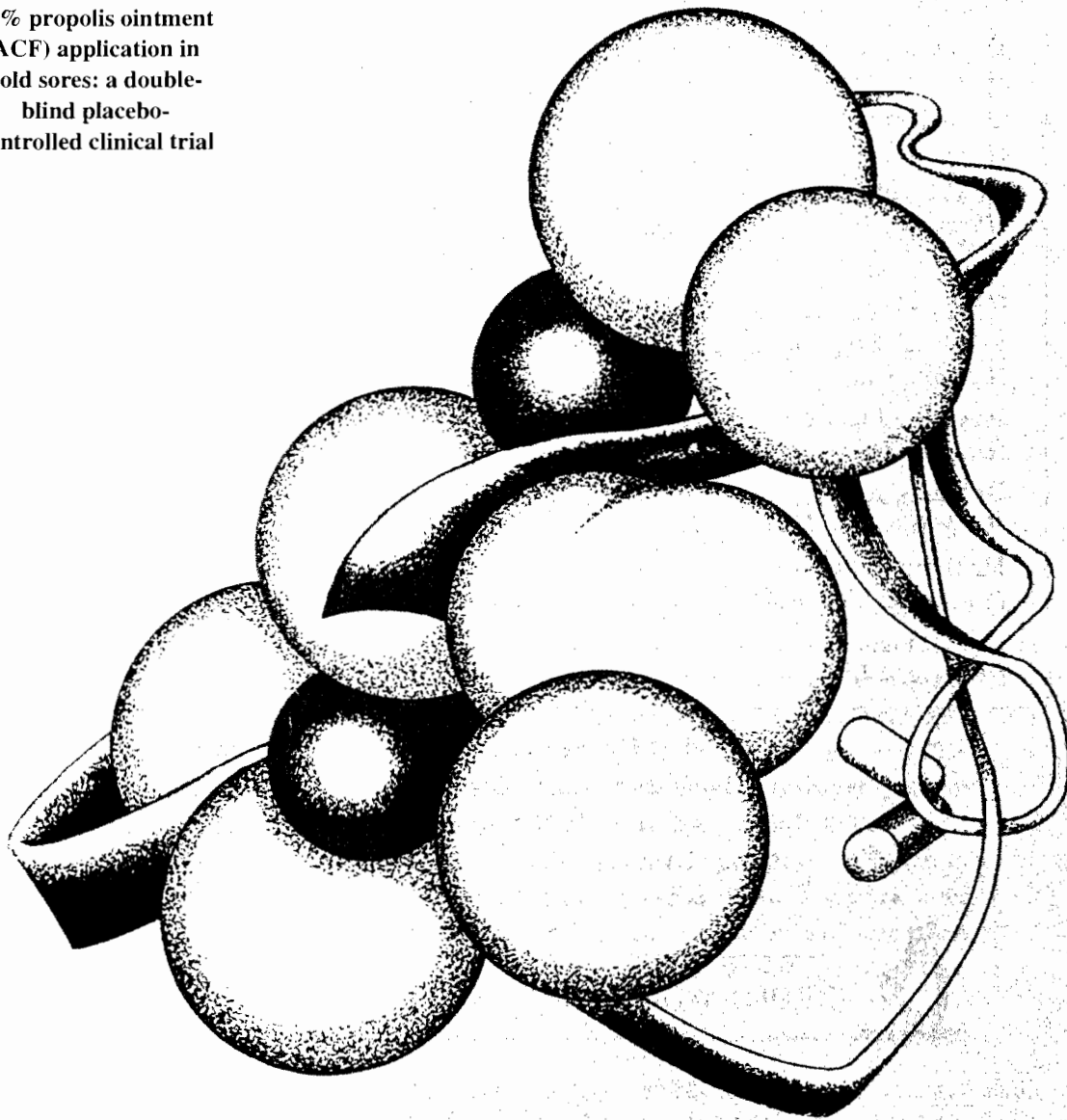


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The effects of Herstat* (3% propolis ointment ACF[†]) application in cold sores: a double-blind placebo-controlled clinical trial

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Keywords: cold sores, herpes labialis, herpes virus, propolis ointment ACF

Summary

A randomised, double-blind placebo-controlled clinical trial was conducted in Sweden to assess the efficacy of Herstat* (3% propolis ointment ACF[†]) in patients with a history of herpes labialis recurrences (cold sores). Patients were block-randomised and instructed to self-initiate the treatment as soon as they had a cold sore, by applying the study medication five times/day. They were instructed to report in a diary (for every application) time of application, cold sore stage and degree of pain.

The main outcome measures were time to healing and time to disappearance of pain. Size of the lesion and time to improvement were also compared. The rate of lesion healing was significantly faster among the patients treated with propolis ointment (mean 6.24 days) compared to those treated with placebo (mean 9.77 days) ($p < 0.00001$, Student's *t*-test for unpaired data, and logrank test). The patients who applied propolis ointment were without pain considerably earlier than those who applied the placebo ointment (logrank test, $p = 0.00671$). There was no overall significant difference between the two groups for time to improvement of the lesion, even if the propolis group appeared to improve faster than the placebo group in the early days of treatment. No differences were found for the size of the lesion. At the end of the treatment, 100% of the patients in the

continued

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propolis ointment group judged their treatment 'very effective' (81.8%) and 'somewhat effective' (18.2%), and almost the reverse was true for the placebo group, where most found it 'hardly effective' (60%) and 'ineffective' (22.9%).

Introduction

Recurrent herpes labialis (also called 'cold sores' or 'fever blisters') is the most frequent clinical manifestation of reactivation of herpes simplex type I virus infection. It is a common infection, and is estimated to occur in 20 to 40% of the population¹.

Following primary infection, the herpes virus is thought to travel through the sensory axons and to establish a chronic latent infection in the ganglia. The reactivation of the latent virus results in the transport of the viral genomes back to the body surface, where replication occurs inside the cells of epidermis and dermis and causes the development of herpetic ulcerations, located most of the time on the vermilion border of the lips and/or on the external facial skin. Usually, the disease is easily recognisable and diagnosed without the need for laboratory tests. Lesion development is rapid, sometimes preceded by a short prodromal period with itching and local tingling sensations. Maximum severity is reached within a few hours to one day from the beginning of the episode. Most of the clinical course represents the phase of lesion resolution, which is analogous to the healing of a small traumatic skin wound, taking 7 to

10 days to resolve completely. Clinically, most recurrences are mild and self-limiting, but in patients with frequent or severe episodes the attacks may be distressing and disfiguring, with pain and sometimes significant psychosocial consequences.

We present here the results of a randomised, double-blind, placebo-controlled study of Herstat* (3% propolis ointment ACF[†]) in the treatment of recurrent episodes of herpes labialis, undertaken in Sweden between October 1999 and December 2000. The rationale for the use of propolis ointment in cold sores is based on its antiviral, antibacterial, anaesthetic, anti-inflammatory and regenerative properties. Studies of propolis ointment ACF in the treatment of herpes labialis and genital herpes have been published elsewhere, and suggested that topical propolis ointment can shorten the healing time of herpetic lesions. Several studies were undertaken in the early 1980s and the present study was undertaken to confirm these earlier published studies^{2, 3} and also unpublished reports (Z Sosnowski, 1982; BA Gorski, 1981; personal communications).

Methods

• Study population and design

This was a randomised, double-blind, placebo-controlled, parallel group study.

Patients were recruited mostly through advertisement in local papers. The study protocol had the approvals of the two Swedish institutional review boards, the

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Ethical Committee (*Forskningsetik-kommittén*) and the Medical Products Agency (*Läkemedelsverket*), and was in accordance with the Declaration of Helsinki (1996, South Africa).

Patients were immunocompetent men and women, aged between 18 and 65, in good general health and sufferers of cold sores, with a clear medical history of recurrent episodes of herpes labialis and with at least three episodes per year. All patients gave written informed consent.

Prohibited concomitant medications were antivirals, antibiotics, vaccines and corticosteroids, and only the study medication could be applied on the lesion area. Analgesics, painkillers and anti-inflammatory drugs were also not allowed. Exclusion criteria were pregnancy, breastfeeding, severe systemic disease, treatment with systemic antibiotics, antivirals or drugs acting on the immune system. Subjects with allergy to propolis or to any of the substances in the study medications were also excluded from the study.

Those patients who met the entry criteria were sequentially assigned, by block-randomisation and with a double-blind procedure, to receive either the 3% propolis ointment or the placebo.

The double-blind study medications were packaged in identical 5 g tubes. The 3% propolis ointment contained also vaseline, liquid paraffin, alcohol and lanolin as excipients. The placebo was a vehicle control ointment containing the same excipients but not the propolis extract, and also containing vanillin and colorants to simulate the yellow-

brown colour and the characteristic pleasant smell of propolis.

• **Assessments**

Treatment was self-initiated. Patients were instructed to apply the assigned study medication on the lesion area, as soon as possible after the beginning of the cold sore episode and then five times per day, until healing, up to 12 days.

Patients were asked to report to the investigator at the start of the treatment and when healing was complete, and were trained to fill a 'cold sore diary' throughout the duration of the study. They were asked to keep a daily record of time of application of the medication, cold sore stage and degree of pain experienced, for every application and for the entire duration of the treatment. The patients received also a mirror and a ruler, and were asked to draw the position of the lesion on the face diagram in the diary at the beginning of the treatment, and to report in the diary the size of the lesion once a day.

Efficacy was evaluated by assessing the effect of the treatment on resolution of the cold sores lesions and on resolution of pain.

Lesion healing was the primary efficacy variable, and the effect on the cold sore healing by the study medication was determined by time to loss of crust and disappearance of the residual local swelling (while a degree of skin redness could still be present). The effect of the study medication on improvement of the lesion was also assessed, and determined by time to reach the stage of crust, when no more ulcers were present.

Pain was evaluated by time to loss of pain. Pain was expressed in a scale from 0 to 4, with 0 = no pain, and 4 = strong pain.

End points assessing the healing of the lesion were analysed using the investigator assessments, who checked patients' condition and diary entries at the first and at the second (final) visits.

For time to reach the stage of crust and for time to loss of pain, patient assessments recorded in the diaries were used. Patients' diary entries were also used to assess if the study medication had any effect in limiting the size of the lesion during the cold sore episode.

These time-to-event data were displayed using the Kaplan-Meier survival plot, and group comparisons were made using the logrank test. At the end of the study, patients were also asked to express their judgement about the study treatment by answering some multiple choice questions. These opinions were also given in double-blind conditions.

Results

• *Patients' characteristics*

A total of 124 patients were enrolled, randomised and entered into the study. Fifty-six were excluded because they did not experience a cold sore attack during the study period (42) or an evaluable cold sore (3). Eleven more patients were not included in the evaluation because of protocol violations (did not complete the treatment/lost of did not fill correctly the diary/did not attend the final visit/developed serious systemic disease).

Subsequently, 68 patients initiated and completed the treatment of a cold sore recurrence during the study period. Of these 68 patients, 33 were randomised to 3% propolis ointment ACF and 35 received the placebo ointment (Table 1).

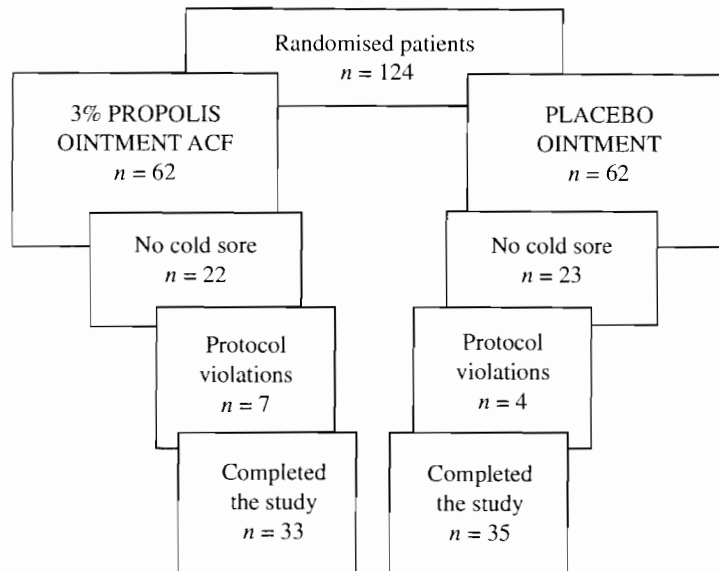
The study population was predominantly female (a total of 54 women, 79.5%, against a total of 14 men, 20.5%), with a mean age of 45 years. There was a gender difference between the groups. The treatment group had only 1 man and 32 women (97%), while the placebo group had 13 men and 22 women (63%). All patients had a clinical history of herpes labialis, with a mean duration of the disease of more than 20 years for both groups (Table 2). The study population came from an area of Sweden with a medium-high socio-economic condition. All patients were educated to at least high school level, and all were in full-time employment.

• *Lesion healing (investigator-assessed)*

The effect of 3% propolis ointment ACF on the resolution of the cold sore lesion is shown in Table 3 and Figure 1.

The rate of lesion resolution was considerably faster among the patients treated with the propolis ointment compared to the patients who applied the placebo ointment ($p < 0.00001$, Student's *t*-test for unpaired data and Mann-Whitney U test), with a difference in the time of healing between the two groups of 3.53 days. The propolis group healed in 6.24 days (mean, $SD = 2.16$), compared to the placebo group that healed in 9.77 days (mean, $SD = 2.39$) (Table 3).

Table 1. Study participant flow



The Kaplan-Meier survival curves for time from beginning of the cold sore to healing confirmed this difference. At day 10, 100% of the propolis patients had healed from the cold sore episode, while the healed placebo patients reached 100% at day 15 ($p < 0.00001$, logrank

test comparison between groups) (Figure 1).

• **Time to improvement (time to reach the crust stage, patient-assessed)**

The patients in the propolis ointment group appeared to reach the crust stage faster than

Table 2. Characteristics of the patient population by treatment group

| Variables | 3% propolis ointment ACF (n = 33) | Placebo ointment (n = 35) | p-value |
|--|--------------------------------------|---------------------------------|-----------------------|
| <i>Demographics:</i> | | | |
| • Men (n) | 1 | 13 | |
| • Women (n) | 32 | 22 | |
| • Age, years (mean, SD) | 46.57 (12.60) | 44.37 (11.36) | 0.450952 |
| <i>History of herpes labialis:</i> | | | |
| • Time with disease, years (mean, SD) | 27.30 (10.53) | 23.86 (12.82) | 0.231746 |
| • No. of episodes per year (mean) | 4.7 episodes | 4.6 episodes | 0.135822 ^a |
| • Usual duration of an episode | 7-9 days for 52% of patients | 7-9 days for 60% of patients | 0.597566 ^b |
| • Main triggers according to patients | Sunlight, stress, menses | Sunlight, stress, illness | |
| • Patients who experienced a prodrome always or most of the time | 90% (30 patients) | 82% (29 patients) | 0.609143 ^c |

^a $p = \chi^2$ analysis, 5x2 contingency table.

^b $p = \chi^3$ analysis, 3x2 contingency table.

^c p = comparison between proportions.

Table 3. Investigator's assessment of cold sore healing

| Time to achieve healing (days) | 3% propolis ointment ACF (n = 33) | Placebo ointment (n = 35) |
|--------------------------------|-----------------------------------|---------------------------|
| Mean (SD) | 6.242 (2.165) | 9.771 (2.399) |
| Median | 6 (2-10) | 10 (3-15) |

Difference between the two treatments: $p < 0.00001$ (Student's *t*-test for unpaired data), $p < 0.00001$ (Mann-Whitney U test).

the placebo group in the early stages from the beginning of treatment, but overall the difference between the two groups did not reach significance, as confirmed by the survival analysis of time to improvement (difference between the two groups, logrank test, $p=0.23016$).

• **Pain resolution (patient-assessed)**

The patients in the 3% propolis ointment group were without pain significantly faster than the patients applying the placebo

ointment. All of the patients in the propolis group experienced no pain already at day 7 from the beginning of the cold sore episode, while 22.9% of the placebo group patients was still reporting pain in their daily diary entries at the same day ($p=0.011$, χ^2 test).

This is evident also from the Kaplan-Meier survival curves of time to loss of pain for both groups, with a logrank test with $p=0.00671$ (Figure 2).

Figure 1. Kaplan-Meier distribution of patients healing by day, by the investigator and measured from time of initiation of treatment until healing of the cold sore. Difference between the two treatments (logrank test): $p < 0.00001$.

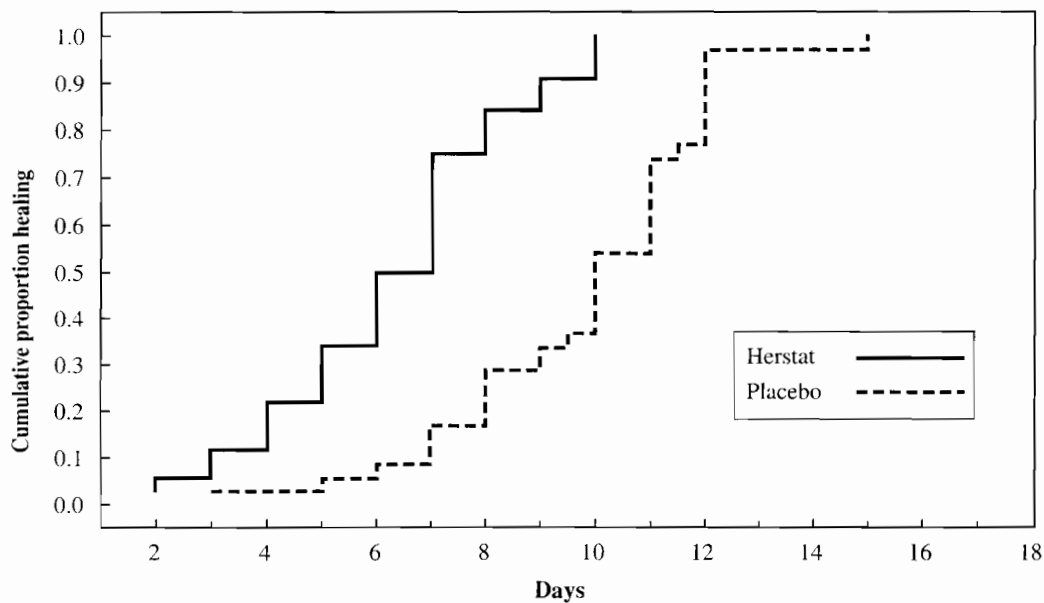
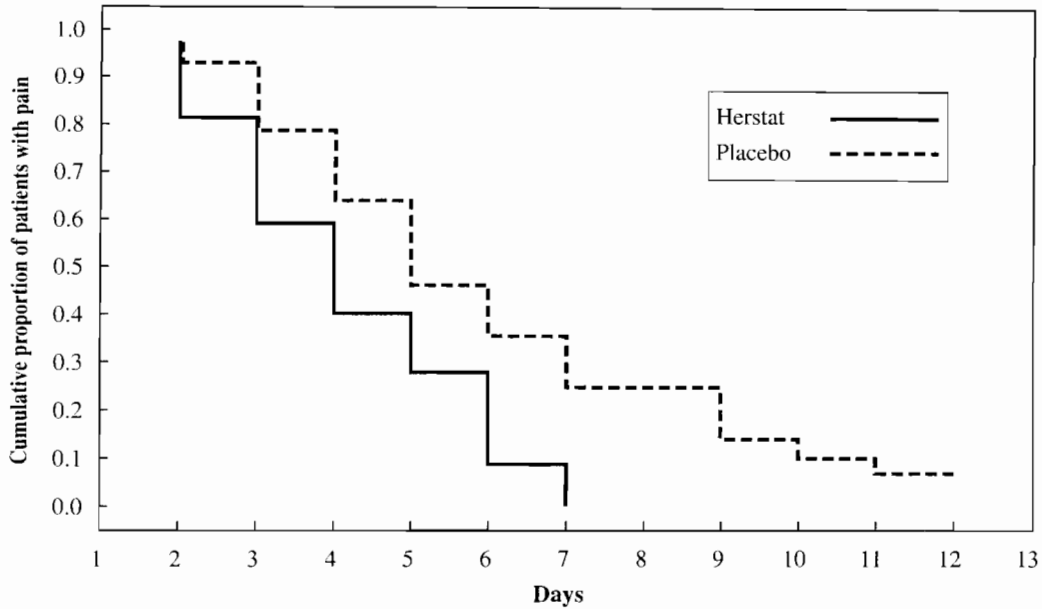


Figure 2. Kaplan-Meier survival curve for time from initiation of treatment to disappearance of pain (patient-assessed). Difference between the two treatments (logrank test): $p = 0.00671$.



• Other results and patients' opinion on study treatments

After analysing the cold sore diary entries, no significant differences by day between the two groups for the size of the cold sore lesion were found. Also the occurrence of prodromes and the occurrence of vesicles and ulcers/crusts by day in the study population did not show significant differences.

At the end of the treatment, patients in both groups were asked to answer some multiple choice questions about the study medication, under double-blind conditions. The answers to these questions reflected patients' opinions about their treatment, and appeared to be coherent with the main study results (Table 4).

Most of the patients in the propolis ointment group found the treatment 'very effective' and, adding the patients who found it

'somewhat effective', it is possible to affirm that all of the propolis-treated patients judged the study medication positively. Almost the reverse is true for the placebo treated patients, with 82.9% of them finding the treatment 'hardly effective' and 'ineffective'. Finally, 93.9% of the propolis treated patients stated that they would use the treatment again, compared to 5.7% of the placebo group patients. The treatment was well tolerated and no specific adverse events were reported in the study. No local reactions were reported. Two patients in the treatment group and one in the placebo group reported a mild headache.

Discussion

The results of this study show that, with the local application of propolis ointment ACF,

Table 4. Patients' answers to the multiple choice questionnaire about the study treatment

| | 3% propolis ointment ACF (n = 33) | Placebo ointment (n = 35) | p value |
|--|--------------------------------------|------------------------------|--------------------|
| <i>Question 1</i> | | | |
| <i>Treatment evaluation</i> | | | |
| • Very effective | 81.8% | 5.7% | 0.000 ^a |
| • Somewhat effective | 18.2% | 11.4% | 0.654 ^a |
| • Hardly effective | 0% | 60% | 0.000 ^a |
| • Ineffective | 0% | 22.9% | 0.011 ^a |
| <i>Question 2</i> | | | |
| <i>Treatment was useful to...</i> | | | |
| • ...accelerate healing | 90.9% | 8.6% | 0.000 ^b |
| • ...accelerate loss of crust | 84.8% | 20% | 0.000 ^b |
| • ...reduce intensity of pain | 100% | 34.3% | 0.000 ^b |
| • ...reduce duration of pain | 93.9% | 14.3% | 0.000 ^b |
| • ...limiting size of the cold sore | 93.9% | 11.4% | 0.000 ^b |
| <i>Question 3</i> | | | |
| <i>The patient would use the treatment again</i> | | | |
| • Yes | 93.9% | 5.7% | 0.000 ^b |

^a p (comparison between proportions)

^b p (χ^2 test)

the healing time of cold sores is shortened by about 3.5 days (mean) compared to the healing time obtained with placebo. The propolis ointment treatment also reduces the local pain, and 100% of propolis treated patients recorded no pain in their diaries at day 7 of treatment while in the placebo group, at the same day, about 23% of the patients were still with pain.

Therapeutically, there are no treatments able to eliminate the herpes simplex virus from the body, and all treatments target the recurrent episode. Apart from the use of systemic antiviral drugs in serious herpes infections or in immunocompromised patients, herpes recurrences are usually treated topically. There have been many clinical trials of topical antiviral drugs for cold sores but, until now, no drug has shown to be really effective at reducing healing time or intensity of pain. The clinical publications

regarding the efficacy of topical acyclovir in the treatment of herpes labialis are mostly inconclusive. The first trials with 5% acyclovir ointment showed no benefits on healing time⁴. Subsequent trials with acyclovir in a cream base produced slightly more positive results, with a modest reduction of healing time^{5,6}; other studies, however, showed no significant differences between treated and control groups^{7,8}. Penciclovir 1% cream has been approved to treat recurrent orolabial herpes simplex virus infections in immunocompetent patients. In a clinical trial involving more than 2,000 patients, penciclovir was shown to be more effective than placebo, with a difference in healing time of less than one day (0.7 days) and with a difference in pain reduction of 0.6 days, if applied within one hour of the beginning of the episode and then repeatedly every two hours⁹. In the treatment of cold sores, time of initiation of therapy appears to

be a critical factor for topical antiviral drugs. The efficacy of topical antivirals seems to be limited to reducing the prodromal stage, but with little effect on blister or ulcer stages and healing time. Antiviral agents like acyclovir or penciclovir must be applied from the very early phase and especially during this phase to be successful, because they are active against the viral replication. When the replication of the herpes simplex virus is effectively reduced, this may be useful in limiting the subsequent development of the cold sore. However, the prodromal phase is short and sometimes absent, easy to miss (it may begin at night time), and the antiviral agent must be applied very frequently.

Propolis ('bee glue') is the resinous substance collected by honeybees from various plant sources and used to seal and keep the beehive sterile. Honeybees collect propolis from the exudates of bud scales of plants and trees, and propolis is a complex mixture of more than 200 different substances. In recent times there has been a new interest in the pharmacological properties of propolis, thanks to modern techniques of analysis and separation that have identified some of the active constituents of propolis, mainly flavonoids and derivatives of caffeic acid^{10,11}.

The propolis extract in Herstat is a patented purified extract of propolis designated ACF (Antiviral Complex of Flavonoids), obtained from a specific region of Canada where species of poplar trees (*populus nigra*) are prolific.

Propolis is active against herpes simplex viruses type 1 and type 2¹²⁻¹⁴. The effects observed *in vitro* consist of a direct

inactivation of the viruses (virucidal activity) and also of the inhibition of virus multiplication inside infected cells (as observed through plaque reduction tests). The antibacterial activity is directed mainly against Gram positive bacteria, including *Staphylococcus aureus* and its methicillin-resistant strain^{15,16}. The anaesthetic activity produces a local effect¹⁷, and it has been related to the anti-inflammatory activity of flavonoids and derivatives of caffeic acid^{18,19}. The effects on tissue regeneration have been demonstrated *in vitro* and also *in vivo*, where the healing of experimental lesions treated with propolis extract was significantly accelerated compared to controls²⁰⁻²². This wide range of effects makes propolis application clinically useful in all phases of the cold sore lesion. The antiviral activity is useful in the early phase, when the pathology is due to the virus replicating inside the dermal and epidermal cells. The antibacterial, anti-inflammatory and regenerating activities of propolis help accelerate the healing of the cold sore in the later phase, when an inflammation process proportional to the quantity of virus produced is likely to impair the process of reepithelisation²³.

The results obtained in this study confirmed the findings of previous reports, where the topical application of propolis ointment ACF consistently reduced healing time of herpetic lesions, both orofacial and genital. Possible limitations of this study are the difference between the two groups for gender composition (97% of women in the propolis group, compared to 63% in the placebo group), and the consideration given to patients' judgement, by means of their diaries.

Is not possible to fully evaluate how much the gender disparity between the two groups might have influenced the final results. However, there is no difference in the healing time of cold sores between the women and men of the placebo group and, further, there are no data in the literature indicating a different reaction of women to local application of propolis. As for the diaries, patients with herpes recurrences are very aware of their condition and are careful observers of any treatment that might improve their condition. The patients' opinion, recorded at the end of the treatment, reflects and confirms the clinical results of the study. The totality of the propolis ointment treated patients found their treatment effective ('very effective', 'somewhat effective'), while a very high percentage of placebo patients judged their treatment ineffective. No treatment-related adverse effects were recorded in this study.

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