Efficacy and Tolerability Assessment of a Topical Formulation Containing Copper Sulfate and *Hypericum perforatum* on Patients With Herpes Skin Lesions: A Comparative, Randomized Controlled Trial

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ABSTRACT

Background: Topical Acyclovir has moderate efficacy on recurrent HSV symptoms, requiring repeat applications for several days. Topical Dynamiclear, which requires only a single dose application, may provide a more effective and convenient treatment option for symptomatic management of HSV.

Objectives: The study assessed the comparative efficacy and tolerability of a single use, topical formulation containing copper sulfate pentahydrate and *Hypericum perforatum* that is marketed as Dynamiclear™ to a topical 5% Acyclovir cream standard preparation and use.

Methods: A prospective, randomized, multi-centered, comparative, open-label clinical study was conducted. A total of 149 participants between 18 and 55 years of age with active HSV-1 and HSV-2 lesions were recruited for the 14-day clinical trial. Participants were randomized into two groups: A (n=61), those receiving the Dynamiclear formulation, and B (n=59), those receiving 5% Acyclovir. Efficacy parameters were assessed via physical examination at baseline (day 1), day 2, 3, 8, and 14. Laboratory safety tests were conducted at baseline and on day 14.

Results: Use of the Dynamiclear formulation was found to have no significant adverse effects and was well tolerated by participants. All hematological and biochemical markers were within normal range for the Dynamiclear group. Statistically, odds for being affected by burning and stinging sensation were 1.9 times greater in the Acyclovir group in comparison to the Dynamiclear group. Similarly, the odds of being affected by symptoms of acute pain, erythema and vesiculation were 1.8, 2.4, and 4.4 times higher in the Acyclovir group in comparison to the Dynamiclear group.

Conclusions: The Dynamiclear formulation was well tolerated, and efficacy was demonstrated in a number of measured parameters, which are helpful in the symptomatic management of HSV-1 and HSV-2 lesions in adult patients. Remarkably, the effects seen from this product came from a single application.

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INTRODUCTION

Herpes simplex virus (HSV) is one of the most common viral diseases in humans. HSV exists as two types, Herpes simplex virus 1 (HSV-1) and Herpes simplex virus 2 (HSV-2), both distinguished by clinical manifestations, biological and serological criteria.[1] Their characteristic feature is a capacity to establish latency in the host after the initial infection and to reactivate periodically. Major symptoms of active infection include prodromal numbness and tingling around the affected area, induration, erythema, itching, burning, pain in the area, and ulcerated lesions.[2,3] In the U.S., over 70–90 percent of adults have the antibody to HSV-1 and approximately 22 percent have the antibody to HSV-2. HSV is the fastest growing infectious disease in the world with 500,000 new cases reported each year. HSV active infections are also associated with enhanced Human Immunodeficiency Virus (HIV) transmission.[4-6]

Oral and topical Acyclovir preparations are used as the current standard of care for treatment of HSV symptoms, including skin lesions. Acyclovir inhibits the replication of herpes virus via inhibition of the viral DNA polymerase, thus preventing the formation of the DNA replication complex and the elongation

of the viral DNA chain. Treatment of recurrent and persistent infections with oral Acyclovir, and with other antivirals including valacyclovir, famciclovir, and penciclovir has resulted in clinical benefit, but also has resulted in the emergence of drug-resistant variants and viruses that are more resistant due to their relative deficiency of viral thymidane kinase or DNA polymerase—especially in immunocompromised individuals.[3,7,8] Although resistant strains of HSV do not currently pose a threat, in a number of decades it is likely that resistant strains will replace wild-type strains in treated individuals.[9]

The primary benefit of topical antiviral treatments thus far has been the reduction in healing time.[10] However, topical treatments do not mitigate the immune-mediated response of the host to the virus. In a study done by Shaw et al, little to no clinical benefit was seen when skin infections were treated with topical Acyclovir.[11] Topical Acyclovir also requires daily application, demands good compliance and is relatively expensive. For these reasons, a novel approach to anti-HSV therapy is desirable. Formulations that enhance natural immune modulation and that cause viral destruction and inactivation would be beneficial. Formulations such as Dynamiclear, which require a single dose application and which do not likely encourage drug-resistant variants or mutation of HSV, may hold promise for this intended effect.[7]

Natural product formulations may serve as effective antiviral and immune modulating agents.[9,12-15] The constituent complexity and diverse mode of action of a natural formulation does not result in the type of mutations seen with single element pharmaceutical formulations.[16] A number of natural products have been shown to be antiviral, to promote tissue repair and to decrease pain. Furthermore, many natural substances have been shown to support local innate immune activity.[16-18] For these reasons, natural products may hold promise in the management of and alteration of the natural progression of HSV recurrence.

Hypericum perforatum, commonly known as St. John's Wort, has been extensively investigated for its antiviral activities against enveloped viruses like HSV and HIV. The bioactive constituent, hypericin, displays multiple modes of actions, including inhibition of new virion budding, prevention of viral uncoating, and inhibition of protein kinase activity required for replication of a number of viruses.[19] *Hypericum perforatum* has additionally been investigated with regard to its ability to relieve neuropathic pain. Antihyperalgesic activity is also associated with the major bioactive constituent hypericin. In studies, hypericin inhibits protein kinase C gamma and epsilon, which are proteins associated with the development of neuropathic pain.[20]

Naturally occurring minerals such as zinc and copper also demonstrate antiviral activity. Research has been conducted on the copper-mediated inactivation of HSV. Copper ions have been shown to inactivate several types of viruses, including members of the Herpesvirus and Arenavirus families, various bacteriophages treated in vitro, and free as well as intracellular HIV.[7,21] Mechanisms of action studies suggest that copper-mediated damage occurs via binding with DNA causing DNA strand breaks. HSV has also been shown to exhibit sensitivity to low concentrations of copper. It is hypothesized that a potential advantage of copper-mediated viral killing resides in broad molecular damage, in that the host cell has the ability to repair much faster for itself than the virus. In addition, rather than suppressing viral replication, copper ions render the viral DNA nonviable for further replication. Interestingly, studies have been done with copper bound to Acyclovir. This complex reportedly exhibited a significant anti-HSV effect.[22] In an in vitro study conducted by Shishkov, et al., a copper complex was found to inhibit the infectivity of free virions.[21] The maximum safe level for topical anhydrous copper sulfate use is 5%, which equates to approximately 2% of copper ions.[23] Skin exposure to copper can cause allergic reactions in some individuals.

The Dynamiclear formulation used in this study contains copper sulfate pentahydrate (5.0%) and *Hypericum perforatum* (0.10%). Unlike the majority of topical treatments marketed for symptomatic relief of HSV lesions, this formulation is recommended as a single application. It has been clinically tested for safety, efficacy, and tolerability by an independent CRO (Apothecaries Ltd, India) using Good Clinical Practice in accordance with the principles that originate in the Declaration of Helsinki. Dynamiclear has been listed on the Australian Register of Therapeutic Goods as a Complementary Medicine since July 2008.

The aim of this clinical study was to investigate the efficacy of a single topical application of the Dynamiclear formulation compared to topical Acyclovir, and to determine Dynamiclear's tolerability in adult patients with recurrent skin, oral, and genital HSV lesions. The trial was a prospective, randomized, multicentered, comparative, open-label clinical study in patients aged between 18 and 55 years old. No blinded comparator product, placebo or active formulation was suitable for the study. Blinding was considered not feasible without placebo, as the formulation and dosage forms of the Dynamiclear formulation and Acyclovir were completely different.

MATERIAL AND METHODS

Population

Men and women between 18 and 55 years of age with active HSV-1 and HSV-2 skin or mucocutaneous lesions were enrolled at eleven centers in India. Prospective study patients were screened by medical history, physical examination, and blood examinations. All patients signed an informed consent form approved by an Indian Ethics Committee (IEC). IEC approval number and date were: SC/111/06version07 31May06. The study was carried out in compliance with the ICH GCP: "Guidelines for Clinical Trials on Pharmaceutical Products in India GCP

Guidelines," which was issued by Central Drugs Standard Control Organization, Ministry of Health, Government of India. The study design and conduct also complied with the ethical principals originated from the Declaration of Helsinki. All changes to the protocol were also approved by the IEC. Enrollment began in June 2006 and lasted through December 2006.

Study Procedures

At the initial visit, patients to be included in the trial gave their written informed consent to participate in the study. Predefined clinically significant limits of change were used to determine laboratory safety parameters. Patients having values outside of these predetermined values were excluded or removed from the study. Participants that qualified for the study were randomly assigned to either Group A: the Dynamiclear formulation containing 5.0% copper sulfate pentahydrate and 0.10% *Hypericum perforatum* in a base of glycerin and water (Global Herbal Supplies Pty Ltd, Australia) or Group B: 5.0% Acyclovir (Zovirax; Glaxo Smithkline, India). The Dynamiclear formulation came from a single batch, while Acyclovir came from one of seven batches. Patients received their Acyclovir on an outpatient basis and were instructed to apply the topical cream five times daily for a period of seven days. Participants using the Dynamiclear formulation received a single topical application at the respective clinic on day 1 of the study. No subsequent application was made for the duration of the study. Subsequent visits occurring on days 2, 3, and 8 included patient diary assessment, physical exam, clinical efficacy evaluation, local tolerability and adverse event assessment. The last visit of the study (day 14) included repeat laboratory tests.

Patients were excluded from the trial if they met the pre-determined exclusion criteria that included a history of hypersensitivity or allergic reaction to any dosage form of study drugs, lesions inside of the oral mucosa, pregnant women and immunodeficient persons.

No concomitant antiviral treatment was permitted. Concomitant medications that were used were recorded by the investigator. None of the patients used steroids or home remedies in the study.

Laboratory

Routine safety laboratory tests that included complete blood cell count with differentials, erythrocyte sedimentation rate, liver enzymes and urinalysis, were conducted by various qualified laboratories in and around the participating clinical sites. Biochemical parameters were evaluated to assess for adverse events and were not used for efficacy measurements.

Efficacy Analysis

The primary efficacy assessments were based on the endpoints measured at predetermined times. Primary endpoints included: appearance of crusting or healed rash of herpes; disappearance of pain (evaluated based on a pain scale: 0=no pain or discomfort, 1=pain can be easily ignored, 2=pain does not interfere with daily activities, 3=pain interferes with concentration or sleep, 4=pain interferes with all but basic needs, 5=pain requires rest or bed rest); and local cutaneous assessments such as erythema, induration, itching, vesiculation and stinging sensation. Treatment began immediately after the participants had been consented into the study. These end points were measured at baseline (day 1) prior to application of preparations, and at day 2 (natural product Dynamiclear group only), day 3, day 8 and day 14. These parameters were either objectively determined by study clinicians or subjectively reported (e.g., presence of pain) by the patient during an interview with the study investigator.

Statistical Analysis

The sample size to be used in the study was calculated based on the postulation that the treatment effect would be 30 percent with a likely 80 percent cure rate in the Dynamiclear group and 50 percent in the comparator group. It was determined that a total of 116 participants were required to reach statistical significance at 80 percent power and $P \leq 0.05$.

Overall efficacy was analyzed using proportion of change over a period of 14 days calculated using the Chi Square test and difference in the proportion of change between the groups (95% CI). To account for the correlated dichotomous response, the generalized estimating equation was used to analyze efficacy parameters. Analysis of covariance (ANCOVA) could not be used with the generalized estimating equation (GEE). The GEE was used to compare the total development of risk between the test preparation and the standard drug over a period of 14 days.

Laboratory parameters were compared between Acyclovir and the Dynamiclear formulation at screening and at the end of the study using *t*-test for independent samples/Wilcox rank sum test based on the nature of the distribution of these parameters. The percentage change was compared between the groups over a period of time using Wilcoxon rank sum test.

RESULTS

Patient Characteristics

From June 2006 to December 2006, 149 patients (104 men and 45 women) entered the study at one of nine of a total of eleven approved sites. Based on serological testing 86 percent of adults presenting had secondary infections and 11 percent were primary infections. Group A (Dynamiclear) final participants n=61 (14 participants were excluded or dropped during the study) and Group B (Acyclovir) final participants n=59 (15 participants were excluded or dropped during the study). The majority of participants fell between the ages of 18 and 34, with the majority being male (70.3%). No statistically significant difference between Group A and Group B occurred in baseline characteristics of age, weight, height and gender. The clinical and laboratory safety pa-

rameters of temperature, blood pressure and pulse rate at all time points remained similar between the two groups. All points remained within normal range.

Reported Herpes Locations

Of the patients recruited for the study, 81 had orofacial lesions, 46 had genital lesions, five had lesions on the chest or abdomen, three had neck lesions and one had a lesion occurring on a limb.

Efficacy

The efficacy evaluation was analyzed using both Intention To Treat (ITT) and Per Protocol (PP) methods. Per protocol data are presented below.

Efficacy Evaluation Based on Per Protocol Analysis

The difference in the percentage of scabbing and crusting in the Acyclovir group (47.4%) versus the Dynamiclear group (42.6%) was not statistically significantly different (*P*=0.595) at day 1. At day 3, the percentage of scabbing and crusting in those using Acyclovir (54.2%) was significantly lower (*P*=0.005) as compared to Dynamiclear, whereas at day 8 the percentage for the Acyclovir group (47.4%) was higher than the Dynamiclear group (35.0%), which was not statistically significant (*P*=0.167). At day 14, the percentage of scabbing and crusting in the Dynamiclear group was 18.0%, and in the Acyclovir group was 18.6%, which was not statistically significant (*P*=0.931).

At day 1, the difference in the percentage of patients suffering with pain was not statistically significant (*P*=0.426) between the groups. The percentage of patients suffering from pain was higher during the study period in the Acyclovir group compared to the Dynamiclear group. Whereas at day 8 the percentage of patients suffering from pain using Dynamiclear (37.7%) was significantly lower (*P*=0.018) compared to those using Acyclovir (59.3%) (Table 1).

TABLE 1.

Mean Pain Scores

Acute Pain	Mean (SE)		Difference in Mean (95% CI) (*P*-value)
	Natural Product (n=61)	Acyclovir (n=59)	
Overall	1.91 (0.08)	2.02 (0.08)	0.11 (-0.05, 0.26)
At day 0	1.87 (0.08)	1.46 (0.08)	
At day 14	0.21 (0.09)	0.31 (0.08)	
Mean Diff.(95% C.I.)	1.36 (1.14, 1.58)	1.15 (0.95, 1.35)	
(*P*-value)	<0.001	<0.001	

[a]The Mean Standard Error Pain Score between Dynamiclear and Acyclovir was 1.91 (0.08) and 2.02 (0.08) respectively, which is not statistically significant (0.11(-0.05, 0.26)). However, the difference in the mean pain score between day 0 and day 14 within each group was found to be statistically significant.

At day 1, the percentage of patients suffering with erythema was not statistically significantly different (*P*=0.730) between the groups. From day 3 onwards, the percentage of patients suffering from erythema was higher in the Acyclovir group compared to the Dynamiclear group, which was statistically significant on day 8 (*P*=0.009) and day 14 *(P*=0.015).

The percentage of patients suffering from itching on day 1 was higher for the Acyclovir group (81.4%) compared to the Dynamiclear group (78.7%), but was not statistically significantly different (*P*=0.715). Throughout the study period the percentage of patients suffering from itching was higher in the Acyclovir group compared to those using Dynamiclear, which was statistically significant on day 8 (*P*=0.044).

The percentage of patients suffering with induration was higher in the Dynamiclear group on day 3 to day 8 as compared to Acyclovir and was not statistically significant (*P*=0.479, *P*=0.386). However, at day 14, there were a higher percentage of patients suffering from induration in the Acyclovir group in comparison to the Dynamiclear group, although the difference was not statistically significant (*P*=0.184).

At day 1, the percentage of patients suffering with vesiculation was not statistically significantly different (*P*=0.275) between the groups. The percentage of patients suffering from vesiculation on day 3, day 8 and day 14 in the Dynamiclear group was 36.1 percent, 9.8 percent, 3.3 percent, and in the Acyclovir group was 76.3 percent, 40.7 percent, and 15.3 percent, respectively, which was statistically significantly different for day 3 and day 8 (*P*=0.001, *P*=0.001) (Tables 2 and 3).

Tolerability

Overall, topical application of Dynamiclear was well tolerated. There was no statistically significant difference among the treatment groups in the frequencies of adverse events. One patient in the investigational product treatment group reported a possible drug related non-serious laboratory adverse event. No patients in the investigational product group necessitated discontinuation from the study.

DISCUSSION

The primary objective of this study was to report the comparative proportion of efficacy in patients treated with Dynamiclear and topical Acyclovir. Both ITT and PP analysis of scabbing and crusting were not statistically significant between the groups. However, between the Dynamiclear and Acyclovir treatments, the changes observed in acute pain, erythema, induration and vesiculation were significantly greater in the Dynamiclear group in the direction of disappearance. In all the efficacy parameters except for induration in PP analysis, the Dynamiclear group showed a higher rate of healing in comparison to the Acyclovir group. The proportion of change of efficacy param-

TABLE 2.

Change in Efficacy Parameters[a]

Efficacy Parameter	Proportion of Change (SE)		Difference in Proportion of Change (95% CI)	OR (95% CI)
	Acyclovir (n=59)	Natural Product (n=61)		
Scabbing & Crusting	0.37 (0.06)	0.27 (0.06)	0.10 (-0.06, 0.26)	0.9 (0.6, 1.5)
Itching	0.26 (0.05)	0.23 (0.05)	0.03 (-0.12, 0.18)	1.7 (0.9, 3.2)
Burning & Stinging	0.27 (0.06)	0.26 (0.06)	0.01 (0.14, 0.17)	2.1 (1.2, 3.8)*
Acute Pain	0.28 (0.06)	0.23 (0.05)	0.05 (-0.11, 0.20)	2.1 (1.2, 3.8)*
Erythema	0.28 (0.06)	0.23 (0.05)	0.05 (-0.10, 0.20)	2.3 (1.2, 4.4)*
Induration	0.30 (0.06)	0.20 (0.05)	0.10 (-0.05, 0.25)	0.9 (0.5, 1.6)
Vesiculation	0.32 (0.06)	0.26 (0.06)	0.06 (-0.10, 0.21)	5.4 (2.8, 10.6)*

[a]The difference in the proportion of change was higher in the Dynamclear group in all the efficacy parameters as compared to the Acyclovir group, but was not statistically significant. The odds for being affected by burning and stinging sensations is 2.1 times more in the Acyclovir group in comparison to the Dynamiclear group. Similarly, the odds of being affected by symptoms of acute pain, erythema and vesiculation are 2.1, 2.3, and 5.4 times higher in the Acyclovir group in comparison to the Dynamiclear group.
*$P<0.05$

TABLE 3.

Efficacy Parameters of Dynamiclear Over Time[a]

Efficacy Parameter	Time (Days) No. (%)					Proportion of change (95% CI)
	1	2	3	8	14	
Scabbing & Crusting						
No	35 (57.4)	17 (27.9)	13 (21.3)	39 (65.0)	50 (82.0)	0.22
Yes	26 (42.6)	44 (72.1)	48 (78.7)	21 (35.0)	11 (18.0)	(0.12, 0.32)
Itching						
No	13 (21.3)	12 (19.7)	22 (36.1)	42 (68.9)	56 (91.8)	0.17
Yes	48 (78.7)	49 (80.3)	39 (63.9)	19 (31.2)	5 (8.2)	(0.08, 0.27)
Burning & Stinging						
No	13 (21.3)	14 (22.9)	23 (37.7)	42 (68.8)	53 (86.9)	0.17
Yes	48 (78.7)	47 (77.1)	38 (62.3)	19 (31.2)	8 (13.1)	(0.08, 0.27)
Acute Pain						
No	4 (6.6)	8 (13.1)	13 (21.3)	38 (62.3)	48 (78.7)	0.18
Yes	57 (93.4)	53 (86.9)	48 (78.7)	23 (37.7)	13 (21.3)	(0.08, 0.27)
Erythema						
No	7 (11.5)	10 (16.4)	21 (34.4)	46 (75.4)	57(93.4)	0.18
Yes	54 (88.5)	51 (83.6)	40 (65.6)	15 (24.6)	4 (6.6)	(0.08, 0.27)
Induration						
No	22 (36.1)	18 (29.5)	21 (34.4)	41 (67.2)	54 (88.5)	0.18
Yes	39 (63.9)	43 (70.5)	40 (65.6)	20 (32.8)	7 (11.5)	(0.08, 0.27)
Vesiculation						
No	3 (4.9)	22 (36.1)	39 (63.9)	55 (90.2)	59 (96.7)	0.19
Yes	58 (95.1)	39 (63.9)	22 (36.1)	6 (9.8)	2 (3.3)	(0.09, 0.29)

[a]The effect of Dynamiclear on scabbing and crusting was revealed on day 2 (72.1) and day 3 (78.7), and a 22% (12%, 32%) change was observed during the study period that was statistically significant. There was significant change ($P<0.05$) in the direction of cure of itching, burning and stinging, acute pain erythema, induration and vesiculation in those patients who received a single application of Dynamiclear.

eters over time showed a faster resolution of all symptoms in the Dynamiclear group, and there was a higher risk of non-resolution of symptoms of burning and stinging, acute pain, erythema, and vesiculation in the Acyclovir group.

> In all the efficacy parameters except for induration in PP analysis, the Dynamiclear group showed a higher rate of healing in comparison to the Acyclovir group.

The secondary objective was to report the proportion of patients receiving the Dynamiclear formulation that developed one or more clinical or laboratory serious drug-related adverse events. In this study, no patient experienced any serious adverse events. One patient in the Dynamiclear treatment group reported a possible drug-related non-serious laboratory adverse event. There were no participants in the Dynamiclear treatment group that necessitated discontinuation from the study.

There is a paucity of HSV clinical trials conducted using naturally occurring substances. One small clinical trial conducted with a topical formulation containing lignin and ascorbic acid showed evidence of efficacy.[24] However, this study differs in content, as it does not contain cooper sulfate or other naturally occurring metals and the above-mentioned product also required multiple daily applications onto affected sites. This current investigation serves as an initial translational study serving to elucidate in vitro antiviral effects of active copper ions. In vitro investigations done with active copper ions showed inactivation and destruction of the herpes virus, suggesting a mechanism for the quick recession of symptoms seen in our investigations. Acyclovir acts to suppress the virus and viral symptoms, generally over a longer period of time. It is likely that *Hypericum perforatum*'s natural analgesic effects played a role in the relatively rapid pain amelioration that was observed with the Dynamiclear formulation used in this study.

CONCLUSIONS

Novel treatment options for HSV must be explored due to increasing rates of infection and of drug-resistant HSV strains. This clinical investigation demonstrates that Dynamiclear may serve as a viable treatment option and may warrant further exploration. The single dose application is advantageous and cost-effective, thus providing a remedy to issues of compliance and affordability. Future studies that record time of onset, reccurrence rate, and selection of primary or secondary infections may provide additional and/or stronger evidence of efficacy. Blinding the study may also help to demonstrate comparative efficacy to clinicians.

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