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Protocol for a randomised, double-blind, placebo-controlled trial of topical herbal and mineral formulation (DynamiclearTM) for treatment of herpes simplex labialis in the community setting.

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TITLE PAGE

ARTICLE TITLE: Protocol for a randomised, double-blind, placebo-controlled trial of topical herbal and mineral formulation (Dynamiclear™) for treatment of herpes simplex labialis in the community setting.

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ABSTRACT

Introduction Herpes Simplex Labialis (HSL) is a common infection that can cause painful lesions on the oral mucosa, commonly referred to as cold sores. Current biomedical treatments include topical aciclovir, which reduces the episode duration by an average of 0.5 days. This study will examine the efficacy and tolerability of an over-the-counter topical treatment, Dynamiclear™ in reducing duration and severity of HSL episodes.

Methods and analysis This prospective, randomised, double-blind, placebo-controlled, multi-centre trial will recruit a minimum of 292 adult participants across Australia and New Zealand who present with a cold sore within 48 hours of onset. They will be randomly allocated in a 2:1 ratio to receive either topical Dynamiclear™ (active) or placebo. Dynamiclear's active ingredients are *Hypericum perforatum*, *Calendula Officinalis* and copper sulfate. A single topical treatment of active or placebo will be applied by a pharmacy-based investigator, and participants will be provided with a viral swab kit to confirm presence of herpes virus 1 or 2 from ulcerated lesions. Participants will receive reminders by email and/or SMS to complete an online daily diary assessing their cold sore lesion using a visual guide, and recording other symptoms on numeric scales until healed. The primary outcome variable is median duration of HSL episode (in days) from presentation to return to normal skin. Secondary outcomes include severity of lesion pain, itching, burning and tingling during the symptomatic phase and proportion of lesions progressing to ulceration.

Ethics and dissemination Australian ethics approval from Western Sydney University Human Research Ethics Committee, ref: H12776. New Zealand Ethics approval from The Health and Disability Ethics Committees (HDEC) ref: 18/CEN/151. Results will be published in a peer-reviewed academic journal, presented at academic meetings and reported to participants

Trial registration number Australia and New Zealand Clinical Trials Registry (ACTRN): ACTRN12618000890235. **Universal Trial Number (UTN)** U1111-1233-2426

Protocol version V8 (16th May 2019)

ARTICLE SUMMARY

Strengths and limitations of this study

- Novel pharmacy research network allows for effective recruitment of HSL sufferers that most often access treatment in the community.
- Single-dose intervention eliminates treatment compliance issues.
- Digital data collection minimises study burden for investigators and participants and ensures real time, accurate data collection.
- Self-report measures for lesion progression verified by second pharmacy visit.

INTRODUCTION

Herpes simplex labialis (HSL)

Herpes simplex labialis (HSL) is a common recurring infection of the labial or perioral skin caused by herpes simplex virus (HSV), also known as cold sores. The majority of cases are caused by HSV-1. Seroprevalence studies indicate that 60% to 90% of adults are infected with HSV-1 and that HSV is the fastest growing infectious disease in the world with 500,000 new cases reported each year[1]. Approximately 20% to 40% of the population experiences recurrent outbreaks of HSL. The frequency of outbreaks ranges from rare episodes every 5 to 10 years, to monthly or more frequent outbreaks[2].

Symptoms of active infection include prodromal numbness and tingling around the affected area, before progression to erythema, itching, burning, pain in the area, and ulcerated lesions. Disease episodes are generally mild and self-limiting[1]; however, the symptoms are uncomfortable and often more importantly aesthetically unpleasant and noticeable to the general public.

Primary herpes simplex infection is most often asymptomatic, however can manifest significant morbidity including general anorexia and malaise, fever, local lymphadenopathy, gingivostomatitis and significant ulcerative lesions; these symptoms may persist for up to three weeks[3]. Following the initial infection, the virus establishes a chronic, latent and life-long infection in sensory nerve ganglia, predominantly the trigeminal ganglion[4]. At a later date, the virus may be reactivated and travel back to the oral mucosa, perioral skin and/or labial surfaces, where it replicates, producing a clinical episode of recurrent herpes labialis. Viral reactivation usually occurs due to stress on the immune system. Triggers include exposure to ultraviolet (UV) light exposure[5], and viral infections such as colds and flu[6]. Recurrent episodes are shorter than the initial episode, with the virus often cleared within three days or less, due to the previously acquired immune response[1]. This immune response contributes to rapid control of recurrent HSL disease episodes but may also be responsible for clinical signs and symptoms such as pain, redness and swelling that can persist for up to a week or longer, even after the virus can no longer be isolated[2].

Current standard treatments for HSL

Oral and topical aciclovir preparations are used as the current standard of care for treatment of HSV. Oral antiviral medications such as aciclovir, valaciclovir, famciclovir and penciclovir limit replication of the herpes simplex virus (HSV) by inhibiting viral DNA polymerase, and shortening the time to healing by approximately ten percent, but have no direct impact on host immune response or associated inflammatory cascade[1].

Topical treatment with aciclovir and other antivirals have also shown clinical benefit but do not mitigate the immune-mediated response of the host to the virus. Topical aciclovir demands good compliance, requiring daily application.

Composition of Dynamiclear™

Dynamiclear™ (Sci-chem International) contains three active ingredients traditionally used to help treat inflamed or infected skin: Hypericum perforatum extract, Calendula officinalis extract and copper sulfate pentahydrate.

Hypericum perforatum, or St. John's Wort, has demonstrated antiviral activities against enveloped viruses such as HSV and human immunodeficiency virus (HIV). The active constituent, hypericin, reduces viral replication, through inhibition of new virion budding, prevention of viral uncoating, and inhibition of protein kinase activity[7]**.** Hypericin has also been found to inhibit protein kinase C gamma and epsilon, which are proteins associated with the development of neuropathic pain[7]. For treatment of HSL skin lesions, *Hypericum perforatum* is likely to be effective for topical application only as ingestion can cause photosensitivity, which may promote HSL reactivation[8].

Calendula Officinalis has been traditionally used for minor skin infections and inflammation[9]. Recent studies have found calendula to be effective in reducing the time to healing in previously non-healing venous leg ulcers[10]. This is likely to occur through the upregulation of genes controlling connective tissue growth factor (CTGF) and α-smooth muscle actin (α-SMA)[11]and the proliferation and migration of fibroblasts[12]. *Calendula Officinalis* may therefore reduce the time to wound healing in HSL lesions that progress to an ulcerative phase.

Copper sulfate is a naturally occurring mineral which has demonstrable antiviral activity. Rather than suppressing viral replication, copper ions render the viral DNA nonviable for further replication. HSV has been shown to exhibit sensitivity to low concentrations of copper, and in vitro research has shown evidence for copper-mediated inactivation of HSV[13].

A non-blinded, active comparator randomised controlled trial[14] of a previous version of Dynamiclear (without Calendula Officinalis) demonstrated lower odds of having pain, redness or progressing to the vesicle stage compared with topical 5% aciclovir in the treatment of orolabial and genital herpes simplex episodes. However, interpretation of these results is limited due to the inclusion of multiple sub-types of HSV, lack of blinding, and use of non-standard outcome measures.

Aim

To assess the efficacy of a single topical application of Dynamiclear™ compared to placebo in the treatment of HSL.

Hypothesis

A single topical application of Dynamiclear™ has greater efficacy than placebo on the severity and duration of HSL

METHODS AND ANALYSIS

Study design

A prospective, randomised controlled, double-blind, multi-site trial to evaluate the efficacy of a single dose of topically applied Dynamiclear™ vs placebo on the duration and severity of HSL.

Sample size calculation

The sample size needed to demonstrate statistically and clinically significant efficacy was determined using the hypothesis that the mean episode duration in the treatment group will be 5.0 days, compared to 6.0 days in the placebo group. This is based on the review of previous studies of aciclovir vs. placebo by Harmenberg et al[1] and a clinically significant difference of one day in episode duration. Given an alpha of 0.05, power of 80% and an allocation ratio of 2:1 for treatment to placebo, 149 participants are required in the treatment group and 75 in the placebo group (224 in total). A recent study of kanuka honey vs aciclovir to treat cold sores, using the New Zealand PRN for recruitment, reported an 11% drop out rate. However, because our study is placebo-controlled, we anticipate a higher dropout rate. Allowing for a 30% drop out rate, 292 participants are required: 194 in the treatment group and 98 in the control group. This sample size will also power the study to detect a moderate effect size on the secondary outcome measures (Cohen's d = 0.4).

Participants

A total of 292 participants aged 18 to 65 presenting to participating pharmacies within 48 hours of onset of prodromal symptoms of HSL and who report at least three prior episodes of HSL will be recruited. Inclusion and exclusion criteria are presented in Box 1.

A Pharmacy based Research Network (PRN) in both New Zealand and Sydney, Australia will be used to register sites for primary recruitment, screening and assessment.

Study centres: 10-15 pharmacies in Sydney, Australia, and 20-25 pharmacies in New Zealand.

Patient and Public Involvement (PPI)

There were no funds or time allocated for PPI so we were unable to involve patients in the study design. We will invite patients at the conclusion of the study to provide feedback on the burden of the intervention, and patients will be involved in developing the dissemination strategies for our findings.

Recruitment and selection

Four parallel recruitment pathways are planned in this study. First, social media will be used to advise participants of the trial, and refer them to a webpage which outlines the inclusion and exclusion criteria and encourage them to present at participating pharmacies within 48 hours of the onset of a cold sore. Second, the New Zealand arm of the PRN has recently completed a different HSL study[15]. The 920 participants who joined that study and consented to being contacted for further studies will be sent an email informing them of this study. Third, all participating PRN pharmacies will have promotional material in their pharmacies and individuals presenting to these pharmacies with a cold sore recurrence within the past 48 hours will be invited to enrol in the study. Fourth, TrialFacts, a paid recruitment service, will be used to advertise and screen for potential participants. Recruitment will begin in June 2019.

Regardless of recruitment pathway, upon presentation at a participating pharmacy, potential participants will be screened and those eligible will be consented by pharmacists and pharmacy technicians. Recruitment staff will ensure that each participant is fully informed about the nature and objectives of the study and possible risks associated with participation.

Box 1: Inclusion and exclusion criteria

Inclusion criteria:

- Age 18-65 years
- Onset of prodromal or clinical symptoms of HSL in the past 48 hours
- Previous clinical history of HSL with at least 3 prior episodes of HSL
- Primary lesion within 1cm of the lip
- Willing to provide informed consent and adhere to study protocol
- Has internet (either by mobile or computer) to complete online forms.

Exclusion criteria:

- History of immunodeficiency, immunosuppression or autoimmune disorder
- Current infection not related to the study condition – e.g. infections such as cold or flu
- Use of other antiviral, anti-inflammatory or steroid medications during or within two weeks prior to the treatment period
- Use of other topical agents (including cosmetics, lip balms, sunscreens) or cosmetic procedures (such as chemical peels or microdermabrasion) on the prodromal or lesion area during the treatment period
- Mechanical disruption (e.g. scrubbing lancing or shaving) of the prodromal or lesion area during the treatment period
- Pregnancy, lactation or planning to become pregnant in the following 14 days
- Participation in another clinical trial within the previous 30 days.

Randomisation

After providing informed consent and satisfying the eligibility criteria, participants will be randomised in a 2:1 ratio to receive either active treatment with Dynamiclear™ (n=194) or the placebo control group (n=98) using the Castor EDC platform[16]. Randomisation is performed using the Castor EDC platform by a researcher independent of the study team. To maintain pharmacist blinding when using a 2:1 ratio, boxes containing the intervention are pre-randomised, and pharmacists are provided with a box code rather than a group allocation. A 2:1 ratio in favour of the active intervention was chosen to maximise recruitment rates as potential participants are actively seeking treatment when attending the pharmacy. Thus, a greater chance of being allocated active treatment is likely to increase recruitment and retention rates. Permuted block randomisation, with a block size of 6 or 9 will be undertaken for each pharmacy to ensure that no patterns or imbalances emerge in group allocation between sites.

Blinding

All pharmacy staff, study team, participants and data analysists are blind to the group allocation. Unblinding will be permitted for either Dr Ee or Dr Semprini, or any other medical monitor, if serious adverse events are reported.

Study interventions

Interventional treatment is applied once only during the first pharmacy visit. Each participant will be allocated one vial of Dynamiclear™ or a placebo that matches Dynamiclear in colour, viscosity, taste and smell. The active and placebo vials are presented in identical packaging. The intervention or placebo will be applied via a cotton-tipped applicator which delivers 0.7mL to the affected area. This will be done either by the trained investigator or by the participant in view of the investigator, and the vial and packaging disposed of immediately. The moistened applicator will be applied for a minimum of 30 seconds, directly on the HSL lesion. No investigational product or packaging will leave the pharmacy site for any reason. Box 2 outlines the composition of both the interventional product and placebo.

Box 2a. Composition of interventional product (Dynamiclear™)

Active ingredients:

- Calendula Officinalis 1:2 liquid extract, 0.05 %w/w
- Hypericum Perforatum 1:2 liquid extract, 0.05 %w/w
- Copper sulphate pentahydrate, 6.4 %w/w

Excipient ingredients:

- Aloe vera
- Glycerol
- Vitamin E (Tocoheryl Acetate)
- Hydroxyethyl cellulose
- Polysorbate 80
- Purified water

Box 2b. Composition of placebo

Masking ingredients:

- Blue dye
- Yellow dye

Excipient ingredients:

- Aloe barbedensis
- Glycerin
- Vitamin E (Tocoheryl Acetate)
- Hydroxyethyl cellulose
- Polysorbate 80
- Purified water
- Sodium benzoate
- Citric acid anydrous (pH adjustor)

Data capture and monitoring

All data will be captured electronically via the secure electronic data capture platform CastorEDC [16]. Each pharmacy will be provided with an iPad (Apple Inc.) which will be used to collect all data during each face to face clinical visit. Signed consent forms will be digitally captured and uploaded directly onto the EDC platform.

Each participant will attend two clinical visits at PRN pharmacies. The first visit will be within 48 hours of symptom onset and will include initial clinical assessment and a single application of either the intervention or placebo in the presence of trained pharmacy staff. A second visit will be triggered when the participant indicates via their online symptom diary that they have returned to normal skin (Stage 7). This visit will involve confirmation of healing by pharmacists and checking for adverse events.

Participants will report symptoms and healing progression in an online daily diary (hosted on Castor EDC) which researchers will monitor for reports of adverse side effects. These diaries will also be used to monitor patient adherence to study requirements.

Participants will receive a follow-up phone call by a member of the research team two weeks after their final pharmacy visit to monitor any adverse events post treatment.

All data will be securely held on the study team database, accessible only by authorised investigators for the purposes of monitoring. The final dataset will be accessible by the coordinating investigators and each locality will retain on-site access to digital copies of source documentations with paper copies stored in secure archives.

Any changes to the study protocol are provided to the HREC and HDEC as per protocol, trial registries will be updated, and updated copies provided to trial sites. A copy of the consent form is included as Supplementary File 1.

Clinical assessments and patient surveys will be completed using Castor clinical trials management software. All the pre-specified outcome measures are outlined in Box 3.

Box 3: Outcome measures

Primary outcome measure:

- Median episode duration in days (participant evaluated)

Secondary outcome measures:

- Median episode duration in days (investigator-evaluated)
- Proportion (%) of lesions progressing to ulceration
- Median duration from wound development (stage 4) to healing (stage 7) in days (participant evaluated)
- Visual Analogue Scale (VAS) ratings of pain, tenderness, tingling and itching during the symptomatic phase
- Proportion (%) of cases of HSV confirmed via swabs
- Adverse event rate
- Patient satisfaction and acceptability

Participant evaluated lesion stage

Participants will be provided with photo images as well as descriptions for each lesion stage via an online web form. We have used this lesion staging system in our previous trial[17]. Participants will be asked to grade the lesion once per day, upon waking, until return to normal skin occurs (Stage 7) or until 14 days from the first pharmacy visit. A reminder SMS and/or email will be sent each morning with an embedded link to the web form. This grading will be submitted via an online web form. When a participant grades their lesion having resolved and the area has returned to normal skin (Stage 7) via the online form they will be contacted to attend for assessment to their nearest study site within 24 hours by phone and email by the research assistant.

Investigator-evaluated lesion stage

Study participants will have their lesion stage graded by trained pharmacists and pharmacy technicians at two stages: (1) on the initial clinical visit for enrolment, and (2) once participants indicate that healing has occurred they will have their lesion stage confirmed by clinicians at a study site within 24 hours (see exceptions below). Investigators will be provided with photo images as well as descriptions for each lesion stage, identical to those provided to the participant. They will assess this independently of the participant's own grading.

Episode duration

The episode duration is defined as follows: if no vesicle formed then duration was from the initiation of treatment to the return to normal skin with cessation of signs or symptoms (aborted lesion); if a

vesicle formed, the duration of episode was measured from the initiation of treatment to the loss of hard crust (Stage 6: residual erythema could be present after loss of hard crust). Duration of wound healing will be determined by the time between the development of an Ulcer (Stage 4) to return to normal skin (Stage 7). This will provide an estimate of the interventions effect on the most cosmetically bothersome and painful stage of the episode.

Participant-graded pain and other symptoms

Participants will grade lesion pain once per day upon waking on a 0-10 numeric rating scale (NRS) from onset of symptoms until healing occurs, with 0 being no pain and 10 the worse pain imaginable. Symptoms of burning, itching and tingling will be measured using a separate 4-point ordinal scale for each symptom as follows: 0 = not present, 1 = mild, 2 = moderate, and 3 = severe. This score will be submitted via the online web portal along with lesion stage assessment.

Participant satisfaction questionnaire

At the second pharmacy visit, participants will be asked to rate their satisfaction with the treatment given using a Likert scale. Questions will include the likelihood of recommendation of the intervention to family and friends, interest in using the intervention again for future cold sores and ranking the intervention relative to other previous treatments they have used (if any) for cold sores.

Laboratory assessments

For confirmation of HSV infection, participants will use the swab kits provided at the initial clinical consultation to take swabs of lesions that reach an ulcerative stage (Stage 4) and do not have evidence of crusting (to avoid interference with the healing process). Samples will be taken by participants at home, stored in their fridge (below 8 degrees Celsius) and shipped for viral culture to a central laboratory within 72 hours from sampling. The presence of HSV will be diagnosed by the appearance of typical cytopathic effects. Isolates will be typed (HSV-1 and HSV-2) by immunofluorescent antibody technique. Those participants who have non-ulcerative (or aborted) lesions will not provide swabs as removing fluid from these can delay healing.

Adverse events

Adverse events will be monitored via the daily online diary, during the second study visit and two weeks after second pharmacy visit.

Adverse events will be graded using the NCI Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE) and reported to the sponsor and HREC. After the end of study and locking of the study database, all adverse events will be summarised.

Data monitoring and stopping guidelines

Data will be monitored for completeness, plausibility and consistency to ensure the integrity and completeness of the data set. Any queries will be resolved by the Chief Investigator or delegated member of the study team. Adverse events will be regularly monitored via the online daily diary, as well as at the second pharmacy visit and at the conclusion of the two-week post trial period. Any serious adverse events will trigger an alert to the Chief Investigator.

Study timeline

The expected duration of the data collection phase of this study will be 12 months, with 14 time points where data is collected. The schedule of events is outlined in Table 1.

Table 1. Timeline of treatment assessments and interventions

Procedure	Site visit 1	Daily via online diary	If lesion reaches stage 4	Site visit 2	Phone call two weeks after site visit 2
Written informed consent	X				
Inclusion and exclusion	X				
Medical history	X				
Randomisation	X				
Treatment or placebo applied	X				
First lesion occurs	X				
Clinical confirmation of lesion stage	X			X	
Participant online diary – lesion stage, pain, stage and other symptoms		X			
Antigen-typing of lesions (HSV1/HSV2)			X		
Participant questionnaire – subjective evaluation of product				X	
Adverse events recorded		X		X	X
Other treatments		X			

Statistical analysis of outcome data

Efficacy analyses will be performed for both the intent-to-treat (ITT) population (all participants) and per-protocol (PP) population (excluding participants with missing data). The primary analysis for the primary outcome will be median duration of episode in days, from the stage at presentation to the clinical site until healing, compared between groups using a two tailed t-test. Secondary outcomes will include Cox proportional hazards for time to healing and time of wound duration, Kaplan-Meier survival plots. NRS pain scores and symptoms will be analysed using a linear mixed model, with time and group as fixed factors. Multilevel analysis will be conducted with different factors including country, gender and number of previous episodes. Results will be documented with *p*-values and 95% confidence intervals. Data will be analysed using SAS and/or SPSS (v24) software.

Participant safety

The product has been listed as a listed medicine on the Australian Register of Therapeutic Goods (ARTG) (AUST L 241934).

The TGA have approved the safety of all ingredients for use in complementary medicines in Australia. A detailed dose justification and safety profile with supporting literature for each of the components of Dynamiclear™ are detailed in Product Information provided by the Sponsor, Sci-Chem, in support of listing on the ARTG.

As the investigational product (Dynamiclear™) is a low-risk product already approved by the Australian Therapeutic Goods Administration (TGA) and available for purchase, a formal data monitoring committee will not be established in Australia or New Zealand. However, the nominated medical representatives for the study, Dr Carolyn Ee, a registered General Practitioner in Australia, and Dr Alex Semprini, a registered medical practitioner, in New Zealand, will review safety on all adverse events. The Dynamiclear™ formulation is considered safe to apply topically at the dosages outlined, however individual components may irritate the skin in sensitive individuals. Copper sulfate, Calendula and Hypericum can all be irritating to the skin and mucosa in some individuals.

Methods for adverse event recording and reporting include the daily online diary in which asks participants to report any adverse events over the previous 24 hours. Adverse events are also recorded

at site visit two by the pharmacist and any post trial events by a phone call two weeks following site visit two. All participants will also be provided with a digital emergency contact card with details of whom to contact in the case of an emergency.

Post-trial care

After the trial has been completed and data analysis undertaken, participants will be advised of their group allocations and the study results. If the intervention is found to be effective, those in the placebo group will be offered one free treatment of the interventional product. All participants will be advised of the availability of the name of the product and its availability to purchase over the counter if they wish to use it in future. Full indemnity insurance is in place for the study sponsor in the case of claims resulting from trial participation.

Ethics, study registration and dissemination

Australian ethics approval from Western Sydney University Human Research Ethics Committee, ref: H12776. New Zealand Ethics approval from The Health and Disability Ethics Committees (HDEC) ref: 18/CEN/151.The trial was prospectively registered with the Australia New Zealand Clinical Trials Registry: ACTRN12618000890235. The Universal Trial Number (UTN) is U1111-1233-2426. Results will be published in international academic journals and presented at conferences. Participating pharmacies will receive summarised results of the publication.

Collaborators: Members of the PRN in Australia (CBD Pharmacies Sydney, ChemistWorks Broadway, Priceline Pharmacy Oxford Street, Cincotta Discount Chemist Belrose, McFadden's Pharmacy St Ives, O'Loughlin's Medical Pharmacy St Ives, Warringah Mall Pharmacy, Donworth Pharmacy, MediADVICE Pharmacy Doonside, MediADVICE Pharmacy Glenmore Park, MediADVICE Pharmacy St Clair, Priceline Pharmacy The Ponds, Figtree Plaza Chemist)

Author Contributions: MA and AS designed and wrote the protocol, CE provided input related to medical components, MA and LM drafted the manuscript while AS and CE provided critical edits and feedback on all versions of the manuscript. All authors reviewed the manuscript critically for important intellectual content, and approved the final manuscript for submission.

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Conflict of Interest: MA and AS received grant support from Sci-Chem International

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Consent Form

Project Title: The effect of a topical treatment containing *Hypericum perforatum* (St John's Wort), *Calendula officinalis* (calendula) and *copper sulfate* on oral herpes.

I hereby consent to participate in the above named research project.

I acknowledge that:

- I have read the participant information sheet (or where appropriate, have had it read to me) and have been given the opportunity to discuss the information and my involvement in the project with the research team.
- The procedures required for the project and the time involved have been explained to me, and any questions I have about the project have been answered to my satisfaction.

I consent to:

☐ *Providing data such as my age, gender and relevant medical history.*

☐ *Having a single vial of topical treatment applied to my cold sore at the pharmacy*

☐ *Coming back to the pharmacy within 24 hours of my lesion healing for visual confirmation*

☐ *If my lesion ulcerates, using a swab at home to take a sample and posting the sample in the envelope provided*

☐ *Providing daily information in an online diary*

I consent for my data and information provided to be used in this project and other related projects for an extended period of time.

Participation is entirely voluntary and you are not obliged to be involved. If you do participate you can withdraw at any time without giving reason.

I understand:

- that my involvement is confidential and that the information gained during the study may be published and stored for other research use but no information about me will be used in any way that reveals my identity but will only be used after additional ethical review.
- that I can withdraw from the study at any time without affecting my relationship with the researcher/s, and any organisations involved, now or in the future.

I would like to receive a summary of the study results when they are available.

Please tick: ☐Yes ☐ No

Participant to sign:

Signed: _____

Name: _____

Date: _____

Pharmacist/researcher to sign:

Signed: _____

Name: _____

Date: _____

This study has been approved by the Human Research Ethics Committee at Western Sydney University. The ethics reference number is H12776.

What if I have a complaint?

If you have any complaints or reservations about the ethical conduct of this research, you may contact the Ethics Committee through Research Engagement, Development and Innovation (REDI) on Tel +61 2 4736 0229 or email humanethics@westernsydney.edu.au.

Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.

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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym – **Pg 1**
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry **Pg 2**
	2b	All items from the World Health Organization Trial Registration Data Set **Pg 2 and Pg 10**
Protocol version	3	Date and version identifier **Pg 2**
Funding	4	Sources and types of financial, material, and other support **Pg 11**
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors **Pg 11**
	5b	Name and contact information for the trial sponsor **Pg 11**
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities **Pg 11**
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) **N/A**
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention **Pg 3-4**
	6b	Explanation for choice of comparators **Pg4**
Objectives	7	Specific objectives or hypotheses **Pg4**

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) **Pg5**

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained **Pg 5**
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) **Pg 6**
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered **Pg 6-7**
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) **N/A**
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) **N/A – single application done by pharmacist**
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial **Pg 6**
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended **Pg 7-9**
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) **Table 1**
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations **Pg 5**
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size **Pg 5**

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions **Pg 6**
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned **Pg 6**
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions **Pg 6**
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how **Pg 6**
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial **Pg 6**
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol **Pg 7-9**
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols **Pg 10**
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol **Pg 9**
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol **Pg 10**
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) **N/A – not planned**
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) **Pg 10**

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed **Pg 10**
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial **Pg 9**
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct **Pg 10**
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor **Pg 9**

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval **Pg 11**
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) **Pg 8**
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) **Pg 5**
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable **N/A**
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial **Pg 8**
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site **Pg 11**
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators **Pg 8**
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation **Pg 10**

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions **Pg 11**
	31b	Authorship eligibility guidelines and any intended use of professional writers **Pg 11**
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code **Pg11**
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates **Appendix 1**
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

The efficacy of a topical herbal and mineral formulation (DynamiclearTM) for the treatment of herpes simplex labialis in the community setting: study protocol for a randomised, double-blind placebo controlled trial.

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TITLE PAGE

ARTICLE TITLE: The efficacy of a topical herbal and mineral formulation (Dynamiclear™) for the treatment of herpes simplex labialis in the community setting: study protocol for a randomised, double-blind placebo controlled trial.

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KEYWORDS: cold sore, herpes simplex, Dynamiclear, hypericum perforatum, St. John's Wort, calendula officinalis, copper sulfate, pharmacy research

ABSTRACT

Introduction Herpes Simplex Labialis (HSL) is a common infection that can cause painful lesions on the oral mucosa, commonly referred to as cold sores. Current biomedical treatments include topical aciclovir, which reduces the episode duration by an average of 0.5 days. This study will examine the efficacy and tolerability of an over-the-counter topical treatment, Dynamiclear™ in reducing duration and severity of HSL episodes.

Methods and analysis This prospective, randomised, double-blind, placebo-controlled, multi-centre trial will recruit a minimum of 292 adult participants across Australia and New Zealand who present with a cold sore within 48 hours of onset. They will be randomly allocated in a 2:1 ratio to receive either topical Dynamiclear™ (active) or placebo. Dynamiclear's active ingredients are *Hypericum perforatum*, *Calendula Officinalis* and copper sulfate. A single topical treatment of active or placebo will be applied by a pharmacy-based investigator, and participants will be provided with a viral swab kit to confirm presence of herpes virus 1 or 2 from ulcerated lesions. Participants will receive reminders by email and/or SMS to complete an online daily diary assessing their cold sore lesion using a visual guide, and recording other symptoms on numeric scales until healed. The primary outcome variable is median duration of HSL episode (in days) from presentation to return to normal skin. Secondary outcomes include severity of lesion pain, itching, burning and tingling during the symptomatic phase and proportion of lesions progressing to ulceration.

Ethics and dissemination Australian ethics approval from Western Sydney University Human Research Ethics Committee, ref: H12776. New Zealand Ethics approval from The Health and Disability Ethics Committees (HDEC) ref: 18/CEN/151. Results will be published in a peer-reviewed academic journal, presented at academic meetings and reported to participants

Trial registration number Australia and New Zealand Clinical Trials Registry (ACTRN): ACTRN12618000890235. **Universal Trial Number (UTN)** U1111-1233-2426

Protocol version V8 (16th May 2019)

ARTICLE SUMMARY

Strengths and limitations of this study

- Novel pharmacy research network allows for effective recruitment of HSL sufferers that most often access treatment in the community.
- Single-dose intervention eliminates treatment compliance issues.
- Digital data collection minimises study burden for investigators and participants and ensures real time, accurate data collection.
- Self-report measures for lesion progression verified by second pharmacy visit.

INTRODUCTION

Herpes simplex labialis (HSL)

Herpes simplex labialis (HSL) is a common recurring infection of the labial or perioral skin caused by herpes simplex virus (HSV), also known as cold sores. The majority of cases are caused by HSV-1. Seroprevalence studies indicate that 60% to 90% of adults are infected with HSV-1 and that HSV is the fastest growing infectious disease in the world with 500,000 new cases reported each year[1]. Approximately 20% to 40% of the population experiences recurrent outbreaks of HSL. The frequency of outbreaks ranges from rare episodes every 5 to 10 years, to monthly or more frequent outbreaks[2].

Symptoms of active infection include prodromal numbness and tingling around the affected area, before progression to erythema, itching, burning, pain in the area, and ulcerated lesions. Disease episodes are generally mild and self-limiting[1]; however, the symptoms are uncomfortable and often more importantly aesthetically unpleasant and noticeable to the general public.

Primary herpes simplex infection is most often asymptomatic, however can manifest significant morbidity including general anorexia and malaise, fever, local lymphadenopathy, gingivostomatitis and significant ulcerative lesions; these symptoms may persist for up to three weeks[3]. Following the initial infection, the virus establishes a chronic, latent and life-long infection in sensory nerve ganglia, predominantly the trigeminal ganglion[4]. At a later date, the virus may be reactivated and travel back to the oral mucosa, perioral skin and/or labial surfaces, where it replicates, producing a clinical episode of recurrent herpes labialis. Viral reactivation usually occurs due to stress on the immune system. Triggers include exposure to ultraviolet (UV) light exposure[5], and viral infections such as colds and flu[6]. Recurrent episodes are shorter than the initial episode, with the virus often cleared within three days or less, due to the previously acquired immune response[1]. This immune response contributes to rapid control of recurrent HSL disease episodes but may also be responsible for clinical signs and symptoms such as pain, redness and swelling that can persist for up to a week or longer, even after the virus can no longer be isolated[2].

Current standard treatments for HSL

Oral and topical aciclovir preparations are used as the current standard of care for treatment of HSV. Oral antiviral medications such as aciclovir, valaciclovir, famciclovir limit replication of the herpes simplex virus (HSV) by inhibiting viral DNA polymerase, and can shorten the time to healing from half a day to just over two days compared to placebo depending on the type of medication and dosage used[1]. Adverse events are generally mild and similar to placebo[7 8].

Topical treatment with aciclovir and other antivirals have also shown modest clinical benefit, reducing average healing time by around half a day compared to vehicle control[1 9]. Topical aciclovir demands good compliance, requiring daily application but is generally well tolerated, with an adverse event rate similar to vehicle control[9].

Composition of Dynamiclear™

Dynamiclear™ (Sci-chem International) contains three active ingredients traditionally used to help treat inflamed or infected skin: Hypericum perforatum extract, Calendula officinalis extract and copper sulfate pentahydrate.

Hypericum perforatum, or St. John's Wort, has demonstrated antiviral activities against enveloped viruses such as HSV and human immunodeficiency virus (HIV). The active constituent, hypericin, reduces viral replication, through inhibition of new virion budding, prevention of viral uncoating, and inhibition of protein kinase activity[10]**.** Hypericin has also been found to inhibit protein kinase C gamma and epsilon, which are proteins associated with the development of neuropathic pain[10]. For treatment of

HSL skin lesions, *Hypericum perforatum* is likely to be effective for topical application only as ingestion can cause photosensitivity, which may promote HSL reactivation[11].

Calendula Officinalis has been traditionally used for minor skin infections and inflammation[12]. Recent studies have found calendula to be effective in reducing the time to healing in previously non-healing venous leg ulcers[13]. This is likely to occur through the upregulation of genes controlling connective tissue growth factor (CTGF) and α-smooth muscle actin (α-SMA)[14]and the proliferation and migration of fibroblasts[15]. *Calendula Officinalis* may therefore reduce the time to wound healing in HSL lesions that progress to an ulcerative phase.

Copper sulfate is a naturally occurring mineral which has demonstrable antiviral activity. Rather than suppressing viral replication, copper ions render the viral DNA nonviable for further replication. HSV has been shown to exhibit sensitivity to low concentrations of copper, and in vitro research has shown evidence for copper-mediated inactivation of HSV[16].

A non-blinded, active comparator randomised controlled trial[17] of a previous version of Dynamiclear (without Calendula Officinalis) demonstrated lower odds of having pain, redness or progressing to the vesicle stage compared with topical 5% aciclovir in the treatment of orolabial and genital herpes simplex episodes. However, interpretation of these results is limited due to the inclusion of multiple sub-types of HSV, lack of blinding, and use of non-standard outcome measures.

Study aims

Primary objective: To evaluate the efficacy of one topical dose of Dynamiclear™ in the treatment of HSL by a reduction in episode duration.

Secondary objectives:

- To assess the effect of Dynamiclear™ on the progression to ulcerative vs non-ulcerative lesions.
- To assess the effect of Dynamiclear™ on the duration of wound healing.
- To assess the effect of Dynamiclear™ on pain, tenderness, tingling and itching during the disease course of HSL.
- To assess patient satisfaction of the Dynamiclear™ product.

Hypothesis

A single topical application of Dynamiclear™ has greater efficacy than placebo on the severity and duration of HSL

METHODS AND ANALYSIS

Study design

A prospective, randomised controlled, double-blind, multi-site trial to evaluate the efficacy of a single dose of topically applied Dynamiclear™ vs placebo on the duration and severity of HSL.

Sample size calculation

The sample size needed to demonstrate statistically and clinically significant efficacy was determined using the hypothesis that the mean episode duration in the treatment group will be 5.0 days, compared to 6.0 days in the placebo group. This is based on the review of previous studies of aciclovir vs. placebo by Harmenberg et al[1] and a clinically significant difference of one day in episode duration. Given an alpha of 0.05, power of 80% and an allocation ratio of 2:1 for treatment to placebo, 149 participants are required in the treatment group and 75 in the placebo group (224 in total). A recent study of kanuka honey vs aciclovir to treat cold sores, using the New Zealand Pharmacy based Research Network (PRN) for recruitment, reported an 11% drop out rate. However, because our study is placebo-controlled, we anticipate a higher dropout rate. Allowing for a 30% drop out rate, 292 participants are required: 194 in the treatment group and 98 in the control group. This sample size will also power the study to detect a moderate effect size on the secondary outcome measures (Cohen's d = 0.4).

Participants

A total of 292 participants aged 18 to 65 presenting to participating pharmacies within 48 hours of onset of prodromal symptoms of HSL and who report at least three prior episodes of HSL will be recruited. Inclusion and exclusion criteria are presented in Box 1.

A PRN in both New Zealand and Sydney, Australia will be used to register sites for primary recruitment, screening and assessment.

Study centres: 10-15 pharmacies in Sydney, Australia, and 20-25 pharmacies in New Zealand.

Patient and Public Involvement (PPI)

There were no funds or time allocated for PPI so we were unable to involve patients in the study design. We will invite patients at the conclusion of the study to provide feedback on the burden of the intervention, and patients will be involved in developing the dissemination strategies for our findings.

Recruitment and selection

Four parallel recruitment pathways are planned in this study. First, social media will be used to advise participants of the trial, and refer them to a webpage which outlines the inclusion and exclusion criteria and encourage them to present at participating pharmacies within 48 hours of the onset of a cold sore. Second, the New Zealand arm of the PRN has recently completed a different HSL study[18]. The 920 participants who joined that study and consented to being contacted for further studies will be sent an email informing them of this study. Third, all participating PRN pharmacies will have promotional material in their pharmacies and individuals presenting to these pharmacies with a cold sore recurrence within the past 48 hours will be invited to enrol in the study. Fourth, TrialFacts, a paid recruitment service, will be used to advertise and screen for potential participants. Recruitment will begin in June 2019.

Regardless of recruitment pathway, upon presentation at a participating pharmacy, potential participants will be screened and those eligible will be consented by pharmacists and pharmacy technicians. Recruitment staff will ensure that each participant is fully informed about the nature and objectives of the study and possible risks associated with participation.

Box 1: Inclusion and exclusion criteria

Inclusion criteria:

- Age 18-65 years
- Onset of prodromal or clinical symptoms of HSL in the past 48 hours
- Previous clinical history of HSL with at least 3 prior episodes of HSL
- Primary lesion within 1cm of the lip
- Willing to provide informed consent and adhere to study protocol
- Has internet (either by mobile or computer) to complete online forms.

Exclusion criteria:

- History of immunodeficiency, immunosuppression or autoimmune disorder
- Current infection not related to the study condition – e.g. infections such as cold or flu
- Use of other antiviral, anti-inflammatory or steroid medications during or within two weeks prior to the treatment period
- Use of other topical agents (including cosmetics, lip balms, sunscreens) or cosmetic procedures (such as chemical peels or microdermabrasion) on the prodromal or lesion area during the treatment period
- Mechanical disruption (e.g. scrubbing lancing or shaving) of the prodromal or lesion area during the treatment period
- Pregnancy, lactation or planning to become pregnant in the following 14 days
- Participation in another clinical trial within the previous 30 days.

Randomisation

After providing informed consent and satisfying the eligibility criteria, participants will be randomised in a 2:1 ratio to receive either active treatment with Dynamiclear™ (n=194) or the placebo control group (n=98) using the Castor EDC platform[19]. Randomisation will be performed using the Castor EDC platform by a researcher independent of the study team. To maintain pharmacist blinding when using a 2:1 ratio, boxes containing the intervention are pre-randomised, and pharmacists are provided with a box code rather than a group allocation. A 2:1 ratio in favour of the active intervention was chosen to maximise recruitment rates as potential participants are actively seeking treatment when attending the pharmacy. Thus, a greater chance of being allocated active treatment is likely to increase recruitment and retention rates. Permuted block randomisation, with a block size of 6 or 9 will be undertaken for each pharmacy to ensure that no patterns or imbalances emerge in group allocation between sites.

Blinding

All pharmacy staff, study team, participants and data analysists are blind to the group allocation. Unblinding will be permitted for either Dr Ee or Dr Semprini, or any other medical monitor, if serious adverse events are reported.

Study interventions

Interventional treatment is applied once only during the first pharmacy visit. Each participant will be allocated one vial of Dynamiclear™ or a placebo that matches Dynamiclear in colour, viscosity, taste and smell. The active and placebo vials are presented in identical packaging. The intervention or placebo will be applied via a cotton-tipped applicator which delivers 0.7mL to the affected area. This will be done either by the trained investigator or by the participant in view of the investigator, and the vial and packaging disposed of immediately. The moistened applicator will be applied for a minimum of 30 seconds, directly on the HSL lesion. No investigational product or packaging will leave the pharmacy site for any reason. Box 2 outlines the composition of both the interventional product and placebo.

Box 2a. Composition of interventional product (Dynamiclear™)

Active ingredients:

- Calendula Officinalis 1:2 liquid extract, 0.05 %w/w
- Hypericum Perforatum 1:2 liquid extract, 0.05 %w/w
- Copper sulphate pentahydrate, 6.4 %w/w

Excipient ingredients:

- Aloe vera
- Glycerol
- Vitamin E (Tocoheryl Acetate)
- Hydroxyethyl cellulose
- Polysorbate 80
- Purified water

Box 2b. Composition of placebo

Masking ingredients:

- Blue dye
- Yellow dye

Excipient ingredients:

- Aloe barbedensis
- Glycerin
- Vitamin E (Tocoheryl Acetate)
- Hydroxyethyl cellulose
- Polysorbate 80
- Purified water
- Sodium benzoate
- Citric acid anydrous (pH adjustor)

Data capture and monitoring

All data will be captured electronically via the secure electronic data capture platform CastorEDC [19]. Each pharmacy will be provided with an iPad (Apple Inc.) which will be used to collect all data during each face to face clinical visit. Signed consent forms will be digitally captured and uploaded directly onto the EDC platform.

Each participant will attend two clinical visits at PRN pharmacies. The first visit will be within 48 hours of symptom onset and will include initial clinical assessment and a single application of either the intervention or placebo in the presence of trained pharmacy staff. A second visit will be triggered when the participant indicates via their online symptom diary that they have returned to normal skin (Stage 7). This visit will involve confirmation of healing by pharmacists and checking for adverse events.

Participants will report symptoms and healing progression in an online daily diary (hosted on Castor EDC) which researchers will monitor for reports of adverse side effects. These diaries will also be used to monitor patient adherence to study requirements.

Participants will receive a follow-up phone call by a member of the research team two weeks after their final pharmacy visit to monitor any adverse events post treatment.

All data will be securely held on the study team database, accessible only by authorised investigators for the purposes of monitoring. The final dataset will be accessible by the coordinating investigators and each locality will retain on-site access to digital copies of source documentations with paper copies stored in secure archives.

Any changes to the study protocol are provided to the HREC and HDEC as per protocol, trial registries will be updated, and updated copies provided to trial sites. A copy of the consent form is included as Supplementary File 1.

Clinical assessments and patient surveys will be completed using Castor clinical trials management software. All the pre-specified outcome measures are outlined in Box 3.

Box 3: Outcome measures

Primary outcome measure:

- Median episode duration in days (participant evaluated)

Secondary outcome measures:

- Median episode duration in days (investigator-evaluated)
- Proportion (%) of lesions progressing to ulceration
- Median duration from wound development (stage 4) to healing (stage 7) in days (participant evaluated)
- Numeric rating scale (NRS) for pain, tenderness, tingling and itching during the symptomatic phase
- Proportion (%) of cases of HSV confirmed via swabs
- Adverse event rate
- Patient satisfaction and acceptability

A systematic review by Harmenberg[1], a recent mini review by Hull[13] and the most recent Cochrane systematic review protocol[20] outline a number of important outcome measures which informed the choices in Box 3. Median duration of the episode, as defined by the time to healing either by the loss of the hard crust in participants with ulcerative lesions or the time to normal skin (complete epithelialisation) in those with aborted lesions, either participant or clinician reported is the most common clinically relevant outcome measure. However, both Harmenberg and Hull argue that using duration of healing alone may miss important clinical effects, for example, if lesion duration is less but the size of the lesion is greater this may not indicate an improvement in the condition from the patient's perspective. The ulcerative stage of the lesion is the most painful and cosmetically bothersome stage of the lesion. Given the difference in disease severity between ulcerative and non-ulcerative lesions, the proportion of these lesions is commonly used as another important outcome measure, which may be of greater concern to HSL sufferers than a small reduction of episode duration often seen in topical treatment. Harmenberg compellingly argues that an important marker in placebo controlled trials is the proportion of participants that develop ulcerative lesions despite use of the study medication, as a reduction in progression to ulceration is of significant clinical benefit to sufferers.[1] Indeed a common feature of antiviral therapies is not a significant reduction in overall duration, but a shortening of the duration of time of wound healing[21], which is still a very important clinical benefit.

Finally, the painful symptoms of burning, tingling and itching are commonly found in participants with HSL, and are usually bothersome and therefore a reduction in either severity or duration of these is an important patient centric outcome.

Participant evaluated lesion stage

Participants will be provided with photo images as well as descriptions for each lesion stage via an online web form. We have used this lesion staging system in our previous trial[22]. Participants will be asked to grade the lesion once per day, upon waking, until return to normal skin occurs (Stage 7) or until 14 days from the first pharmacy visit. A reminder SMS and/or email will be sent each morning with an embedded link to the web form. This grading will be submitted via an online web form. When a participant grades their lesion having resolved and the area has returned to normal skin (Stage 7) via the online form they will be contacted to attend for assessment to their nearest study site within 24 hours by phone and email by the research assistant.

Investigator-evaluated lesion stage

Study participants will have their lesion stage graded by trained pharmacists and pharmacy technicians at two stages: (1) on the initial clinical visit for enrolment, and (2) once participants indicate that healing has occurred they will have their lesion stage confirmed by clinicians at a study site within 24 hours (see exceptions below). Investigators will be provided with photo images as well as descriptions for each lesion stage, identical to those provided to the participant. They will assess this independently of the participant's own grading.

Episode duration

The episode duration will be defined as follows: if no vesicle formed then duration was from the initiation of treatment to the return to normal skin with cessation of signs or symptoms (aborted lesion); if a vesicle formed, the duration of episode was measured from the initiation of treatment to the loss of hard crust (Stage 6: residual erythema could be present after loss of hard crust). Duration of wound healing will be determined by the time between the development of an Ulcer (Stage 4) to return to normal skin (Stage 7). This will provide an estimate of the interventions effect on the most cosmetically bothersome and painful stage of the episode.

Participant-graded pain and other symptoms

Participants will grade lesion pain once per day upon waking on a 0-10 numeric rating scale (NRS) from onset of symptoms until healing occurs, with 0 being no pain and 10 the worse pain imaginable. Symptoms of burning, itching and tingling will be measured using a separate 4-point ordinal scale for each symptom as follows: 0 = not present, 1 = mild, 2 = moderate, and 3 = severe. This score will be submitted via the online web portal along with lesion stage assessment.

Participant satisfaction questionnaire

At the second pharmacy visit, participants will be asked to rate their satisfaction with the treatment given using a Likert scale. Questions will include the likelihood of recommendation of the intervention to family and friends, interest in using the intervention again for future cold sores and ranking the intervention relative to other previous treatments they have used (if any) for cold sores.

Laboratory assessments

For confirmation of HSV infection, participants will use the swab kits provided at the initial clinical consultation to take swabs of lesions that reach an ulcerative stage (Stage 4) and do not have evidence of crusting (to avoid interference with the healing process). Samples will be taken by participants at home using a supplied dry swab that will be gently rubbed on the lesion in a circular motion for 5 seconds, then placed in a sterile outer tube, stored in their fridge (below 8 degrees Celsius) and shipped for viral culture to a central laboratory within 72 hours from sampling by courier. The presence of HSV will be diagnosed by the appearance of typical cytopathic effects. Isolates will be typed (HSV-1 and

HSV-2) by immunofluorescent antibody technique. Those participants who have non-ulcerative(or aborted) lesions will not provide swabs as removing fluid from these can delay healing.

Adverse events

Adverse events will be monitored via the daily online diary, during the second study visit and two weeks after second pharmacy visit.

Adverse events will be graded using the NCI Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE) and reported to the sponsor and HREC. After the end of study and locking of the study database, all adverse events will be summarised.

Data monitoring and stopping guidelines

Data will be monitored for completeness, plausibility and consistency to ensure the integrity and completeness of the data set. Any queries will be resolved by the Chief Investigator or delegated member of the study team. Adverse events will be regularly monitored via the online daily diary, as well as at the second pharmacy visit and at the conclusion of the two-week post trial period. Any serious adverse events will trigger an alert to the Chief Investigator.

Study timeline

The expected duration of the data collection phase of this study will be 12 months, with 14 time points where data will be collected. The schedule of events is outlined in Table 1.

Table 1. Timeline of treatment assessments and interventions

Procedure	Site visit 1	Daily via online diary	If lesion reaches stage 4	Site visit 2	Phone call two weeks after site visit 2
Written informed consent	X				
Inclusion and exclusion	X				
Medical history	X				
Randomisation	X				
Treatment or placebo applied	X				
First lesion occurs	X				
Clinical confirmation of lesion stage	X			X	
Participant online diary – lesion stage, pain, stage and other symptoms		X			
Antigen-typing of lesions (HSV1/HSV2)			X		
Participant questionnaire – subjective evaluation of product				X	
Adverse events recorded		X		X	X
Other treatments		X			

Statistical analysis of outcome data

Efficacy analyses will be performed for both the intent-to-treat (ITT) population (all participants) and per-protocol (PP) population (excluding participants with missing data). The analysis for the primary outcome of median duration of episode as reported by the participant, from the stage at presentation to the clinical site until healing and for the secondary outcomes of the median investigator rated duration, and median duration of wound development will all be analysed using a Mann-Whitney U-

test. Differences between groups in proportion of lesions progressing to ulceration, proportion of confirmed HSV ,adverse events rates and participant satisfaction rates will be compared using Chi-square tests.

Differences in NRS ratings for pain, tenderness, tingling and itching will be analysed using linear mixed model analysis of variance with group and time as fixed effects. In mixed model analysis subject will be used as a random effect. Secondary outcomes will include Cox proportional hazards for time to healing and time of wound duration and Kaplan-Meier survival plots. Multilevel analysis may be conducted with different factors including country, gender and number of previous episodes. Results will be documented with p-values and 95% confidence intervals. Data will be analysed using SAS and/or SPSS (v24) software.

Participant safety

The product has been listed as a listed medicine on the Australian Register of Therapeutic Goods (ARTG) (AUST L 241934).

The Australian Therapeutic Goods Administration (TGA) have approved the safety of all ingredients for use in complementary medicines in Australia. A detailed dose justification and safety profile with supporting literature for each of the components of Dynamiclear™ are detailed in Product Information provided by the Sponsor, Sci-Chem, in support of listing on the ARTG.

As the investigational product (Dynamiclear™) is a low-risk product already approved by the TGA and available for purchase, a formal data monitoring committee will not be established in Australia or New Zealand. However, the nominated medical representatives for the study, Dr Carolyn Ee, a registered General Practitioner in Australia, and Dr Alex Semprini, a registered medical practitioner, in New Zealand, will review safety on all adverse events. The Dynamiclear™ formulation is considered safe to apply topically at the dosages outlined, however individual components may irritate the skin in sensitive individuals. Copper sulfate, Calendula and Hypericum can all be irritating to the skin and mucosa in some individuals.

Methods for adverse event recording and reporting include the daily online diary in which asks participants to report any adverse events over the previous 24 hours. Adverse events are also recorded at site visit two by the pharmacist and any post trial events by a phone call two weeks following site visit two. All participants will also be provided with a digital emergency contact card with details of whom to contact in the case of an emergency.

Post-trial care

After the trial has been completed and data analysis undertaken, participants will be advised of their group allocations and the study results. If the intervention is found to be effective, those in the placebo group will be offered one free treatment of the interventional product. All participants will be advised of the availability of the name of the product and its availability to purchase over the counter if they wish to use it in future. Full indemnity insurance is in place for the study sponsor in the case of claims resulting from trial participation.

Ethics, study registration and dissemination

Australian ethics approval from Western Sydney University Human Research Ethics Committee, ref: H12776. New Zealand Ethics approval from The Health and Disability Ethics Committees (HDEC) ref: 18/CEN/151.The trial was prospectively registered with the Australia New Zealand Clinical Trials Registry: ACTRN12618000890235. The Universal Trial Number (UTN) is U1111-1233-2426. Results will be published in international academic journals and presented at conferences. Participating pharmacies will receive summarised results of the publication.

Collaborators: Members of the PRN in Australia (CBD Pharmacies Sydney, ChemistWorks Broadway, Priceline Pharmacy Oxford Street, Cincotta Discount Chemist Belrose, McFadden's Pharmacy St Ives, O'Loughlin's Medical Pharmacy St Ives, Warringah Mall Pharmacy, Donworth Pharmacy, MediADVICE Pharmacy Doonside, MediADVICE Pharmacy Glenmore Park, MediADVICE Pharmacy St Clair, Priceline Pharmacy The Ponds, Figtree Plaza Chemist)

Author Contributions: MA and AS designed and wrote the protocol, CE provided input related to medical components, MA and LM drafted the manuscript while AS, NS and CE provided critical edits and feedback on all versions of the manuscript. All authors reviewed the manuscript critically for important intellectual content, and approved the final manuscript for submission.

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Conflict of Interest: MA and AS received grant support from Sci-Chem International

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Consent Form

Project Title: The effect of a topical treatment containing *Hypericum perforatum* (St John's Wort), *Calendula officinalis* (calendula) and *copper sulfate* on oral herpes.

I hereby consent to participate in the above named research project.

I acknowledge that:

- I have read the participant information sheet (or where appropriate, have had it read to me) and have been given the opportunity to discuss the information and my involvement in the project with the research team.
- The procedures required for the project and the time involved have been explained to me, and any questions I have about the project have been answered to my satisfaction.

I consent to:

☐ *Providing data such as my age, gender and relevant medical history.*

☐ *Having a single vial of topical treatment applied to my cold sore at the pharmacy*

☐ *Coming back to the pharmacy within 24 hours of my lesion healing for visual confirmation*

☐ *If my lesion ulcerates, using a swab at home to take a sample and posting the sample in the envelope provided*

☐ *Providing daily information in an online diary*

I consent for my data and information provided to be used in this project and other related projects for an extended period of time.

Participation is entirely voluntary and you are not obliged to be involved. If you do participate you can withdraw at any time without giving reason.

I understand:

- that my involvement is confidential and that the information gained during the study may be published and stored for other research use but no information about me will be used in any way that reveals my identity but will only be used after additional ethical review.
- that I can withdraw from the study at any time without affecting my relationship with the researcher/s, and any organisations involved, now or in the future.

I would like to receive a summary of the study results when they are available.

Please tick: ☐Yes ☐ No

Participant to sign:

Signed: _____

Name: _____

Date: _____

Pharmacist/researcher to sign:

Signed: _____

Name: _____

Date: _____

This study has been approved by the Human Research Ethics Committee at Western Sydney University. The ethics reference number is H12776.

What if I have a complaint?

If you have any complaints or reservations about the ethical conduct of this research, you may contact the Ethics Committee through Research Engagement, Development and Innovation (REDI) on Tel +61 2 4736 0229 or email humanethics@westernsydney.edu.au.

Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym – **Pg 1**
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry **Pg 2**
	2b	All items from the World Health Organization Trial Registration Data Set **Pg 2 and Pg 10**
Protocol version	3	Date and version identifier **Pg 2**
Funding	4	Sources and types of financial, material, and other support **Pg 11**
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors **Pg 11**
	5b	Name and contact information for the trial sponsor **Pg 11**
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities **Pg 11**
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) **N/A**
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention **Pg 3-4**
	6b	Explanation for choice of comparators **Pg4**
Objectives	7	Specific objectives or hypotheses **Pg4**

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) **Pg5**
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained **Pg 5**
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) **Pg 6**
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered **Pg 6-7**
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) **N/A**
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) **N/A – single application done by pharmacist**
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial **Pg 6**
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended **Pg 7-9**
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) **Table 1**
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations **Pg 5**
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size **Pg 5**
Methods: Assignment of interventions (for controlled trials)		
Allocation:		

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions **Pg 6**
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned **Pg 6**
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions **Pg 6**
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how **Pg 6**
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial **Pg 6**
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol **Pg 7-9**
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols **Pg 10**
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol **Pg 9**
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol **Pg 10**
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) **N/A – not planned**
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) **Pg 10**

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed **Pg 10**
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial **Pg 9**
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct **Pg 10**
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor **Pg 9**

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval **Pg 11**
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) **Pg 8**
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) **Pg 5**
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable **N/A**
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial **Pg 8**
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site **Pg 11**
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators **Pg 8**
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation **Pg 10**

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions **Pg 11**
	31b	Authorship eligibility guidelines and any intended use of professional writers **Pg 11**
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code **Pg11**
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates **Appendix 1**
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

The efficacy of a topical herbal and mineral formulation (DynamiclearTM) for the treatment of herpes simplex labialis in the community setting: study protocol for a randomised, double-blind placebo controlled trial.

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Keywords:	Herbal medicine < THERAPEUTICS, Infectious diseases & infestations < DERMATOLOGY, COMPLEMENTARY MEDICINE, Clinical trials < THERAPEUTICS

TITLE PAGE

ARTICLE TITLE: The efficacy of a topical herbal and mineral formulation (Dynamiclear™) for the treatment of herpes simplex labialis in the community setting: study protocol for a randomised, double-blind placebo controlled trial.

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ABSTRACT

Introduction Herpes Simplex Labialis (HSL) is a common infection that can cause painful lesions on the oral mucosa, commonly referred to as cold sores. Current biomedical treatments include topical aciclovir, which reduces the episode duration by an average of 0.5 days. This study will examine the efficacy and tolerability of an over-the-counter topical treatment, Dynamiclear™ in reducing duration and severity of HSL episodes.

Methods and analysis This prospective, randomised, double-blind, placebo-controlled, multi-centre trial will recruit a minimum of 292 adult participants across Australia and New Zealand who present with a cold sore within 48 hours of onset. They will be randomly allocated in a 2:1 ratio to receive either topical Dynamiclear™ (active) or placebo. Dynamiclear's active ingredients are *Hypericum perforatum*, *Calendula Officinalis* and copper sulfate. A single topical treatment of active or placebo will be applied by a pharmacy-based investigator, and participants will be provided with a viral swab kit to confirm presence of herpes virus 1 or 2 from ulcerated lesions. Participants will receive reminders by email and/or SMS to complete an online daily diary assessing their cold sore lesion using a visual guide, and recording other symptoms on numeric scales until healed. The primary outcome variable is median duration of HSL episode in days (participant evaluated) from presentation to return to normal skin. Secondary outcomes include severity of lesion pain, itching, burning and tingling during the symptomatic phase and proportion of lesions progressing to ulceration.

Ethics and dissemination Australian ethics approval from Western Sydney University Human Research Ethics Committee, ref: H12776. New Zealand Ethics approval from The Health and Disability Ethics Committees (HDEC) ref: 18/CEN/151. Results will be published in a peer-reviewed academic journal, presented at academic meetings and reported to participants

Trial registration number Australia and New Zealand Clinical Trials Registry (ACTRN): ACTRN12618000890235. **Universal Trial Number (UTN)** U1111-1233-2426

Protocol version V8 (16th May 2019)

ARTICLE SUMMARY

Strengths and limitations of this study

- Novel pharmacy research network allows for effective recruitment of HSL sufferers that most often access treatment in the community.
- Single-dose intervention eliminates treatment compliance issues.
- Digital data collection minimises study burden for investigators and participants and ensures real time, accurate data collection.
- Self-report measures for lesion progression verified by second pharmacy visit.
- Self-reported outcomes for days in-between pharmacy visits are subjective and may over or underestimate lesion progression or symptom severity.

INTRODUCTION

Herpes simplex labialis (HSL)

Herpes simplex labialis (HSL) is a common recurring infection of the labial or perioral skin caused by herpes simplex virus (HSV), also known as cold sores. The majority of cases are caused by HSV-1. Seroprevalence studies indicate that 60% to 90% of adults are infected with HSV-1 and that HSV is the fastest growing infectious disease in the world with 500,000 new cases reported each year[1]. Approximately 20% to 40% of the population experiences recurrent outbreaks of HSL. The frequency of outbreaks ranges from rare episodes every 5 to 10 years, to monthly or more frequent outbreaks[2].

Symptoms of active infection include prodromal numbness and tingling around the affected area, before progression to erythema, itching, burning, pain in the area, and ulcerated lesions. Disease episodes are generally mild and self-limiting[1]; however, the symptoms are uncomfortable and often more importantly aesthetically unpleasant and noticeable to the general public.

Primary herpes simplex infection is most often asymptomatic, however can manifest significant morbidity including general anorexia and malaise, fever, local lymphadenopathy, gingivostomatitis and significant ulcerative lesions; these symptoms may persist for up to three weeks[3]. Following the initial infection, the virus establishes a chronic, latent and life-long infection in sensory nerve ganglia, predominantly the trigeminal ganglion[4]. At a later date, the virus may be reactivated and travel back to the oral mucosa, perioral skin and/or labial surfaces, where it replicates, producing a clinical episode of recurrent herpes labialis. Viral reactivation usually occurs due to stress on the immune system. Triggers include exposure to ultraviolet (UV) light exposure[5], and viral infections such as colds and flu[6]. Recurrent episodes are shorter than the initial episode, with the virus often cleared within three days or less, due to the previously acquired immune response[1]. This immune response contributes to rapid control of recurrent HSL disease episodes but may also be responsible for clinical signs and symptoms such as pain, redness and swelling that can persist for up to a week or longer, even after the virus can no longer be isolated[2].

Current standard treatments for HSL

Oral and topical aciclovir preparations are used as the current standard of care for treatment of HSV. Oral antiviral medications such as aciclovir, valaciclovir, famciclovir limit replication of the herpes simplex virus (HSV) by inhibiting viral DNA polymerase, and can shorten the time to healing from half a day to just over two days compared to placebo depending on the type of medication and dosage used[1]. Adverse events are generally mild and similar to placebo[7 8].

Topical treatment with aciclovir and other antivirals have also shown modest clinical benefit, reducing average healing time by around half a day compared to vehicle control[1 9]. Topical aciclovir demands good compliance, requiring daily application but is generally well tolerated, with an adverse event rate similar to vehicle control[9].

Composition of Dynamiclear™

Dynamiclear™ (Sci-chem International) contains three active ingredients traditionally used to help treat inflamed or infected skin: Hypericum perforatum extract, Calendula officinalis extract and copper sulfate pentahydrate.

Hypericum perforatum, or St. John's Wort, has demonstrated antiviral activities against enveloped viruses such as HSV and human immunodeficiency virus (HIV). The active constituent, hypericin, reduces viral replication, through inhibition of new virion budding, prevention of viral uncoating, and inhibition of protein kinase activity[10]**.** Hypericin has also been found to inhibit protein kinase C gamma and epsilon, which are proteins associated with the development of neuropathic pain[10]. For treatment of

HSL skin lesions, *Hypericum perforatum* is likely to be effective for topical application only as ingestion can cause photosensitivity, which may promote HSL reactivation[11].

Calendula Officinalis has been traditionally used for minor skin infections and inflammation[12]. Recent studies have found calendula to be effective in reducing the time to healing in previously non-healing venous leg ulcers[13]. This is likely to occur through the upregulation of genes controlling connective tissue growth factor (CTGF) and α-smooth muscle actin (α-SMA)[14]and the proliferation and migration of fibroblasts[15]. *Calendula Officinalis* may therefore reduce the time to wound healing in HSL lesions that progress to an ulcerative phase.

Copper sulfate is a naturally occurring mineral which has demonstrable antiviral activity. Rather than suppressing viral replication, copper ions render the viral DNA nonviable for further replication. HSV has been shown to exhibit sensitivity to low concentrations of copper, and in vitro research has shown evidence for copper-mediated inactivation of HSV[16].

A non-blinded, active comparator randomised controlled trial[17] of a previous version of Dynamiclear (without Calendula Officinalis) demonstrated lower odds of having pain, redness or progressing to the vesicle stage compared with topical 5% aciclovir in the treatment of orolabial and genital herpes simplex episodes. However, interpretation of these results is limited due to the inclusion of multiple sub-types of HSV, lack of blinding, and use of non-standard outcome measures.

Study aims

Primary objective: To evaluate the efficacy of one topical dose of Dynamiclear™ in the treatment of HSL by a reduction in episode duration.

Secondary objectives:

- To assess the effect of Dynamiclear™ on the progression to ulcerative vs non-ulcerative lesions.
- To assess the effect of Dynamiclear™ on the duration of wound healing.
- To assess the effect of Dynamiclear™ on pain, tenderness, tingling and itching during the disease course of HSL.
- To assess patient satisfaction of the Dynamiclear™ product.

Hypothesis

A single topical application of Dynamiclear™ has greater efficacy than placebo on the severity and duration of HSL

METHODS AND ANALYSIS

Study design

A prospective, randomised controlled, double-blind, multi-site trial to evaluate the efficacy of a single dose of topically applied Dynamiclear™ vs placebo on the duration and severity of HSL.

Sample size calculation

The sample size needed to demonstrate statistically and clinically significant efficacy was determined using the hypothesis that the mean episode duration in the treatment group will be 5.0 days, compared to 6.0 days in the placebo group. This is based on the review of previous studies of aciclovir vs. placebo by Harmenberg et al[1] and a clinically significant difference of one day in episode duration. Given an alpha of 0.05, power of 80% and an allocation ratio of 2:1 for treatment to placebo, 149 participants are required in the treatment group and 75 in the placebo group (224 in total). A recent study of kanuka honey vs aciclovir to treat cold sores, using the New Zealand Pharmacy based Research Network (PRN) for recruitment, reported an 11% drop out rate. However, because our study is placebo-controlled, we anticipate a higher dropout rate. Allowing for a 30% drop out rate, 292 participants are required: 194 in the treatment group and 98 in the control group. This sample size will also power the study to detect a moderate effect size on the secondary outcome measures (Cohen's d = 0.4).

Participants

A total of 292 participants aged 18 to 65 presenting to participating pharmacies within 48 hours of onset of prodromal symptoms of HSL and who report at least three prior episodes of HSL will be recruited. Inclusion and exclusion criteria are presented in Box 1.

A PRN in both New Zealand and Sydney, Australia will be used to register sites for primary recruitment, screening and assessment.

Study centres: 10-15 pharmacies in Sydney, Australia, and 20-25 pharmacies in New Zealand.

Patient and Public Involvement (PPI)

There were no funds or time allocated for PPI so we were unable to involve patients in the study design. We will invite patients at the conclusion of the study to provide feedback on the burden of the intervention, and patients will be involved in developing the dissemination strategies for our findings.

Recruitment and selection

Four parallel recruitment pathways are planned in this study. First, social media will be used to advise participants of the trial, and refer them to a webpage which outlines the inclusion and exclusion criteria and encourage them to present at participating pharmacies within 48 hours of the onset of a cold sore. Second, the New Zealand arm of the PRN has recently completed a different HSL study[18]. The 920 participants who joined that study and consented to being contacted for further studies will be sent an email informing them of this study. Third, all participating PRN pharmacies will have promotional material in their pharmacies and individuals presenting to these pharmacies with a cold sore recurrence within the past 48 hours will be invited to enrol in the study. Fourth, TrialFacts, a paid recruitment service, will be used to advertise and screen for potential participants. Recruitment will begin in June 2019.

Regardless of recruitment pathway, upon presentation at a participating pharmacy, potential participants will be screened and those eligible will be consented by pharmacists and pharmacy technicians. Recruitment staff will ensure that each participant is fully informed about the nature and objectives of the study and possible risks associated with participation.

Box 1: Inclusion and exclusion criteria

Inclusion criteria:

- Age 18-65 years
- Onset of prodromal or clinical symptoms of HSL in the past 48 hours
- Previous clinical history of HSL with at least 3 prior episodes of HSL
- Primary lesion within 1cm of the lip
- Willing to provide informed consent and adhere to study protocol
- Has internet (either by mobile or computer) to complete online forms.

Exclusion criteria:

- History of immunodeficiency, immunosuppression or autoimmune disorder
- Current infection not related to the study condition – e.g. infections such as cold or flu
- Use of other antiviral, anti-inflammatory or steroid medications during or within two weeks prior to the treatment period
- Use of other topical agents (including cosmetics, lip balms, sunscreens) or cosmetic procedures (such as chemical peels or microdermabrasion) on the prodromal or lesion area during the treatment period
- Mechanical disruption (e.g. scrubbing lancing or shaving) of the prodromal or lesion area during the treatment period
- Pregnancy, lactation or planning to become pregnant in the following 14 days
- Participation in another clinical trial within the previous 30 days.

Randomisation

After providing informed consent and satisfying the eligibility criteria, participants will be randomised in a 2:1 ratio to receive either active treatment with Dynamiclear™ (n=194) or the placebo control group (n=98) using the Castor EDC platform[19]. Randomisation will be performed using the Castor EDC platform by a researcher independent of the study team. To maintain pharmacist blinding when using a 2:1 ratio, boxes containing the intervention are pre-randomised, and pharmacists are provided with a box code rather than a group allocation. A 2:1 ratio in favour of the active intervention was chosen to maximise recruitment rates as potential participants are actively seeking treatment when attending the pharmacy. Thus, a greater chance of being allocated active treatment is likely to increase recruitment and retention rates. Permuted block randomisation, with a block size of 6 or 9 will be undertaken for each pharmacy to ensure that no patterns or imbalances emerge in group allocation between sites.

Blinding

All pharmacy staff, study team, participants and data analysists are blind to the group allocation. Unblinding will be permitted for either Dr Ee or Dr Semprini, or any other medical monitor, if serious adverse events are reported.

Study interventions

Interventional treatment is applied once only during the first pharmacy visit. Each participant will be allocated one vial of Dynamiclear™ or a placebo that matches Dynamiclear in colour, viscosity, taste and smell. The active and placebo vials are presented in identical packaging. The intervention or placebo will be applied via a cotton-tipped applicator which delivers 0.7mL to the affected area. This will be done either by the trained investigator or by the participant in view of the investigator, and the vial and packaging disposed of immediately. The moistened applicator will be applied for a minimum of 30 seconds, directly on the HSL lesion. No investigational product or packaging will leave the pharmacy site for any reason. Box 2 outlines the composition of both the interventional product and placebo.

Box 2a. Composition of interventional product (Dynamiclear™)

Active ingredients:

- Calendula Officinalis 1:2 liquid extract, 0.05 %w/w
- Hypericum Perforatum 1:2 liquid extract, 0.05 %w/w
- Copper sulphate pentahydrate, 6.4 %w/w

Excipient ingredients:

- Aloe vera
- Glycerol
- Vitamin E (Tocoheryl Acetate)
- Hydroxyethyl cellulose
- Polysorbate 80
- Purified water

Box 2b. Composition of placebo

Masking ingredients:

- Blue dye
- Yellow dye

Excipient ingredients:

- Aloe barbedensis
- Glycerin
- Vitamin E (Tocoheryl Acetate)
- Hydroxyethyl cellulose
- Polysorbate 80
- Purified water
- Sodium benzoate
- Citric acid anydrous (pH adjustor)

Data capture and monitoring

All data will be captured electronically via the secure electronic data capture platform CastorEDC [19]. Each pharmacy will be provided with an iPad (Apple Inc.) which will be used to collect all data during each face to face clinical visit. Signed consent forms will be digitally captured and uploaded directly onto the EDC platform.

Each participant will attend two clinical visits at PRN pharmacies. The first visit will be within 48 hours of symptom onset and will include initial clinical assessment and a single application of either the intervention or placebo in the presence of trained pharmacy staff. A second visit will be triggered when the participant indicates via their online symptom diary that they have returned to normal skin (Stage 7). This visit will involve confirmation of healing by pharmacists and checking for adverse events.

Participants will report symptoms and healing progression in an online daily diary (hosted on Castor EDC) which researchers will monitor for reports of adverse side effects. These diaries will also be used to monitor patient adherence to study requirements.

Participants will receive a follow-up phone call by a member of the research team two weeks after their final pharmacy visit to monitor any adverse events post treatment.

All data will be securely held on the study team database, accessible only by authorised investigators for the purposes of monitoring. The final dataset will be accessible by the coordinating investigators and each locality will retain on-site access to digital copies of source documentations with paper copies stored in secure archives.

Any changes to the study protocol are provided to the HREC and HDEC as per protocol, trial registries will be updated, and updated copies provided to trial sites. A copy of the consent form is included as Supplementary File 1.

Clinical assessments and patient surveys will be completed using Castor clinical trials management software. All the pre-specified outcome measures are outlined in Box 3.

Box 3: Outcome measures

Primary outcome measure:

- Median episode duration in days (participant evaluated)

Secondary outcome measures:

- Median episode duration in days (investigator-evaluated)
- Proportion (%) of lesions progressing to ulceration
- Median duration from wound development (stage 4) to healing (stage 7) in days (participant evaluated)
- Numeric rating scale (NRS) for pain, tenderness, tingling and itching during the symptomatic phase
- Proportion (%) of cases of HSV confirmed via swabs
- Adverse event rate
- Patient satisfaction and acceptability

A systematic review by Harmenberg[1], a recent mini review by Hull[13] and the most recent Cochrane systematic review protocol[20] outline a number of important outcome measures which informed the choices in Box 3. Median duration of the episode, as defined by the time to healing either by the loss of the hard crust in participants with ulcerative lesions or the time to normal skin (complete epithelialisation) in those with aborted lesions, either participant or clinician reported is the most common clinically relevant outcome measure. However, both Harmenberg and Hull argue that using duration of healing alone may miss important clinical effects, for example, if lesion duration is less but the size of the lesion is greater this may not indicate an improvement in the condition from the patient's perspective. The ulcerative stage of the lesion is the most painful and cosmetically bothersome stage of the lesion. Given the difference in disease severity between ulcerative and non-ulcerative lesions, the proportion of these lesions is commonly used as another important outcome measure, which may be of greater concern to HSL sufferers than a small reduction of episode duration often seen in topical treatment. Harmenberg compellingly argues that an important marker in placebo controlled trials is the proportion of participants that develop ulcerative lesions despite use of the study medication, as a reduction in progression to ulceration is of significant clinical benefit to sufferers.[1] Indeed a common feature of antiviral therapies is not a significant reduction in overall duration, but a shortening of the duration of time of wound healing[21], which is still a very important clinical benefit.

Finally, the painful symptoms of burning, tingling and itching are commonly found in participants with HSL, and are usually bothersome and therefore a reduction in either severity or duration of these is an important patient centric outcome.

Participant evaluated lesion stage

Participants will be provided with photo images as well as descriptions for each lesion stage via an online web form. We have used this lesion staging system in our previous trial[22]. Participants will be asked to grade the lesion once per day, upon waking, until return to normal skin occurs (Stage 7) or until 14 days from the first pharmacy visit. A reminder SMS and/or email will be sent each morning with an embedded link to the web form. This grading will be submitted via an online web form. When a participant grades their lesion having resolved and the area has returned to normal skin (Stage 7) via the online form they will be contacted to attend for assessment to their nearest study site within 24 hours by phone and email by the research assistant.

Investigator-evaluated lesion stage

Study participants will have their lesion stage graded by trained pharmacists and pharmacy technicians at two stages: (1) on the initial clinical visit for enrolment, and (2) once participants indicate that healing has occurred they will have their lesion stage confirmed by clinicians at a study site within 24 hours (see exceptions below). Investigators will be provided with photo images as well as descriptions for each lesion stage, identical to those provided to the participant. They will assess this independently of the participant's own grading.

Episode duration

The episode duration will be defined as follows: if no vesicle formed then duration was from the initiation of treatment to the return to normal skin with cessation of signs or symptoms (aborted lesion); if a vesicle formed, the duration of episode was measured from the initiation of treatment to the loss of hard crust (Stage 6: residual erythema could be present after loss of hard crust). Duration of wound healing will be determined by the time between the development of an Ulcer (Stage 4) to return to normal skin (Stage 7). This will provide an estimate of the interventions effect on the most cosmetically bothersome and painful stage of the episode.

Participant-graded pain and other symptoms

Participants will grade lesion pain once per day upon waking on a 0-10 numeric rating scale (NRS) from onset of symptoms until healing occurs, with 0 being no pain and 10 the worse pain imaginable. Symptoms of burning, itching and tingling will be measured using a separate 4-point ordinal scale for each symptom as follows: 0 = not present, 1 = mild, 2 = moderate, and 3 = severe. This score will be submitted via the online web portal along with lesion stage assessment.

Participant satisfaction questionnaire

At the second pharmacy visit, participants will be asked to rate their satisfaction with the treatment given using a Likert scale. Questions will include the likelihood of recommendation of the intervention to family and friends, interest in using the intervention again for future cold sores and ranking the intervention relative to other previous treatments they have used (if any) for cold sores.

Laboratory assessments

For confirmation of HSV infection, participants will use the swab kits provided at the initial clinical consultation to take swabs of lesions that reach an ulcerative stage (Stage 4) and do not have evidence of crusting (to avoid interference with the healing process). Samples will be taken by participants at home using a supplied dry swab that will be gently rubbed on the lesion in a circular motion for 5 seconds, then placed in a sterile outer tube, stored in their fridge (below 8 degrees Celsius) and shipped for viral culture to a central laboratory within 72 hours from sampling by courier. The presence of HSV will be diagnosed by the appearance of typical cytopathic effects. Isolates will be typed (HSV-1 and

HSV-2) by immunofluorescent antibody technique. Those participants who have non-ulcerative(or aborted) lesions will not provide swabs as removing fluid from these can delay healing.

Adverse events

Adverse events will be monitored via the daily online diary, during the second study visit and two weeks after second pharmacy visit.

Adverse events will be graded using the NCI Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE) and reported to the sponsor and HREC. After the end of study and locking of the study database, all adverse events will be summarised.

Data monitoring and stopping guidelines

Data will be monitored for completeness, plausibility and consistency to ensure the integrity and completeness of the data set. Any queries will be resolved by the Chief Investigator or delegated member of the study team. Adverse events will be regularly monitored via the online daily diary, as well as at the second pharmacy visit and at the conclusion of the two-week post trial period. Any serious adverse events will trigger an alert to the Chief Investigator.

Study timeline

The expected duration of the data collection phase of this study will be 12 months, with 14 time points where data will be collected. The schedule of events is outlined in Table 1.

Table 1. Timeline of treatment assessments and interventions

Procedure	Site visit 1	Daily via online diary	If lesion reaches stage 4	Site visit 2	Phone call two weeks after site visit 2
Written informed consent	X				
Inclusion and exclusion	X				
Medical history	X				
Randomisation	X				
Treatment or placebo applied	X				
First lesion occurs	X				
Clinical confirmation of lesion stage	X			X	
Participant online diary – lesion stage, pain, stage and other symptoms		X			
Antigen-typing of lesions (HSV1/HSV2)			X		
Participant questionnaire – subjective evaluation of product				X	
Adverse events recorded		X		X	X
Other treatments		X			

Statistical analysis of outcome data

Efficacy analyses will be performed for both the intent-to-treat (ITT) population (all participants) and per-protocol (PP) population (excluding participants with missing data). The analysis for the primary outcome of median duration of episode as reported by the participant, from the stage at presentation to the clinical site until healing and for the secondary outcomes of the median investigator rated duration, and median duration of wound development will all be analysed using a Mann-Whitney U-

test. Differences between groups in proportion of lesions progressing to ulceration, proportion of confirmed HSV ,adverse events rates and participant satisfaction rates will be compared using Chi-square tests.

Differences in NRS ratings for pain, tenderness, tingling and itching will be analysed using linear mixed model analysis of variance with group and time as fixed effects. In mixed model analysis subject will be used as a random effect. Secondary outcomes will include Cox proportional hazards for time to healing and time of wound duration and Kaplan-Meier survival plots. Multilevel analysis may be conducted with different factors including country, gender and number of previous episodes. Results will be documented with p-values and 95% confidence intervals. Data will be analysed using SAS and/or SPSS (v24) software.

Participant safety

The product has been listed as a listed medicine on the Australian Register of Therapeutic Goods (ARTG) (AUST L 241934).

The Australian Therapeutic Goods Administration (TGA) have approved the safety of all ingredients for use in complementary medicines in Australia. A detailed dose justification and safety profile with supporting literature for each of the components of Dynamiclear™ are detailed in Product Information provided by the Sponsor, Sci-Chem, in support of listing on the ARTG.

As the investigational product (Dynamiclear™) is a low-risk product already approved by the TGA and available for purchase, a formal data monitoring committee will not be established in Australia or New Zealand. However, the nominated medical representatives for the study, Dr Carolyn Ee, a registered General Practitioner in Australia, and Dr Alex Semprini, a registered medical practitioner, in New Zealand, will review safety on all adverse events. The Dynamiclear™ formulation is considered safe to apply topically at the dosages outlined, however individual components may irritate the skin in sensitive individuals. Copper sulfate, Calendula and Hypericum can all be irritating to the skin and mucosa in some individuals.

Methods for adverse event recording and reporting include the daily online diary in which asks participants to report any adverse events over the previous 24 hours. Adverse events are also recorded at site visit two by the pharmacist and any post trial events by a phone call two weeks following site visit two. All participants will also be provided with a digital emergency contact card with details of whom to contact in the case of an emergency.

Post-trial care

After the trial has been completed and data analysis undertaken, participants will be advised of their group allocations and the study results. If the intervention is found to be effective, those in the placebo group will be offered one free treatment of the interventional product. All participants will be advised of the availability of the name of the product and its availability to purchase over the counter if they wish to use it in future. Full indemnity insurance is in place for the study sponsor in the case of claims resulting from trial participation.

Ethics, study registration and dissemination

Australian ethics approval from Western Sydney University Human Research Ethics Committee, ref: H12776. New Zealand Ethics approval from The Health and Disability Ethics Committees (HDEC) ref: 18/CEN/151.The trial was prospectively registered with the Australia New Zealand Clinical Trials Registry: ACTRN12618000890235. The Universal Trial Number (UTN) is U1111-1233-2426. Results will be published in international academic journals and presented at conferences. Participating pharmacies will receive summarised results of the publication.

Collaborators: Members of the PRN in Australia (CBD Pharmacies Sydney, ChemistWorks Broadway, Priceline Pharmacy Oxford Street, Cincotta Discount Chemist Belrose, McFadden's Pharmacy St Ives, O'Loughlin's Medical Pharmacy St Ives, Warringah Mall Pharmacy, Donworth Pharmacy, MediADVICE Pharmacy Doonside, MediADVICE Pharmacy Glenmore Park, MediADVICE Pharmacy St Clair, Priceline Pharmacy The Ponds, Figtree Plaza Chemist)

Author Contributions: MA and AS designed and wrote the protocol, CE provided input related to medical components, MA and LM drafted the manuscript while AS, NS and CE provided critical edits and feedback on all versions of the manuscript. All authors reviewed the manuscript critically for important intellectual content, and approved the final manuscript for submission.

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Conflict of Interest: MA and AS received grant support from Sci-Chem International

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Consent Form

Project Title: The effect of a topical treatment containing *Hypericum perforatum* (St John's Wort), *Calendula officinalis* (calendula) and *copper sulfate* on oral herpes.

I hereby consent to participate in the above named research project.

I acknowledge that:

- I have read the participant information sheet (or where appropriate, have had it read to me) and have been given the opportunity to discuss the information and my involvement in the project with the research team.
- The procedures required for the project and the time involved have been explained to me, and any questions I have about the project have been answered to my satisfaction.

I consent to:

☐ *Providing data such as my age, gender and relevant medical history.*

☐ *Having a single vial of topical treatment applied to my cold sore at the pharmacy*

☐ *Coming back to the pharmacy within 24 hours of my lesion healing for visual confirmation*

☐ *If my lesion ulcerates, using a swab at home to take a sample and posting the sample in the envelope provided*

☐ *Providing daily information in an online diary*

I consent for my data and information provided to be used in this project and other related projects for an extended period of time.

Participation is entirely voluntary and you are not obliged to be involved. If you do participate you can withdraw at any time without giving reason.

I understand:

- that my involvement is confidential and that the information gained during the study may be published and stored for other research use but no information about me will be used in any way that reveals my identity but will only be used after additional ethical review.
- that I can withdraw from the study at any time without affecting my relationship with the researcher/s, and any organisations involved, now or in the future.

I would like to receive a summary of the study results when they are available.

Please tick: ☐Yes ☐ No

Participant to sign:

Signed: _____

Name: _____

Date: _____

Pharmacist/researcher to sign:

Signed: _____

Name: _____

Date: _____

This study has been approved by the Human Research Ethics Committee at Western Sydney University. The ethics reference number is H12776.

What if I have a complaint?

If you have any complaints or reservations about the ethical conduct of this research, you may contact the Ethics Committee through Research Engagement, Development and Innovation (REDI) on Tel +61 2 4736 0229 or email humanethics@westernsydney.edu.au.

Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym – **Pg 1**
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry **Pg 2**
	2b	All items from the World Health Organization Trial Registration Data Set **Pg 2 and Pg 10**
Protocol version	3	Date and version identifier **Pg 2**
Funding	4	Sources and types of financial, material, and other support **Pg 11**
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors **Pg 11**
	5b	Name and contact information for the trial sponsor **Pg 11**
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities **Pg 11**
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) **N/A**
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention **Pg 3-4**
	6b	Explanation for choice of comparators **Pg4**
Objectives	7	Specific objectives or hypotheses **Pg4**

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) **Pg5**
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained **Pg 5**
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) **Pg 6**
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered **Pg 6-7**
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) **N/A**
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) **N/A – single application done by pharmacist**
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial **Pg 6**
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended **Pg 7-9**
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) **Table 1**
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations **Pg 5**
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size **Pg 5**
Methods: Assignment of interventions (for controlled trials)		
Allocation:		

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions **Pg 6**
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned **Pg 6**
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions **Pg 6**
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how **Pg 6**
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial **Pg 6**
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol **Pg 7-9**
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols **Pg 10**
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol **Pg 9**
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol **Pg 10**
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) **N/A – not planned**
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) **Pg 10**

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed **Pg 10**
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial **Pg 9**
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct **Pg 10**
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor **Pg 9**

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval **Pg 11**
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) **Pg 8**
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) **Pg 5**
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable **N/A**
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial **Pg 8**
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site **Pg 11**
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators **Pg 8**
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation **Pg 10**

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions **Pg 11**
	31b	Authorship eligibility guidelines and any intended use of professional writers **Pg 11**
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code **Pg11**
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates **Appendix 1**
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.