Topical tripelennamine 1% cream versus diphenhydramine 1% cream for the relief of histamine-induced itching: A proof concept study in healthy adults.

Principal Investigator: Adwoa O. Nornoo, PhD

Sponsor: Adwoa O. Nornoo, PhD

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Summary of Changes from Previous Version:

Affected Section(s)	**Summary of Revisions Made**	**Rationale**
1.0 Protocol Summary 1.1 Synopsis	The study is extended from 6 months to 10 months.	Thirty-three subjects have been recruited to date. The target number is 34. The study duration is being extended to allow for more subjects to be recruited and to analyze the data.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

Investigators and clinical trial research assistants who are responsible for the conduct, management, or oversight of the clinical trial have completed Human Subjects Protection and ICH GCP Training (Faculty and Graduate Students – Behavioral, Social Sciences, Education through the CITI Program).

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form will be obtained before any participant is enrolled. Any amendment to the protocol will be submitted for review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

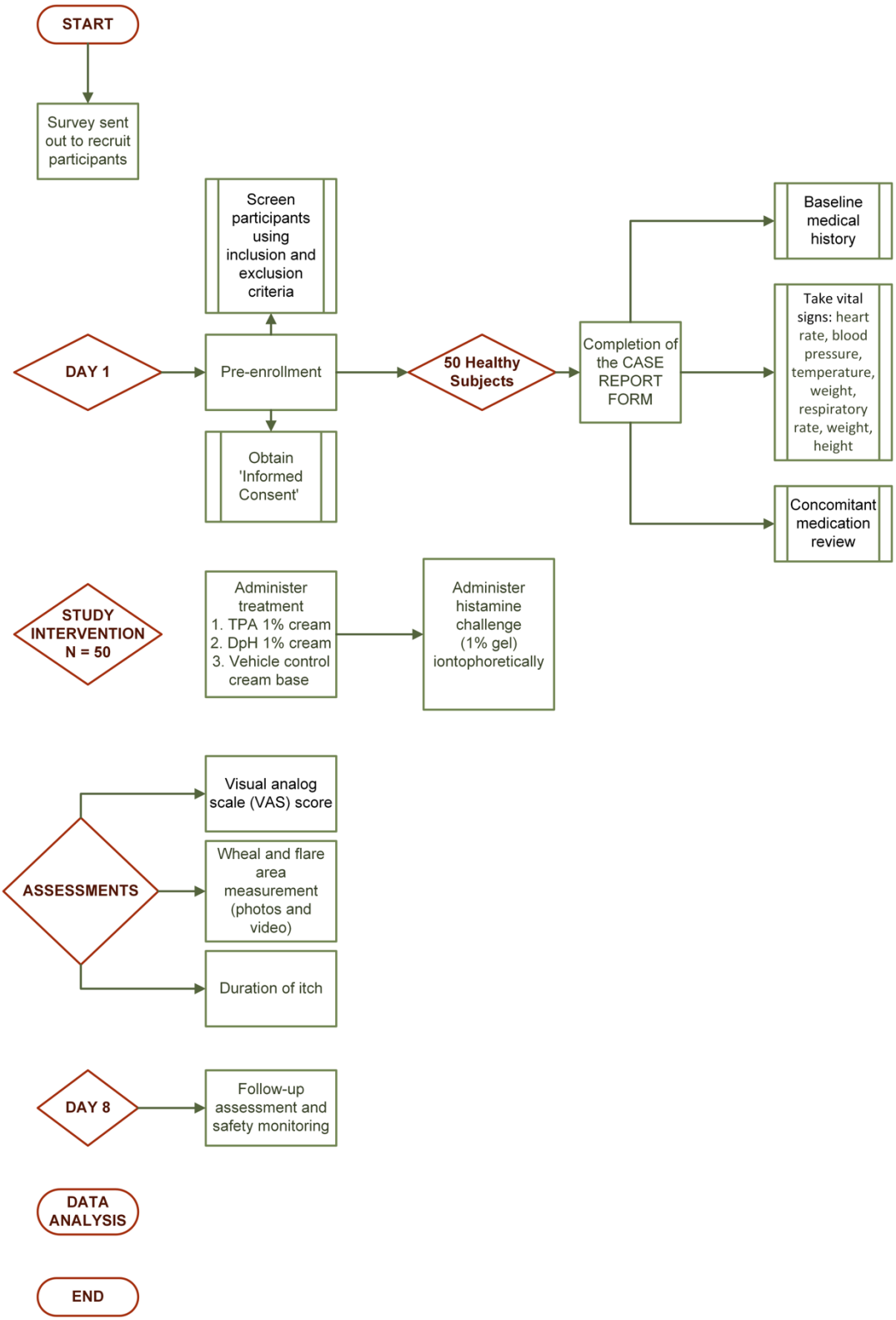
1.1 SYNOPSIS

Title:	Topical tripelennamine cream compared to diphenhydramine cream for the relief of histamine-induced itching: A proof of concept study in healthy adults.
Study Description:	There is an unmet need for effective topical anti-pruritic medications for acute itch, as currently there are only few over-the-counter topical drug products that have a direct effect on itch. The goal of this study is to determine the effectiveness of tripelennamine (TPA) 1% cream compared to diphenhydramine 1% cream to relieve histamine-induced itching using a visual analogue scale for pruritus (VAS). The antihistamines and vehicle control cream base will be applied to the skin and then the skin will be challenged with histamine delivered via iontophoresis. The hypothesis is that TPA with the histamine challenge will have a significantly lower sensation of itching when compared to diphenhydramine or vehicle control cream base.
Principal Investigator	Adwoa O. Nornoo, BPharm, MS, PhD
Co-Principal Investigator	Harm Maarsingh, PhD

Co-Investigator (Medical Director)	Jennifer Fleigelman, DNP, APRN, FNP-C, CNE
Primary Outcome	To determine the effectiveness of tripelennamine 1% cream compared to diphenhydramine 1% cream in relieving histamine-induced itching using a visual analogue scale for pruritus (VAS) as an endpoint.
Secondary Outcome	To determine the effectiveness of tripelennamine 1% cream compared to diphenhydramine 1% cream in relieving the histamine-induced triple response by measuring the following as endpoints: • The presence or absence of wheals. • Flare area expansion. • The duration of itch.
Trial Design	Single-center, double-blinded, vehicle-controlled randomized study
Trial Center	Lloyd L. Gregory School of Pharmacy, Palm Beach Atlantic University 1314 S Dixie Highway, West Palm Beach, FL 33401
Number of Subjects	Fifty (50) healthy subjects to reach a sample size of 36 subjects who complete the study.
Inclusion criteria	1. Healthy subjects 18-50 years old 2. Must be in general good health with no disease or physical conditions that would impair evaluation of itch and pain perception. 3. No history of chronic itch or pain. 4. Must abstain from the use of any systemic or topical antihistamine, steroid, or pain relief medications from the week prior to the study till the completion of the study. 5. Must abstain from the use of moisturizers on the arms 24 hours before study visit.
Exclusion criteria	1. Individuals under 18 or over 50 years of age. 2. Inability to complete the required measures. 3. The presence of an itchy skin disease. 4. Diagnosis of disease that would affect itch or pain perception (e.g. neuropathies). 5. Currently enrolled in any investigational study in which the subject is receiving any type of drug, biological, or non-drug therapy. 6. Use of oral, topical analgesics, or other medications known to interfere with itch or pain perception in the week prior to the study

	and throughout the study (e.g. antihistamines, anesthetics, opioids, neuroleptics, etc.). 7. Use of emollients on the arms a week prior to the study and throughout the study. 8. Known allergies to tripelennamine and/or diphenhydramine. 9. Pregnant women. (Women of childbearing potential will undergo an hCG pregnancy test).
Description of Study Intervention: trial drugs, dose, route of administration, frequency of administration	• Tripelennamine 1% topical cream, single 0.2g dose • Diphenhydramine 1% topical cream, single 0.2g dose • Vehicle control – cream base, single 0.2g dose Treatment will be followed by a histamine challenge. All treatment to be applied topically over a 16cm^2 area on the volar forearm on four locations: upper and lower part of left arm and upper and lower part of right arm.
Study Duration	10 months
Statistical analysis	Normality assessment will be tested by visual inspection and Sharpiro-Wilks tests. A repeated measure (between–within) ANOVA will be used for the primary outcome analysis and post hoc comparisons will be conducted using the Bonferroni correction in a manner similar to Dunnett's test.

1.2 SCHEMA


START
Survey sent out to recruit participants
Screen participants using inclusion and exclusion criteria
DAY 1
Pre-enrollment
50 Healthy Subjects
Completion of the CASE REPORT FORM
Baseline medical history
Take vital signs: heart rate, blood pressure, temperature, weight, respiratory rate, weight, height
Concomitant medication review
Obtain 'Informed Consent'
STUDY INTERVENTION N = 50
Administer treatment
1. TPA 1% cream
2. DpH 1% cream
3. Vehicle control cream base
Administer histamine challenge (1% gel) iontophoretically
ASSESSMENTS
Visual analog scale (VAS) score
Wheal and flare area measurement (photos and video)
Duration of itch
DAY 8
Follow-up assessment and safety monitoring
DATA ANALYSIS
END

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	**Timeline**
Advertisement for study participants and research assistants	1 month prior to study
Inclusion and exclusion criteria checklist (part of Informed Consent)	1 week prior to study
Pregnancy test (if applicable)	1 week prior to study
Informed consent signed	Study day
Complete Case Report Forms (CRFs) -Demographics -Vital signs -Concomitant medication review -Baseline medical history	Study day
Selection of test sites on the volar surface of two arms	Study day
Administer study intervention -Treatment application: TPA, DPH, vehicle control cream base -Histamine challenge	Study day
Measurements -VAS -Wheal/flare area: photos and videos -Duration of itch	Study day
Adverse event review and evaluation	1 week post study day
Data analysis	After study day for 1 month
Other assessments	

2 INTRODUCTION

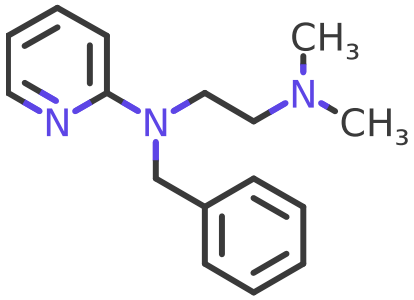
2.1 STUDY RATIONALE

There is an unmet need for effective topical anti-pruritic medications for acute itch, as currently there are only few over-the-counter topical drug products that have a direct effect on itch. The goal of this study is to determine the effectiveness of tripelennamine 1% cream compared to diphenhydramine 1% cream in relieving histamine-induced itching using a visual analogue scale for pruritus (VAS). The antihistamine creams and vehicle control cream base will be applied to the skin and then the skin will be challenged with histamine delivered via iontophoresis. The hypothesis is that TPA will have a significantly lower sensation of itching when compared to diphenhydramine or the vehicle control cream base.

2.2 BACKGROUND

Histamine is the main mast cell mediator released initially during allergic reactions. It is responsible for several allergy symptoms such as itching, sneezing, runny nose and watery eyes. If histamine is administered intradermally, an inflamed red spot appears within 15 seconds (due to local vasodilation), followed by the appearance of an erythematous flare around the red spot

within 1 minute (due to axon reflex-mediated vasodilation) and lastly a light wheal with palpable edema (due to increased capillary permeability) within a few minutes. This is known as the 'triple response of Lewis'. These responses, including pruritus, can be induced in non-atopic subjects making intradermal histamine administration a useful model to explore therapeutic actions of drugs affecting histamine (1). Massive release of various mast cell mediators in response to allergens (such as in severe peanut allergies) can lead to anaphylaxis, which may be fatal (2). These responses to histamine are mediated by binding to its specific cell surface H1 receptors in the skin. In the skin, histamine is released by the skin mast cells and basophil lymphocytes, and its well-known effects are itching, increase in skin blood perfusion and augmentation of microcapillary permeability (3). Itch is as a result of 2 separate pathways; histaminergic itch is transmitted by nociceptive mechano-insensitive C-fibres (CMi) responsive to histamine, while non-histaminergic itch is transmitted by polymodal nociceptive mechano-sensitive C-fibres (PmC) responsive to, for example, mucunain from cowhage (4). Histamine excites the subset of pruriceptive CMi-fibres expressing H1 and H4 receptors. These CMi-fibres terminate within and below the stratum granulosum of the skin's epidermis.



Tripelennamine hydrochloride (MW 299.36) competitively blocks peripheral histamine H1 receptors, thereby limiting histamine's effects, including bronchoconstriction, vasodilation, increased capillary permeability, and spasmodic contractions of gastrointestinal smooth muscle and allergic symptoms like itch. Tripelennamine (TPA) an aromatic amine is a first-generation histamine H1 receptor antagonist with a molecular weight of 255.36 g/mole and a logP = 3.3. Due to its tertiary amine structure, TPA crosses the blood-brain barrier and blocks central receptors. In addition, this agent binds and inhibits several other targets, including muscarinic cholinergic receptors, resulting in anticholinergic activity. Regarding the analgesic action of antihistamines such as TPA, there is evidence suggesting that histaminergic and serotoninergic central pathways are involved in nociception and that TPA can modulate their responses, thereby providing analgesic actions. Moreover, although not a mu-opioid receptor agonist by itself, TPA has been shown to potentiate the action of opioids, particularly in 'recreational use' (5). The main side effects of oral first-generation antihistamines include drowsiness/sedation (due to action on central H1- and M-receptors) and thickening of bronchial secretions, and typical anticholinergic responses such as dry mouth and palpitations (6).

The selectivity of TPA in blocking H1-receptor over other pharmacological targets (IC50-values/Ki-values) is as follows: ***H1 receptors***: IC50 = 40 nM (human H1, www.bindingdb.org); Ki = 7 nM (bovine H1, (7)) and 35 nM (rat, (8)) – this is >1,000-fold more selective compared to H2, H3 and H4 receptors making it a selective H1-antagonist (over other histamine receptors). ***Muscarinic receptors***: Ki = 1,300 nM (bovine, (7)), making TPA >100-fold selective for H1 compared the muscarinic receptors. In comparison, diphenhydramine only has a 20-fold selectivity in the same study, which is in line with data reported on bindingdb.org (Ki-values ranging from ~100 nM to ~260 nM for M1, M2, M3, M4 and M5-receptors vs ~15 for H1). Inhibition of the reuptake of serotonin (Ki: ~400 nM), dopamine (Ki: ~1.9 µM) and norepinephrine (Ki: ~1.7 µM), leading to increased levels and subsequent actions of these neurotransmitters. ***Sodium channels***: TPA has local anesthetic actions by inhibiting sodium channels, although the Ki is unknown (3)(4).

There are a few clinical trials testing the effectiveness of oral and topical TPA to relieve itching and pain. In a clinical trial involving mosquito bites, treatment with TPA 2% cream topically alleviated pruritus in 80% of cases of subjects with reaction to mosquito bites (9). In one study, TPA was administered intravenously, orally and topically to healthy subjects. TPA was able to block the effect of histamine introduced into the skin at different concentrations via electrophoresis in a delayed manner (10). In a head-to-head study with Benadryl® given orally in 241 patients (various skin diseases in a dermatology clinic), TPA had a superior antipruritic effect compared to Benadryl® with fewer side effects (11). Finally, TPA was amongst several antihistamines tested for suppression of pollen/mold-induced wheal suppression, duration of suppression and side effects. TPA ranked 3rd out of five in terms of wheal suppression, 2nd in terms of duration of wheal suppression and 2nd in terms of the least amount of side effects (12).

There is an unmet need for effective topical anti-pruritic medications for acute itch, as currently there are only few over-the-counter topical drug products that have a direct effect on itch. The goal of this study is to determine the effectiveness of tripelennamine 1% cream compared to diphenhydramine 1% cream to relieve histamine-induced itching using a visual analogue scale for pruritus (VAS). The antihistamines and vehicle control cream base will be applied to the skin and then the skin will be challenged with histamine delivered via iontophoresis. The hypothesis is that TPA will have a significantly lower sensation of itching when compared to diphenhydramine or vehicle control cream base.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Tripelennamine 1% Topical Cream
After oral administration, subjects may experience (6):

- slight to moderate drowsiness and thickening of bronchial secretions (>10%)
- headache, fatigue, nervousness, dizziness, appetite increase, weight increase, nausea (1-10%)
- diarrhea, abdominal pain, dry mouth, arthralgia, and pharyngitis (< 1%)
- edema, palpitations, hypotension, depression, sedation, paradoxical excitement, insomnia, angioedema, photosensitivity, rash, urinary retention, hepatitis, myalgia, paresthesia, tremor, blurred vision, bronchospasm, and epistaxis

Topical administration – data on adverse drug reactions to TPA is scarcely available. However, it is expected that the incidence and severity of adverse reactions to topical application of tripelennamine will be less than those reported for oral administration.

Diphenhydramine 1% Topical Cream (13)
After topical administration, subjects may experience mild urticaria and rash. Extremely rare severe adverse reactions include anaphylaxis and/or angioedema. Also, burning, crusting, or peeling of dry skin could occur at the site of application.

Histamine 1% Topical Gel
No specific serious risks or adverse effects are associated with the application of histamine to the skin. Histamine application will cause brief itching and tingling, with a typical triple response with the development of wheal and flare reactions. The wheal and flare response usually disappear about one hour after the histamine is applied to the skin. We do not expect a tachyphylaxis reaction or an additive effect from multiple histamine applications because we will apply only a

low dose once in the same site. Histamine iontophoresis does not cause any significant changes to blood pressure or heart rate in healthy study subjects (14). Subjects may experience a burning/tingling sensation due to iontophoresis. Other immediate adverse reactions include dizziness and headache (<10%) and heaviness of the lower limbs (<15%), whereas 24 hours post treatment some itching and wheals were still observed in a subset of patients.

In the event of an allergic reaction, the study will be stopped and appropriate treatment administered (use of an EpiPen®). Following the use of an EpiPen®, the subject will be referred to the emergency room and monitored by physicians.

3 OBJECTIVES AND ENDPOINTS

<table>
<tr><th>OBJECTIVES</th><th>ENDPOINTS</th><th>JUSTIFICATION FOR ENDPOINTS</th></tr>
<tr><td>Primary</td><td></td><td></td></tr>
<tr><td>The goal of this study is to qualitatively determine the effectiveness of tripelennamine 1% cream compared to diphenhydramine 1% cream in relieving histamine-induced itching.</td><td>A visual analog score will be used as the primary endpoint measure to assess intensity of itching induced by the iontophoretically delivered histamine (1% topical gel).

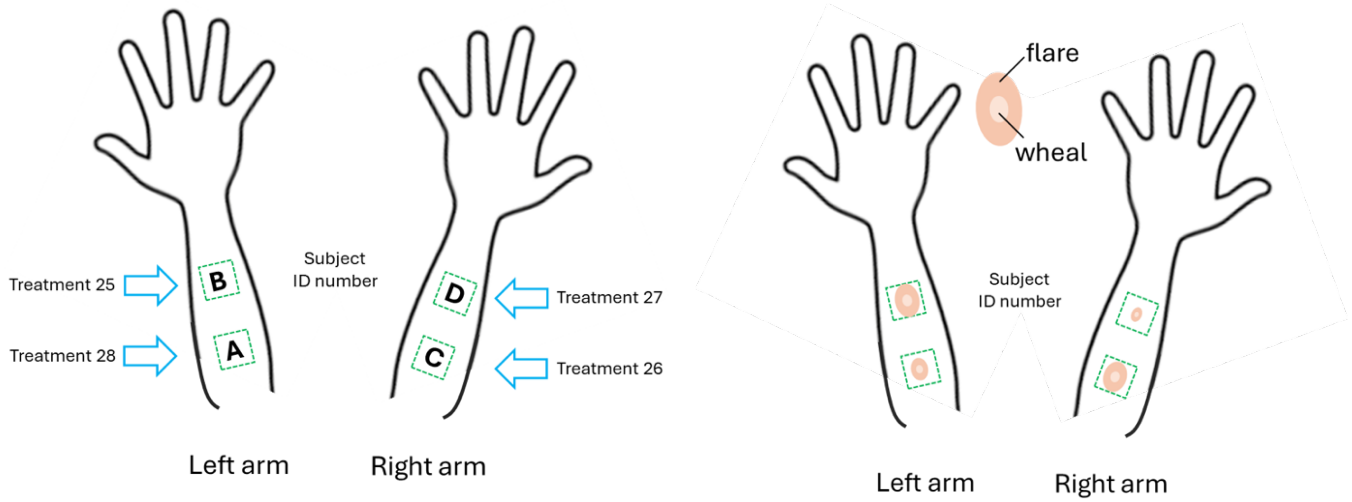
<table><tr><th>VAS scoring</th><th>Interpretation</th></tr><tr><td>0 points</td><td>No pruritus</td></tr><tr><td>1-3 points</td><td>Mild pruritus</td></tr><tr><td>4-6points</td><td>Moderate pruritus</td></tr><tr><td>7-8 points</td><td>Severe pruritus</td></tr><tr><td>≥9 points</td><td>Very severe pruritus</td></tr></table></td><td>The VAS score is a validated test for the assessment of pruritus and the potential effectiveness of treatments, such as antihistamines.</td></tr>
<tr><td>Secondary</td><td></td><td></td></tr>
<tr><td>The goal of this study is to quantitatively determine the effectiveness of tripelennamine 1% cream compared to diphenhydramine 1% cream in relieving histamine-induced itching.</td><td>The presence or absence of a wheal, flare expansion and the duration of itch will be used as a secondary end point measures. Photographs and videos will be taken at timed intervals and the flare areas measured by planimetry.</td><td>The wheal and flare are part of the triple response to histamine. The presence or absence of a wheal and the flare area over time are objective measurements for the response to histamine and the potential effectiveness of the treatments.</td></tr>
</table>

4 STUDY DESIGN

4.1 OVERALL DESIGN

There is an unmet need for effective topical anti-pruritic medications for acute itch, as currently there are only few over-the-counter topical drug products that have a direct effect on itch. The goal of this study is to determine the effectiveness of tripelennamine 1% cream compared to diphenhydramine 1% cream in relieving histamine-induced itching using a visual analogue scale for pruritus (VAS). The VAS is a 0–10 scale, with 0 weighted as “no itch” and 10 weighted as “worst itch imaginable”. The antihistamines and will be applied to the skin and then the skin will be challenged with histamine delivered via iontophoresis and compared to histamine iontophoresis when the vehicle control cream base is applied. The hypothesis is that TPA will have a significantly lower sensation of itching induced by histamine when compared to diphenhydramine or vehicle control cream base as measured using the VAS score test. This will be a single center, double-blinded, vehicle-controlled randomized study in healthy subjects. To detect medium effects of the treatments with a given *alpha* of 0.05 and an error probability of 0.05, with a power of 0.95, the number of participants needed is 50 (10 within post hoc pairwise comparisons).

Subjects who satisfy the inclusion and exclusion criteria are assigned a unique randomized Subject ID number upon enrollment. Subjects will participate in the study lasting approximately 3 hours. During the visit, 4 x 4 cm square test areas will be demarcated on the volar forearms, 2 on each arm, each separated by 4 cm. Each area is pretreated for 15 min with 0.2g of the topical cream formulations spread evenly over the test area with a spatula: TPA 1%, DPH 1% or vehicle control cream base. The vehicle control cream base will serve as a control for each of the antihistamine creams, hence will be placed on both arms to compare the response to histamine. The 4 treatment locations will be randomized (A-D) and will be recorded with the subject ID number (making sure that the vehicle control cream base is applied to each arm).



After 15 min of pretreatment on one arm, any remaining cream is wiped away and histamine will be applied to these sites. Measurements will be taken before proceeding to the next site on the other arm. Itch intensity will be assessed via a VAS and recorded periodically until all itch sensation has subsided for a maximum of 2 hours. Itch duration, the presence/absence of a wheal

and flare area expansion will also be recorded. Photographs and videos will be taken of the wheal and flare for area measurements using planimetry.

To induce acute histaminergic itch, iontophoresis of 0.2g of a 1% histamine topical gel will be placed in an anode delivery electrode and placed on the test area, while a cathode grounding electrode will be placed on the subject's arm at a certain distance (< 10 cm away) from the test area (but not in the test area). The iontophoresis device, a battery-powered stimulus isolation unit (ActivaDose II device, Activa Tek, Taiwan) that generates a current of no more than 500 µA for 60 s will be used.

4.2 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed the 3-hour study on only one day including the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3. The end of the study is defined as the completion of the last procedure shown in the SoA.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form.
2. Stated willingness to comply with all study procedures and availability for the duration of the study.
3. Male or female aged 18-50 years old.
4. In good general health as evidenced by medical history, with no disease or physical conditions that would impair evaluation of itch perception.
5. No history of chronic itch.
6. Must abstain from the use of any systemic or topical antihistamine, steroid, or pain relief medications from the week prior to the study till the completion of the study.
7. Must abstain from the use of moisturizers on the arms 24 hours before study visits.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Individuals under 18 or over 50 years of age.
2. Inability to complete the required measures.
3. The presence of an itchy skin disease.
4. Diagnosis of diseased that would affect itch or pain perception (e.g. neuropathies).
5. Currently enrolled in any investigational study in which the subject is receiving any type of drug, biological, or non-drug therapy.

6. Use of oral, topical analgesics, or other medications known to interfere with itch perception in the week prior to the study and throughout the study (e.g. antihistamines, anesthetics, opioids, neuroleptics, etc.).
7. Use of emollients on the arms a week prior to the study and throughout the study.
8. Known allergies to tripelennamine and diphenhydramine.
9. Pregnancy (all women will undergo an hCG pregnancy test).

5.3 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information will be required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information to be used includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

5.4 STRATEGIES FOR RECRUITMENT AND RETENTION

Subjects in this study will be recruited through appropriate IRB-approved flyer advertising around Palm Beach Atlantic University. The flyer shall be advertised in the Gregory School of Pharmacy's 'Queue' and Palm Beach Atlantic University's 'Wave'. Application links will be included in these flyers for participants to sign up (study participants). Research assistants will also be recruited through flyer advertising amongst Pharmacy and Nursing students. We anticipate most subjects will be pharmacy and nursing students, both populations are ethnically and racially diverse. Fifty subjects, likely mostly women (because of the discipline of their study major), will be recruited to reach a target minimal enrollment of 36. We anticipate an enrollment of 10-20 subjects per month. Study participants who complete the study, will be compensated with a $50 gift card at the completion of their participation.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Tripelennamine (TPA) 1% topical cream
Diphenhydramine (DpH) 1% topical cream
Vehicle control topical cream base
Histamine 1% topical gel

6.1.2 DOSING AND ADMINISTRATION

This will be a single center, double-blinded, vehicle-controlled randomized study in healthy subjects. To detect medium effects of the treatments with a given *alpha* of 0.5 and an error probability of 0.05, with a power of 0.95, the number of participants needed is 50 (10 within post hoc pairwise comparisons).

Subjects who satisfy the inclusion and exclusion criteria are assigned a unique Subject ID number upon enrollment. Subjects will participate in the study lasting approximately 3 hours. During the visit, 4 x 4 cm square test areas will be demarcated on the volar forearms, 2 on each arm, each separated by 4 cm. Each area is pretreated for 15 min with 0.2g of the topical cream formulations spread evenly over the test area with a spatula: TPA 1%, DPH 1% or vehicle control cream base. The vehicle control cream base will serve as a control for each of the antihistamine creams, hence will be placed on both arms to compare the response to histamine. The 4 treatment locations will be randomized (A-D) and will be recorded with the subject ID number (making sure that the vehicle control cream base is applied to each arm).

After 15 min of pretreatment on one arm, any remaining cream is wiped away and histamine will be applied to these sites. Measurements will be taken before proceeding to the next site on the other arm. Test areas will alternate between the left and right arm. Itch intensity will be assessed via a VAS and recorded periodically until all itch sensation has subsided for a maximum of 2 hours. Itch duration, the presence/absence of a wheal and flare area expansion will also be recorded. Photographs and videos will be taken of the wheal and flare for area measurements using planimetry.

To induce acute histaminergic itch, iontophoresis of 0.2g of a 1% histamine topical gel will be placed in an anode delivery electrode and placed on the test area, while a cathode grounding electrode will be placed on the subject's arm at a certain distance (< 10 cm away) from the test area (but not in the test area). The iontophoresis device, a battery-powered stimulus isolation unit (ActivaDose II device, Activa Tek, Taiwan) that generates a current of no more than 500 μA for 60 s will be used.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

Histamine 1% topical gel will be formulated in the Gregory School of Pharmacy in the 'Pharmaceutical analysis' laboratory (GH 212) by the principal investigator. A 'Master Compounding Formulation Record' will be completed. The coded and blinded formulations will be distributed by the research assistants to study participants.

Tripelennamine 1% topical cream will be supplied by the sponsor. Diphenhydramine 1% topical cream and the vehicle control cream base will be purchased from retail stores.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

A. Tripelennamine 1% Topical Cream – FOR INVESTIGATIONAL USE ONLY

Composition: Active ingredients – Tripelennamine HCL 1% Inactive ingredients - Acrylates/Acrylamide Copolymer, Allantoin, Aloe Barbadensis Leaf Extract, Caprylic/Capric Triglyceride, Cetyl Alcohol, Dimethicone, di-Alpha Tocopheryl Acetate, Ethylhexylglycerin Glycerin, Glyceryl Stearate, Mineral Oil, Panthenol, PEG 100 Stearate, Phenoxyethanol, Polysorbate 80, Polysorbate 85, Sodium Polyacrylate, Stearic Acid, Triethanolamine, Water

Appearance: soft white, slightly opaque cream.

Packaging: one ounce (28g) white opaque plastic cream tube. To be stored at 20°C to 25°C (68°F to 77°F)

Label: pbz OTC Pyribenzamine™ Powerful Itch Relief Antihistamine Cream (Kingsway Pharmaceuticals dba NFI Consumer Products)

For External Use Only
Store at room temperature
Do not use beyond: Month/Day/Year
Lot Number: xxxxxxx

B. Diphenhydramine 1% Topical Cream – FOR INVESTIGATIONAL USE ONLY

Composition: Active ingredients - Diphenhydramine hydrochloride 1%, Zinc acetate 0.1%
Inactive ingredients - Cetyl alcohol, diazolidinyl urea, glycerl stearate, methylparaben, PEG-100 sterate, Polyoxyl 40 stearate, propylene glycol, propyparaben, purified water.

Appearance: soft white, slightly opaque cream.

Packaging: 1 OZ (28g) white opaque plastic cream tube. To be stored at 20°C to 25C (68F to 77F)

Label: Itch Relief Cream – Topical Analgesic Skin Protectant (CVS Health™)

For External Use Only
Store at room temperature
Do not use beyond: Month/Day/Year
Lot Number: xxxxxxx

C. Vehicle Control Topical Cream Base – FOR INVESTIGATIONAL USE ONLY

Composition: Water, Glycerin, Caprylic/Capric Triglyceride, Cetyl Alcohol, Cetearyl Alcohol Dimethicone, Ceteareth-20, Stearyl Alcohol, Ceramide NP, Ceramide AP, Ceramide EOP, Beta-Sitosterol, Sucrose Stearate, Sucrose Distearate, Sodium Hyaluronate, Cholesterol, Polysorbate 20, Polyglyceryl-3 Diisostearate, Dipotassium Phosphate, Sodium Lauroyl Lactylate, Phenoxyethanol, Phytosphingosine, Caprylyl Glycol, Hexylene Glycol, Disodium EDTA, Carbomer, Xanthan Gum.

Appearance: soft white, slightly opaque cream.

Packaging: 12 FL OZ (355mL) white opaque plastic cream tube. To be stored at 20°C to 25C (68F to 77F)

Label: Moisturizing Lotion – For normal to dry skin (CVS Health™)

For External Use Only
Store at room temperature
Do not use beyond: Month/Day/Year

Lot Number: xxxxxxx

D. Histamine 1% Topical Gel - Gel Formulations For each 100g	
Ingredient	Amount (g)
Normal saline (0.9% w/v sodium chloride)	96.5
Histamine dihydrochloride	1.0
Hydroxymethyl propyl cellulose (HPMC)	2.5

Histamine dihydrochloride is dissolved in the normal saline. While mixing the HPMC is added slowly until the gel forms. The gel will be transferred to a tight, light resistant container and stored at 4°C in GH 212 in a fridge. Labelling will be as follows:

Histamine 1% Topical Gel – FOR INVESTIGATIONAL USE ONLY
For External Use Only
Store in the refrigerator
Do not use beyond: Month/Day/Year
Lot Number: xxxxxxx

6.2.3 PRODUCT STORAGE AND STABILITY

Beyond Use Dates - As per USP 795 standards on compounding with a water-containing base, ~~these~~ the histamine formulations has an expiration date of 30 days. A 'Beyond Use Date' will be placed on the label.

The manufactured products each have an expiration date of 2 years.

Storage conditions - The formulations will be stored at room temperature or refrigerated (4°C) in a locked laboratory in the Gregory School of Pharmacy (GH 212) that only study personnel will have access with a key or card. Ambient temperature and min/max of the laboratory will be recorded using a HOBO® MX temp/RH logger. Logs of formulation delivery and use per subject will be kept.

6.2.4 PREPARATION

See Section 6.2.2

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

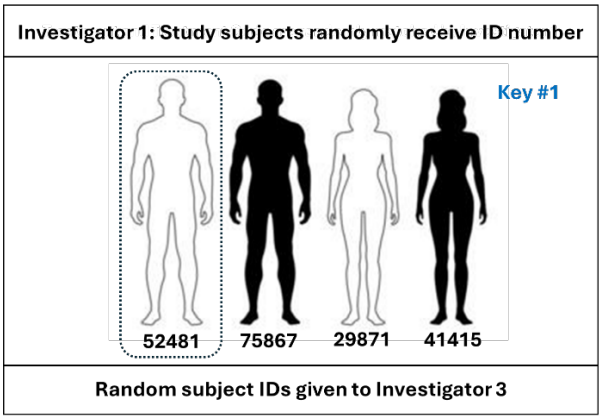
Study subjects will receive a randomly generated Subject ID number upon enrollment. This number will be used on all subsequent study forms (Informed Consent, Case Report Form, etc.) to de-identify the study subjects at the end of the study. Bias is prevented as there is only one study group, and all study subjects will receive the same 3 treatments at the same time on the 4 skin areas on the two volar forearms, so there is no need to randomize the subjects into different groups. The vehicle control cream base will be placed on both arms.

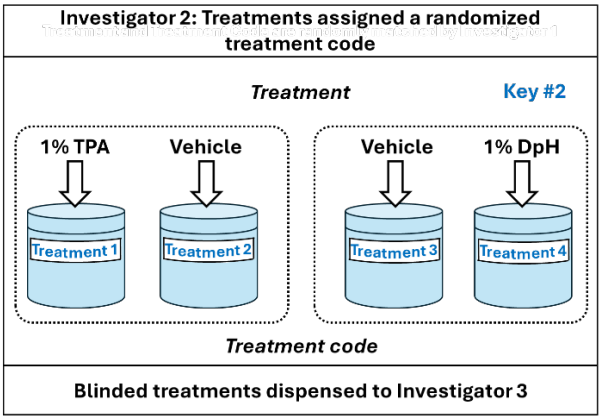
The 3 treatments include:
- Tripelennamine 1% topical cream, single 0.2g dose
- Diphenhydramine 1% topical cream, single 0.2g dose

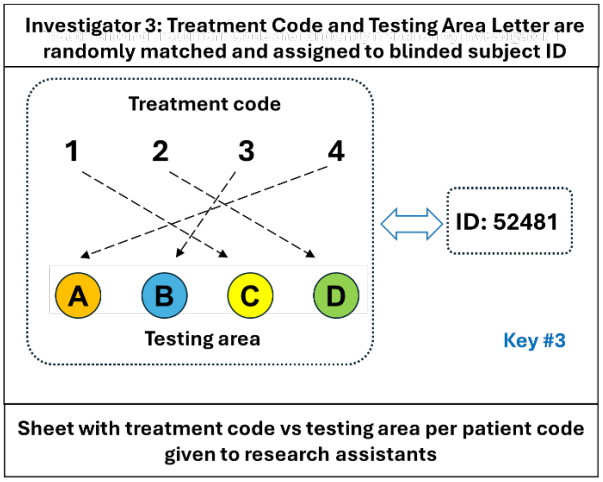
- Vehicle control cream base, single 0.2g dose

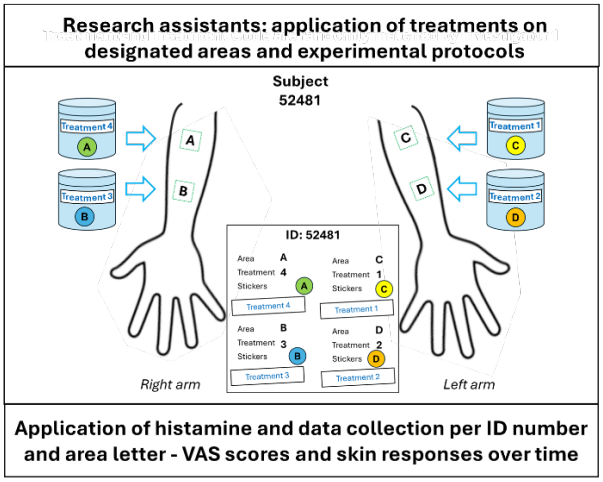
Randomization of the treatments over the 4 testing areas (2 on each forearm, vehicle control cream base will be on both arms) will occur as follows:

- To blind the treatments, each treatment will be given a unique sequential number ('treatment code') that will be randomly assigned in sets of 4 by Investigator 1 (see diagram). All treatments will look identical because the same cream base will be used for all.
- Investigator 3 will randomly assign the blinded 'treatment codes' in sets of 2 to each of the 4 testing areas (A-D) using an online random combination generator (commentpicker.com/combination-generator). These 'treatment codes' will be linked to the Qualtrics generated Subject ID numbers.
- Research assistants will receive 4 containers that are labeled with the treatment codes and a list that shows which number should be applied to what testing area on each subject. Treatments will always be applied in the same order on the different testing area, alternating between the left and right arm (order A to C to B to D). Upon application, the sticker with the treatment number will be transferred from the container to the table under the appropriate area letter and assigned 'treatment code' to ensure that treatments have been properly applied to the assigned areas.
- Histamine iontophoresis will always be applied 15 minutes after the application of the cream. There will be at least a 30-minute interval between each histamine iontophoresis.









Only the principal investigator (PI) will know what actual treatment is linked to which 'treatment code' (key #1) and will keep this information in a file that is not accessible to the other investigators during the time of the study. The PI is blinded for what Subject ID will receive what treatment. A list matching the name of the subject to the Subject ID number will not be accessible to the other investigators during the time of the study (key #2). The co-investigator (Co-I) will record the medical history and vital signs of the subjects electronically that only display the de-identified Subject ID. Only the subjects that meet the inclusion/exclusion criteria will receive a Subject ID and be enrolled in the study to receive the randomized treatments that will be given to the co-principal investigator (Co-PI).

The Co-PI will randomly assign the blinded 'treatment codes' to the 4 different testing areas. Only the Co-PI will know the blinding of the 'treatment codes' over the testing areas and will assign this to a Subject ID (key #3). This information is not accessible to the other investigators during the time of the study (key #3). The Co-PI is blinded to what treatments match what treatment codes.

Research assistants will record the VAS scores and the photos/videos of the skin for each of the testing areas in forms/files that display the Subject ID and the testing area. All analysis of responses will be performed on blinded data. The keys to the different 'treatment codes' (key #1), testing areas and Subject ID (key #3) will only be revealed at the completion of the analysis of the VAS scores and the skin reactions to unblind the data set and to allow for statistical analysis. Interim analysis of all data to determine if the study has reached statistical significance with adequate power, will occur when 36 study subjects have completed the study. If this is the case, no further study subjects will be included.

None of the investigators involved will have all the information needed to unblind the data. All the pretreatment areas will remain blinded to all. Breaking of the blind prior to completion of the study may occur earlier only when more severe skin reactions compared to vehicle control cream base are observed. Since tripelennamine and diphenhydramine are antihistamines, lower rather than higher skin reactions are expected compared to the vehicle control cream base. The research assistants will notify the PI of each instance that a study subject experiences a VAS score that is 3 points higher than the vehicle control cream base area for at least 5 timepoints. When this has been reported for a sixth study subject, the PI will pause the study – any instance where histamine iontophoresis has already been applied, recordings will be completed. When treatments have already been applied, but histamine iontophoresis has not yet started, the test will be stopped, and the subjects will not receive any histamine iontophoresis. The PI, the Co-PI and the Co-I will meet, and the codes can only be broken when at least two of them agree that the criteria for breaking the blinding have been met.

6.4 STUDY INTERVENTION COMPLIANCE

All study research assistants will be trained on the procedures of the study. The PI and Co-PI will monitor adherence to all protocols.

6.5 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications and supplements.

Study treatments are for a local effect only. Restricted medications have been listed in the exclusion criteria.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

If a subject experiences an adverse event assessed at ≥ Grade 2 according to CTCAE v5, the subject will not receive additional treatments and will be followed until the adverse event resolves or stabilizes. Any new clinically relevant finding will be reported as an adverse event (AE). Subjects discontinued due to an AE will be followed until the AE is resolved, a reasonable explanation is provided for the event, or the subject is referred to his/her own primary medical doctor. The specific AE in question will be recorded on the appropriate source document.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

The following medical and other reasons justify a premature termination (by subject or investigator) of the investigational treatments:

- Withdrawal of informed consent
- SAEs
- Patient's request
- Investigator's judgment

If a subject withdraws from the study, all efforts will be made to complete a final evaluation, if possible, including the reason for withdrawal. Withdrawals due to non-attendance or non-compliance must be followed up to obtain the reason for non-attendance or non-compliance. Withdrawals due to intercurrent illness or adverse events (AEs) will be fully documented with supplementary information where available and appropriate. Subjects discontinued due to an AE will be followed until the AE is resolved, a reasonable explanation is provided for the event, or the subject is referred to his/her own primary medical doctor. The specific AE in question will be recorded on the appropriate source document.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

Subjects in this study will be recruited through appropriate IRB-approved flyer advertising around Palm Beach Atlantic University. Flyers shall be advertised in the Gregory School of Pharmacy's 'Queue' and Palm Beach Atlantic University's 'Wave'.

Subjects will be asked to participate in the study for approximately a 3-hour long visit. All subjects shall receive written and verbal information concerning the clinical trial. This information will emphasize that participation in the clinical trial is voluntary and that the subject may withdraw from the clinical trial at any time and for any reason. All subjects will be given an opportunity to

ask questions and will be given sufficient time to consider before consenting. The signed and dated informed consent to participate in the clinical trial will be obtained prior to any clinical trial related procedure being carried out. Information on prior and concomitant medications will be collected during the study visit. A history of prior medications in the past week will be collected for any systemic or topical antihistamine, steroid, or pain relief therapies (i.e. any medications known to interfere with itch perception). These will also be collected as concomitant medications.

Following a signed informed consent, obtained in the presence of the investigator or designee, the inclusion and exclusion criteria (including pregnancy status) will be confirmed, and vitals (weight, respiratory rate, heart rate, temperature, blood pressure) will be taken. The screening will be performed the same day as the treatment.

This will be a single center, double-blinded, vehicle-controlled randomized study in healthy subjects to test the efficacy of the tripelennamine 1% topical cream compared to diphenhydramine 1% topical cream for relieving histamine-induced itch. To detect medium effects of the treatments with a given α of 0.5 and an error probability of 0.05, with a power of 0.95, the number of participants needed is 36 (10 within post hoc pairwise comparisons). Fifty study subjects will be recruited to account for any experimental failure (withdrawal of consent before all conditions have been studied, non-responders to histamine challenge, withdrawal due to AEs, decision by the investigators' team to discontinue the study for a study subject)

Four 4 x 4 cm^2 test areas will be mapped on the subject's volar forearms (two squares on the left arm and two squares on the right arm). These squares will be separated from each other by 4 cm. Only one ~~two~~ area~~s~~ will be tested at a time to allow for adequate VAS scoring. There will be at least a 30-minute wait period between the iontophoresis of one area and the start of the histamine iontophoresis in the next area. Testing will alternate between the left and right arm. The placement of the treatments in these test areas will be randomized, with each arm having a vehicle control cream base. Each test area will be pretreated by applying 0.2g of test cream (with a spatula) of either one of the test formulations (1% TPA; 1% DpH) and the vehicle cream base control to the skin area based on the randomization scheme. The treatment will be allowed to permeate the skin for 15 minutes prior to any other study procedure commencing at the test site. Fifteen minutes after pretreatment, the area will be wiped clean with a dry lab wipe and itch will be induced by iontophoresis of 0.2g of a histamine 1% topical gel. The gel will be placed in an anode delivery electrode placed on the test area, while a cathode grounding electrode is placed on the subject's arm at a certain distance (at least 10 cm away) from the test area (but not in the test area). The iontophoresis device, a battery- powered stimulus isolation unit (*ActivaDose II device, Activa Tek, Taiwan*) generates a current of no more than 500 μA for 60 sec. Subjects will rate their itch intensity every 60 seconds until the itch sensation has completely subsided using a visual analog scale (VAS), from 0 (no itch) to 10 (most unbearable itch). Study subjects will be instructed to report any adverse events up to one week after the study is completed.

The investigator(s) will make the following assessments on each patient during the study:
1. Itch intensity and duration after histamine itch induction – VAS score

2. Presence and severity of wheal formation (1-mild; 2-moderate; 3-severe) and area measurement using planimetry of the flare. Video will be recorded and photos taken aid in the taking these measurements.

If a subject experiences an adverse event assessed at ≥ Grade 2 according to CTCAE v5, the subject will not receive additional treatments and will be followed until the adverse event resolves or stabilizes. Study stoppage will occur if 36 subjects complete the study or if a severe adverse event occurs. Interim analysis will be used to analyze data for statistical significance and power. If study reaches significance with adequate power, the study may be concluded before 36 subjects complete the study.

8.2 SAFETY AND OTHER ASSESSMENTS

Safety and tolerability assessments will consist of ongoing monitoring and recording of all adverse (AE) and serious adverse event (SAE) reports, the regular monitoring of signs and symptoms of cutaneous irritation, and regular measurements of vital signs. Safety assessments will consist of monitoring and recording protocol-defined adverse events (AEs) and serious adverse events (SAEs) and other protocol-specified tests that are deemed critical to the safety evaluation of the trial creams.

Local and systemic adverse events will be monitored during the study. Dermatologic evaluations for rash, erythema (outside of administration area), urticaria, eruptions, and epidermal necrolysis will be performed throughout the study visit. Anaphylaxis and hypersensitivity (erythema multiforme) will also be monitored. The PI is responsible for identifying, documenting and reporting adverse events to the IRB.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

An adverse event is the development of an undesirable medical condition, or the deterioration of a pre-existing medical condition following or during exposure to a medicinal product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (e.g., chest pain), signs (e.g., tachycardia), or the abnormal results of an investigation (e.g., laboratory findings).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

The definitions of serious adverse events (SAEs) are given below. The PI is responsible for ensuring that all staff involved in the trial are familiar with the content of this section. An SAE or reaction is defined as any untoward medical occurrence that: results in death, is immediately life-threatening, requires at least a 24-hour inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect. The definition of SAE also includes any important medical event. Medical and scientific judgment should be exercised in deciding whether expedited reporting is

appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the previous definition. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse. Hospitalization during the trial for a pre-planned surgical or medical procedure (one which was planned prior to entry in the trial), does not require reporting as a serious adverse event.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

If a subject experiences an adverse event assessed at ≥ Grade 2 according to CTCAE v5, the subject will not receive additional treatments and will be followed until the adverse event resolves or stabilizes.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

A pre-existing medical condition is one that is present at the start of the trial. Such conditions will be recorded on in the case report form (CRF). A pre-existing medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens during the trial. When recording such events on an AE log, it is important to convey the concept that the pre-existing condition has changed by including applicable descriptors. The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate source document. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study will be be documented appropriately regardless of relationship. Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. Changes in the severity of

an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. The medical director (Co-I) will record all reportable events with start dates occurring any time after informed consent is obtained.

8.3.5 ADVERSE EVENT REPORTING

All adverse events of any patient during the course of the trial will be reported in the source document, and the medical director (Co-I) will give their opinion as to the relationship of the adverse event to trial drug treatment (i.e., whether the event is related or unrelated to trial drug administration). If the adverse event is serious and related to the drug treatment, it will be reported within 24 hours to the IRB as per PBA policy. Other untoward events occurring in the framework of a clinical trial are also to be recorded as AEs (i.e., AEs that occur prior to assignment of trial treatment that are related to a protocol-mandated intervention, including invasive procedures such as biopsies, medication washout, or no treatment run-in).

All AEs, regardless of seriousness or relationship to trial treatment, spanning from the start of trial treatment until study completion are to be recorded in the source document. If an abnormal vital sign is associated with clinical signs and/or symptoms, the sign or symptom should be reported as an AE, and the associated vital sign should be considered additional information that must be collected on the relevant source document.

All adverse events resulting in discontinuation from the trial will be followed until resolution or stabilization. Subjects will be followed for AEs for 7 calendar days after discontinuation or completion of protocol-specific treatment. All new AEs occurring during this period will be reported and followed until resolution unless, in the opinion of the investigator, these values are not likely to improve because of the underlying disease. In this case, the medical director (Co-I) must record their reasoning for this decision in the subject's medical record and as a comment on the source document.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

It is important to distinguish between "serious" and "severe" adverse events, as the terms are not synonymous. Severity is a measure of intensity; however, an AE of severe intensity need not necessarily be considered serious. For example, nausea which persists for several hours may be considered severe nausea but may not be considered a SAE. On the other hand, a stroke which results in only a limited degree of disability may be considered only a mild stroke but would be considered a SAE. Severity and seriousness will be independently assessed when recording AEs on the CRF and SAEs on the appropriate source document.

Adverse events classified by the medical director (Co-I) as serious require documentation and reporting to the IRB according to PBA policies and procedures in order to comply with regulatory requirements. Serious adverse events may occur at any time from the signing of the informed consent form start of trial treatment through the 7-day follow-up period after the last trial treatment. To report a SAE, the source document should be completed with the necessary information. All SAEs and medically confirmed deaths (regardless of causality assessment) occurring on trial treatment or within 7 days of last trial treatment must be reported on the source document and followed until resolution (with autopsy report if applicable). The medical director (Co-I) will report SAEs and follow-up information to the IRB according PBA policies.

Investigators will use correct medical terminology/concepts when recording AEs or SAEs on the SAE and AE logs. All colloquialisms and abbreviations will be avoided. All AEs, including those that meet SAE reporting criteria, should be recorded on the source document and be reported accordingly.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Primary and Secondary Efficacy Endpoint(s): Normality assessment will be tested by visual inspection and Sharpiro-Wilks tests. A repeated measure (between–within) ANOVA will be used for the primary endpoint and post hoc comparisons will be conducted using the Bonferroni correction in a manner similar to Dunnett's test.

9.2 SAMPLE SIZE DETERMINATION

To detect medium effects of the treatments with a given alpha of 0.5 and an error probability of 0.05, with a power of 0.95, the number of participants needed is 36 (10 within post hoc pairwise comparisons). Normality assessment will be tested by visual inspection and Sharpiro-Wilks tests. A repeated measure (between–within) ANOVA will be used and post hoc comparisons will be conducted using the Bonferroni correction in a manner similar to Dunnett's test.

9.3 POPULATIONS FOR ANALYSES

The analysis data set will include:

- Intention-to-Treat (ITT) Analysis Dataset (i.e., all subjects)

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

The VAS scores over time will be grouped per treatment condition (vehicle control cream base, tripelennamine1%, and diphenhydramine1%). Differences in VAS scores (itch intensity) and duration of itch between the different treatments will be determined. Similarly, differences in area of the flare and the presence and severity of wheal reactions between the 3 treatment groups will be determined. The measurements for the vehicle control cream will be set as 100%.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

- Primary Efficacy Endpoint(s): Normality assessment will be tested by visual inspection and Sharpiro-Wilks tests. A repeated measure (between–within) ANOVA will be used and post hoc comparisons will be conducted using the Bonferroni correction in a manner similar to Dunnett's test.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

- Secondary Efficacy Endpoint(s): Normality assessment will be tested by visual inspection and Sharpiro-Wilks tests. A repeated measure (between–within) ANOVA will be used and post hoc comparisons will be conducted using the Bonferroni correction in a manner similar to Dunnett's test.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to administering study intervention. The following consent materials are submitted with this protocol:

- Electronic Informed Consent Form

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB) approved, and the participant will be asked to review and sign the document (electronically and a paper copy provided if requested) the day of the study in Gregory Hall 212, Palm Beach Atlantic University, WPB, FL 33401. The PI (Adwoa O. Nornoo, PhD) or Co-PI (Harm Maarsingh, PhD) will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be sent electronically to the participants for their records. The informed consent process will be conducted and documented in survey sent by Qualtrics and signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that their grades in any particular course (for students) or staff evaluation forms (for faculty or staff) WILL NOT be affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, and the IRB. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor and IRB.

Discontinuation from Trial Treatment

If a subject experiences an adverse event assessed at ≥ Grade 2 according to CTCAE v5, the subject will not receive additional treatments and will be followed until the adverse event resolves or stabilizes.

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor. All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records for the participants in this study. The clinical study site will permit access to such records. The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored in 21 CFR Part 11 compliant data storage platform. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique Subject ID. All de-identified study

data (paper and electronic) will be collected on study source documents and logs. All de-identified paper data, including records of subjects, source documents, and will be kept in the Gregory School of Pharmacy under lock for 6 years after the completion of the study. All electronic records will be stored in a 21 CFR Part 11 compliant data storage platform available to the PI and Co-PI for a period of 6 years after the completion of the study and then deleted.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

De-identified data collected for this study will be analyzed and stored in a 21 CFR Part 11 compliant data storage platform that is password protected. A confidentiality statement regarding data collected will be included in the informed consent. When the study is completed, access to study data and/or samples will be available through a 21 CFR Part 11 compliant data storage platform (password protected).

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	**Co-Principal Investigator**	**Co-Investigator (Medical Director)**
Adwoa O. Nornoo, BPharm, MS, PhD	Harm Maarsingh, MS, PhD	Jennifer Fleigelman, DNP, ARNP, FNP-C, CNE
Chair and Professor, Department of Pharmaceutical Sciences	Professor of Pharmaceutical Sciences	Assistant Professor of Nursing
Palm Beach Atlantic University	Palm Beach Atlantic University	Palm Beach Atlantic University
901 S Flagler Drive, West Palm Beach, FL 33401	901 S Flagler Drive, West Palm Beach, FL 33401	901 S Flagler Drive, West Palm Beach, FL 33401
561-803-2739	561-803-2746	561-803-2837
adwoa_nornoo@pba.edu	harm_maarsingh@pba.edu	jennifer_fleigelman@pba.edu

10.1.6 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical trial site will perform internal quality management of study conduct, data and documentation and completion. An individualized quality management plan will be developed to describe a site's quality management. Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated for clarification/resolution. Those monitoring will verify that the clinical trial is conducted, and data are generated, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)). The investigational site will provide direct access to all trial related source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.7 DATA HANDLING AND RECORD KEEPING

10.1.7.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

The clinical trial site shall maintain appropriate medical and research records for this trial, in compliance with ICH GCP and IRB requirements for the protection of confidentiality of participants. Data collection (VAS scores, wheal/flare measurement and duration of itch) is the responsibility of the research assistants under the supervision of the PIs. The Co-I (Medical Director) is responsible for collecting protected health information (electronic Case Report Form, eCRF) that is de-identified with a unique Subject ID generated by Qualtrics. The PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data collected. All source documents will be completed electronically to ensure accurate interpretation of data. Data recorded in the eCRF derived from source documents should be consistent with the data recorded on the source documents. Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) will be entered into a survey electronically (Qualtrics a 21 CFR Part 11-compliant data capture system). The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

10.1.7.2 STUDY RECORDS RETENTION

All de-identified study data (paper and electronic) will be collected on study source documents and logs. All de-identified paper data, including records of subjects, source documents will be kept in the Gregory School of Pharmacy under lock for 6 years after the completion of the study. All electronic records will be stored in a 21 CFR Part 11 compliant data storage platform available to the PI for a period of 6 years after the completion of the study and then deleted.

10.1.8 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study research assistants. As a result of deviations, corrective actions will be developed and implemented promptly. It will be the responsibility of the PI to use continuous vigilance to identify and report deviations to the IRB. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The PI is responsible for knowing and adhering to the reviewing IRB requirements.

10.1.9 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

All information provided regarding the trial, as well as all information collected/documented during the course of the trial, will be regarded as confidential. The PIs and Co-I will publish the results of the trial.

10.1.10 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest. The 'Conflict of Interest Disclosure Form for Clinical Trial Investigators' will be completed prior to trial participation from all PIs and Co-I who are involved in the trial.

10.2 ABBREVIATIONS

AE	Adverse Event
ANOVA	Analysis of Variance
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
CRF	Case Report Form
CTCAE v5	Common Terminology Criteria for Adverse Events version 5
DpH	Diphenhydramine
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
GH	Gregory Hall
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
hCG	Human Chorionic Gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
HPMC	Hydroxymethyl propyl cellulose
ICH	International Conference on Harmonization
IRB	Institutional Review Board
ISO	International Organization for Standardization
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SDS	Safety Data Sheet
SOA	Schedule of Activities

TPA	Tripelennamine
US	United States
VAS	Visual Analog Scale

10.3 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
1	01/17/2025	NA	NA
2	02/11/2025	The treatments will be tested sequentially, instead of simultaneously as previously stated.	Sequential treatment testing will allow for a more accurate determination of the VAS scores for each treatment. Assessing the itch of two different sites simultaneously may not be feasible, as the stronger itch in one area may make it harder to adequately assess the itch in the area that itches less. Therefore, treatments followed by histamine iontophoresis will alternate between the left and right arm.
3	2/21/2025	Sponsor to supply tripelennamine 1%w/w cream. Diphenhydramine and vehicle control cream base obtained from retail stores.	A more therapeutically relevant comparison would be to compare the final marketed tripelennamine cream product against comparable products of diphenhydramine cream on the market.
4	8/22/2025	The study is extended from 6 months to 10 months.	Thirty-three subjects have been recruited to date. The target number is 34. The study duration is being extended to allow for more subjects to be recruited and to analyze the data.

11 REFERENCES

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12 APPENDICES

Adverse Event Tracking Log 

Informed Consent 

Recruitment Flyer 



Case Report Form