Clinical Study Report

Controlled study to investigate the efficacy of the insect bite healer BR 60 against pruritus and swelling after mosquito bites (MüPruritus)

Kontrollierte Studie zur Untersuchung der Wirksamkeit des Insektenstichheilers BR 60 gegen Pruritus und Schwellung nach Moskitostichen (MüPruritus)

Short Title	Insektenstichheiler BR 60 gegen Pruritus und Schwellung
Protokoll Nr. DRKS Nr.	202304 DRKS00033004 (8.11.23)
Version	2.0b
Date	11.6.2024
Investigator	M. Sc. Sergej Sperling
Sponsor	Prof. Dr. med. Thomas Kurscheid
Data Analysis	Prof. Dr. Martin Sieber

Signature Investigator 

Signature Sponsor 

Signature Data Analysis 

INSTITUT PROF. KURSCHEID

CSR MÜPRURITUS

1. Summary

Insect bite-related pruritus is a prevalent health concern globally. The objective of this clinical study was to evaluate the effectiveness of the Beurer Insect Bite Healer (technical type BR 60) in mitigating the itching sensation following a mosquito bite.

The research was structured as an open-label, single-arm trial, focusing on the effectiveness of the BR 60 device in alleviating pruritus, and assessing its safety by monitoring swelling and redness for up to 60 minutes post-bite. The primary endpoint was the differential in pruritus severity on a 10-point Visual Analog Scale (VAS) over one hour, comparing responses to bites from the Yellow fever mosquito (Aedes aegypti) and the Southern house mosquito (Culex quinquefasciatus).

Efficacy and safety were gauged using two distinct laboratory-reared mosquito strains by comparing intraindividual VAS scores for pruritus, swelling, and redness between untreated control bites and those treated with the BR 60 heat pen.

22 subjects were enrolled, 15 females (68%) and 7 males (32%), with an average age of 27.95 years (SD = 5.81). The mean Body Mass Index (BMI) was 22.51 (SD 3.33.) A consistent reduction in pruritus was observed across species and at all time points. The primary endpoint was achieved, with a statistically significant decrease in itch intensity in the responder analysis set (RAS) due to the treatment observed within the first 60 minutes ($p<0.01$). Control group bites resulted in an average itch exposure (AUC) of 161 (SD = 72) over 60 minutes, while treated bites had a substantially lower average AUC of 97 (SD = 57). All participants who responded exhibited lower pruritus scores in the treated bites compared to the untreated bites. Notably, at the 2-minute mark post-bite, the mean pruritus score for control bites was 4.30 VAS, which stood in stark contrast to the mean score of 2.40 observed in the treated bites. In 61% of the observations the treated bites achieved at least a 50% reduction in pruritus at the first measurement point compared to the non-treated bites, 49% of the observations showed that the treated bites had at least a 2-point lower pruritus score than the non-treated bites at the first measurement point compared (2 min) to the non-treated bites.

. Those results potentially support the BR 60 device's potential for providing prompt symptomatic relief and for potentially preventing the chronicity of pruritus and subsequent dermatological complications.

The BR 60 was found to be well-tolerated, with no adverse events reported throughout the study. This safety profile was corroborated by the absence of significant differences in erythema and redness between the control and treated bites.

While the open-label design of the study necessitates consideration of a potential placebo effect, the definitive and consistent pattern of the results suggests that the observed benefits are attributable to the therapeutic action of the Beurer Insect Bite Healer BR 60 rather than any psychological expectation.

2. Synopsis

Title	Controlled study to investigate the effectiveness of the insect bite healer BR 60 against pruritus and swelling after mosquito bites
Short title	Insect bite healer BR 60 against pruritus and swelling
Study Objectives	Investigation of the efficacy of the insect bite healer BR 60 against pruritus and swelling after mosquito bites
Testing	Insect bite healer BR 60 for mosquito bites using laboratory strains of • Yellow fever mosquito (Aedes aegypti) and • southern house mosquito (Culex quinquefasciatus)
Indication	Insect bite
Design studies	Unblinded, controlled (2 timely stings; one sting is treated, one sting is not treated)
Study duration	1 hour per patient
Inclusion criteria	• Consent to the study • >18 years • Knowledge about your own reaction to insect bites • Willingness to be bitten twice by the two mosquito laboratory strains
Exclusion criteria	• Allergies to insect bites • Acute skin diseases in the region • Acute illnesses (especially with a tendency to thromboembolic diseases and malignant neoplasms) • Chronic pain conditions that have not been clarified, regardless of the body region • Diabetes • Sensory disorder with reduced pain sensation (e.g. metabolic disorder)
Cancellation criteria	Unbearable pain (then cooling of the skin area)
Number of patients	22 (each stung by A. aegypti, and by the southern house mosquito (laboratory strains)) (2 subjects were replaced due to no pruritus response to the bites)
Primary endpoint	Difference in pruritus severity between treated and untreated prick on a 10-point VAS score over 1 hour (AUC)
Secondary endpoint	• Difference in **pruritus** severity between treated and untreated bites on a 10-point VAS score over 1 hour (AUC) Yellow fever mosquito (Aedes aegypti) or southern house mosquito (Culex quinquefasciatus) (10 patients each) • Difference in **pruritus** severity between treated and untreated bites on a 10-point VAS score at time points 2, 7, 11, 15, 40, 60 minutes (AUC) for all mosquitoes and each individual mosquito

	• Difference in **swelling** between treated and untreated stitch on a 10 point VAS score over 1 hour (AUC) • Difference in **redness** between treated and untreated sting on a 10-point VAS score over 1 hour (AUC) Photo documentation (use in PI/IC) & Comments Participants (use in PI/IC)

2.1. Timetable of activities

Schedule of activities: Inclusion in the study at the beginning before the insect bite. The questionnaire asks about the assessment of pruritus, swelling and redness of the sting site of untreated and treated stings before and after application of the insect bite healer (BR 60) at the time points 0, 2, 7, 11, 15, 40 and 60 minutes after the sting.

Start of study		**Study realisation**					
		2 min.	7 Min.	11 min.	15 Min.	40 Min.	60 min.
Screening visit	**Stitch**	**Application BR 60**					
Study clarification		Application questionnaire (pruritus, swelling, redness) (time 2, 7, 11, 15, 40, 60 min.)					
Declaration of consent							
Inclusion and exclusion criteria							
Screening questionnaire **Study questionnaire**		**Study questionnaire**					

3. ABBREVIATIONS

AE Adverse Event
AUC Area Under the Curve
BfArM Federal Institute for Drugs and Medical Devices
BR 60 Beurer Insect Bite Healer BR 60
BMI Body Mass Index
CRF Case Report Form
VAS Visual Analog Scale

4. Introduction and Rational of the Study

The aim of this study was to test the effectiveness of the "Beurer Insect Bite Healer BR 60" (BR 60) in relieving pruritus after insect bites.
Pruritus or itching describes the predominant symptom of acute or chronic skin diseases. 1. Pruritus, redness and swelling are pathological changes of the skin as a result of local reactions of the immune system to invading exogenous factors, caused for example by an insect bite or sting. The strength of the immune reaction to the sting varies depending on the quality of the injected secretion and the degree of sensitization of the patient. 2. Cooling the sting site with cooling pads or essential oils such as menthol can lead to a temporary improvement, but rarely to complete relief of the symptom. Antihistamines or corticosteroids are also used for treatment 1. These are associated with side effects such as tiredness, drowsiness or headaches if given orally. For this reason, many sufferers also look for alternative therapies to get their skin reaction to insect bites under control. One non-drug therapy option is the application of heat to the affected skin area. The application of heat has a positive effect on the immune response and has been shown to relieve pain. 3.The BR 60 product is designed to relieve itching and swelling caused by insect bites or stings by generating heat (local hyperthermia). 4. The device has 2 different application times (3 or 6 seconds), which can be selected depending on the sensitivity of the skin. The device is pressed onto the puncture site and activated by pressing a button. The ceramic heating plate then heats up to 50 °C ± 2 °C. The aim of this study is to determine the effectiveness of the "Beurer Insect Bite Healer (technical type BR 60)" by means of an intervention study.

5. Aim of the Study

5.1. STUDY OBJECTIVES

The objective of the study was to investigate the efficacy of the "Beurer Insect Bite Healer BR 60" medical device in terms of relieving pruritus and swelling. The following endpoints were investigated:

5.1.1. Primary Endpoint

The primary endpoint of the study was the difference in pruritus intensity between treated and untreated bites on a 10-point Pruritus Visual analog scale (VAS)score over 1 hour (AUC).

5.1.2. Secondary Endpoints

Secondary endpoints included:

- Difference in pruritus intensity between treated and untreated bites on a 10-point VAS score over 1 hour (AUC) for Yellow fever mosquito (Aedes aegypti) and Southern house mosquito (Culex quinquefasciatus)
- Difference in pruritus intensity between treated and untreated bites on a 10-point VAS score at times 2, 7, 11, 15, 40, 60 minutes (AUC) for all mosquitoes and each mosquito species individually
- Difference in swelling between treated and untreated bites on a 10-point VAS score over 1 hour (AUC)
- Difference in redness between treated and untreated bites on a 10-point VAS score over 1 hour (AUC)

6. STUDY DESIGN

6.1. Study Design Summary

The study was an interventional, unblinded, monocentric, single-arm trial. Due to the heat development of the BR 60 and the associated difficulty of blinding participants, the study was conducted unblinded. It was assumed that the assessment of swelling and erythema would not be subject to the placebo effect. The study duration was approximately 1 hour per patient. The study was considered completed when the data of the last patient had been recorded.

6.2. Study Population

The study population consisted of 20 individuals with pruritus and swelling as a reaction to insect bites.

6.3. Inclusion Criteria

Participants could only take part in the study if the following criteria were met:

- Willingness to participate in the study
- Older than 18 years
- Knowledge of own reaction to insect bites
- Willingness to be bitten twice by laboratory strains of mosquitoes

6.4. Exclusion Criteria

Participants were excluded from the study if one or more of the following points applied to them:

- Allergies to insect bites
- Acute skin diseases in the region
- Acute diseases (especially those prone to thromboembolic diseases and malignant neoplasms)
- Unexplained chronic pain conditions regardless of body region
- Diabetes
- Sensory disturbances with reduced pain perception (e.g., metabolic disorders)

6.5. Patient Recruitment

Patient recruitment was carried out in Regensburg. The mosquito bites were conducted by the experienced laboratory head of Biogents AG, who was also available for queries. 22 Patients were recruited to the study between the 03.12.23 – 22.12.23.

6.6. Informed Consent

All participant personally signed the latest version 1.2 (22.08.23) of the consent form before any study-specific procedures of any kind were carried out. The details from the consent form and participant information were presented to the participants both orally and in writing by the investigator. The written consent was declared through a dated signature by the participating person and by the person who presented the consent form and obtained the signature. A copy of the signed consent form was to be handed to the participant. The original of the signed consent form remained at the study site in Regensburg.

6.7. Study Procedures

Written consent was obtained before conducting any study-specific procedures. The screening questionnaire was used to check whether the patient met all inclusion criteria and exhibited no exclusion criteria. Suitable patients could participate in the study and were assigned a study number. They received the "Beurer Insect Bite Healer BR 60" device and questionnaires (CRF) for self-completion. Photodocumentation of the skin area to be examined was carried out at times 0, 2, 7, 11, 15, 40, and 60 minutes.

6.8. Study Responsibilities

Sponsor	Prof. Dr. Thomas Kurscheid Goethestraße 29a 50968 Köln
Funding	Firma Beurer GmbH (Manufacturer) Söflinger Str. 218 89077 Ulm
Investigator	M. Sc. Sergej Sperling (Labor Head) Biogents AG Weissenburgstr. 22 93055 Regensburg
CRO	Biogents AG Weissenburgstr. 22 93055 Regensburg

Study Documentation	Prof. Dr. Sieber Hochschule Bonn-Rhein-Sieg von Liebig Straße 20 53359 Rheinbach
Data Analysis & Report Writing	Prof. Dr. Sieber Hochschule Bonn-Rhein-Sieg von Liebig Straße 20 53359 Rheinbach

6.9. TREATMENT OF STUDY PARTICIPANTS

Due to the heat development of the BR 60 and the associated difficulty of blinding participants, the study was conducted unblinded. The application of the BR 60 was performed by the participants themselves at the site (Biogents AG, Weissenburgerstr. 22, 93055 Regensburg) according to the instructions for use. Initially, participants were bitten under controlled conditions by two mosquitoes of different laboratory strains, the yellow fever mosquito (Aedes aegypti) and the Southern house mosquito (Culex quinquefasciatus), respectively.

6.10. STATISTICS

The number of study participants was determined based on the following estimates: In the literature, comparable medical devices report an average reduction of pain by approximately 4 points on the VAS score with a standard deviation of 5,2 points on the VAS. We consider a difference of a decrease of 1,2 points or less on the VAS pain score in such a study to be not significant. The significance level is set at 0.05, and the power at 90%. For sample size calculation using a two-sided paired t-test was used concluding a sample size of 20 participants. With a dropout rate (drop out) of 10%, the sample size needed for the study is set to include 22 patients. Two data sets were used for the analysis. Two Analysis sets were defined:

(i) the **full analysis set (ITT),** including all experimental treatments including 22 Patients and 44 observations , or

(ii) **the pruritus responder analysis set (RAS)** 21 Patients and 41 observations (3 observations in which observations showed no pruritus in the control or treated areas were excluded

The area under the curve (AUC) was calculated with the trapezoid rule $((VAS_n+VAS_{n+1})/2*(T_{n+1}-T_n))$ in the text we refer to “AUC” in short for the Unit “Pruritus*60min” with the help of Excel for Mac Version 16.81 (Microsoft, WA, USA). Statistical analysis was performed using Excel for Mac Version 16.81 (Microsoft, WA, USA). ChatGPT Version 4 and DEEPLE were used as writing assistants.

7. Ethical Considerations and Study Approval

The ethical approval for the study was sought from the Ethical Committee at the Bavarian Chamber of Physicians (Bayerische Landesärztekammer). Following the submission of the application on September 18, 2023, the committee granted a favorable opinion on October 24, 2023, file number 23040 fs/al. (Formularnummer: 00072856
DMIDS Antragsnummer: 00014704)

Subsequently, the study was communicated to the Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte, BfArM) in accordance with Article 74, Paragraph 1 of the Regulation (EU) 2017/745. The filing, under the file number 94.2.13-5660-14704, was completed on October 27, 2023.

The study was officially registered with the German Clinical Trials Register (Deutsches Register Klinischer Studien, DRKS) on November 8, 2023, under the identifier DRKS00033004. Detailed information about the study can be accessed through the DRKS website at DRKS - Trial DRKS00033004

8. Funding

The study was funded by the product's manufacturer, Beurer GmbH.

9. Patient Population & Demographics:

A total of 22 patients and 44 observations were included in the study (ITT), 15 of whom were female and 7 male. This corresponds to a ratio of approximately 68% female to 32% male participants.
In average, participants were 27,95 years old with a standard deviation of 5,81 years. The youngest participant was 20 years old, and the oldest was 41 years old. The mean height of the study population was 1,73 meters with a standard deviation of 0.10 meters. The shortest participant measured 1,60 meters in height, while the tallest reached 1,93 meters. Regarding body mass, the average weight across all patients was 67,55 kilograms, accompanied by a standard deviation of 11,26 kilograms. The weight ranged from 53 kilograms as the lightest to 90 kilograms as the heaviest participant. The average Body Mass Index (BMI) was calculated to be 22,51, with a standard deviation of 3,33. The minimum and maximum BMI values observed in the study were 17.36 and 29,39, respectively.

	All	Female	Male
Participants	22	15	7
	100%	68%	32%
Average Age (years)	27,95	27,40	29,14
Minimum Age (years)	20.00	20.00	23,00
Maximum Age (years)	41,00	41,00	36,00

	All	Female	Male
Standard Deviation of Age (years)	5,81	5,60	6.52
Average Height (m)	1,73	1,68	1,84
Minimum Height (m)	1,60	1,60	1,75
Maximum Height (m)	1,93	1,81	1,93
Standard Deviation of Height (m)	0.10	0.06	0.06
Average Weight (kg)	67,55	63,87	75,43
Minimum Weight (kg)	53,00	53,00	60.00
Maximum Weight (kg)	90.00	80.00	90.00
Standard Deviation of Weight (kg)	11,26	10.13	9,90
Average BMI	22,51	22,54	22,43
Minimum BMI	17,36	17,36	16,79
Maximum BMI	29,39	26,34	29,39
Standard Deviation of BMI	3,33	3,21	3,83

Table 1: Demographic Overview:This table represents the average physical parameters of the participants segregated by gender, which is a fundamental part of the demographic profile in a study report. The counts reflect the number of participants in each category, while the mean and standard deviation provide insights into the central tendency and dispersion of weight, height, and BMI within each gender group.

For the female participants:
Among the female participants, the mean age was about 27 years with a standard deviation of 5,6 years, with the youngest participant being 20 years old and the oldest 41 years old. The average height was 1,68 meters with a standard deviation of 0.06 meters. The heights ranged from 1,60 to 1,81 meters. The average weight was 63,87 kilograms with a standard deviation of 10.13 kilograms and varied between 53 and 80 kilograms. The average BMI was calculated to be 22,54, with a standard deviation of 3,21 and a range of 17,36 to 26,34.
For the male participants:
The group of male participants included 7 individuals, corresponding to a mean age of 29,14 years with a standard deviation of 6.52 years. The age of the male participants varied from 23 to 38 years. The average height was 1,84 meters with a standard deviation of 0.06 meters and a height range of 1,75 to 1,93 meters. The average weight was found to be 75,43 kilograms, with a standard deviation of 9,90 kilograms and weights between 60 and 90 kilograms. The average BMI for men was 22,43, with a standard deviation of 3,83 and a range of 16,79 to 29,39,
The study population predominantly consisted of young individuals, with an overall average age of 27,95 years, indicating a relatively youthful cohort. The participants' average weight and height fell within normal ranges, with an average BMI indicative of a generally healthy population. Notably, the average BMI of 22,51 across all participants reflects a group well within the standard parameters for a normal body weight status.

10. Results

10.1. Efficacy Outcomes

10.1.1. Primary Endpoint: The primary endpoint of the study was the difference in pruritus intensity between treated and untreated bites on a 10-point VAS score over 1 hour (AUC).

The differences in the exposure to pruritus after stinging with both mosquito species was observed between the control bites and the treated bites. The control bites had an average itch exposure (AUC) of 161 (SD = 72) Pruritis*60 min over 60 minutes, whereas the treated bites showed a significantly lower average AUC of 97 (SD = 57), indicating a significant decrease in itch intensity due to the treatment (p<0,005) (RAS). All participants (n=21) showing a response showed a nominal lower pruritus score AUC in the treated bites vs. the untreated bites. The average decrease in itch scores between the control and treated bites was 64, suggesting the treatment's effectiveness in reducing itch severity over the observed period (Figure 1).

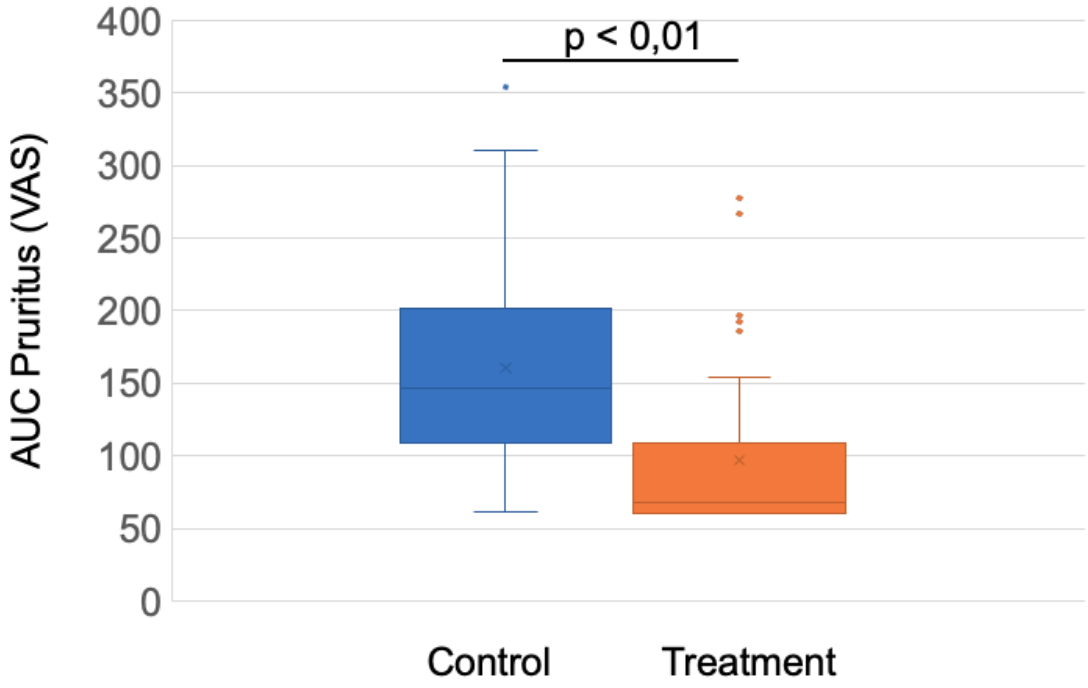

Figure 1: Boxplot Comparison of exposure to itch scores over a 60-minute period (AUC) between control (blue) and Beurer Insect Bite Healer BR 60 treatment (orange) bites, measured on a 10-point VAS following a mosquito bite by either yellow fever mosquito (Southern house mosquito (Culex quinquefasciatus) and (Aedes aegypti) The central box represents the interquartile range (IQR), the line inside the box marks the median, and the 'whiskers' extend to the most extreme non-outlier data points. A total of 41 comparisons are shown (RAS). 3 observations were excluded due to missing pruritus response of the respective subject.

10.1.2. Secondary Efficacy Outcome

*10.1.2.1. Relative Reduction of treatment vs control Difference in pruritus intensity between treated and untreated bites on a 10-point VAS score over 60 min (Pruritus*60 min (AUC) for Yellow fever mosquito (Aedes aegypti) and Southern house mosquito (Culex quinquefasciatus)*

The pruritus exposure or AUC (Pruritus*60 min) after a bite from *Culex quinquefasciatus*: For the control bites, the average pruritus score was 158, with a maximum reported score of 300 and a minimum of 76 and a standard deviation of 61 in the RAS population. The average pruritus score was 150, with a maximum reported score of 300 and a minimum of 60 a standard deviation of 65 in the ITT population.

In the treated bites, which received intervention with the insect bite healer BR 60, the average score was significantly lower ($p<0,01$): the average pruritus score was 92, with a maximum reported score of 195 and a minimum of 50 and a standard deviation of 49 in the RAS population. The average pruritus score was 88, with a maximum reported score of 195 and a minimum of 60 a standard deviation of 48 in the ITT population (Figure 2, Table 3)

The pruritus exposure or AUC after a bite from *Aedes aegypti*: For the control bites, the average pruritus score was 163, with a maximum reported score of 355 and a minimum of 62 and a standard deviation of 83 in the RAS population. The average pruritus score was 158, with a maximum reported score of 355 and a minimum of 60 a standard deviation of 84 in the ITT population.

In the treated bites, which received intervention with the insect bite healer BR 60, the average score was significantly lower ($p<0,005$) after a bite from *Aedes aegypti*: the average pruritus score was 101, with a maximum reported score of 278 and a minimum of 60 and a standard deviation of 65 in the RAS population. The average pruritus score was 99, with a maximum reported score of 278 and a minimum of 60 a standard deviation of 64 in the ITT population (Figure 2, Table 4)

In both mosquito bites resulted in significantly less pruritus exposure after treatment than the control bite. No difference in the effect of the insect bite healer BR 60 between the bites of both mosquito species.

This comparison suggests that the treatment with an insect bite healer BR 60 was associated with a lower average pruritus score, which implies an improvement in symptoms compared to the control bites. The reduction in both the average pruritus scores and the range of scores in the treated bites highlights the potential effectiveness of the insect bite healer BR 60 in managing the discomfort caused by mosquito bites.

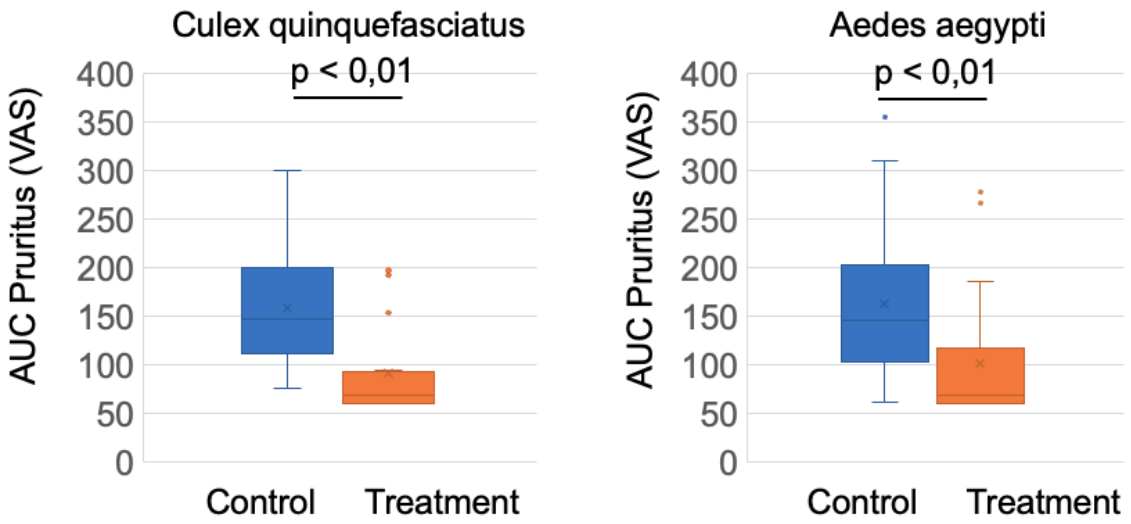

Figure 2: Boxplot comparison of exposure itch scores over a 60-minute period (AUC) between control and treated bites, measured on a 10-point VAS after bites with Southern house mosquito (Culex quinquefasciatus) (left) and Yellow fever mosquito (Aedes aegypti) (right). The bites were treated with insect bite healer BR 60 (orange) or served as control (untreated) (blue The central box represents the interquartile range (IQR), the line inside the box marks the median, and the 'whiskers' extend to the most extreme non-outlier data points. Results for responders to stinging are given (RAS) .

10.1.2.2. Relative Reduction of treatment vs control

As aforementioned, the comparisons between the treatment and control bites, a reduction in pruritus was observed in the treated bites after the application of the insect bite healer BR 60. This trend was consistent for both *Culex quinquefasciatus* and Aedes aegypti mosquito bites. The treatment consistently mitigated the intensity of pruritus as evidenced by the lower VAS scores at each time point post-exposure, compared to the control bites which received no such intervention.

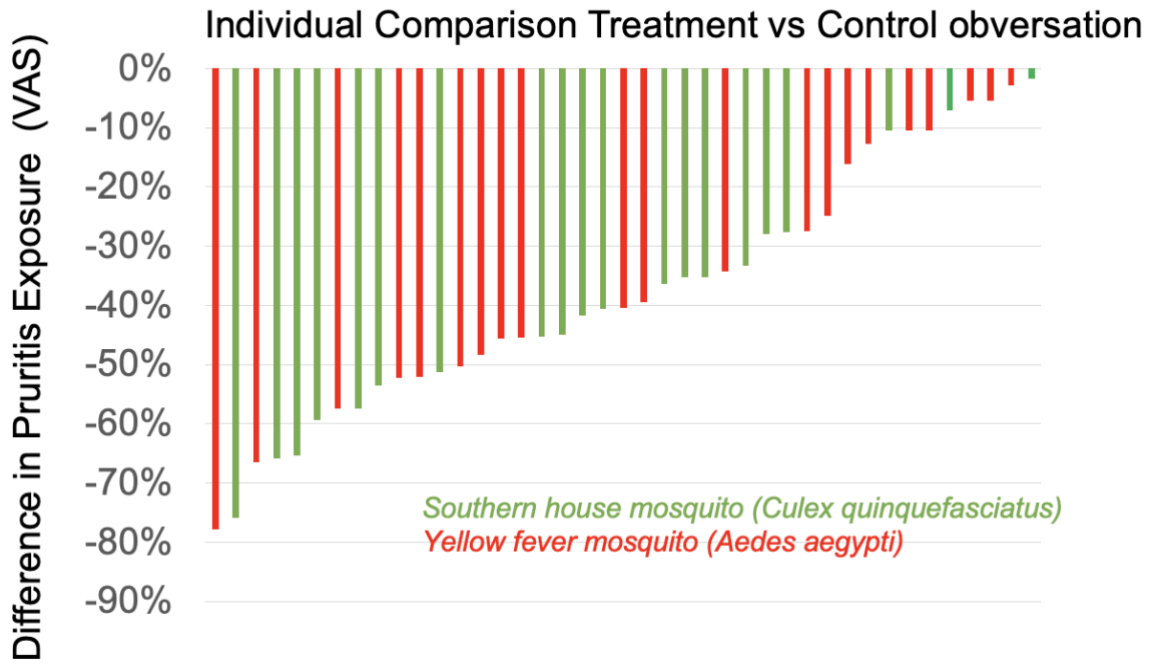

Figure 3: This bar graph illustrates the individual patient responses difference to the treatment as a percentage reduction in pruritus AUC over a 60-minute observation period (RAS). Each bar represents a single subject, with the length of the bar indicating the magnitude of pruritus reduction relative to baseline measures after bites with and Southern house mosquito (Culex quinquefasciatus) (green) and Yellow fever mosquito (Aedes aegypti) (red). *Negative percentages reflect a decrease in pruritus intensity post-treatment in the treatment vs control bites.*

In the analysis of pruritus reduction in the RAS population, a notable proportion of subjects exhibited a significant decrease in itch intensity following treatment with insect bite healer BR 60. 2 subjects (approximately 4,88% of those analysed) demonstrated a pruritus exposure reduction (AUC) of 75% or more over 60 min, indicating a substantial response to the intervention. A larger subset, consisting of a total of 13 subjects (32%), experienced a reduction of at least 50%, which is often considered clinically relevant. Furthermore, a total of 31 subjects (76%) achieved a pruritus reduction of 25% over 60 min, suggesting that the treatment was generally effective in reducing the severity of itching to some degree across the study population. All subjects in the study showed some reduction in pruritus after treatment; none of the participants had an increase in itch scores, which indicates that the treatment was effective to some extent for the entire cohort in terms of itch reduction (Figure 3, Figure 4).

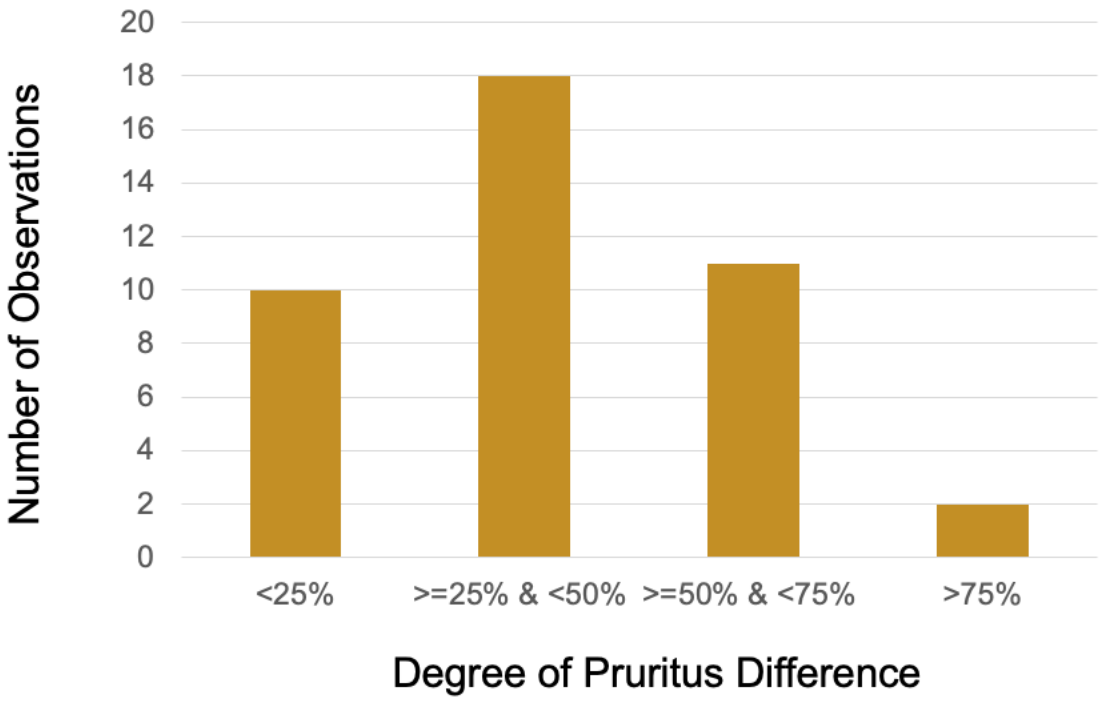

Figure 4: The histogram illustrates the effectiveness of the treatment across the RAS study cohort. The histogram visually demonstrates the distribution of pruritus reduction percentages among the subjects, categorized into four bites: greater than 75%, between 50% to 75%, between 25% to 50%, and less than 25%. The x-axis represents the categorized bites of pruritus reduction percentages, while the y-axis denotes the number of subjects within each category. This graphical representation provides a clear view of the treatment's impact, with a notable majority of subjects experiencing a pruritus reduction of at least 25%.

10.1.2.3. Pruritus score over time

In this study, the pruritus scores of subjects exposed to *Culex quinquefasciatus* and *Aedes aegypti* were monitored over a 60-minute period. A total of 22 subjects underwent 44 evaluations to assess the efficacy of an insect bite healer BR 60 treatment on pruritus induced by *Culex quinquefasciatus* or *Aedes aegypti* bites. A rapid increase in pruritus was observed during the first two minutes. At 7 minutes after the sting, pruritus levels decreased over time. A minimum value was observed at 60 minutes. This was observed in both the treatment and control bites and for both mosquito species.

At baseline, both bites exhibited a baseline pruritus VAS score of 1, After 2 minutes, the control bites presented a VAS score of 4,08 (± SD 1,94), while the treated bites had a lower VAS score of 2,31 (± SD 1,73). At the 7-minute mark, the VAS score for the control bites was measured at 3,92 (± SD 1,93), in contrast to the treated bites, which had a score of 2,35 (± SD 1,77) in the ITT population.

Progressing to 11 minutes post-exposure, the control bites' pruritus score further decreased to 3,59 (± SD 1,75), and the treated bites' score was 2,01 (± SD 1,59). By the 15-minute interval, the control bites' score was observed at 3,32 (± SD 1,88), while the treated bites continued to show a more significant reduction with a score of 1,89 (± SD 1,53). At 40

minutes, the control bites' pruritus VAS score stood at 2,03 (± SD 1,22), compared to the treated bites, which exhibited a score of 1,27 (± SD 0.66).

Finally, at the 60-minute endpoint, the control bites' score decreased to 1,40 (± SD 0.73), and the treated bites' score was 1,10 (± SD 0.30) (Table 2).

Time (min)	0	2	7	11	15	40	60	AUC
Control	1	4,08	3,92	3,59	3,32	2,03	1,40	154
SD Control	0	1,94	1,93	1,75	1,88	1,22	0,73	74
Treatment	1	2,31	2,35	2,01	1,89	1,27	1,10	94
SD Treatment	0	1,73	1,77	1,59	1,53	0,66	0,30	56
		p<0,001	p<0,001	p<0,001	p<0,001	p<0,001	p<0,001	p<0,001

Table 2: This table presents the mean pruritus scores at various time points over a 60-minute period for subjects exposed to Culex quinquefasciatus and Aedes aegypti in the ITT population. The 'Control' row reflects scores for the untreated bites, while the 'Treatment' row shows scores for the bites treated with the insect bite healer BR 60. Standard deviations for both bites are provided, illustrating the variability within each bites. A two-sided paired Student's t-test was employed to assess the statistical significance between bites at each time point, with p-values less than 0.01 indicating a significant difference in pruritus scores favouring the treated bites.SD Standard Deviation

Throughout all time points, the treated bites consistently demonstrated lower pruritus VAS scores compared to the control bites. Statistical analysis using a two-sided paired Student's t-test indicated significant differences between the bites at each time point (p<0.01), validating the treatment's effectiveness in mitigating pruritus over the course of the study (Figure 5)

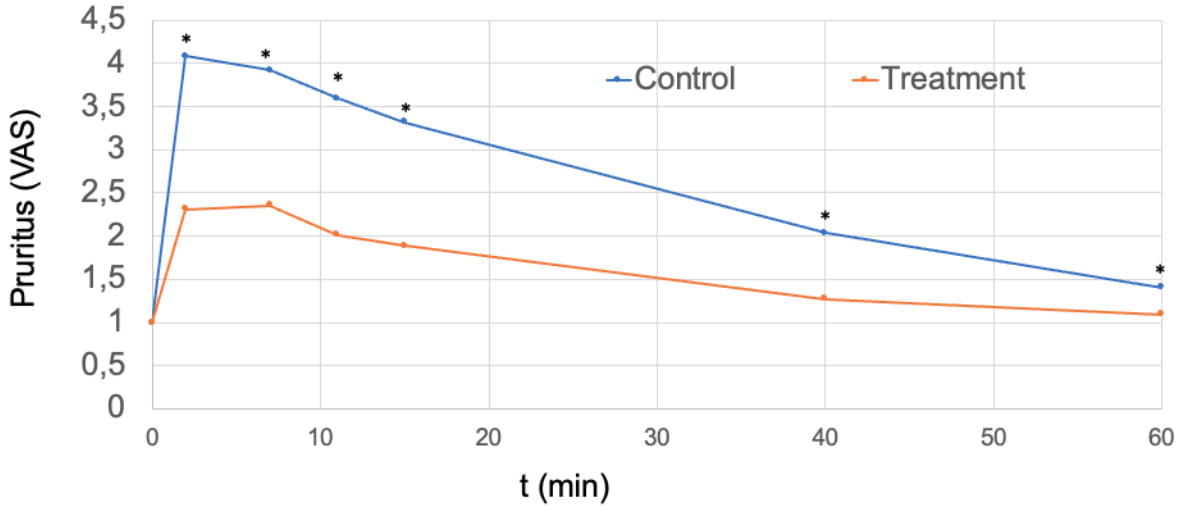

Figure 5: This line graph presents the average comparative trajectory of the average pruritus scores over a 60-minute period following exposure to Culex quinquefasciatus and Aedes aegypti bites. A total of 22 subjects with 44 observations are represented. The blue line denotes the control bites, which did not receive any treatment, while the orange line represents the treated bites, which was administered with an insect bite healer BR 60. (ITT)

10.1.2.4. Pruritus scores at various time points over 60 minutes for subjects exposed to Culex quinquefasciatus & Aedes aegypti

Culex quinquefasciatus:

22 subjects with 22 evaluations were exposed to two *Culex quinquefasciatus* bites to compare treatment (insect bite healer BR 60) to control. Initially, both the control and treated bites started with a baseline pruritus VAS score of 1, After 2 minutes, the control bites reported a VAS score of 4,00 (± SD 1,83), while the treated bites, which was treated with an insect bite healer BR 60, observed a lower VAS score of 2,25 (± SD 1,54). The difference was significantly different (p<0,001) in the ITT population.

7-minute after the bites, the control bites' score slightly decreased to 3,84 (± SD 1,77), whereas the treated bites' score was 2,32 (± SD 1,70) again was significantly different (p<0,001). By 11 minutes, there was a further reduction in the control bites' VAS score to 3,50 (± SD 1,60), and the treated bites' score was 1,89 (± SD 1,48). At the 15-minute interval, the VAS score was 3,23 (± SD 1,60) in the control, and score of 1,70 (± SD 1,32) after treatment. By 40 minutes, the average VAS decreased to 1,98 (± SD 1,12) and 1,23 (± SD 0.53) on the control and treatment bite respectively.

At the 60-minute endpoint, the average VAS further decreased on both bites to o 1,38 (± SD 0.65) and 1,05 (± SD 0.22) on the control and treated bites respectively. At each time point, a two-sided paired Student's t-test revealed significant differences between the control and treated bites (p<0.01), indicating the treatment's significant effect on reducing pruritus scores throughout the observation period (Table 3, Figure 6).

Time (min)	**0**	**2**	**7**	**11**	**15**	**40**	**60**	**AUC**
Control	1	4,00	3,84	3,50	3,23	1,98	1,38	149
SD Control	0	1,83	1,77	1,60	1,60	1,12	0,65	65
Treatment	1	2,25	2,32	1,89	1,70	1,23	1,05	88
SD Treatment	0	1,54	1,70	1,48	1,32	0,53	0,22	48
		p<0,001	p<0,001	p<0,001	p<0,001	p<0,001	p<0,001	p<0,002

Table 3: This table presents the mean pruritus scores at various time points over a 60-minute period for subjects exposed to Culex quinquefasciatus (ITT). The 'Control' row reflects scores for the untreated bites, while the 'Treatment' row shows scores for the bites treated with the insect bite healer BR 60. Standard deviations for both bites are provided, illustrating the variability within each bites. A two-sided paired Student's t-test was employed to assess the statistical significance between bites at each time point, with p-values less than 0.01 indicating a significant difference in pruritus scores favoring the treated bites.

Aedes aegypti:

In a study of pruritus response following an *Aedes aegypti* mosquito bite, 22 subjects underwent 22 evaluations. Starting with a baseline pruritus VAS score of 1, the control bites' score increased to the average VAS 4,16 (± SD 2,09) at the 2-minute mark. In contrast, the treated bites, which received an insect bite healer BR 60 intervention, reported a lower average VAS score of 2,36 (± SD 1,94) at the 2-minute mark.

As time progressed to the 7-minute point, the control bites had an average VAS score of 4,00 (± SD 2,12), while the treated bites showed a score of 2,39 (± SD 1,88). By the 11-minute interval, the control bites' score slightly declined to an average pruritus VAS score of 3,68 (± SD 1,92), and the treated bites' score was 2,14 (± SD 1,73).

Continuing to the 15-minute observation, the average pruritus VAS score was 3,41 (± SD 2,15) and 2,07 (± SD 1,73) on the control and treatment bit respectively. At 40 minutes, the control bites' score further reduced to 2,09 (± SD 1,34), while the treated bites recorded a score of 1,32 (± SD 0,78).
Finally, at 60 minutes, the control bites' VAS score was 1,43 (± SD 0.81), and the treated bites' score had decreased to 1,14 (± SD 0.35). Statistical analysis using a two-sided paired Student's t-test indicated significant differences between the control and treated bites at all time points ($p<0.01$) (Table 4).

Time (min)	**0**	**2**	**7**	**11**	**15**	**40**	**60**	**AUC**
Control	1	4,16	4,00	3,68	3,41	2,09	1,43	158
SD Control	0	2,09	2,12	1,92	2,15	1,34	0,81	84
Treatment	1	2,36	2,39	2,14	2,07	1,32	1,14	100
SD Treatment	0	1,94	1,88	1,73	1,73	0,78	0,35	64
		p<0,001	p<0,001	p<0,001	p<0,001	p<0,001	p<0,001	p<0,001

Table 4: This table presents the mean pruritus scores at various time points over a 60-minute period for subjects exposed to Aedes aegypti (ITT). The 'Control' row reflects scores for the untreated bites, while the 'Treatment' row shows scores for the bites treated with the insect bite healer BR 60. Standard deviations for both bites are provided, illustrating the variability within each bites. A two-sided paired Student's t-test was employed to assess the statistical significance between bites at each time point, with p-values less than 0.01 indicating a significant difference in pruritus scores favoring the treated bites.

In the investigation of the pruritus response to bites from both *Culex quinquefasciatus* and *Aedes aegypti*, the study comprising 22 subjects revealed consistent patterns in the pruritus scores across the control and treated bites, respectively. (Figure 6).

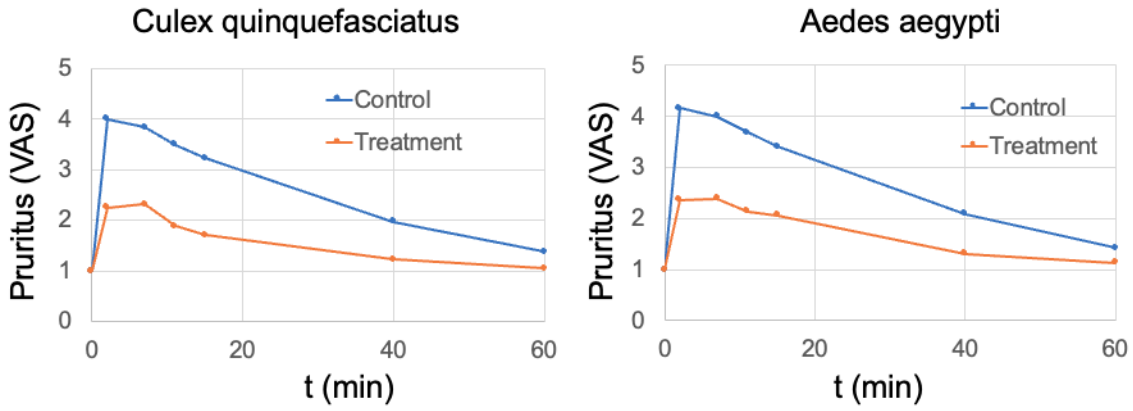

Figure 6: This line graph presents the comparative trajectory of pruritus scores over a 60-minute period following exposure to Culex quinquefasciatus and Aedes aegypti bites. A total of 22 subjects with 44 observations (ITT) are represented. The blue line denotes the control bites, which did not receive any treatment, while the orange line represents the treated bites, which was administered with an insect bite healer BR 60.

10.1.2.1. Onset on pruritus reduction

Use of the Insect bite healer BR 60 demonstrated a rapid onset of the reduction of pruritus. The primary increase in pruritus was reduced significantly by treatment with the Insect bite

healer BR 60. After two minutes, the mean pruritus score for the control bite in the RAS population was 4,30 (± SD 1,81), indicating the average level of pruritus reported by subjects without intervention. In the treated bites, the mean pruritus score in the RAS population was significantly lower at 2,40 VAS (± SD 1,80), demonstrating the effect of the insect bite healer BR 60 in significantly reducing itch intensity ($p<0.01$) (Figure 7). This was the highest difference between control and treated bites. The analysis reflects a mean difference of 1,90 VAS between the control and treated bites, meaning that, on average, subjects in the treated bites reported almost half the itch intensity compared to those in the control bites.

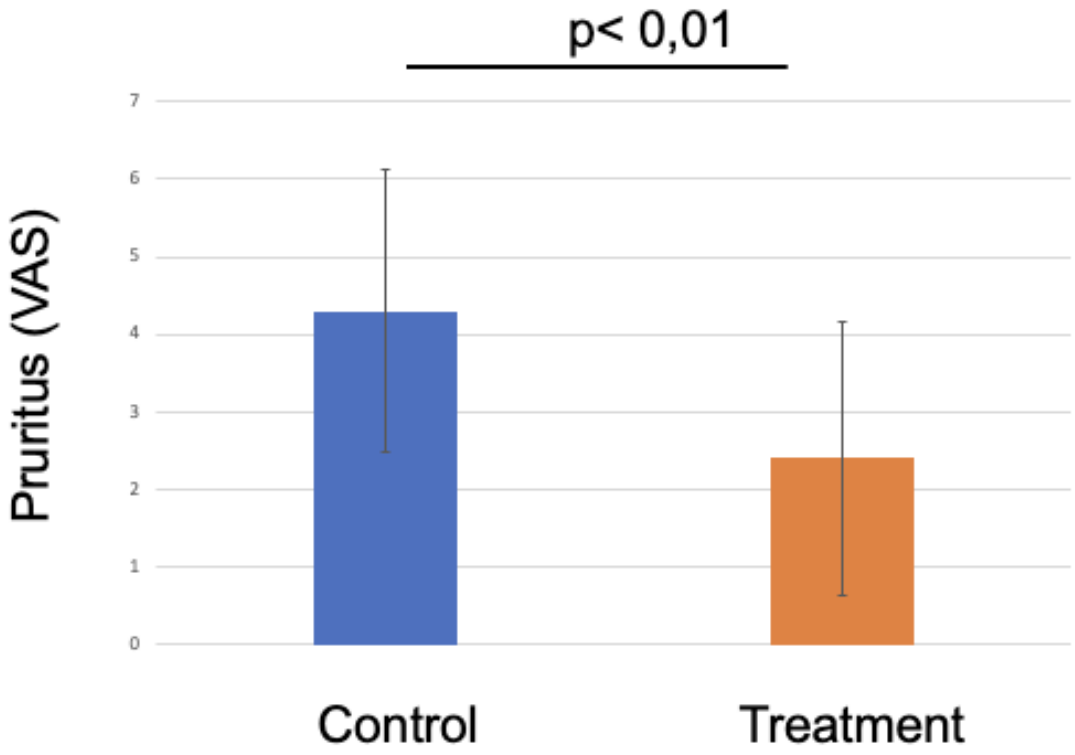

Figure 7: Reduction of pruritus after 2 minutes of insect bite healer BR 60 treatment. Blue bar represents untreated control Culex quinquefasciatus and Aedes aegypti bites and the orange bar represents insect bite healer BR 60 treated Culex quinquefasciatus and Aedes aegypti bites from the responder dataset (n=41) (RAS).

In 61% of the observations (25 observations of 41 observations) the treated bites achieved at least a 50% reduction in pruritus at the first measurement point compared to the non-treated bites. In 78 % of the observations (32 observations of 41 observations) the treated bites achieved at least a 50% reduction in pruritus at the first measurement point compared to the non-treated bites.

49% (20 observations of 41 observations) of the observations showed that the treated bites had at least a 2-point lower pruritus score (VAS) than the non-treated bites at the first measurement point compared (2 min) to the non-treated bites. 80% (33 observations of 41 observations) of the observations showed that the treated bites had at least a 1-point lower pruritus score (VAS) than the non-treated bites at the first measurement point compared (2 min) to the non-treated bites. 17% (7 observations of 41 observations) of the observations showed that the treated bites the same pruritus score (VAS) than the non-treated bites at the first measurement point compared (2 min) to the non-treated bites.

This also reflects by an almost 50% reduction in average pruritus (Figure 8) in the RAS population.

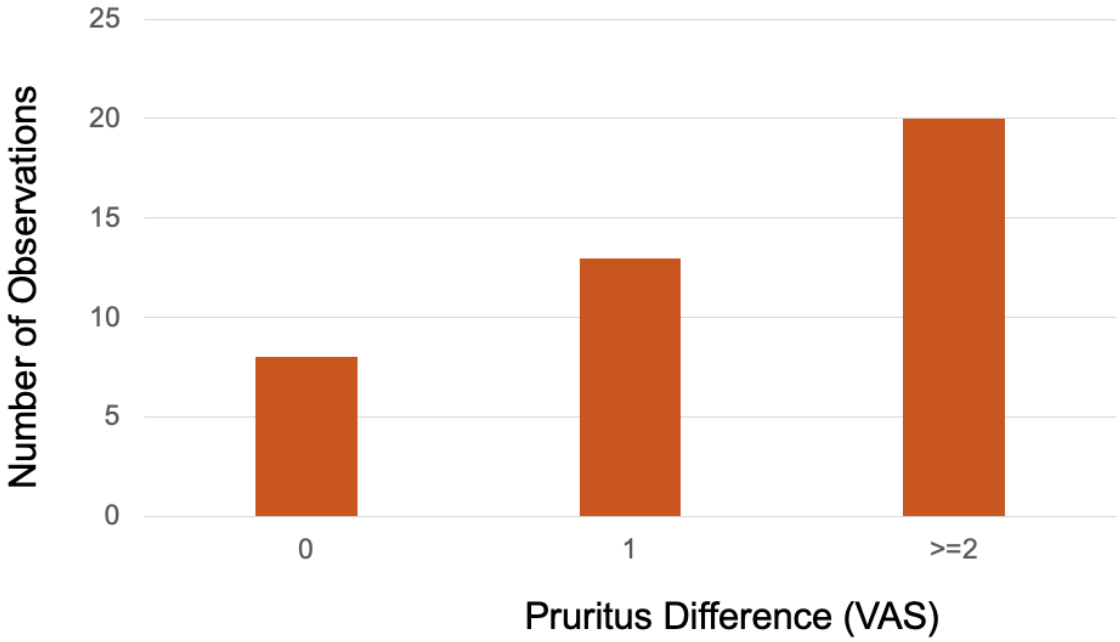

Figure 8: Pruritus Reduction per Individual Comparison (Treatment vs. Control) after Culex quinquefasciatus and Aedes aegypti bites. The graph reflects 41 comparisons between Control and Insect bite healer BR 60 treated bites. In about 50% of the comparisons (49%), there was at least a 2-point improvement, representing a 50% reduction (RAS).

These results suggest that the insect bite healer BR 60 treatment was effective in reducing the pruritus experienced by subjects within the first 2 minutes of the mosquito bite compared to those who received no treatment.

10.1. Safety Outcomes

The observations from the study indicate that the insect bite healer BR 60 treatment was very well tolerated by all subjects, with no adverse events reported. This high tolerability is further substantiated by the absence of any significant difference in erythema, as measured by swelling and redness, between the treatment and control bites.

10.1.1. Swelling

Following the bites from Culex quinquefasciatus and Aedes aegypti, swelling was observed in both the control bites and the bites treated with the insect bite healer BR 60. The average exposure (AUC) to swelling, measured on a 10-point VAS score, in the treated bites was 163, with a standard deviation (± 87), while in the untreated control bites, it was 170, with a standard deviation (± 92). The difference in average exposure to swelling between the two bites was not statistically significant. A large interindividual variation in swelling response was noted among subjects for both types of mosquito bites, with the minimum swelling observed at 60 and the maximum swelling at 471 (Figure 9).

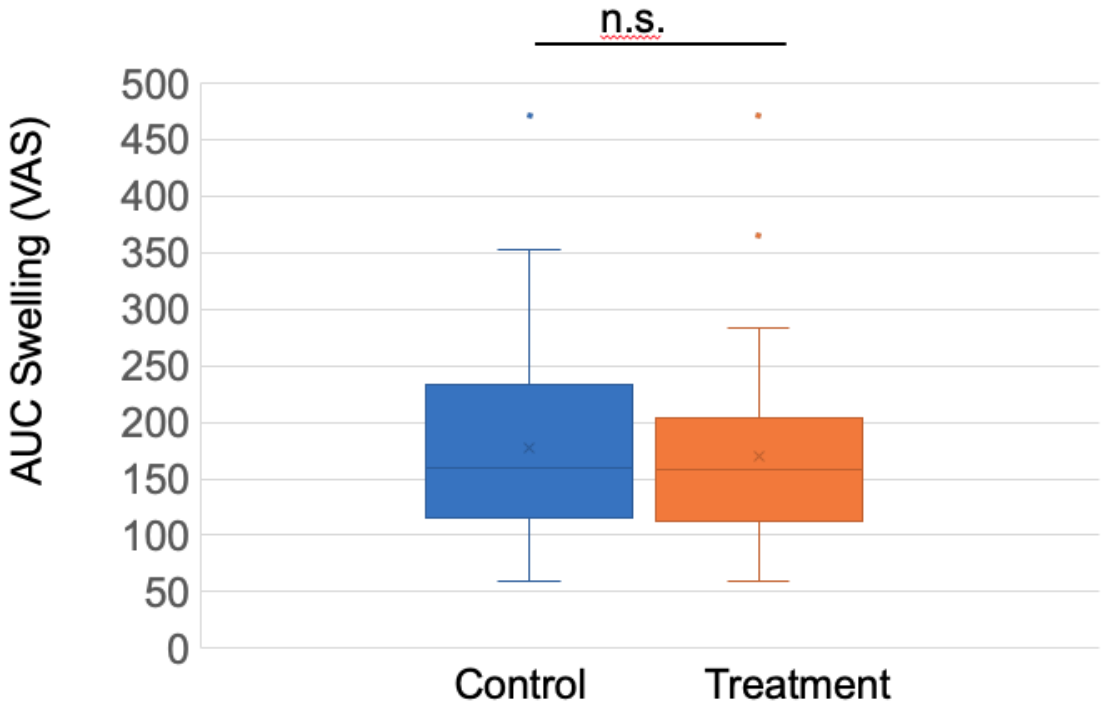

Figure 9: This boxplot illustrates the difference in swelling (measured as Area Under the Curve, AUC) over a 60-minute period following the bites of Culex quinquefasciatus and Aedes aegypti with a insect bite healer BR 60 versus untreated control bites. The blue box represents the control bites, showing the range of swelling responses without treatment, while the orange box depicts the responses after treatment with the insect bite healer BR 60. The central 'X' marker in each box indicates the mean AUC value. Whiskers extend to the furthest points that are not considered outliers, and the outliers are marked with dots. The comparison highlights the effectiveness of the insect bite healer BR 60 in reducing the swelling AUC as compared to the control, with the treated bites demonstrating a lower mean and a more compact interquartile range, indicative of less variability in response to treatment.

In contrast to pruritus, the swelling observed in both bites peaked at 7 minutes post-bite, with VAS scores of 3,57 (standard deviation ± 1,97) for the control bites and slightly lower at 3,52 (standard deviation ± 1,86) for the insect bite healer BR 60-treated bites.

Throughout these intervals, the swelling responses post-bite gradually subsided from their peak at 7 minutes to lower levels at 60 minutes, all the while remaining above the baseline VAS score. Swelling scores gradually decreased until reaching 1,93 (standard deviation ± 1,20) in the control bites and 1,82 (standard deviation ± 1,08) in the treated bites at 60 minutes post-bite. Despite this decline, these values remained above the initial VAS score of 1 for both bites.

After 2 minutes post-bite, the control bites exhibited a VAS swelling score of 2,81 (standard deviation ± 1,66), and the treated bites showed a VAS score of 2,95 (standard deviation ± 1,63). Moving to the 11-minute mark, the control bites swelling score increased to 3,52 (standard deviation ± 2,08), and the treated bites had a swelling score of 3,50 (standard deviation ± 2,00). At 15 minutes post-bite, the swelling VAS score for the control bites was 3,51 (standard deviation ± 2,08), with the treated bites not far behind at 3,34 (standard deviation ± 1,89). By 40 minutes, the control bites swelling score had decreased to 2,67 (standard deviation ± 1,52), and the treated bites had a score of 2,49 (standard deviation ± 1,48).

By 60 minutes, the control bites swelling score had decreased to 1,93 (standard deviation ± 1,20), and the treated bites had a score of 1,82 (standard deviation ± 1,08) (Table 5, Figure 10).

Time (min)	0	2	7	11	15	40	60	AUC
Control	1	2,81	3,57	3,52	3,51	2,67	1,93	170
SD Control	0	1,66	1,97	2,08	2,08	1,52	1,20	92
Treatment	1	2,95	3,52	3,50	3,34	2,49	1,82	163
SD Treatment	0	1,63	1,86	2,00	1,89	1,48	1,08	87
		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 5: This table presents the mean pruritus scores (VAS) at various time points over a 60-minute period for subjects exposed to Culex quinquefasciatus and Aedes aegyptiin the ITT population (22 subjects 44 observations) and the overall exposure (AUC) The 'Control' row reflects scores for the untreated bites, while the 'Treatment' row shows scores for the bites treated with the insect bite healer BR 60. Standard deviations for both bites are provided, illustrating the variability within each bites. A two-sided paired Student's t-test was employed to assess the statistical significance between bites at each time point, with p-values less than 0.01 indicating a significant difference in pruritus scores favoring the treated bites.

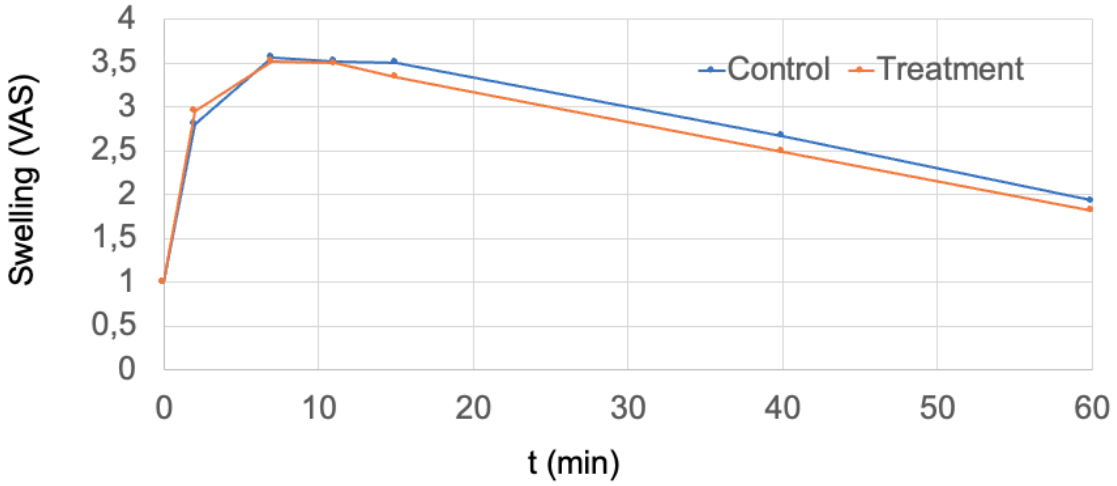

Figure 10: This line graph presents the comparative trajectory of average swelling scores over a 60-minute period following exposure to Culex quinquefasciatus and Aedes aegypti bites. A total of 22 subjects with 44 observations are represented (ITT). The blue line denotes the control bites, which did not receive any treatment, while the orange line represents the treated bites, which was administered with an insect bite healer BR 60.

No observable differences were apparent in the timing or magnitude of swelling following the bites of *Culex quinquefasciatus* and Aedes aegypti. The exposure (AUC) to redness in the control bites from Culex quinquefasciatus was 156, whereas the treated bites exhibited a slightly higher AUC of 149, Similarly, for Aedes aegypti, the control bites had an AUC for swelling of 183, while the treated bites showed a marginally lower AUC of 177 (data not shown) (ITT).
Swelling was also noted in subjects who did not exhibit pruritus in response to mosquito bites.

10.1.2. Redness

Following the bites from *Culex quinquefasciatus and Aedes aegypti*, redness was observed in both control untreated and in bites treated with the insect bite healer BR 60. The average exposure (AUC) to redness, measured on a 10-point score, in the insect bite healer BR 60 treated bites was 186, with a standard deviation (SD ± 81), while in the untreated control bites, it was 188, with a standard deviation (SD ± 99). The difference in average exposure to swelling between the control and treated bites was not statistically significant. A large interindividual variation in swelling response was noted among subjects for both types of mosquito bites, with the minimum swelling observed at 61 and the maximum swelling at 460 (Figure 11)

No discernible differences were noted with respect to the timing and extent of swelling resulting from the bites of Culex quinquefasciatus and Aedes aegypti.

Swelling and redness were also noted in subjects who did not exhibit pruritus in response to mosquito bites.

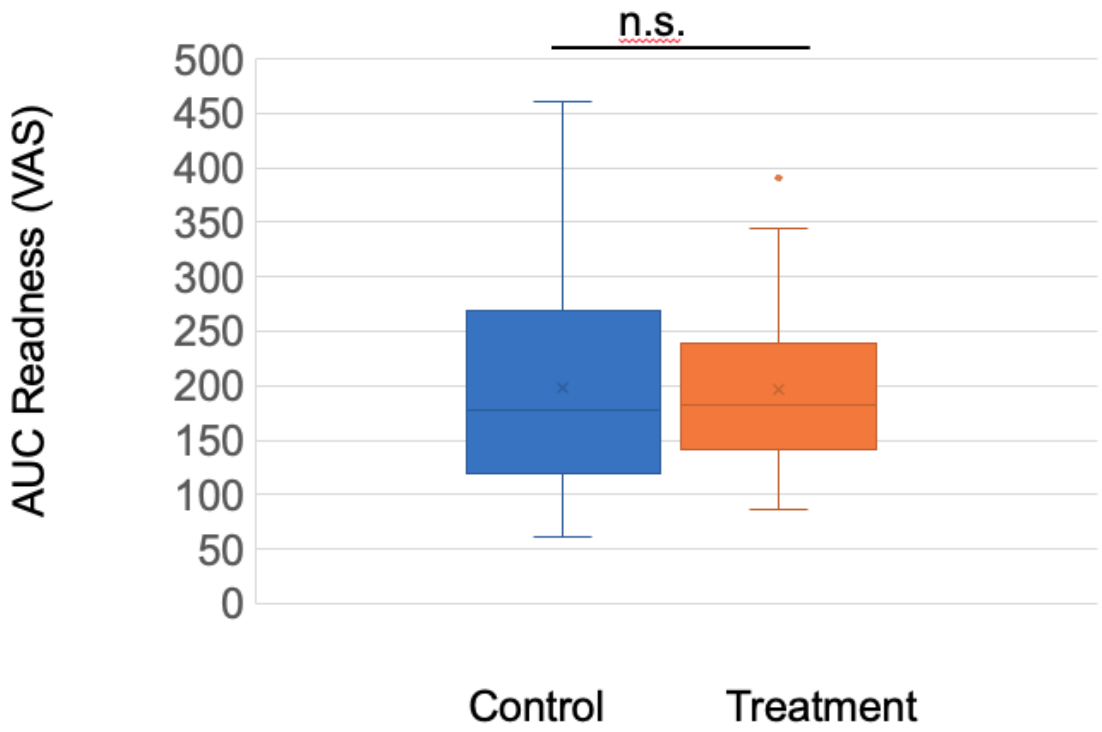

Figure 11: This boxplot illustrates the difference in the average redness (measured as Area Under the Curve, AUC) over a 60-minute period following the bites of Culex quinquefasciatus and Aedes aegypti with the insect bite healer BR 60 versus untreated control bites ITT). The blue box represents the control bites, showing the range of swelling responses without treatment, while the orange box depicts the responses after treatment with the insect bite healer BR 60. The central 'X' marker in each box indicates the mean AUC value. Whiskers extend to the furthest points that are not considered outliers, and the outliers are marked with dots. The comparison highlights the effectiveness of the insect bite healer BR 60 in reducing the swelling AUC as compared to the control, with the treated bites demonstrating a lower mean and a more compact interquartile range, indicative of less variability in response to treatment.

The response to mosquito bites in terms of redness, as measured by VAS scores, showed similar trends in both the control and the insect bite healer BR 60-treated bites. Initially, both bites started with a baseline redness score of 1,

At 2 minutes post-bite, the control bites exhibited a redness score of 3,22 with a standard deviation (SD) of ±1,72, while the treated bites had a marginally higher score of 3,55 (SD ±1,44). By the 7-minute mark, the control bites' score had risen to 3,50 (SD ±1,91), and the treated bites matched this with a score of 3,78 (SD ±1,66).

The redness peaked at 11 minutes in both bites, with the control bites reaching a score of 3,78 (SD ±2,24) and the treated bites scoring slightly higher at 3,83 (SD ±1,98). However, by 15 minutes, the scores began to converge, with the control bites showing a score of 3,63 (SD ±2,09) and the treated bites at 3,80 (SD ±1,80).

Thereafter, a gradual decline in redness was noted. At 40 minutes, the control bites' score was 3,14 (SD ±1,69) compared to the treated bites' score of 2,94 (SD ±1,38). By the 60-

minute endpoint, the control bites' redness had decreased to 2,39 (SD ±1,11), while the treated bites had a further reduction to 2,17 (SD ±0.88) (Table 6, Figure 12).

Time (min)	0	2	7	11	15	40	60	AUC
Control	1	3,22	3,50	3,78	3,63	3,14	2,39	188
SD Control	0	1,72	1,91	2,24	2,09	1,69	1,11	99
Treatment	1	3,55	3,78	3,83	3,80	2,94	2,17	186
SD Treatment	0	1,44	1,66	1,98	1,80	1,38	0,88	81
		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 6: This table presents the mean pruritus scores at various time points over a 60-minute period for subjects exposed to Culex quinquefasciatus and Aedes aegypti (ITT). The 'Control' row reflects scores for the untreated bites, while the 'Treatment' row shows scores for the bites treated with the insect bite healer BR 60. Standard deviations for both bites are provided, illustrating the variability within each bites. A two-sided paired Student's t-test was employed to assess the statistical significance between bites at each time point, with p-values less than 0.01 indicating a significant difference in pruritus scores favoring the treated bites.

Throughout all time points, no significant differences (n.s.) were observed between the control and treated bites, indicating that the insect bite healer BR 60 treatment did not significantly alter the course of redness development compared to the natural progression observed in the control bites. Both bites showed a gradual resolution of redness over time, maintaining above-baseline VAS scores throughout the observation period.

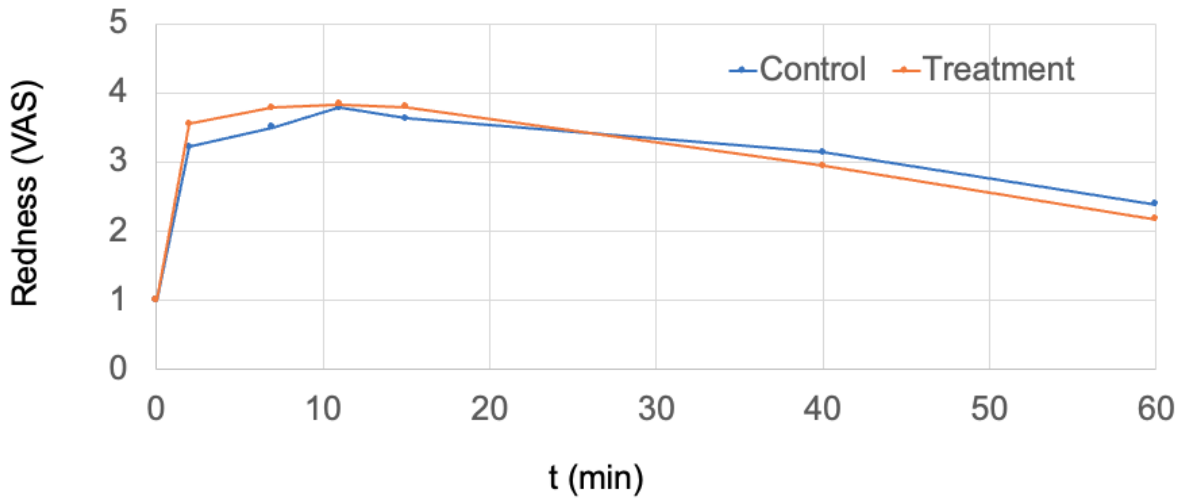

Figure 12: This line graph presents the comparative trajectory of pruritus scores over a 60-minute period following exposure to Culex quinquefasciatus and Aedes aegypti bites. A total of 22 subjects with 44 observations are represented. The blue line denotes the control bites, which did not receive any treatment, while the orange line represents the treated bites, which was administered with an insect bite healer BR 60.

No observable differences were apparent in the timing or magnitude of redness following the bites of *Culex quinquefasciatus* and *Aedes aegypti*. The exposure (AUC) to redness in the control bites from Culex quinquefasciatus was 184, whereas the treated bites exhibited a slightly higher AUC of 185, Similar, for Aedes aegypti, the control bites had an AUC for redness of 204, while the treated bites showed a marginally lower AUC of 187 (Figure 12) in the ITT set.

Redness was also noted in subjects who did not exhibit pruritus in response to mosquito bites.

11. Conclusion

The objective of the clinical study was to evaluate the efficacy of BR 60 Insect Bite Healer in reducing pruritus following a mosquito bite. The study design was an open-label, single-arm study involving 22 subjects and assessing pruritus, swelling and redness in response to insect bites up to 60 minutes post bite. The efficacy and safety of the BR 60 head pen was tested with two different mosquito strains, Culex quinquefasciatus and Aedes aegypti.

The demographic distribution of the study population reflected a normal weight and BMI range, with a gender distribution of 68% female and 32% male participants, providing a relevant cross-section of the general population to assess the efficacy of the device.

The results showed a rapid, significant reduction in pruritus intensity immediately after application of the Insect Bite Healer (technical type BR 60), with this effect maintained at all subsequent time points measured. At the 2-minute post-bite mark, the mean pruritus score for control bites was 4.30, in sharp contrast to the 2.40 mean score observed in the treated bites. This significant early effect is clinically important as it disrupts the typical cycle of pruritus and subsequent scratching behaviour, which can exacerbate skin damage and further increase pruritus. This suggests that the Insect Bite healer (technical type BR 60) can break the above-mentioned pruritus-scratch vicious circle and further reduce pruritus and also the risk of infection through damaged skin.

Remarkably, over 50% (61%) of the bites treated resulted in a 50% reduction in pruritus at the first measurement point, highlighting the potential of the BR 60 device to not only provide immediate symptomatic relief, but also to prevent chronicity of pruritus and associated dermatological damage.

A very consisted reduction of pruritus was observed cross mosquito species - Culex quinquefasciatus and Aedes aegypti - suggests its broad applicability to different pruritic stimuli.

The reduction was immediate after application of the Insect Bite Healer BR 60, indicating a potential antipruritic effect. Although heat-induced protein denaturation is widely discussed, heat-induced denaturation at the bite site (activated at 50°C) is unlikely, but recent studies suggest that heat may act directly on neural circuits to suppress both histaminergic and non-histaminergic itch.
It's important to note that BR 60 was well tolerated, with no adverse events including local skin irritation marked by increased redness and erythema were reported in the treatment bites compared to the control bites (besides the redness and erythema caused by the insect bite itself) . This was evidenced by no significant changes in erythema and redness between control and treated bites.

Swelling and redness were also noted in subjects who did not show pruritus in response to mosquito bites. This phenomenon suggests that even in the absence of typical pruritus, there are observable physiological responses such as local oedema and erythema, highlighting the complexity of individual responses to mosquito bites.

In particular, the open-label nature of the study invites consideration of the potential for a placebo effect. Nevertheless, the clear and consistent pattern of results lends weight to the argument that the observed benefits are due to the intervention rather than psychological expectation.
In conclusion, the Beurer Insect Bite Healer BR 60 appears to be an effective, immediate-acting acting and safe tool for the relief of mosquito bite pruritus, with implications for improving patient comfort and preventing secondary complications associated with insect bites. Further studies, possibly with a blinded design, would be valuable in confirming these findings and exploring the wider utility of this device in different clinical scenarios.

12. Literature