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Title: Evaluation of topical ibuprofen and steroid in the reduction of local reactions and symptoms from an *Aedes aegypti* mosquito bites

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Abstract

The rapid development of a wheal, erythema and itching are all symptoms associated with the Type I reaction to mosquito bites. Such bites are a frequent problem in tropical travellers and may lead to local infections following scratching. Numerous products are available to consumers for the relief of these symptoms, and include topical after-bite treatments and physical devices. There have been only a few randomised control trials carried out to test products that may relieve pruritus and erythema and therefore, the benefit topical treatments provide consumers is not conclusive. Furthermore, corticosteroids and ibuprofen used as topical anti-inflammatory preparations have been suggested for the treatment of bites, but there is little evidence supporting their role in symptom relief.

In this study, forty participants were recruited to take part in a double-blinded, randomised control trial to investigate the use of over-the-counter topical preparations of a non-steroidal anti-inflammatory (NSAI) gel containing 5% ibuprofen and a steroidal Clobetasone cream (0.05%) used as after-bite treatments. On each of two visits, participants received a single, controlled bite on each forearm from a laboratory-reared mosquito. Blinded, single-measured application of a treatment was applied in turn to each arm; one containing the active compound and the other the placebo preparation. A standardised measurement of wheal size, erythema size and itching were recorded during 90 minutes following the bite and participants were followed up within 48 hours to monitor for adverse events.

Participants varied in their reaction sizes. Neither of the active compounds reduced the bite response in terms of wheal size, erythema size or itching over 90 minutes of the test when compared to placebo. The average size of the wheal after 5 and 15 minutes was larger (xx mm) for the bites treated with ibuprofen or corticosteroid compared to the placebo. A longer-term benefit (> 90mins) would not have been detected in this design.

Key words: mosquito, bite, after-bite, relief, itching, corticosteroids, ibuprofen, symptoms, pruritus, wheal, erythema

Introduction

The rapid development of localised, red, itchy swellings are typical clinical symptoms associated with mosquito bites (Peng and Simmons, 2004). These symptoms develop as part of the body's response to proteins present in mosquito saliva (Hudson et al. 1958; Hudson et al. 1960; King et al., 2011) and are mediated by IgE antibodies and histamine (Konishi, 1990; Reunala et al., 1994; Horsmanheimo et al., 1996; Peng and Simons, 1997; Peng et al., 2004). Mediators released by mast cells during the IgE-mounted response cause the development of the wheal and flare/erythema reaction which are characteristic symptoms of the body's early (type I) reaction to a mosquito bite (McNeil, 1996). These mediators also cause the stimulation of sensory nerves, inducing the itching (pruritus) which typically accompanies the visible reaction (Melanby, 1946; Hudson et al. 1958; McNeil, 1996).

Mosquito bites can be irritating and if scratched may lead to skin infections (Lederman et al., 2008; Morris-Jones and Morris-Jones, 2012). Topically-applied products designed to treat mosquito bites are reputed to relieve the early symptoms of itching and redness. Despite the fact that several such products are on sale to consumers around the world, there has been little robust clinical evaluation of their efficacy (BMJ, 2012). Most studies have concentrated on oral antihistamines as treatment for the relief of mosquito bite symptoms (e.g. Karppinen et al. 2002; Karppinen et al. 2006; Reunala et al. 1990). Limited studies of topical treatments include evaluations of homeopathic creams (Hill et al. 1995; 1996), ammonium solution (Zhai et al., 1998) and antihistamine (McKiel et al., 1954; O'Rourke and Murnaghan, 1953), with variable results.

Ibuprofen gels are typically used for targeting pain relief and to reduce inflammation of joints, muscles or tendons (Jorge et al., 2011). Ibuprofen is absorbed quickly into the skin and into the synovial fluid. Ibuprofen works by blocking the production of cyclo-oxygenase, which in turn prevents the production of prostaglandins, which are produced in damaged tissues and cause inflammation and pain. Due the fast action and analgesic properties, topical ibuprofen may also help relieve the symptoms associated with mosquito bites.

Topical corticosteroids are artificially manufactured versions of naturally released corticosteroid hormones that are produced in the body to reduce inflammation among other things. Like ibuprofen, topical corticosteroids reduce the production of prostaglandins by blocking the production of arachidonic acid. Without arachidonic acid, many of the inflammation-causing chemicals are not produced (Kragballe, 1989). Corticosteroids also reduce inflammation by constricting the capillaries, which reduces redness and swelling. Topical corticosteroids are recommended for the treatment of inflamed, itchy skin, symptoms associated with eczema for example, but have also been recommended for relieving the symptoms of insect bites (NHS choices online).

In order to inform best practice, the aim of this study was to determine if the application of an ibuprofen gel or a corticosteroid cream is effective for the relief of the early symptoms of mosquito bites. We describe a double-blind, placebo-controlled, complete cross-over study which evaluated

these products by applying active or placebo to volunteers each of whom received controlled mosquito bites on their forearms.

Materials and Methods

Participants

Forty volunteers (27 women, 13 men; mean age 31 years, age range 22-58 years) were enrolled from the staff and students of the London School of Hygiene and Tropical Medicine (LSHTM). All subjects were asked to complete a questionnaire prior to taking part to exclude participants who may have a history of hypersensitivity to mosquitoes, bees, or wasps or allergy to the trial medication or its ingredients. Several days prior to the experiment, participants were given a screening bite using laboratory-reared female *Aedes aegypti* mosquito to exclude participants who lacked a visible, immediate reaction. This was included as an amendment after the first thirteen participants had completed the study, of which 4 displayed minimal or no immediate reaction.

All participants were followed up in person or by email to check whether they had experienced any adverse events. No adverse events were reported for this trial.

The study was approved by the NHS Ethics Committee (Ref No. 11/LO/1898), LSHTM ethics committee (Ref No. 6044) and the Medicines and Healthcare products Regulatory Agency (MHRA, Ref No. 17072/0005/001) and written informed consent was obtained from all volunteers.

Study protocol

This was a controlled, double-blind, single centre study. Two actives (0.5% ibuprofen gel and 0.05% Clobetasone cream) and their matching placebos (Aguagel lubricating jelly and Cetomacgrogol emulsifying ointment) were manufactured by St Mary's Pharmaceutical Unit, Cardiff and tested for their early relief of mosquito bite symptoms.

During each of two visits, volunteers received a single mosquito bite on the underside of each forearm (Figure S1). Each bite was administered using a single five to seven-day-old, non blood-fed female *St. aegypti* mosquito, held in a small cage against the forearm for two minutes. Immediately after the mosquito had fed, one gram of the cream or gel containing the active was applied to one arm and the matching placebo was applied to the other according to a randomization schedule. To account for any potential effect of participants' dominant arm, arm (right or left) was included in the randomisation and blinded treatments were pre-assigned to be applied to the right or left arm. The volunteer's bite reaction was assessed at 1, 5, 15, 30, 60 and 90 minutes following mosquito challenge using callipers to measure the width and length of the wheal and erythema (Figure S2) as well as a 100 mm visual analog scale (VAS) to measure pruritus, ranging from 0 (no itching) to 100 (intense itching).

Statistical Analyses

The three variables (wheal size, erythema size and itching) were analysed using a repeated measures analysis of a linear mixed model that considered an autoregressive correlation between measurements of a given individual at a given visit. This was done using a power error correlation as implemented in PROC MIXED within the software SAS Version 9.2, Copyright SAS Institute Inc. The model fitted was:

$$y = \mu + Arm + Treatment + Time + Time*Arm + Time*Treatment + Subject + Visit(Subject) + e$$

where μ is the overall mean, *Arm* corresponds to the arm been tested (left or right), *Treatment* is a factor for the treatment levels (A, B, C, D), *Time* is a factor that identifies each of the measurement times, and *Time*Arm* and *Time*Treatment* are interactions. The factors *Subject* identifies each patient and the factor *Visit(Subject)* identifies each visit within a subject. All of these factors, with the exception of *Subject* and *Visit(Subject)* were considered fixed. In addition, as indicated earlier, the errors were considered correlated within the same measurements from a given level of *Visit(Subject)* using a power error structure. In order to test the significance of the fixed effects, approximate F-test were used with the Kenward-Rogers degrees of freedom correction. In addition, the covariates *Age Group*, *Gender* and *Weight* were evaluated individually in order to determine if they need to be incorporated in the fitted models. Finally, for each time point the difference between treatments was evaluated using another approximated F-test. Whenever F-tests were significant, multiple comparisons between treatments across all time points compared with a given time point were performed, using the least significance difference (LSD) with a significance level of 5%.

In order to approximate to a normal distribution, the wheal and erythema area were transformed using a log-transformation using the following expressions: log(Wheal_Area+1) and log(Erythema_Area+100).

Results

Thirty-nine subjects completed the study according to the protocol and one subject completed the first visit only. Age, gender and weight variables were not included as covariates in the analyses as these did not show any significant effect on the response.

Volunteer's reactions were measured immediately after the mosquito had fed and at six further time points in the subsequent 90 minutes. Thirty-five volunteers exhibited wheal, erythema and itching symptoms. Four volunteers showed no itching at all throughout the trial, erythema was absent in three volunteers, and all volunteers developed at least a small wheal reaction to the bite. Wheal, erythema and itching responses were all significantly correlated but there was a lot of variability between participants in their reactions to mosquito bites (Subject variance: residual variance (erythema) = 0.576/0.232; Subject variance: residual variance (Wheal) = 0.485: 0.304).

Mosquito bite reactions; wheal area, erythema area and itching were all significantly correlated with time ($p<0.0001$). The red rash or erythema that surrounds the puncture wound peaked in size in all treatment allocations 5 minutes after the bite and then reduced steadily over the course of the 90-minute assessment period (Figure 1). Neither after-bite treatment was effective at reducing the erythema area of volunteers' mosquito bites Table 1; Figure 1).

The central, raised wheal started to appear immediately in some volunteers and, on average, grew in size until between 15 to 30 minutes, after which point started to recede (Figure 2). Overall, neither ibuprofen gel nor corticosteroid was effective at reducing wheal size. In fact, wheal size in each group was compared separately for each time point, wheal area of bites treated with placebo was reduced compared with wheal areas of bites treated with active substances at 5 and 15 minutes (p-value=0.0116 and 0.0448, respectively; Table2; Figure 2).

Participants reported itching almost immediately following the mosquito bite (Table 3; Figure 3). Maximum itching was recorded at the earliest assessment time point (1 minute) and rapidly decreased thereafter (Table 3; Figure 3). Neither the corticosteroid cream nor the ibuprofen reduced itching overall, but there is some evidence of a treatment effect on itching at 5 minutes (p-value=0.076; Table 3; Figure 3).

Due to the large variability between participants, no treatment effects were detected using the repeated measures analysis, suggesting that overall, the topical application of ibuprofen or corticosteroid does not reduce the immediate symptoms of mosquito bites.

Discussion

The immunological mechanism after a mosquito bite relates to sensitisation to the salivary proteins and IgE and IgG antibody meditated lymphocyte proliferation. Typically a wheal and flare develops within 20 minutes of a bite and a pruritic indurated papule present for between 24-48hrs later but may persist for weeks (Peng and Simons, 2007). Local mast cell activation results in the release of vasoactive amines, including histamine and leukotriene C4 (Horsmanheimo et al., 1996) which induce vascular permeability and the subsequent of wheal and flare. (Demeure et al., 2005). There is also a Non-IgE mediated mast cell degranulation. Mice studies suggest the mechanism leading to characteristic itch associated with the bite may be from the release of 5-lipoxygenase metabolite(s) in mosquito allergy-associated itching (Kuraishi et al., 2007).

The rational for selecting 5% ibuprofen and 0.5% Clobetasone in this study was that these products were available without a prescription, and accessible to anyone, and as a topical formulation meant they could be applied immediately after a bite and not require to be ingested. As nuisance biting is very common amongst the population, and in particular in travellers to areas where there are 'aggressive feeders', having an accessible and easy-to-apply agent was a foremost consideration. The other consideration was reducing the pruritus and itch rather than the wheal and erythema so anti-inflammatory and anti-pruritic effect were considered in the selection.

Traditional topical treatments (ammonia (Zhai et al., 1998), phenol, camphor, menthol) act through a direct cooling effect on the skin and/or through a direct anaesthetic effect on the peripheral sensory system. The mechanism of action of most homeopathic and phythotherapeutic preparations is unknown, anti-inflammatory effects are likely to play a role in this context (Hill et al., 1995).

This double-blind, placebo-controlled, complete cross-over study with 40 participants showed that neither 0.5% ibuprofen nor 0.5% Clobetasone relieved the early symptoms of bites from *Stegomyia aegypti* mosquitoes. To make a comparison, similar studies where oral antihistamine was given as

treatment, wheal size was reduced by up to 60% compared with placebo and pruritis reduced by up to 62% compared with placebo (Karppinen et al., 2002; Karppinen et al., 2006).

Other clinical trials of topical treatments that have also failed to show a significant benefit of topical treatment including McKiel et al. (1956), where the antihistamine cream pyribenzamine had no effect on immediate reactions to *Aedes aegypti* bites, and also Hill and van Haselen (1996) and Hill et al., (1995) where in both cases homeopathic gels failed to significantly reduce bite symptoms.

The lack of effect in terms on erythema size reduction and pruritus observed in this study was extended for wheal size, where in fact, wheals were bigger after 5 and 15 minutes for bites treated with the actives compared with bites treated with placebo. No previous studies into the use of topical ibuprofen or corticosteroids have been published, making it is difficult to compare with other findings. Two case reports have been published where adults taking oral anti-inflammatories for osteoarthritis suffered significant increased sensitivity to bee and wasp stings only while on this medication (Bernard and Kersley, 1986), suggesting that treatment with anti-inflammatories may in rare cases be contra-indicative. The exact mode of action of ibuprofen is unknown, the roles of the different prostaglandins involved in the inflammatory response vary and have even shown to have an inhibitory effect where certain types of prostaglandins reduce inflammation (Crunkhorn and Willis, 1971; Kaur et al., 2010). It is possible that at the time points assessed during this study, the actives actually augmented the immune reaction.

The timing of when the measurements were taken in this study must also be considered as a factor influencing the results as it is possible the time points selected for assessments did not capture any perceived benefit. Reactions to mosquito bites vary in size and timing (Hudson et al., 1958). Some people have no reaction at all while others have an immediate response (type I) or a delayed response after approximately 24h (type IV) or both responses (Melanby, 1946; Hudson et al., 1958; Peng et al., 2004). The variety in reaction size and timing was also observed here and is demonstrated by the large confidence intervals surrounding the response variable means. It is possible that the large variability between individuals in their reactions to mosquito bites may have masked any reduction of symptoms within individuals once the data had been pooled for analysis. In addition, the assessment period included in this study was 90 minutes following mosquito challenge. In terms of the irritation caused by a mosquito bite, this is a relatively short period of time. If there were any lasting benefits (e.g. on type IV reactions) of immediate treatment of the insect bite with ibupfrofen or corticosteroid, this was not measured here.

This study highlights the lack of evidence supporting the use of topical treatments for type I reactions to mosquito bites. In particular, the timing and frequency of their application should be investigated.

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whole body extracts of mosquito species in Thailand. *Asian Pacific Journal of Allergy and Immunology*, **28**, 162-169.

Figure S1. Female *Aedes aegypti* mosquito feeding on a volunteer's arm through a mesh barrier.

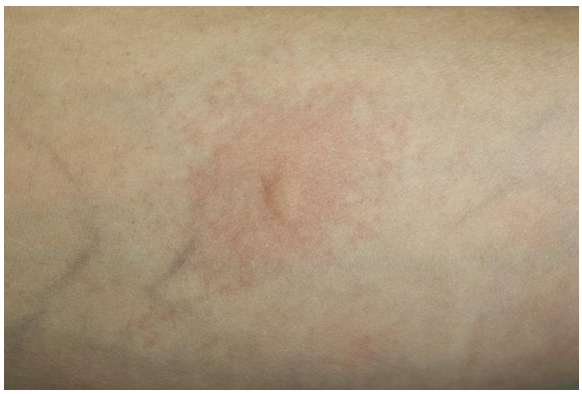

Figure S2. Wheal (raised, centre) and erythema (red rash) reaction 5 minutes after a single *Aedes aegypti* bite

Table 1. Wheal area mm^2 (mean±95%CI)

	Treatment Means (95%CI)			
Time	Steroid	Placebo (steroid)	Ibuprofen	Placebo (ibuprofen)
1	16.2 (11.9-22.1)	13.5 (9.8-18.4)	16.2 (11.9-21.9)	15.8 (11.5-21.5)
5	30.6 (22.6-41.2)	20.4 (15.0-27.6)	28.2 (20.9-37.9)	24.2 (17.8-32.7)
15	35.7 (26.5-48.1)	26.9 (19.9-36.3)	36.0 (26.7-48.3)	28.6 (21.1-38.6)
30	34.2 (25.3-46.0)	28.9 (21.4-39.0)	33.4 (24.8-44.8)	29.4 (21.7-39.7)
60	27.4 (20.2-37.0)	24.8 (18.2-33.5)	27.4 (20.3-36.9)	27.1 (20.0-36.6)
90	20.9 (15.3-28.3)	20.1 (14.7-27.2)	18.3 (13.5-24.8)	23.3 (17.1-31.5)

Table 2. Erythema area mm^2 (mean±95%CI)

	Treatment Means (95%CI)			
Time	Steroid	Placebo (steroid)	Ibuprofen	Placebo (ibuprofen)
1	329 (216-480)	299 (195-441)	420 (285-603)	366 (243-531)
5	475 (325-679)	530 (365-752)	588 (410-830)	502 (344-716)
15	443 (301-635)	501 (344-713)	514 (354-729)	494 (338-705)
30	411(277-591)	425 (287-610)	459 (314-655)	383 (256-555)
60	301(196-443)	300 (195-442)	318 (209-464)	284 (183-420)
90	191 (115-294)	186 (111-288)	151 (85-238)	167 (97-262)

Table 3. Itching (mean±95%CI)

	Treatment Means (95%CI)			
Time	Steroid	Placebo (steroid)	Ibuprofen	Placebo (ibuprofen)
1	39.4 (33.3-45.6)	36.1 (30.0-42.2)	35.4 (29.4-41.5)	30.1 (23.9-36.5)
5	36.7 (30.5-42.8)	34.1 (27.9-40.2)	33.4 (27.4-39.5)	29.6 (23.4-35.8)
15	30.9 (24.8-37.0)	29.6 (23.5-35.8)	28.4 (22.4-34.5)	23.8 (17.6-30.0)
30	19.2 (13.1-25.4)	18.9 (12.7-25.0)	15.3 (9.3-21.3)	15.7 (9.5-21.8)
60	8.9 (2.7-15.1)	9.9 (3.7-16.1)	8.2 (2.1-14.2)	8.6 (2.4-14.8)
90	5.5 (-0.6-11.7)	4.4 (-1.8-10.5)	3.4 (-2.6-9.5)	4.8 (-1.4-11.0)

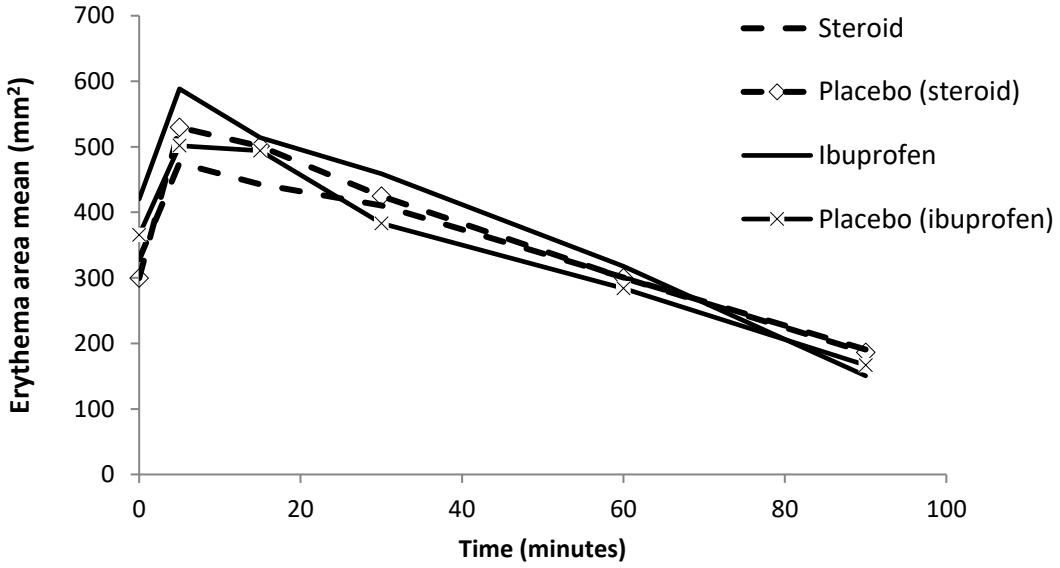

Figure 1. Erythema area over time following a single bite with *St. aegypti* and after-bite treatment. Values presented are mean areas of the erythema ellipse of 40 participants, calculated from measurements of erythema width and length.

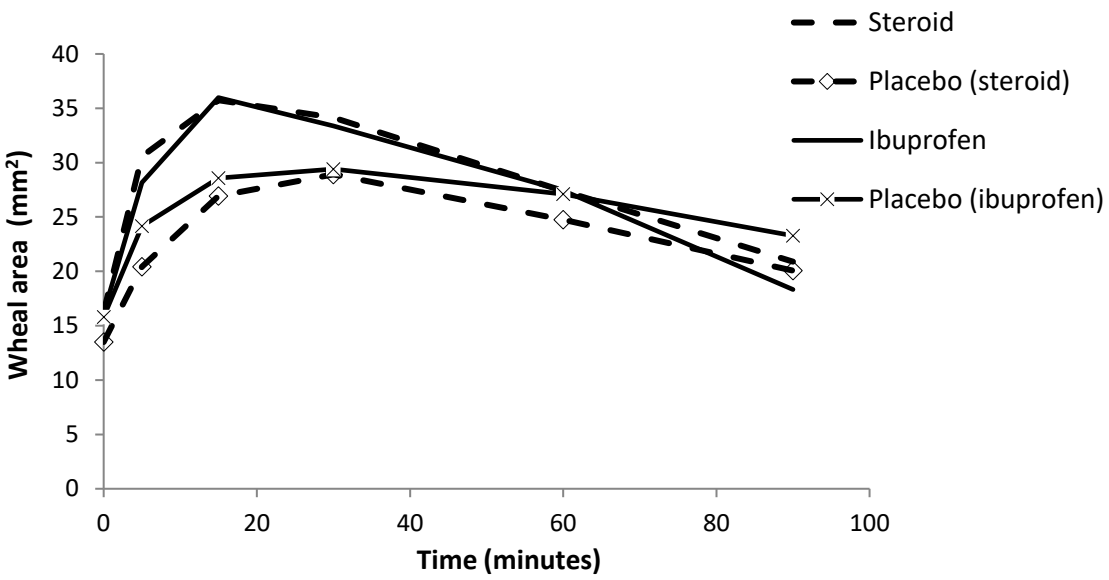

Figure 2. Wheal area over time following a single bite with *St. aegypti* and after-bite treatment. Values presented are mean areas of the wheal ellipse of 40 participants, calculated from measurements of wheal width and length.

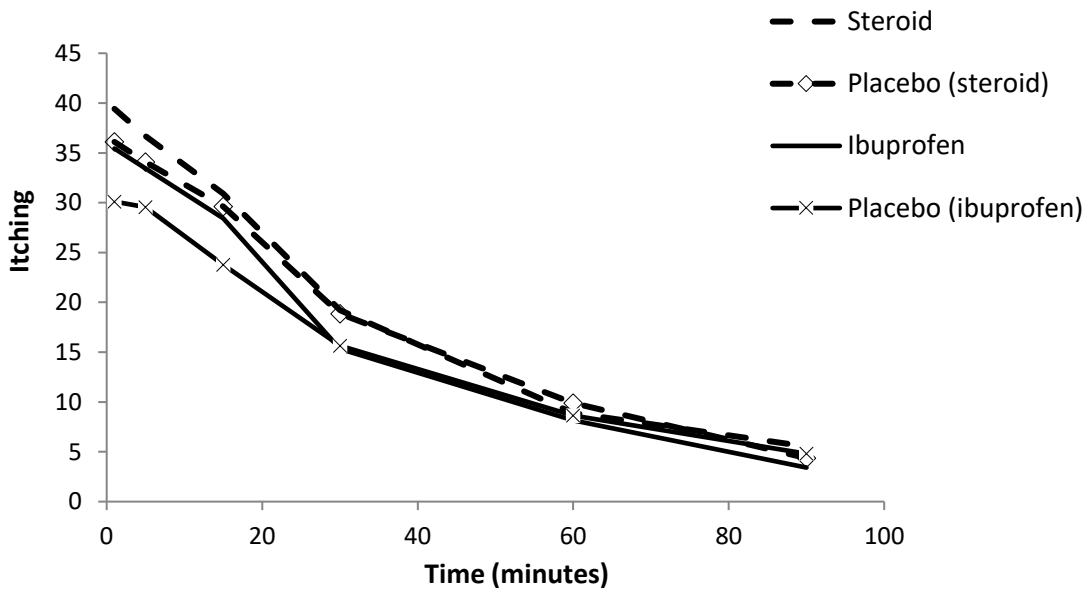

Figure 3. Itching score over time following a single bite with *St. aegypti* and after-bite treatment. Values presented are means from 40 participants. Itching was graded on a scale of 0 – 100.

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Secondary Sources

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