

Topical *Channa striatus* 5% cream for inflammatory skin conditions: A phase I randomized double-blind, controlled trial

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INTRODUCTION

Channa striatus or better known as the haruan fish is an indigenous, snakehead, striped, air breathing freshwater fish of Malaysia. This versatile fish inhabits all types of fresh water bodies from small ponds to rice fields and rivers and is found throughout Pakistan and India to Southeast Asia

Background: *Channa striatus* or better known as the haruan fish is an indigenous, snakehead, striped, air breathing freshwater fish of Malaysia and has long been consumed traditionally during post-operative and post-partum period to speed recovery. Most medical properties of the haruan are contributed to its high content of essential fatty and amino acids. This was a phase I clinical trial to examine the safety of *Channa striatus* (CS) 5% cream with patch testing and cumulative dosing for up to 4 weeks on the healthy skin.

Methods: This double-blind randomized controlled trial was conducted in two stages. Eighty-three participants who fulfilled the inclusion criteria underwent patch testing. The allergens, aqueous and CS 5% cream, were patch tested simultaneously but separately on each arm of the same participant. The participants with a negative patch test started stage 2, whereby they applied randomly assigned creams on both forearms for a duration of 1 month.

Results: A total of 83 participants were screened successfully and underwent patch testing. One participant in the cohort developed a positive patch test to CS 5% and was excluded from stage 2. Two participants experienced mild side effects that resolved with mild topical steroid. The incidence of allergic contact dermatitis and irritant contact dermatitis was 1.2% and 2.4% in the participants, respectively.

Conclusion: The uniquely Malaysian *Channa striatus* 5% cream has a good safety profile. Information on the safety and tolerability obtained from this study can be used to design larger phase II studies in patients with inflammatory skin conditions.

Trial registration: Registered with National Medical Research Register of Malaysia, ID: NMRR-14-1901-22292

Keywords: safety, haruan extract, *Channa striatus*, anti-inflammatory agent

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and Southern China ¹. In Malaysia, the haruan has long been consumed traditionally during post-operative and post-partum period to prompt wound healing, ease pain, and boost energy by the sick.

Animal studies have shown promising results for its wound healing ^{2,3} properties as well as anti-inflammatory ^{4,5}, antinociceptive ⁶ and antimicrobial effects ⁷. The haruan is also recently being

studied for its use in osteoarthritis⁸, depression⁹ and hypertension¹⁰. Most medical properties of the haruan are attributed to its high content of essential fatty acids and amino acids that have anti-inflammatory properties^{4,5,11-13}.

This is the first clinical trial using topically applied *Channa striatus* (CS) cream. Assessment of skin irritation and sensitisation potential is required by regulatory authorities for new topical drugs. The current study used patch testing and cumulative irritation testing, which are both effective predictive tests to determine the allergic contact dermatitis potential of the topically applied CS 5% cream¹⁴⁻¹⁶.

PARTICIPANTS AND METHDOS

This prospective, double blind, randomized controlled trial was done to evaluate the safety of CS 5% versus aqueous cream as the vehicle in a healthy population over a 5-week period. This study consisted of 2 stages with patch testing in stage 1 and cumulative irritation testing over 4 weeks in stage 2.

Study design

The 5-week study consisted of 2 stages. In the first stage, 83 participants who fulfilled the inclusion criteria underwent a patch test. The vehicle, which was aqueous cream, and the potential allergen, CS 5%, were patch tested separately on each arm of the same participant. The allergen was randomly selected by an uninvolved staff using simple randomization through flipping a coin. Both the participants and researchers were blind to the type of the allergen tested. The patch test reading was done on day 2 (48 hrs) and day 4 (96 hrs).

Following successful completion of patch testing, the participants with a negative patch test were carefully instructed to apply CS 5% and aqueous cream on a 5 x 5 cm area on each forearm on a daily basis (repeat open application test) on the same site at night for a month. The participants were clinically evaluated for itching, erythema, papules, and erosions every week for a period of one month. The study medication could be continued or stopped to reflect normal clinical practice. The study medications with jars or containers were weighed pre and post treatment at week 0, 1, 2,

3 and 4. Adverse effects were assessed at each weekly visit. The participants were also told to fill up a safety leaflet on a daily basis and to record any change in their skin.

Participants

We recruited 83 participants among the healthy population who fulfilled the inclusion criteria and attended the research clinic in UITM, Malaysia. In our study, healthy participants were chosen as they were more robust, free of other medicines, more likely to respond uniformly, and better at completing this trial. As this was a phase 1 clinical trial, the number of the required participants was between 50 to 200 participants¹⁷. The study protocol was reviewed and approved by the local medical ethics committee and the study was performed in accordance with the Good Clinical Practice guidelines. Written informed consent was obtained from all participants.

Eligibility Criteria

Male and female individuals aged 16-30 years with a normal appearing healthy skin were included in this study. The participants were divided into 2 age groups: adolescent (16 to 19 years old) and young adults (20 to 30 years old).

The participants who were pregnant or breast-feeding, were diagnosed with atopic eczema or other primary or secondary dermatoses, had chronic or recurrent skin infection, received anti histamine, and those who had any chronic systemic illness such as diabetes mellitus, asthma, hypertension, and malignancy, were on systemic medications, or were allergic to any component of the haruan extract were excluded from this study.

Interventions

Experimental intervention: *Channa striatus* cream (CS)

The CS cream prototype was prepared using scientific methods, starting from the preparation of the haruan fresh fillet and preparation of the haruan water extract (HWE) to the final step of CS cream preparation. The CS cream used in this study was formulated at a concentration

of 5%. For the 200ml formulation used by the participants, 10 ml HWE was mixed with 190 ml aqueous cream. All preparations were homogenized with a homogenizer using emulsifying agents (Wigger Hauser D-500, Germany) at 3500 rpm for a period of 10 minutes. Aqueous cream was used as the base. More than 60% of the ingredients of CS cream were locally sourced from Malaysia. The formulation contained minimum chemical additives, followed halal production requirements and was non-steroidal. The formulation itself was an innovation, and its manufacturing was outsourced to the Biotechnology Research Center (IBRC), SIRIM Bhd at Shah Alam, Malaysia. The facility at SIRIM Bhd has been awarded Cosmetic Good Manufacturing Procedure (Cosmetic GMP), and the haruan cream prototype was registered and notified by National Pharmaceutical Control Bureau (NPCB), Ministry of Health of Malaysia on 17 March 2014.

Control intervention: Aqueous cream

The aqueous cream preparation was manufactured by the same company; i.e. Pharmaniaga. Its contents were also follows: emulsifier 9.0%, white soft paraffin 15.0%, liquid paraffin 6.0%, chlorocresol 0.1%, glycerine 5.0% and purified water 64.9%

Outcome assessment

The patch test chambers were made from

polypropylene and manufactured by Van der Bend Co., the Netherlands. Each chamber contained a chromatographic filter paper (Figure 1). The plaster material could be written on and was made from hypo-allergenic, medical, non-woven polyester. All materials used complied with accepted medical quality standards. A standard 'patch test chambers reading plate' was used during patch test reading. A standard patch test information leaflet was given to each patient.

Patch testing was done according to the European Society of Contact Dermatitis Guidelines, with modifications made to the size of the chamber used for occluding and applying the allergen. The test concentration of the allergen was 5% as the concentration of the tested allergen is recommended to be between 0.01% to 10%¹⁸. The reaction sites were interpreted based on the method recommended by the International Contact Dermatitis Research Group (ICDRG)¹⁹.

Safety Issues

The participants kept a daily safety calender during the study, which included any adverse event. Each participant recorded the time of onset and resolution of any such event, and the events were rated as mild, moderate, or severe.

This trial was registered with National Medical Research Register of Malaysia, ID: NMRR-14-1901-22292

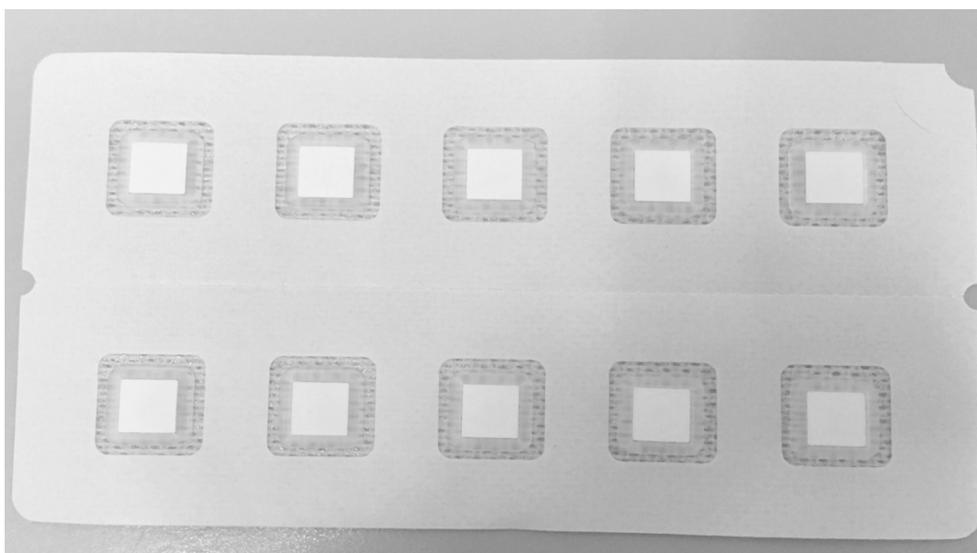


Figure 1. Van der Bend patch test chambers

RESULTS

Eighty-three participants were screened successfully and underwent patch testing. Table 1 shows the demographic and baseline characteristics of the participants. The mean age of the participants was 24.9 years. One participant developed a 2+ positive patch test to CS 5% cream. Another participant was lost to follow up at the first week. After applying the creams, two participants experienced mild side effects at the site of CS 5% cream application. Case 1 developed mild erythema and itching on day 3 after using 0.25gm and Case 2 developed few papules and itching on day 28 after using 5.04gm. A total of 81 participants completed the study. Case 1 was known to have mild food allergy towards seafood, but not particularly to the haruan fish. The mean use of CS and vehicle was 10.24 and 12.77gm, respectively. Figure 2 illustrates the cumulative use of CS 5% cream over 4 weeks.

One out of 83 (1.2%) participants developed allergic contact dermatitis to CS 5% cream as proven by a 2+ positive patch test. It resolved with elimination of the allergen and application of mild topical steroids.

Over the course of the study, 79/81 (97.5%) of the participants did not report any adverse events. Two out of 81 (2.4%) participants developed irritant reaction to CS 5% cream. The skin reaction in both participants resolved with mild topical steroid within 48 hours. There were no localized or systemic severe adverse effects in all participants.

To summarize, the incidence of allergic contact

Table 1. Demographic and baseline characteristics of study participants.

Characteristic	N (%)
Female sex	54 (65.1)
Race	
Malay	70 (84.3)
Chinese	2 (2.4)
Indian	2 (2.4)
Others	9 (10.8)
Household income (Malaysian Ringgit)	
<1000	20 (24.1)
1000≤ and ≤5000	61 (73.5)
>5000	2 (2.4)
Family history of atopy	18 (21.7)
Food allergy	10 (12.0)
History of drug induced eczema	2 (2.4)
History of contact dermatitis	6 (7.2)

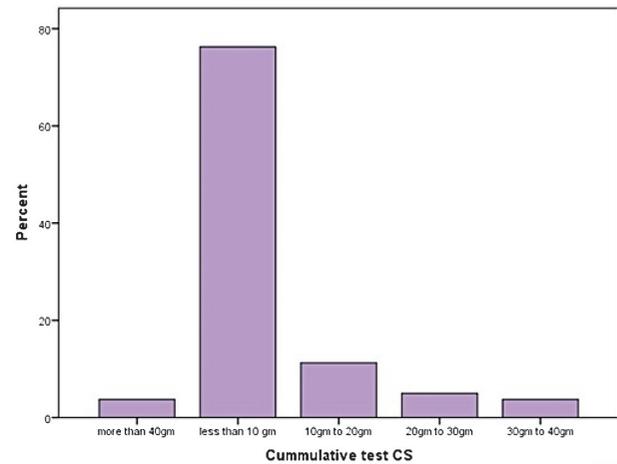


Figure 2. Cumulative dosing of *Channa striatus* cream after 4 weeks. The mean on the amount of *Channa striatus* 5% cream used for 4 weeks was 10.24 gm.

dermatitis and irritant contact dermatitis to CS cream 5% was 1.2% and 2.4%, respectively.

DISCUSSION

Our overall objective was to develop safe, effective, and cost efficient agents for the treatment of inflammatory skin conditions. As of today, the most widely used and available treatment is topical corticosteroids, the use of which has a bad reputation because of its local and systemic side effects. Some adverse effects include skin atrophy, striae, perioral dermatitis, acne, purpura, and hypertrichosis²⁰. Systemic reactions such as hyperglycemia, glaucoma, and adrenal insufficiency²¹ have also been reported following chronic topical application. The demand for non steroidal topical treatments brought about the development of topical calcineurin inhibitors like pimecrolimus (1.0%) and tacrolimus (0.03 and 0.1%), which have been marketed primarily for the treatment of atopic dermatitis. Although widely available, they received a black box warning by the Food and Drug Administration in 2005, raising patient and parental concerns²².

In the pursuit of these goals, we set to determine if CS 5% cream was a safe and tolerable treatment in the general population with normal skin. This phase 1 trial was part of the development of CS 5% cream for topical use in patients with mild to moderate atopic eczema. A phase II trial of CS 5% cream is being pursued to assess the efficacy of this formulation in participants with mild to moderate

eczema. The duration of our study mirrored similar safety studies done for topical creams developed for chemoprotection for skin cancer prevention²³ and ongoing studies for atopic dermatitis²⁴ with a study period of 4 weeks.

Mild, treatment-related adverse events were observed in 3.6 % of the participants. In this study, however, it is important to point out that all the adverse events were very mild and resolved within 48 hours with mild topical steroid application. The adverse events could be attributed to environmental causes even though the participants considered them related to the cream. Irritant contact reactions seen in 2 participants could be due to the vehicle. As the participants only developed an irritant reaction on the site of CS 5% cream application, the occurrence of these adverse events was surprising. However, based on this information, these types of adverse events need to be followed carefully with subsequent clinical testing of the formulation in larger studies of longer duration.

As for other limitations, the participants were mainly limited to the district of Selayang, Sungai Buloh, and the surrounding areas in Malaysia. There was a discrepancy in the number of male and female participants. Not many young participants were included in the study. The racial distribution also did not reflect the actual racial demography in Malaysia.

The results of this study indicate that the topical formulation of *Channa striatus* 5% is safe when applied once daily for 30 days. This discovery gives us the platform to embark on further research on the use of *Channa Striatus* for the treatment of inflammatory skin conditions such as seborrheic and atopic dermatitis.

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