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Antipruritic properties of topical *Channa striatus* extract on stratum corneum disruption-itch mice model

Irfan Fauzi *, Ahmad Akira Omar Farouk and Enoch Kumar Perimal

Faculty of Medicine & Health Sciences, Universiti Putra Malaysia, Serdang, Selangor Darul Ehsan.
*Correspondence: imuhd98@yahoo.com; Tel.: +601126821255

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Abstract: Itch is an unpleasant sensation that provokes a strong desire to scratch. In some cases, antihistamines are ineffective in treating chronic itch and produce side effects. *Channa striatus* fish extract, which can alleviate pain, is also used in this experiment to alleviate the itch. This experiment aims to determine the possible antipruritic effect of topical *Channa striatus* extract (CSE) on stratum corneum disruption (SCD)-itch mice model. Experiments were divided into 6 groups of male ICR mice which were positive, negative, vehicles, 3% CSE, 7% CSE, and 10% CSE groups. Rostral back of mice was induced with chemicals acetone, diethyl ether (1:1) and water (AcetoneEthylWatermethod) daily for five consecutive days. All groups were treated with specific selective treatments. The scratching behaviour of the mice was observed by videotape. The skin moisture and oiliness scoring was measured daily using a skin analyzer. The skin was isolated for RNA extraction and measured for tumour necrosis factor-alpha (TNF- α) gene expression level using real-time RT PCR. All treatment groups of *Channa striatus* extract cream had shown a significant reduction in scratching time, increase in skin moisture and skin oiliness. The TNF- α gene expression level had inconsistent results and needed a more extensive sample. The *Channa striatus* extract cream may be able to alleviate itch compared to the control parameters.

Keywords: Antipruritic; *Channa striatus* extract (CSE); Chronic itch; Tumor necrosis factor-alpha (TNF- α)

1.0 INTRODUCTION

Itch or pruritus can be defined as an unpleasant sensation that provokes a strong desire to scratch (Yosipovitch et al., 2003). It is understood that chronic itch is a complex sensory experience with many similarities to pain. Also, a mechanism-based definition of itch has been proposed that separates itch induced in a healthy nervous system by peripheral (pruriceptive) and central (neurogenic) mechanisms from itch caused by diseased neurons (neuropathic). However, the pathophysiology of most clinical itch conditions is unclear. Acute itch is a sensation that can usually stop or relieve by shortly scratching near the area of itching and usually not longer than 6 weeks. Chronic itch is difficult to abolish and generally longer than 6 weeks. Local scratching often provides little relief and can exacerbate the problem (Ikoma et al., 2006). Unlike the pain sensation, where an organism will try to withdraw from that unpleasant stimulus, an itch compels the affected to seek out the source and respond with a scratch (Nutten, 2015). The specificity and selectivity theory suggested that itch and pain pathways were the

same but not identical (McMahon & Koltzenburg, 1992). Some itch-producing agents have been found to activate nociceptive primary afferent fibre and thus generate nociceptive sensation (LaMotte et al., 2014). However, when it comes to itch-related diseases, either acute or chronic itch, antihistamines and corticosteroids are the current medication among physicians. Antihistamine is ineffective against chronic itch, especially on dry skin itch and eczema. Corticosteroid has common side effects such as increased skin fragility, petechial and skin atrophy (Grundmann & Ständer, 2012).

Currently, there are many problems with medications faced by people suffering from chronic itch due to the side effects that prevent certain patients with certain health conditions from consuming them. Other problems are due to the drug availability and the high cost of the available drugs. Therefore, it is warranted to search for alternatives to treat all types of dry skins with fewer side effects that are also effective and readily available. Today, natural product-based remedies have gained the attention of many researchers as these types of remedies have many medicinal purposes and be considered safe and cheap. The animal-based natural product is one of the families within the natural products that have been studied well. *Channa striatus* fish, striped snakehead locally known as Haruan, is a freshwater fish species indigenous to Malaysia (Mat Jais, 2007; Mohsin & Ambak, 1983). Studies have shown that *Channa striatus* exhibited anti-inflammatory and antinociceptive activities. This is due to its high arachidonic acid content, acting as a precursor for prostaglandin production. Prostaglandins stimulate the synthesis of mucus that is required as a barrier against those truculent factors (Zakaria et al., 2008, Zakaria et al., 2004). According to a previous study, there is an elevation of TNF-α when pain is presented. It may also be elevated when an itch sensation is present (Leung & Cahill, 2010; Polgreen et al., 2016). The inflammatory response is regulated by many chemical mediators that form a complex regulatory network, including chemokines, vasoactive amines, eicosanoids, and cytokines (Medzhitov, 2010). The increased production of inflammatory cytokines and chemokines is thought to play a significant role in the pathogenesis of atopic dermatitis (Homey et al., 2006). Hence the experiment on TNF-α on itch is conducted as a screening experiment for extensive detail parameters ahead. This experiment is based on the hypothesis that *Channa striatus* extract (CSE) may have antipruritic activity on stratum corneum disruption - itch mice model. The significance of this research will be the discovery of the potential of CSE cream as an effective and nonsteroidal treatment option for atopic dermatitis and any other chronic inflammatory skin disorders.

2.0 MATERIALS AND METHODS

2.1 Experimental animals

Adult male, IcrTac (ICR) mice weighing 25-30 grams have been used in this experiment. The animals were kept for 3 days before use under room temperature (27±2°C; 70-80 % humidity; 12h light/darkness cycle) in the Animal House, Faculty of Medicine and Health Sciences, UPM in accordance with the IACUC guidelines (Ref no: UPM/IACUC/AUP-R001/2017) for the care of laboratory animals and ethics. The mice were supplied with a commercial pellet diet and water *ad libitum* up to the end of the experiments. A total of 48 mice were divided into 6 groups (3 control groups and 3 treatment groups), with 8 mice per group **(Table 1)**.

Table 1. Experimental group design (8 mice per group x 6 groups = 48 mice)

Group no.	Group name	Treatment day 6
1	Positive control	AEW + 1% Corticosteroid
2	Negative control	AEW
3	Vehicle control	AEW + Aqueous cream
4	3% CSE cream	AEW + 3% CSE cream
5	7% CSE cream	AEW + 7% CSE cream
6	10% CSE cream	AEW + 10% CSE cream

2.2 *Channa striatus* extract (CSE) cream

The CSE cream had been produced using *Channa striatus* fish extract and was received as a ready-to-use product for this study. Briefly, the preparation of the CSE extraction process is based on the conventional laboratory extraction of organic solvents (Mat Jais et al., 1997; Dahlan-Daud et al., 2010). This process provided solid and liquid portions of the extract. The solid part of the extract was oven dried to discard water completely and ground into a dried superfine powder. The CSE powder was stored at room temperature (27°C). Preparation of the CSE cream used 3g, 7g and 10 g of the stock super fine powder, which was homogenized with 97g, 93g and 90g of aqueous cream as the base, to give the three different concentrations of 3%, 7% and 10% CSE cream in a total of 100g (Miyamoto et al., 2002). These percentages were used for standardization purposes and not more than 10% CSE on cream products.

2.3 Behavioral assessment of stratum corneum disruption-itch mice model

The itch model was carried out according to the method described by Miyamoto et al. (2002). Mice were shaved on the rostral back about 2cm x 2cm using a shaver and acclimatized (27±2°C; 70-80% humidity; 12h light/darkness cycle) for 3 days in the Animal House. Dry skin itch by stratum corneum disruption has been induced by treating the rostral back with chemicals; acetone, diethyl ether (1:1) and water (AEW). The mixture of acetone and diethyl ether (1:1) solutions was applied for 15 seconds and continued with distilled water for 30 seconds using cotton wool. These two steps were repeated twice daily (9.00 and 16.00) for 5 consecutive days. Treatment compounds of aqueous cream (vehicle control), 1% Corticosteroid (positive control), 3 %, 7 % and 10 % of CSE creams (treatment groups) were applied topically at 0.5 g/mice at the rostral back right after 5 days of AEW treatment, and let sitting for 30 minutes. Mice were placed in custom chambers on an elevated metal mesh floor and scratching-time (s) were recorded for 30 minutes **(Figure 1)**. The skin moisture and oiliness of the mice were recorded before and after treatment according to the respective groups.

Figure 1. Custom chamber on the metal mesh.

2.4 Gene expression of tumour necrosis factor-alpha

Rostral-back tissues were harvested right after day 6 of behavioural assessment and immediately kept submerged in the RNA stabilization reagent RNAlater (Qiagen, Germany) and stored at 4°C overnight and later transferred to −20°C for more extended storage. According to the manufacturer's protocol, RNA extraction was performed using RNeasy Mini Kit (Qiagen, Germany). Twenty milligrams of the back tissue previously stored in RNAlater were thoroughly homogenized using TissueRuptor (Qiagen, Germany). The concentration and purity of RNA product have been determined by using a micro-volume spectrophotometer (Quawell, USA) by measuring the absorbance at 260nm and 280nm, respectively. Samples with purity > 1.9 were selected for RNA quantification. For RNA quantification, 40 ng of sample RNA and 2.5 µl of pre-validated gene-specific primers (Mm_Tnfaip1_1_SG) (Qiagen, Germany), 10X QuantiTect Primer Assay (Qiagen, Germany) was mixed with the supplied materials in the QuantiFast SYBR Green RT-PCR kit (Qiagen, Germany) to make a total mixture of 25 µl in a 0.2 ml microcentrifuge tube, according to the manufacturer's protocol. The pre-validated primers used can be referred to as follows: TNF-α (Mm_Tnfaip1_1_SG) (Qiagen) and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (Mm_Gapdh_3_SG). All reactions were operated by Rotor-Gene 6000 real-time cycler (Corbett Research), which was set at the following settings; (1) 50°C for 10min, (2) 95°C for five minutes, (3) 95°C for 10s, (4) 60°C for the 30s for a total number of 40 cycles. Melting curve analyses were performed by slowly increasing the temperature from 65°C to 95°C. Raw data in the quantification cycle (Cq) was analyzed by relative quantification using the Comparative method (ΔΔCq) (Livak & Schmittgen, 2001). GAPDH gene was selected as the reference gene after confirming that the gene expression was stable and not influenced by any treatment given.

2.5 Data analysis

All the data will be expressed as mean ± Standard Error Mean (SEM) and analyzed by one-way ANOVA followed by Dunnet's test. Differences will be considered as significant when the p-value is less than 0.05.

3.0 RESULTS

3.1 Behavioral assessment of stratum corneum disruption-itch mice model

3.1.1 Itch mice model

The effectiveness of the SCD-itch model was measured by recording and observing the average scratching time (s) of mice from all groups for five consecutive days. The average scratching time of mice in every group for five consecutive days after receiving a repeated dose of acetone, diethyl ether (1:1) and water (AEW) at 9.00am and 4.00pm daily **(Figure 2)**. The highest reading was exhibited on Day 5 where the scratching time (s)

(mean±SEM) was 69.52±4.085 and was followed by 50.19±3.659 (Day 4), 43.69±1.595 (Day 3), 34.33±1.714 (Day 2) and 20.53±1.079 (Day 1). The average scratching time (s) from Day 2 to Day 5 was significantly higher than on Day 1.

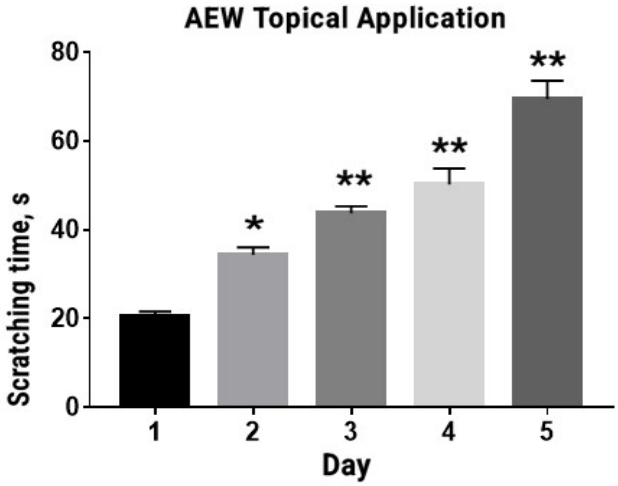

Figure 2. The average scratching time of each group was significantly higher when mice received repeated AEW induction twice daily for 5 consecutive days. Results are shown as mean ± SEM and compared by one-way ANOVA with Dunnet's test. * p<0.01, ** p<0.0001.

3.1.2 Scratching time after CSE cream treatment

The scratching time of the mice was recorded and observed for 30 minutes after a single treatment application according to the respective groups; positive control, negative control, vehicle control, 3%, 7% and 10% of CSE cream groups. The vehicle control group exhibited the highest scratching time (s) duration compared to other groups **(Figure 3)**. There was no significant difference between the vehicle control and negative control groups. The lowest scratching time(s) (mean ± SEM) was shown by 34.9 ± 5.485 (positive) followed by 39.35 ± 1.986 (10%), 42.02 ± 5.536 (7%) and65.57 ± 7.686 (3%). All the groups were significantly lower when compared to the negative and vehicle control groups.

3.2 Skin condition after treatment

3.2.1 Skin moisture scale

The skin moisture scale of the mice was recorded before and after the treatment according to the respective groups using a skin analyzer; positive control, negative control, vehicle control, 3%, 7% and 10% of CSE creams. The negative control group exhibited the lowest moisture scale compared to the other treatment groups **(Figure 4)**. Results were significantly higher in reading on the moisture scale (0 to 10) (mean ± SEM) in three different concentrations of CSE cream in comparison to the negative group, which were 5±0.516 (3%), 4.167±0.307 (7%), 4.167±0.167 (10%), and 5.167±0.703 (positive).

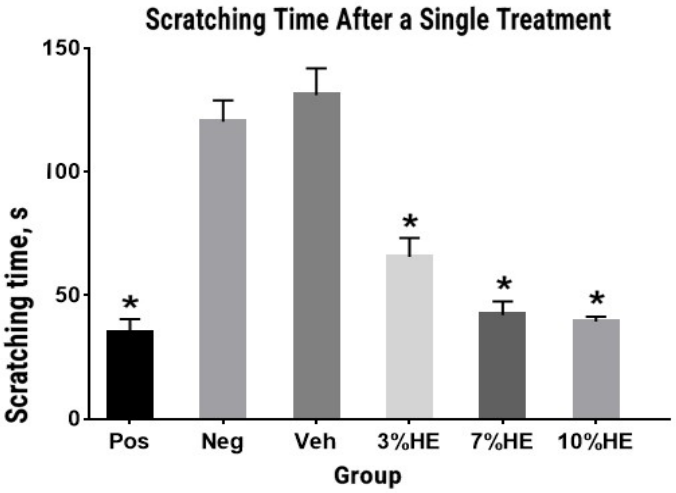

Figure 3. The scratching time for 30 minutes after a single treatment of 3% CSE, 7% CSE and 10% CSE were significantly lower compared to the negative and vehicle control group. Results are shown as mean ± SEM and compared by one-way ANOVA with Dunnet's test. *** p<0.0001. HE (Haruan extract) refers to CSE (*Channa striatus* extract); Pos (positive group); Neg (negative group); Veh (vehicle group).

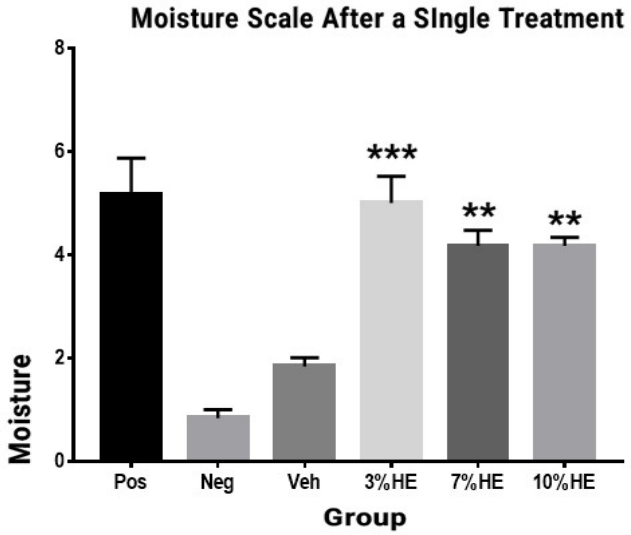

Figure 4. The skin moisture of each mice in every group was recorded on a scale of 1 to 10. The moisture scale after 30 minutes of a single treatment of 3% CSE, 7% CSE and 10% CSE were significantly higher compared to the negative and vehicle control group. Results are shown as mean ± SEM and compared by one-way ANOVA with Dunnet's test. ** p<0.001, *** p<0.0001. HE (Haruan extract) refers to CSE (*Channa striatus* extract); Pos (positive group); Neg (negative group); Veh (vehicle group).

3.2.2 Skin oiliness scale

The oiliness scale of the mice was recorded before and after treatment according to the respective groups by using a skin analyzer; positive control, negative control, vehicle control, 3 %, 7 % and 10 % of CSE creams**.** The negative control and vehicle groups exhibited the lowest oiliness scale compared to the other treatment groups, and there was no significant difference between both groups **(Figure 5)**. Reading of the oiliness scale (0 to 10) (mean ± SEM) were significantly higher in three different concentrations of CSE cream in comparison to the negative group, which were 6.833 ± 1.014 (3%), 4.667 ± 0.422 (7%), and 4.833 ± 0.401 (10%).

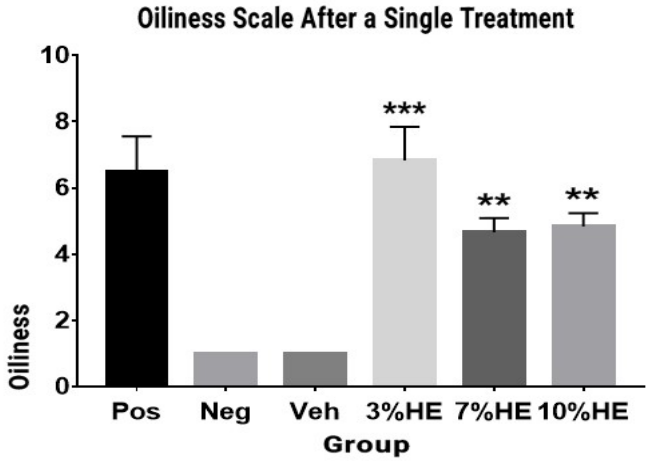

Figure 5. The skin oiliness of mice in every group was recorded on a scale of 1 to 10. The scale of oiliness after 30 minutes of a single treatment of 3% CSE, 7% CSE and 10% CSE were significantly higher compared to the negative and vehicle control groups. Results are shown as mean ± SEM and compared by one-way ANOVA with Dunnet's test (** p<0.001, *** p<0.0001). HE (Haruan extract) refers to CSE (*Channa striatus* extract); Pos (positive group); Neg (negative group); Veh (vehicle group).

3.3 Gene expression level of tumour necrosis factor-alpha (TNF-α)

The topical application of 10% CSE cream extract showed the highest expression of TNF-α in comparison to the other groups; 151.167 (fold change; $2^{-\Delta\Delta Cq}$) **(Figure 6)**. On the contrary, 3% and 7% CSE creams had down-regulated the expression level of TNF-α when directly compared to the vehicle group.

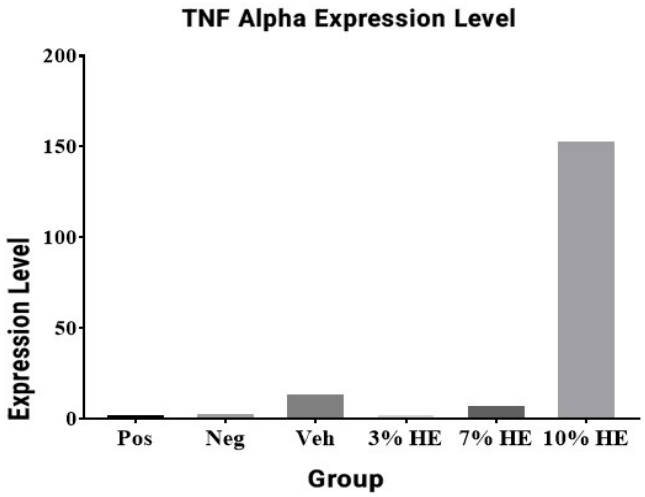

Figure 6. TNF-α gene expression levels of dry skin itch groups were determined using real-time RT PCR and directly compared between groups (n=4-5). Each point represents the treatment/vehicle fold ratios after normalization to GAPDH. HE (Haruan extract) refers to CSE (*Channa striatus* extract); Pos (positive group); Neg (negative group); Veh (vehicle group).

4.0 DISCUSSION

There are two well-known theories of itch signal transmission that have been proposed. First, the intensity theory suggested that low-level nociceptor activation could initiate the sensation of itch, whereas a higher frequency should give rise to pain sensation. Second, the specificity and selectivity theory suggested that itch and pain pathways were the same but not identical (McMahon & Koltzenburg, 1992). Some itch-producing agents have been found to activate nociceptive primary afferent fibre and thus generate nociceptive sensation (LaMotte et al., 2014). Chronic itch has been a major concern nowadays as it causes a reduction in the quality of life. To see the effectiveness and changes by *Channa striatus* extract against dry skin itch, it had to be prepared into cream or emollient and applied topically onto the itchy skin. Therefore SCD-itch, one of the chronic itch mice models, was used.

In this study, the effectiveness of *Channa striatus* extract cream as a topical antipruritic (SCD-itch) agent was demonstrated by the (1) reduction of the rostral back-scratching time (s) of the mice and (2) suppression of histological features of common dry skin itch criteria such as skin moisture and skin oiliness. Interestingly, about 10% of the *Channa striatus* extract cream had produced an antipruritic activity with similar potential to that of steroid-based corticosteroid 1 % cream. This finding may also demonstrate the effectiveness of more than 10% concentration of *Channa striatus* extract cream in reducing pruritis.

It is still largely unknown which bioactive compound(s) of *Channa striatus* are involved in the antipruritic pathway. Since itch and pain may have the same

pathway as suggested (McMahon & Koltzenburg, 1992), anti-inflammatory may also play its role in antipruritic activities. It has been suggested that high contents of docosahexaenoic acid (DHA) in *Channa striatus* could have contributed to the anti-inflammatory action (Zuraini et al., 2006). Notably, DHA was shown to suppress the production of some inflammatory mediators, including TNF-α and COX-2 (James et al., 2000). Several major fatty acids, including palmitic acid, stearic acid and linoleic acid, are abundant in Channa striatus. These fatty acids have contributed to the anti-inflammatory effect of Channa striatus by inhibiting both COX-1 and COX-2 (Ringbom et al., 2001). Besides, profiling of the *Channa striatus* traditional extract revealed four unknown major fractions, which are believed to be bioactive compounds of the *Channa striatus* (Dahlan-Daud et al., 2010; Zakaria et al., 2007). These unknown fractions are also believed to be peptides or polypeptides, which could have been combined from the essential amino acids such as glycine, arginine, alanine and proline in *Channa striatus*. More studies should be conducted into the identification of the bioactive compounds as *Channa striatus* is a promising candidate for future nutraceutical and pharmaceutical products. However, in this study, the whole extraction from the *Channa striatus* was used to formulate CSE cream since its bioactive compounds are still unknown.

The presence of arachidonic acids and glycine contents of CSE are involved in the antinociceptive pathway (Kapoor et al., 2006; Zakaria et al., 2005; Zuraini et al., 2006). The presence of the amino and fatty acids, which act as the major component of skin collagen and phospholipid membranes, may help break the itch-scratch cycle (Mohd Shafri & Mat Jais, 2012; Wu, 2013). They also act as precursors in producing eicosanoids, prostaglandins, thromboxane, prostacyclin and leukotrienes (Kim et al., 2002). These might be involved in repairing the dry skin membrane since a higher concentration of CSE in the aqueous cream was a more effective treatment against scratching time. The fatty acid is the substitute for the natural loss of skin lipid and structural elements of the stratum corneum (Agner, 2016). This can be proven by an increase in the scale of skin moisture and skin oiliness of the three treatment groups, which were 3%, 7% and 10% of CSE creams. Glycine, the major amino acid in *Channa striatus* (Zuraini et al., 2006) is believed to work synergistically with arachidonic acid from the fatty acid group in the healing process. CSE can promote the wound healing in conditions like a patient with post-operative surgical procedures and post-partum mothers (Mat Jais et al., 1994; Mat Jais et al., 1997). Thus, CSE may aid in reducing the effect of pruritus that causes redness on the skin besides helping to retain skin structures.

TNF-α is a primary inflammatory cytokine and will increase when there is a pain sensation. It has been postulated that both itch and pain have a similar mechanism of action as both are involved in the action of afferent C-fibers (McMahon & Koltzenburg, 1992; LaMotte et al., 2014). Thus, in this experiment, both vehicle and negative control groups should express the highest reading of TNF-α, while its expression is vice versa for the positive group and treatment groups **(Figure 6)**. In contrast, the highest concentration of CSE cream, 10% of the concentration, showed the highest reading. This result may occur due to this specific type of itch, as only two random representatives from each group were collected for this screening test. Therefore, the result is inconclusive in proving that a high CSE cream concentration exhibits the lowest TNF-α level expression in SCD-itch mice. However, due to the high sensitivity of the real-time PCR, this finding have to be interpreted with caution, and more technical replication will produce more reliable data.

In the future, more parameters should be measured to understand better the antipruritic properties and their relation to pain and its association with CSE at different concentrations. The study should include histological findings to determine the gross alteration of skin structure, amounts of specific parameters caused by chronic and acute itch models and its association with CSE.

5.0 CONCLUSIONS

Our results demonstrate that topical *Channa striatus* extract (CSE) cream has ability to alleviate pruritus (SCD-itch) scratching time and improve the skin conditions depending on the parameter concentrations, thus suggesting that it may be an effective treatment option for atopic dermatitis and various types of inflammatory skin diseases.

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and AA All authors have read and agreed to the published version of the manuscript.

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