Efficacy of Snakehead Fish (*Channa striatus*) Extract Supplementation in Allergic Rhinitis Patients: A Randomized Double Blind Placebo Controlled Trial

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ABSTRACT

**Objective:** The purpose of this study is to determine the effect of CS on Total Nasal Symptoms Score (TNSS), serum eosinophil and interleukin-4 (IL-4) in patients with allergic rhinitis.

**Design:** Randomized double-blind placebo controlled study

**Material and methods:** A 6-week, randomized, double-blinded placebo-controlled, parallel group comparative study was conducted. The patients received 500mg/day of CS extract or placebo. Patients were assessed at weeks 0, 2 and 6 for AR symptoms using TNSS. Serum eosinophil and IL-4 were taken at the first and last visit.

**Results:** A total of 54 patients were recruited. However, 46 patients completed the trial. There was no significant reduction of TNSS score, serum eosinophil and IL-4 in CS compared to the placebo group (p < 0.05). Within group analysis reported a significant decrement of TNSS, serum eosinophil and IL-4 in the CS group (p < 0.05) at week 6. However, only a significant decrement of TNSS was observed in the placebo group.

**Conclusion:** Adjunctive supplementation of CS accelerated the beneficial therapeutic effect in AR patients.

KEY WORDS

allergic rhinitis, allergy, *Channa striatus*, snakehead fish

INTRODUCTION

Globally, the prevalence of Allergic Rhinitis (AR) is increasing and becoming a major concern due to the increasing health care cost\(^1\). Allergic rhinitis occurs due to an Immunoglobulin E (Ig E) - mediated inflammatory reaction and eosinophil infiltration following exposure of the nasal mucosa to an allergen\(^2\). Cells mediators, cytokines, chemokines, neutrophides, as well as adhesion and molecules cells cooperate in a complex network provoking symptoms of nasal hyperreactivity\(^3\). Its symptoms are comprised of sneezing, rhinorrhea and nasal obstruction. Patients may have impairments of Quality of life (QOL) due to sleep disorders, emotional problems and from impairment in activities functioning\(^4\).

Characteristic history, skin prick test and serum allergen specific Ig E give the best predictive value in diagnosing AR\(^5\). However, many studies have investigated the diagnostic value of other allergy markers such as eosinophil count and eosinophil cationic protein (ECP). These markers have been reported to be useful for the diagnosis and prediction of severity of AR\(^6\). A study to identify the cut-off values for the numbers of eosinophil count for use in the diagnosis of AR in the absence of other allergic diseases had been conducted\(^6\). They reported the cut-off values in serum sample which is 4.0% for the eosinophil count. The sensitivity, specificity and odds ratio of eosinophil count are 57.5%, 72.0% and 3.47\(^6\). Various studies have used eosinophil counts as one of the parameters to assess effectiveness of interventions in clinical trial\(^7\).

Interleukin 4 (IL-4) is one of the cardinal cytokine in driving sensitization to allergens in AR\(^8\). IL-4 plays a central role in the IgE synthesis, the development of Th-2-like cells, and co-ordination as well as the persistence of airway inflammatory process in allergic disorders\(^8\). The decrease in IL-4 is reported to be correlated with the decrease of specific IgE antibodies following long-term immunotherapy\(^8\).

Recently, there has been proliferation of information regarding complementary and alternative medicine (CAM) practices especially in AR\(^9\). This growing interest is due to the fact that conventional medicine has limitations and "belief" that CAM have some clinical benefits with
mineral or no harm to the health\(^9\). A survey by the American Academy of Allergy, Asthma and Immunology members reported that 81% of respondents had patients who use CAM and herbal medicine is the most common of CAM used, followed by vitamins, probiotics and fish oil supplements\(^9\).

*Channa striatus* (CS) is a freshwater fish found in South-east Asia countries, which is popular as food and for its medicinal properties, such as enhancing wound healing, relieving pain, and boosting energy in the sick\(^2\). Literature review disclosed that this natural remedy has the potential to promote wound healing, and act as anti-inflammatory and anti-nociceptive properties\(^4\). Biochemical analysis of the fish reported that it contained 17 essential amino acids and polyunsaturated fatty acids\(^16\). The amino acids that are present include glutamic acid, aspartic acid, glycine and lysine\(^16\). CS also contains eicosapentaenoic Acid, docosahexanoic acid (DHA) which could have contributed to its anti-inflammatory action\(^11\). Studies on CS demonstrated that its anti-inflammatory activity acts through the inhibition of various media - tors such as Prostaglandin E2\(^7\), Tumour Necrosis Factor (TNF)-\(\alpha\), Interleukin (IL)-10\(^18\) and interferon\(^\gamma\). Based on this activity, it is used in ameliorating knee osteoarthritis (OA) which has been explored both in vivo and in clinical trials\(^7\)\(^18\).

In view of CS anti-inflammatory properties, this study was conducted to investigate its therapeutic potential and its immune-modulatory effect in allergic rhinitis patients. Hence, this study aims to investigate the effectiveness of CS extract as a supplementation on nasal symptoms, serum eosinophil and interleukin-4 (IL-4) in allergic rhinitis patients.

### METHODOLOGY

#### Study design and patient selection

A double-blinded, randomized, placebo controlled study was conducted among AR patients at the Otorhinolaryngology Clinic, Universiti Sains Malaysia from February until June 2014. All procedures performed in this study were in accordance with the ethical standards of the Human Research Ethics Committee Universiti Sains Malaysia [Ref. no: USM / JEPeM / 273.3(9)] and with the Helsinki Declaration of 1975, as revised in 2008. The written informed consent was obtained from all participants in the study.

Eligible participants are both males and females aged between 18-50 years old, diagnosed with AR based on clinical examination, who are tested positive with skin prick test and were treated with intranasal corticosteroid. The exclusion criteria were as follows: patients with concurrent rhino-sinusitis or other nasal pathology, history of nasal surgery, disabling co-morbid condition such as severe hematologic disorders, renal disease, liver disease, neoplasms or nursing mothers, history of allergy to Channa, history of surgical intervention for conditions related to AR, currently taking systemic corticosteroid or adrenergic therapy.

#### Study Intervention

Eligible patients were invited for a screening visit where history taking, clinical assessment and skin prick test for allergic rhinitis were performed. Those who were on oral anti-histamine were asked to stop taking their medication at least two weeks prior to participation in this study as a wash-out period. Those who had consumed CS regularly or taken traditional medication were asked to withhold their intake 1 month prior to the study. Patients who fulfilled the criteria were randomized at a ratio of 1:1 to 500 mg/day oral CS or placebo group by using a computer-generated table in blocks of four. The allocation concealment was minimized by labelling the bottle containing the investigational products with the randomization numbers and the subjects were prescribed investigational product sequentially following the time of participation in the study. Only one co-investigator who prepared the product knew of the randomization scheme, and the blinding was maintained throughout the study until the last patient follow-up. The patients were instructed to take the treatment for the duration of 6 weeks. The CS extract and the placebo were available as 250-mg capsules and were identical to ensure proper blinding. Patients were instructed to take four capsules per day, irrespective of their groups. Both also were also treated with intranasal corticosteroid, mometasone furoate at a dose of 200mcg daily.

Clinical visits were carried out at week 2 and week 6 after the treatment was initiated. The patients were assessed for nasal symptoms using Total Nasal Symptoms Score (TNSS) at each visits. Total Nasal Symptoms Score is based on four main nasal symptoms of allergic rhinitis which are nasal obstruction, nasal itchiness, rhinorrhea and nasal itching\(^19\) with each individual symptom rated on a 4-point scale (0 = none, 1 = mild, 2 = moderate, 3 = severe)\(^20\). This nasal score had a minimum score of 0 and maximum total score of 12. Blood samples for eosinophil count and IL-4 were taken at the first visit and after 6 weeks of treatment.

#### Skin prick test

Skin prick test were performed on the volar side of the forearm using the commercially available kit (ALK-Abello, Madrid, Spain). The extract of the allergens were applied in droplets on the skin with appropriate interval around 1.5cm from each other. The skin was then pricked through the droplets using separate lancet on each allergen. The reaction was observed after 10-15 minutes, and was considered positive if the measured diameter was equal or larger than the control (histamine) or

### Table 1. Demographics and baseline characteristic of the subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CS group Mean (SD) or (%)</th>
<th>Placebo group Mean (SD) or (%)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36.2 (10.7)</td>
<td>33.2 (9.1)</td>
<td>0.300</td>
</tr>
<tr>
<td>Female</td>
<td>66.7</td>
<td>68.2</td>
<td></td>
</tr>
<tr>
<td>Eosinophil count (x10^6/L)</td>
<td>0.35 (0.13)</td>
<td>0.33 (0.14)</td>
<td>0.710</td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>1.13 (0.99)</td>
<td>0.75 (0.55)</td>
<td>0.110</td>
</tr>
<tr>
<td>TNSS</td>
<td>7.63 (1.91)</td>
<td>6.86 (1.86)</td>
<td>0.180</td>
</tr>
</tbody>
</table>

*Independent T test

### Table 2. Mean difference of the outcomes of the study from baseline to week-6 between the treatment groups.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Placebo Mean (SD)</th>
<th>CS 500mg Mean (SD)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNSS</td>
<td>1.13 (0.99)</td>
<td>1.79 (1.35)</td>
<td>0.070</td>
</tr>
<tr>
<td>Serum Eosinophil count (x10^6/L)</td>
<td>0.02 (0.05)</td>
<td>0.05 (0.10)</td>
<td>0.230</td>
</tr>
<tr>
<td>Serum interleukin-4 level (pg/ml)</td>
<td>-0.03 (0.34)</td>
<td>0.12 (0.56)</td>
<td>0.280</td>
</tr>
</tbody>
</table>

*p value from Independent T test

### Table 3. Comparison of TNSS, serum Eosinophil count and IL-4 in the CS and placebo groups at baseline and end of supplementation period (week – 6)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Placebo Mean (SD)</th>
<th>CS Mean (SD)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNSS</td>
<td>6.86 (1.86)</td>
<td>7.60 (1.90)</td>
<td>5.80 (1.04)*</td>
</tr>
<tr>
<td>Serum eosinophil count (x10^6/L)</td>
<td>0.33 (0.14)</td>
<td>0.35 (0.14)</td>
<td>0.30 (0.12)*</td>
</tr>
<tr>
<td>Serum interleukin-4 level (pg/ml)</td>
<td>0.75 (0.55)</td>
<td>1.13 (1.00)</td>
<td>1.01 (0.62)*</td>
</tr>
</tbody>
</table>

Paired T test, *p < 0.05

### Table 4. Biochemical safety analysis

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Baseline Mean (SD)</th>
<th>Post-treatment Mean (SD)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (iU/L)</td>
<td>23.58 (1.50)</td>
<td>22.16 (1.21)</td>
<td>0.170</td>
</tr>
<tr>
<td>ALT (iU/L)</td>
<td>22.33 (1.99)</td>
<td>20.96 (2.00)</td>
<td>0.170</td>
</tr>
</tbody>
</table>

*p value from Paired T test

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The dose of CS was based on previous clinical trials conducted which reported a dose of 500mg/day which was effective and safe. The CS extract preparation has been reported in literature. The orally administered freeze dry CS extract, and the placebo (maltodextrin) was provided by the School of Pharmacy, Universiti Sains Malaysia.

### Outcome measurement

The main outcome measures of the study were change of the AR symptoms score, serum IL-4 and eosinophil count from baseline to week 6. The secondary outcome measure was a change of the AR symptoms score according to the division of symptoms intensity. The patients were divided into three groups according to their nasal symptoms score with TNSS of 0-2 points which was considered to be very mild symptoms, 3-6 points was considered as mild, 7-9 points was considered as moderate and 10-12 points was considered as severe.

Blood for eosinophil count was analyzed using the automated blood analyzer (Sysmex XE-5000, USA). The serum level of IL-4 was determined with the already commercially available ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the company instruction.

### Assessment of safety

The assessment of safety was based on adverse events and biochemistry parameters (creatinine, aspartate transaminase (AST), and alanine transaminase (ALT)) tests that were conducted at baseline and final visit. If any of these analyzed parameters are abnormal (urea and creatinine > normal values; AST and ALT > 3X of the upper limit of normal values), the patients will be informed and withdrawn from the study.

### Statistical analysis

Analyses were performed using SPSS for Windows version 20.0. Randomized groups were compared for any possible differences at the baseline using independent T test for numerical data and chi-square test for the categorical data. The calculation for mean difference of the AR symptoms score, serum IL-4 and Eosinophil count from baseline to week 6 between the CS and control groups were done using independent T test (if normal distribution was confirmed by Shapiro-Wilk's test) or its equivalent nonparametric test (Mann-Whitney U test). Changes of AR symptoms score, serum IL-4 and Eosinophil count from baseline to week 6 within the CS and placebo group was analysed with paired T test. The value of p < 0.05 was considered statistically significant.

### RESULTS

In the study period between February 2014 and October 2014, a total of 54 patients were recruited and randomized into two groups, to receive either CS extract (n = 28) or placebo (n = 26). Out of 54 patients recruited, 8 of them failed to complete the study (4 in CS group and 4 in placebo group) due to their noncompliance (n = 7) and involvement in motor vehicle accidents (n = 1). Both groups were comparable in the demographic data (Table 1).

Table 2 showed the mean difference of TNSS, serum Eosinophil count and IL-4 from baseline to week 6 between the treatment groups. There was no significant difference in the change of TNSS, eosinophil count and serum IL-4 for CS and placebo groups. There was a significant difference in their nasal score. (p = 0.03 and p = 0.04 respectively).

Table 3 showed the changes of serum Eosinophil count and IL-4 and TNSS in CS and placebo at baseline and end of supplementation period (week 6). The supplementation of CS significantly decreased serum Eosinophil count and IL-4 and TNSS at week 6 compared to baseline (p < 0.05). However, in the placebo group, only TNSS significantly increased at week 6 compared to baseline (p < 0.05).

Table 4 shows a comparison of safety biochemical parameters among the treatment groups. No significant differences in the laboratory parameters were observed at baseline and post-treatment, and all parameters were within normal limits. There was no side effects reported in this study.

### DISCUSSION

AR sufferers generally endure the disturbing symptoms almost every day, and it has a serious impact on their life quality. It costs a burden in their social life and financial aspect. The results of this study demonstrated that within the CS group, the nasal symptoms and inflammatory markers showed significant changes between the baseline and week 6. Compared to the placebo group, only nasal symptoms was significant. However, when compared between the two groups, the results did not show significant changes.

The results of this study were parallel to the study done in post caesarian women which showed the administration of the CS which caused a significant reduction in high sensitivity C-Reactive Protein (p = 0.001) and total white cell count (p < 0.001). A randomized, double-blind study to assess the effect of CS extract supplementation for 12 weeks among pulmonary TB patients also reported significant reductions of TNF-α, IFN-γ, and IL -10 at week 12 compared to the baseline in the CS group. In both studies, it was reported that the inflammatory markers reduction was more pronounced in the CS group but did not reach a statistically different variation in comparison with placebo groups.

The results of this study revealed that the CS extract has some immunomodulation effects on the production of inflammatory markers such as IL-4 and Eosinophil count. There are a few reasons for the insignificant results in this study. One of the reasons is that both intervention and control groups had low level of serum interleukin-4 and eosinophil count at baseline. Thus, not much changes can be observed at week 6. In this study, serum interleukin-4 level was chosen as an outcome because it represents immune-modulation in both early and late phase reaction. Notably, serum eosinophil represents immune-modulation in the late phase reaction and systemic activation in allergic rhinitis. The mean value of baseline serum eosinophil in this study was in agreement with other studies. The mean baseline serum eosinophil in this study was between 330-350 cells/μl. In India, it was reported that the mean serum was 250 cells/μl and 650cells/μl in China. However, the mean baseline serum IL-4 in this study was low compared to other studies in AR patients. The mean serum IL-4 in this study was almost similar to the normal population.

These significant results may be explained by the use of intra nasal steroid treatment. Even if the systemic bioavailability of mometason furoate nasal spray is less than 0.1%, we still could not exclude it as the reason for the low IL-4 and Eosinophils levels. There are evidence that the absorbed corticosteroid could affect the production of cytokines. The use of corticosteroids has been proven to involve in the inhibition of transcription of various cytokines such as IL-1, IL-3, IL-4, IL-5, IL-6, IL-8, TNF-α and IFN-γ.

For the subjective measurement of the effect of the CS extract on allergic rhinitis patients, there was a greater reduction of nasal symptoms in CS compared to placebo group. However, it was not significant. Further analysis disclosed that patients with moderate and severe symptoms expressed a significant reduction in their nasal score but not for those with mild symptoms. This is probably because, those with mild symptoms at the baseline had low nasal score. Thus, no significant difference was noted.

Nutraaceuticals and functional foods such as CS contain multiple active compounds that target multiple pathways compared to pharmacological treatments that usually have a single mode of action. This could be the reason for their partial lack of clinical efficacy in AR. This is because a nutritional compound has limited effects on its biological target and significant differences are reached over time through a build-up effect in which daily benefits add up day after day. This study is of a very short duration of six weeks. The time window for the CS extract is probably longer which is similar to the other time window for intervention using nutraaceuticals and functional foods.

The CS have N-3 fatty acids such as eicosapentaenoic Acid (EPA),...
and docosahexanoic acid (DHA). Studies have showed that the DHA and EPA have a modulating effect on the eosinophil chemotactic and chemokinetic response to Leukotriene B4 (LTB4)19). In the human body, the ingested DHA and EPA will be integrated into neutrophil and monocyte cell membranes and change its arachidonic acid make-up, hence inhibit the LTB4, formation from arachidonic acid. The LTB4, action in AR is related to its role in inducing Ig E production by mononuclear cells and E-cells. LTB4 also plays its role in allergic rhinitis by becoming a chemo-attractant to the eosinophils20).

There has been a considerable debate in the use of omega 3 fatty acids for the prevention and treatment of symptoms of eczema, rhinitis and asthma21). Some studies demonstrated its benefit while several others concluded that it was non-beneficial. On the other hand, there are only a little evidence to recommend patients with allergic disorders and to include a high amount of omega-3 in their diet in order to control their symptoms22).

However, there are other possible mechanisms for the CS anti-inflammatory effect. The CS extract has fatty acids such palmitic acid, stearic acid and linoleic acid23). The effects of linoleic acid on COX-2-catalyzed prostaglandin biosynthesis had been reported24). Oleic and linoleic acid supplementation had been shown to suppress the activity of cyclooxygenase synthase in 30 healthy volunteers25).

There are a few limitations in this study. The number of subjects is small, the study duration is short and the number of biomarkers is limited. It is suggested that further studies with bigger sample size, longer duration and the use of more inflammatory markers can be conducted.

CONCLUSION

The results of this study suggested that adjunctive supplementation of CS extract did show an additional immune-modulation effect in AR patients. Larger clinical studies are required to verify these initial results.

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